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Validation and Monitoring of Carminic Acid using HPLC-DAD and LC-MS/MS in Processed Foods

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ABSTRACT - Carmine and cochineal extract are vibrant red colored pigments derived from cochineal insects, containing carminic acid as their primary component. These colorants are commonly used in widely consumed food products including candies and fish cakes. Carminic acid has recently been associated with allergic reactions linked to specific proteins. This study aimed to develop and authenticate a method for quantifying carminic acid using high-performance liquid chromatography with a diode array detector (HPLC-DAD) and a C18 UG120 column. Conditions included a water-trifluoroacetic acid (TFA) 0.1% (A) and acetonitrile-TFA 0.1% (B) mobile phase, with a flow rate of 1.0 mL/min and a column temperature of 30°C. Calibration curves (0.2-50 mg/L) show good linearity ($r^2 \geq 0.9999$). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) facilitates qualitative analysis, with a 0.05 mg/kg limit of detection (LOD) and a 0.15 mg/kg limit of quantification (LOQ). Intra-day and inter-day measurements exhibited accuracy (87.3-97.1%, recovery) and precision (0.48-8.90%, relative standard deviation, RSD. Measurement uncertainty was also estimated. The developed method is applicable for the effective monitoring of carmine and cochineal extracts in diverse food types, this is crucial for understanding and mitigating the potential health concerns associated with carminic acid.

Key words: Carminic acid, Food additive, Measurement uncertainty, Validation

Recently, research papers related to the quantitative analysis, intake evaluation, and exposure assessment of food additives in various foods have been continuously published. These results serve as a scientific basis for the development of analytical methods, the precise analysis of food additive compounds in processed foods, and the establishment of usage standards[1-3].

Cochineal extract and carmine are added as food additives in various crimson-colored foods, including beverages, cakes, sausages, fish cakes, macaroons, Campari, strawberry milk, snacks, and strawberry jam[4,5]. Carminic acid, with a molecular formula of $C_{22}H_{20}O_{13}$, is the primary component of cochineal extract and carmine, which are anthraquinone-based pigments[6]. When quantifying these two items, cochineal extract contains more than 1.8% carminic acid, and carmine contains more than 50.0% carminic acid based on Korean food additive codes[7].

The main ingredient of cochineal extract and carmine is derived from the dried female insects of cochineal (Coccus cacti), which are parasitic on the cactus *Nopalea cochenillifera*[8]. *Dactylopius coccus*, a species of scaly insect, predominantly feeds on the cladodes (leaves) of Opuntia cacti and is found in South America[9].

The reference standard was set at 5 mg/kg or less by the European Food Safety Authority[10] and also by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives[11]. The committees inferred that cochineal extract, carmine, and carminic acid in food could potentially cause allergic reactions in some individuals. In the United States, according to regulations established by the Food and Drug Administration (FDA)[12], cochineal extract and carmine are not subject to certification and can generally be safely utilized in colored foods (21 CFR 73.100), ingested and externally applied drugs (21 CFR 73.1100). Additionally, carmine is generally authorised for use in cosmetics, including formulations designed for use in the ocular region, in compliance with good manufacturing practices (GMP).

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In analytical technology, UV-Vis spectrometry[13], ultra performance liquid chromatography (UPLC)[14], H-quantitative nuclear magnetic resonance spectroscopy (H-qNMR), and HPLC with a photodiode array detector (PDA) and ultraviolet (UV)[15], HPLC with PDA[16], enzyme-linked immunosorbent assay (ELISA) and HPLC[17], HPLC-MS/MS (Lech et al., 2015), UPLC-MS/MS[18], fourier transform infrared spectroscopy (FT-IR) in attenuated total reflectance (ATR) mode, and ultraviolet-visible spectroscopy (VIS)[19] were found to be useful in analyzing carminic acid.

The UPLC method, developed by Taujenis and Olšauskaitė[14], used a binary mobile phase (0.1% TFA in water, 0.1% TFA in acetonitrile) and was performed as a gradient method with a runtime of 6 minutes. The HPLC method, as described by Nishizaki et al.[15], utilized a mixture of water, methanol, and TFA (600:400:1, v/v/v) for the isocratic method and had a runtime of 20 minutes. Additionally, Lech et al.[20] employed the HPLC-MS/MS method with (A) 1.5% (v/v) formal acid in water and (B) methanol in a 30-minute runtime.

