pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 40, No. 2, pp. 87~95 (2025)
https://doi.org/10.13103/JFHS.2025.40.2.87

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Quantitative Analysis and Intake Exposure Assessment of Laccaic Acid in Processed Foods Using HPLC-DAD

Ji-Eun Kang[1], Ga-Yeong Lee[1], Choong-In Yun[2], Young-Jun Kim[1,2]*

[1]*Department of Food Science and Biotechnology, Seoul National University of Science and Technology, Seoul, Korea*
[2]*Research Institute of Food and Biotechnology, Seoul National University of Science and Technology, Seoul, Korea*

(Received March 31, 2025/Revised April 21, 2025/Accepted April 21, 2025)

ABSTRACT - Lac color, a water-soluble red food colorant, is obtained from stick lac, a secretion of the insect *Laccifer lacca*. Its main component is anthraquinone-based laccaic acid, which consists of types A, B, C, D, and E. The objectives of this study were to validate the quantitative analysis of laccaic acid using high-performance liquid chromatography with diode array detection and to calculate intake exposure. The proposed analytical method was evaluated for various parameters, including specificity, linearity, limit of detection, limit of quantification, accuracy, and precision, and the matrix effect was also considered. The calibration curve showed high linearity ($R^2 \geq 0.999$) across all matrices, and the method detection limit and method quantification limit of laccaic acid were confirmed to be 1.93 mg/kg and 5.86 mg/kg, respectively. In intra-day and inter-day recovery experiments, accuracy was 91.5-100.9%, while precision was 0.8-2.6% RSD, both meeting the AOAC criteria. The matrix effect values ranged from -1.35% to 2.96%. To evaluate laccaic acid intake by food type, various foods distributed throughout Korea were sampled and analyzed. The range of laccaic acid intake levels was 0.0000-0.0027 mg/kg bw/day for the average Korean population (*per capita*) and 0.0000-0.0306 mg/kg·bw/day for consumers. The developed analytical method is applicable to a wide range of food types, enabling effective monitoring of lac color extracts. It can also be utilized for risk assessment through intake evaluation.

Key words: Lac color, Laccaic acid, HPLC, Validation, Intake exposure

Color plays an important role in food, and adding color makes the food look more appealing and stimulates the appetite. As a result, consumer preference for the food also increases[1)]. Food colorings are added to maintain the organoleptic characteristics and color of foods during processing and storage[2)]. Lac color, a water-soluble red food colorant, is derived from stick lac and obtained from the secretion of the insect *Laccifer lacca*.[3)]. The red color is known to come from a water-soluble dye. As a food additive, lac color can be used in foods other than natural foods such as meat, fish and shellfish, fruits, vegetables, seaweed, beans, tea, coffee, red pepper powder, red pepper threads, kimchi, red pepper paste, and vinegar[4)]. Based on a market survey of foods sold in large supermarkets and online shopping malls, lac color is primarily used for coloring products such as candies, beverages, sausages, and ham.

Despite its wide use in various types of foods as a food additive for coloring purposes, toxicity studies of laccaic acid have not been well reported. The U.S. Food and Drug Administration (US FDA) and the EU do not approve lac color as a food additive and it is not permitted in foods. In Japan, it is listed on the Food Additives List of the Ministry of Health, Labor and Welfare and it can be used in all foods, but there are no standards for its use[5)]. Although toxicity data on laccaic acid has historically been limited, recent studies have begun to address this gap. A nonclinical toxicity study by Park et al.[6)] reported no toxicity or antigenicity of lac dye following oral administration up to 500 mg/kg, and Lim et al.[7)] further confirmed the absence of antigenic responses in a guinea pig model.

Lac color is composed of anthraquinones compounds such as laccaic acid A, B, C, D, and E, with laccaic acid A and B being the main components[8)]. In a study by Shin et al.[9)], laccaic acid A, B, C, and E were qualitatively analyzed using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS), and each component was individually quantified in food samples using high-performance liquid chromatography-photo diode array (HPLC-PDA). There are various analytical instruments used

*Correspondence to: Young-Jun Kim, Department of Food Science and Biotechnology, Seoul National University of Science and Technology, Seoul 01811, Korea
Tel: +82-2-970-6734, Fax: +82-2-970-9736
E-mail: kimyj@seoultech.ac.kr

Copyright © The Korean Society of Food Hygiene and Safety. All rights reserved. The Journal of Food Hygiene and Safety is an Open-Access journal distributed under the terms of the Creative Commons Attribution