Therefore, this study aimed to validate an commonly available HPLC-DAD analysis method for quantifying carminic acid in both liquid and solid (fat-containing) food matrices. The objectives of the study were 1) to improve an HPLC-DAD method for quantifying carminic acid in food, 2) to estimate measurement uncertainty, and 3) to employ the validated analytical method to quantify carminic acid content a wide variety of food products to confirm its applicability.

Materials and Methods

Chemicals and reagents

The carminic acid standard (≥95%; CAS No. 1260-17-9) was procured from Sigma-Aldrich (Saint Louis, MO, USA). All other chemicals used in the preparation of the mobile phase and extraction, such as trifluoroacetic acid (TFA) (99%) and formic acid (98.2%), were also obtained from Sigma-Aldrich. Acetonitrile, water, methanol, and hexanes (95% n-hexane) were purchased as HPLC-grade solvents from JT Baker Chemical Co. (Radnor, PA, USA). Hydrochloric acid (36.1%) was supplied by JUNSEI (Tokyo, Japan).

Sample collection

Samples were collected by assigning weights to each food type to ensure a statistically significant sample size based on data from Production performance of food, etc. 2018 to 2020 (MFDS&NFSI)[21], Product Manufacturing Report and Import Declaration Form (MFDS&NFSI)[22], the raw data supporting the high-frequency food types calculated using the 8th KNHANES for the years 2019 to 2021 (KHIDI)[23]. Processed foods available in the market were randomly sampled, and specifically those identified with labelling indicating the presence of the target food additives were included for analysis. In the case of the samples, all of them included carmine or cochineal extract as food additives, falling within the categories of food types permitted to use these food additives according to the Korean food additive codes[7].

For sample monitoring using the proposed method in this study, a total of 162 food products, including 39 candies, 9 fruit/vegetable beverages, 12 confectioneries, 11 other beverages, 12 bacons, 18 sausages, 11 seasoned jeoktal, 20 hams, and 30 surimi, were obtained either from a local marketplace or through online channels. All samples contained carmine or cochineal extract as food additives. After the samples were collected, they were homogenized and stored at -20ºC.

Preparation of standard solution and samples

Preparation of standard solution and samples for HPLC-DAD analysis

The preparation of standard solutions and samples followed procedures outlined in previous publications[14,24] with some modifications. The carminic acid stock solution (500 mg/kg) was prepared by transferring 10 mg of carminic acid into a 20 mL volumetric flask and adding a solution composed of 50% methanol in water and 10% hydrochloric acid in water (4:1, v/v) to achieve a final volume of 20 mL. Calibration standard solutions with varying concentrations of carminic acid (0.2-50 mg/kg) were then prepared by successive dilutions using the same solution.

For each homogenized food sample (approximately 1 g), a 50 mL conical tube was used, and a solution of 50% methanol in water and 10% hydrochloric acid in water (4:1, v/v) was added to 20 mL. After mechanical shaking for 30 minutes (280 rpm), the sample was mixed with 10 mL of hexane. Following centrifugation of the extract at 5000 rpm for 5 minutes, the hexane layer was removed, the solution layer was decanted, and the remaining solution was filtered through a 0.45 μm syringe filter before undergoing HPLC analysis.

Preparation of standard solution and samples for LC-MS/MS analysis

For the qualitative analysis of carminic acid, LC-MS/MS was employed. The formulation of standard solutions and samples for LC-MS/MS analysis followed a procedure similar to that used for HPLC-DAD. The stock solution of carminic acid for 100 mg/kg was prepared by transferring 2 mg of carminic acid into a 20 mL volumetric flask. A solution composed of 50% methanol in water was then added to fill up a final volume of 20 mL. Optimization was conducted using a concentration of 1 mg/kg. Standard

solutions with concentrations ranging from 0.01 to 1 mg/kg of carminic acid were obtained through subsequent dilutions with the previously mentioned solution.

For each homogenized food sample (approximately 1 g), a 50 mL conical tube was used, and a solution of 50% methanol in 20 mL of water was added. After using mechanical shaker (MMV-1000W, EYELA, Tokyo, Japan) or 30 minutes at 280 rpm, the sample was combined with 10 mL of hexane. Following centrifugation for 5 minutes at 5000 rpm, the hexane layer was removed, the solution layer was decanted, filtered through a 0.22 μm syringe filter, and subjected to LC-MS/MS analysis.

Analytical instrument

HPLC-DAD analytical instruments

The HPLC analytical methods were implemented following the procedures outlined by Taujenis and Olšauskaitė[14] and Kunkely and Vogler[24] with some modifications. Samples were analyzed using an Agilent 1200 series HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a DAD detector, column compartment, pump, and autosampler. Analytes were separated on a Capcell Pak C18 UG120 column (250 mm×4.6 mm, 5 μm, OSAKA-Soda, Osaka, Japan) maintained at 30°C. The wavelength was monitored at 280 nm, and the mobile phase consisted of water with 0.1% TFA (A) and acetonitrile with 0.1% TFA (B). The gradient program was set as follows: 0-6 min, 90% A; 6-28 min, 90-50% A; 28-28.1 min, 50-20% A; 28.1-37 min, 20% A; 37-37.1 min, 20-90% A. The injection volume and flow rate were 10 μL and 1.0 mL/min, respectively. Carminic acid was identified by examining absorption spectra and comparing retention times with the standard solution.

LC-MS/MS analytical instruments

For The LC-MS/MS analytical methods were employed in accordance with the procedures outlined by Yang, et al.[25], with some modifications. The samples conducted analysis using a Thermo Scientific Vanquish system coupled with a TSQ Quantis Mass Spectrometer (Thermo Fisher Scientific, Germering, Germany). Separation of analytes was carried out on a Unison UK C18 column (100 mm×2.0 mm, 3 μm, IMTAKT, Kyoto, Japan) at a temperature of 30°C. The binary mobile phases consisted of 0.1% formic acid (FA) in water (A) and 0.1% FA in acetonitrile (B). The gradient elution profile was as follows: 0-1 min, 5% B; 1-3 min, from 5% to 70% B; 3-4 min, from 70% to 90% B; maintaining 90% B for 6 min; 6-6.5 min, from 90% B to 5% B. The gradient was delivered at a flow rate of 0.2 mL/min, and the injection volume was set at 5 μL.

Peaks were subjected to tandem mass spectrometry (MS/MS) analysis using highly-selective reaction monitoring (H-SRM) mode. Data for carminic acid were collected using selected reaction monitoring with the following transitions in negative ion mode: m/z 491 (RF lens; 226 V) to 447 (collision energy; 19 V), 327 (collision energy; 27 V), and 357 (collision energy; 26 V). Among precursor ions, m/z 447 was selected as the quantitative ion and m/z 327 and 357 used as the qualification ions. The optimized parameter settings for H-SRM were as follows: spray voltage -2500 V; sheath gas pressure 50; aux gas pressure 10; sweep gas pressure 1; ion transfer tube temperature 300°C; and vaporizer temperature 350°C.

Method validation

The suggested method was employed to validate its applicability for single-laboratory (in-house) usage, in accordance with FDA, International Conference on Harmonization (ICH) guideline Q2 (R1)[26], and the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) guideline[27]. The specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision were determined.

Specificity is accurate measurement of the analyte in the presence of interference, such as excipients, synthetic precursors, known (or possibly) degradation products, and enantiomers that may be included in the sample matrix. By comparing spectrum and chromatogram of blank and standard solutions, it was confirmed the method's suitability for the specific determination of carminic acid.

Linearity refers to a linear measurement within a specific range in correlation with the concentration or quantity of the compound you want to analyze and is assessed based on function of the concentration or content of the analyte. In this experiment, a calibration curve using a regression line (y=ax+b) was drawn using a peak area values of six concentrations within the specified range of 0.2-50 mg/L, with seven replicates and linearity is evaluated with a regression coefficient (r^2).

LOD refers to the minimum amount or concentration of the compound to be analyzed present in the sample. LOQ means the minimum amount or concentration of the compound to be analyzed in samples that can be expressed as quantitative values with appropriate precision and accuracy. LOD and LOQ were determined through the repetitive analysis of the three lowest concentrations of calibration curves, prepared in accordance with ICH Guideline Q2 (R1)[26], seven times. The computation involved dividing the standard deviation (SD) of the y-intercept of the calibration curve by the mean value of the slope. Subsequently, LOD and LOQ were obtained by

multiplying the result by 3.3 and 10, respectively.

Accuracy, in this study, is defined as the closeness of measurements to established or known values. Precision refers to the closeness or dispersion among individual measurements when evaluating a sample obtained by repeatedly collecting a uniform sample under constant conditions. It is evaluated using statistical methods such as SD and relative standard deviation (RSD%).

Accuracy and precision are verified through repeatability, which involves repeated measurements at short intervals under the same. Candies and surimi were chosen as the matrices because both 'cochineal extract' and 'carmine' are commonly used samples. Candy is high in sugar, and surimi is a protein and fat-rich sample, which was thought to be sufficient to represent the samples. The compounds under analysis, containing three concentrations, were assessed by calculating the RSD of the recovery rates for both intra-day and inter-day analyses. Six replicates were performed for one day of intra-day precision. Three replicates were conducted for three days of inter-day precision[27].

Inter-laboratory validation

Inter-laboratory validation was conducted to assess accuracy and precision. This involved comparing the results obtained from analyzing an identical candy sample across three different laboratories (Lab A, Lab B, and Lab C) using the same analytical method. For a recovery experiment, 1, 5, and 10 mg/kg of carminic acid standard were added to the candy sample. The recovery rate and RSD% were determined through three repeated experiments, confirming the accuracy and precision of the analysis.

Measurement uncertainty estimation

The concept of measurement uncertainty, as outlined in the ISO/IEC 17025:2017 standard[28] titled 'General Requirements for

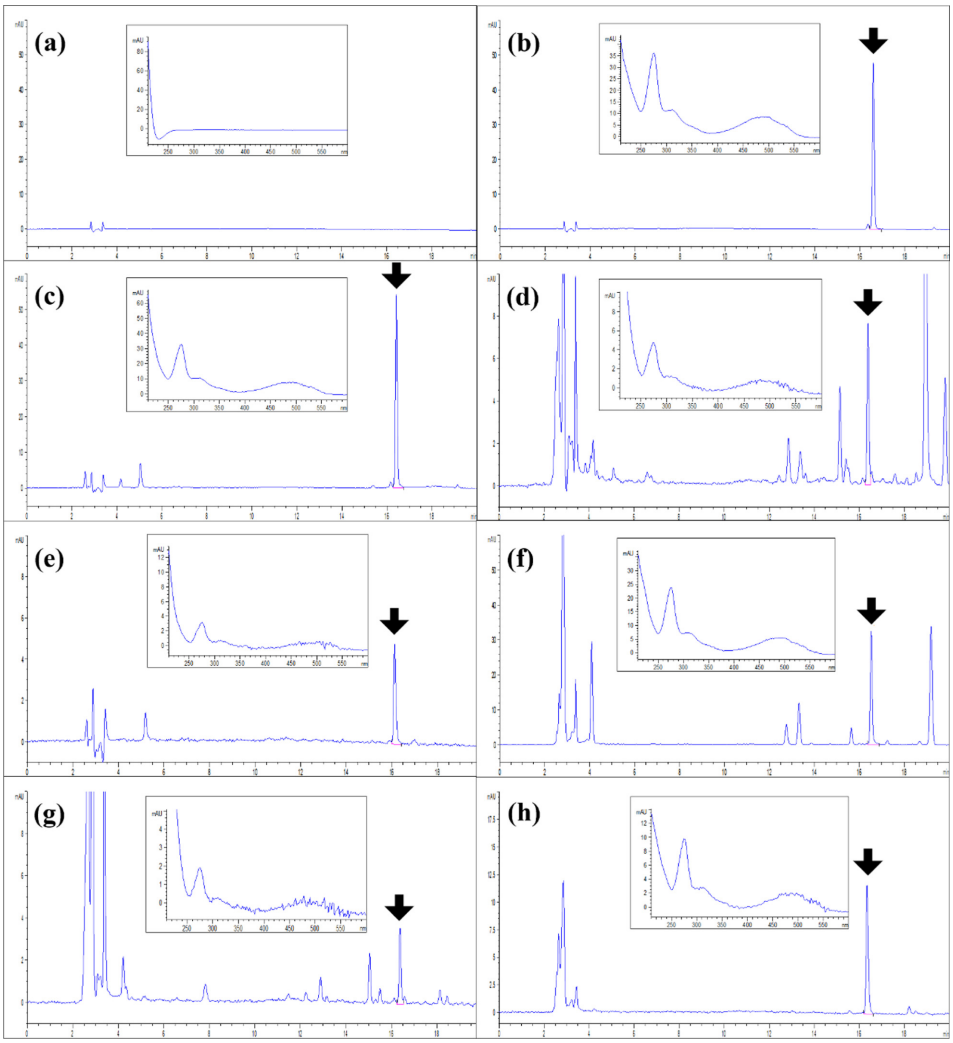

Fig. 1. Chromatograms using HPLC-DAD of the (a) Blank, (b) 10 mg/L carminic acid standard solution, (c) Candies, (d) Confectioneries, (e) Other beverages, (f) Seasoned *jeotgal*, (g) Hams, and (h) Surimi.

the Competence of Testing and Calibration Laboratories,' pertains to the degree of variation in values that can reasonably be attributed to the measured value. The assessment of uncertainty values involves comparing inter-laboratory data, and measurement reliability is established based on the outcomes of this comparison. In this study, uncertainty estimation utilized statistical methods and mathematical processing, as referenced by EURACHEM (A Focus for Analytical Chemistry in Europe) [29]. Measurement uncertainty was assessed for standard stock solution preparation (uSSS), calibration curve (uCal.), sample preparation (uSP), and repeated measurement of samples (uRP). Additionally, the expanded uncertainty (Uc) was estimated by applying the coverage factor (k) of 2 at the 95% confidence level.

Results and Discussion

Method validation

Specificity

In Fig. 1, a comparison was made among the chromatograms of the blank, carminic acid standard solution (10 mg/L), and the samples. Specificity was confirmed by validating that no interfering compound was observed at the peak retention times of carminic acid in each sample. The retention time of carminic acid was 16-17 minutes, respectively.

Furthermore, chromatograms obtained through LC-MS/MS analysis of the carminic acid standard solution (0.1 mg/L) and samples were compared in Fig. 2. Specificity was confirmed by verifying the absence of any interfering compounds at the peak retention times corresponding to carminic acid in each sample. The retention time for carminic acid was determined to be 4.5 minutes, respectively.

Linearity, LOD, and LOQ

Calibration curves were established by conducting repeated analyses (six times) of carminic acid at six concentrations ranging from 0.2 to 50 mg/L. The coefficient of determination (r^2) is 0.9999, meeting the minimum

Table 1. Calibration parameter results of carminic acid

Parameters	Carminic acid
Range (mg/L)	0.2-50
Coefficient of determination (r^2)	0.9999
Slope	30.72
Intercept	-1.20
LOD[1] (mg/L)	0.05
LOQ[2] (mg/L)	0.15

[1] Limit of detection, [2] Limit of quantification.

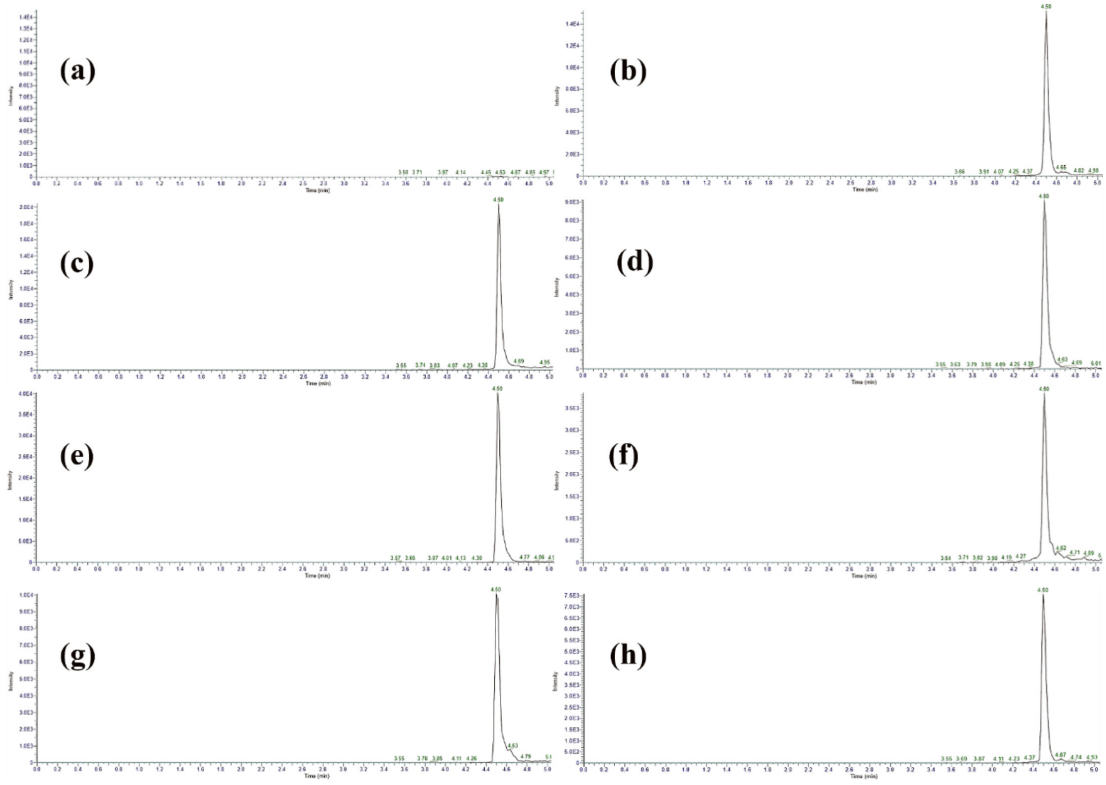

Fig. 2. Chromatograms using LC-MS/MS of the (a) Blank, (b) 0.1 mg/L carminic acid standard solution, (c) Candies, (d) Confectioneries, (e) Other beverages, (f) Seasoned *jeotgal*, (g) Hams, and (h) Surimi.

standards set by the FDA of ≥0.995[30]. The LOD and LOQ were determined to be 0.05 and 0.15 mg/L, respectively. These results align closely with the LOD and LOQ values of 0.4 and 1.0 mg/L for carminic acid reported in previous studies[31]. Detailed results of the calibration parameters are presented in Table 1.

Accuracy and Precision

Table 2 presents the results of intra- and inter-day accuracy and precision. The accuracy results for both intra- and inter-day assessments, determined by spiking candies and surimi with carminic acid (1, 5, and 10 mg/kg), were 90.8-97.1% and 87.3-96.7%, respectively. The precision results for both intra- and inter-day evaluations, calculated for the same analyte, yielded RSD values ranging from 0.48% to 8.90% and 0.63% to 3.03%, respectively. These results were within the acceptable range according to the AOAC guideline[27].

Inter-laboratory validation

The recovery rate experiment of a candy sample spiked with carminic acid was conducted in three laboratories, and the results were compared. The outcomes are presented as recovery±SD (%), and Table 3 includes the average results from each laboratory along with the corresponding precision (RSD). The recovery rates were 96.32-97.84% in Lab A, 95.56-97.64% in Lab B, and 93.17-97.27% in Lab C, with the corresponding precision ranging from 1.11% to 2.61% RSD, respectively. These results meet the standards outlined in the AOAC guidelines[27]. Moreover, the accuracy and precision of the suggested analytical method were confirmed through this validation procedure.

Measurement uncertainty

The evaluation of measurement uncertainty in this study was conducted through the recovery test. Uncertainty values related to uSSS, uSP, uCal, and uRP were taken into consideration. As indicated in Table 2, the expanded uncertainty ranged from 2.0% to 5.0% in candies and from 3.8% to 8.2% in surimi. The results adhered to the acceptable limit defined by the CODEX standard (≤22%)[32]. Among all the samples, the expanded uncertainty decreased with the rising concentration of carminic acid. The outcomes presenting the impact of measurement uncertainty contributions on the expanded uncertainty are illustrated in Fig. 3.

No substantial variance was noted in uSP and uSSS during the computation of factors influencing uncertainty for the spiking concentration of each sample. However, as the concentration of added carminic acid decreased, it was verified that the uncertainty of uCal was 3.26% in candies and 2.84% in surimi. Furthermore, it was confirmed that the uncertainty of uRP was 2.14% to 4.07% in surimi as the concentration of added carminic acid was lowered. Therefore, a greater level of proficiency is essential for the researcher when establishing the calibration curve at the lowest concentration during sample analysis.

Application

To validate its applicability, the suggested HPLC analysis method was employed to analyze various products labeled as ‘cochineal extract’ or ‘carmine’ distributed in Korea.

Table 2. Validation results of accuracy, precision, relative expanded uncertainty of carminic acid

Matrix	Added standard (mg/kg)	Intra-day[1]		Inter-day[2]		Relative expanded uncertainty (%)
		Accuracy[3] (%)	Precision (%RSD)	Accuracy[3] (%)	Precision (%RSD)	
Candies	1	95.6±0.5	0.48	96.7±1.4	1.48	5.0
	5	97.1±0.9	0.90	96.4±0.6	0.63	2.2
	10	96.2±0.7	0.75	96.4±0.6	0.63	2.0
Surimi	1	90.8±8.1	8.90	87.3±2.7	3.03	8.2
	5	93.9±4.5	4.79	91.7±2.4	2.65	4.4
	10	94.9±5.1	5.42	92.1±2.2	2.38	3.8

[1]Analysis was conducted six time/day, [2]Analysis was conducted three times on three days, [3]Average±SD.

Table 3. Inter-laboratory reproducibility results of carminic acid

Sample	Added standard (mg/kg)	Recovery±SD (%)			Average±SD (%)	RSD (%)
		Lab. A	Lab. B	Lab. C		
Candies	1	96.32±2.87	97.64±0.18	93.17±0.91	95.71±2.50	2.61
	5	96.57±0.40	95.56±0.40	93.83±0.11	95.32±1.23	1.29
	10	97.84±0.96	95.96±0.78	97.27±0.60	97.02±1.08	1.11

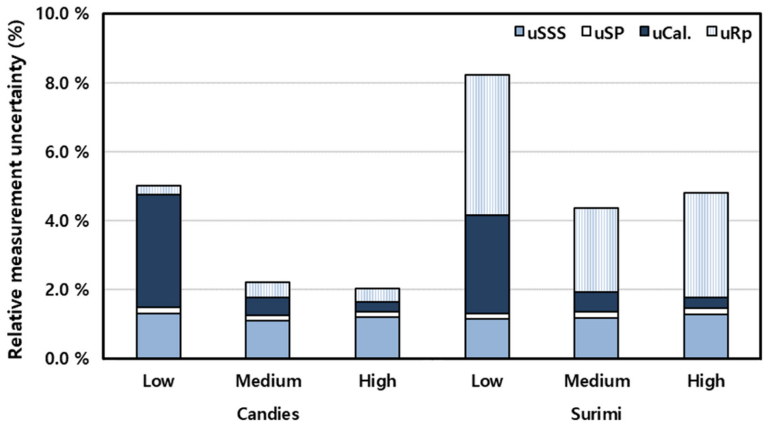

Fig. 3. Contributions of measurement uncertainty to the relative expanded uncertainty of carminic acid spiked in Candies and Surimi.

Table 4. Concentration (mg/kg) of carminic acid in foods

Food category	Total no. of sample	Positive no. of sample	Range		Total Average[1)] (mg/kg)	Positive Average[2)] (mg/kg)
			Min (mg/kg)	Max (mg/kg)		
Products cochineal extract labeled						
Candies	18	16	N.D.[3)]	638.16±0.68	51.44±147.58	57.87±155.84
Fruit/Vegetable beverages	9	9	0.23±0.01	6.63±0.12	3.87±1.58	3.87±1.58
Confectioneries	12	11	N.D.	33.75±0.53	13.89±12.16	15.16±11.89
Other beverages	11	10	N.D.	102.59±0.69	21.31±28.73	23.44±29.36
Bacons	12	10	N.D.	7.77±0.22	2.95±3.14	3.54±3.12
Sausages	18	14	N.D.	19.03±0.35	3.12±6.03	4.02±6.61
Seasoned jeoktal	11	11	3.27±0.21	151.54±0.68	20.05±43.80	20.05±43.80
Hams	20	15	N.D.	17.10±0.46	2.51±5.17	3.34±5.76
Surimi	10	9	N.D.	25.38±0.46	8.79±7.96	9.77±7.79
Products carmine labeled						
Candies	21	20	N.D.	239.36±2.65	64.22±60.93	67.43±60.66
Surimi	20	15	N.D.	61.16±1.08	7.39±13.52	9.86±14.90

[1)]Average and standard deviation of all samples, [2)]Average and standard deviation of detected samples, [3)]not detected (below LOD).

These products included candies, fruit/vegetable beverages, confectioneries, other beverages, bacons, sausages, seasoned jeoktal (Korean traditional food), hams, and surimi. Each sample underwent three repeated analyses, and the average value was obtained and calculated as the actual content (mg/kg). The results are presented in Table 4. Samples that were not detected were expressed as not detected (N.D.), and the experiment confirmed that 140 out of 162 samples were detected. Food samples labelled as containing carmine or cochineal extract were purchased; however, some samples were reported as "Not Detected". This classification as undetectable was due to the very low levels of carmine or cochineal extract present in the products, which were below the LOD when analyzed using HPLC-DAD.

Products labeled as 'cochineal extract', such as candies, fruit/vegetable beverages, confectioneries, other beverages, bacons, sausages, seasoned jeoktal, hams, and surimi, contained carminic acid concentrations of 51.44 mg/kg (N.D.-638.16 mg/kg), 3.87 mg/kg (0.23-6.63 mg/kg), 13.89 mg/kg (N.D.-33.75 mg/kg), 21.31 mg/kg (N.D.-102.59 mg/kg), 2.95 mg/kg (N.D.-7.77 mg/kg), 3.12 mg/kg (N.D.-19.03 mg/kg), 20.05 mg/kg (3.27-151.54 mg/kg), 2.51 mg/kg (N.D.-17.10 mg/kg), and 8.79 mg/kg (N.D.-25.38 mg/kg), respectively. Products labelled as 'carmine', specifically candies and surimi, contained carminic acid concentrations of 64.22 mg/kg (N.D.-239.36 mg/kg) and 7.39 mg/kg (N.D.-61.16 mg/kg). These findings were consistent with the data reported in the literature. For example, for surimi and sugaring products, the quantities of carminic acid were N.D.-28.9±0.3 mg/kg and N.D.-754.7 ±2.8 mg/kg[31)]. This study verified the suitability of the

proposed analytical method for quantifying carminic acid in various foods. Additionally, the samples mentioned above were identified through LC-MS/MS analysis as indicated by our analysis.

Conclusion

This study developed and validated a preparation method, as well as an HPLC-DAD and LC-MS/MS method for the quantitative and qualitative analysis of carminic acid—a component of cochineal extract and carmine, widely used as red colorants in foods. The analysis method carried out validation for specificity, linearity, accuracy, and precision. In the process, it was confirmed that even lower concentrations could be analysed compared to previous studies. The validation data for the method demonstrated compliance with standard validation guidelines. Additionally, present study conducted an estimation of the primary influential factors contributing to the measurement uncertainty, confirming the reliability of the analysis results.

Furthermore, the study showcased the applicability of the proposed method for quantifying carminic acid in diverse food products labeled as ‘cochineal extract’ and ‘carmine’, including candies and surimi. This method, utilizing HPLC-DAD, has been demonstrated to be suitable for routine analysis, enabling analysis down to lower concentrations, and confirming applicability to various commercial products, thus proving its suitability for rapid and quality control analysis of carmine acid.

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국문요약

식품첨가물 카민과 코치닐 추출물은 붉은 색을 띄는 색소로, 주로 코치닐 곤충에서 유래하며 카민산이 주요 성분으로 사탕, 어묵과 같이 널리 소비되는 식품에 일반적으로 사용되고 있다. 최근 카민산은 특정 단백질과 관련된 알레르기 반응과 연관이 있는 것으로 보고되고 있다. 본 연구는 highperformance liquid chromatography with a diode array detector (HPLC-DAD)와 C18 UG120 컬럼을 사용하여 카민산을 정량시험법을 개발하고 밸리데이션을 수행하였다. HPLC 최적 분석조건은 이동상 (A)물-트리플루오로아세트산(trifluoroacetic acid, TFA) 0.1% 및 (B)아세토니트릴-TFA 0.1%, 유속 1.0 mL/min, 컬럼온도 30°C에서 수행하였으며, 검량선(0.2-50 mg/L) 범위에서 우수한 결정계수(r^2≥0.9999)를 보였다. 또한, liquid chromatography-tandem mass spectrometry (LC-MS/MS)를 활용한 정성 분석을 통해 limit of detection (LOD) 0.05 mg/k, limit of quantification (LOQ) 0.15 mg/kg을 보였다. 또한, 일내 및 일간 밸리데이션에서 정확도(87.3-97.1%, 회수율)와 정밀도(0.48-8.90%, 상대표준편차)이었으며, 측정 불확도 또한 추정하였다. 개발된 분석법은 다양한 식품 유형에 적용 가능하며, 카민과 코치닐 추출물의 모니터링을 통해 카민산과 관련된 잠재적인 문제가능성을 확인하는 데 활용될 수 있을 것으로 사료된다.

Conflict of interests

The authors declare no potential conflict of interest.

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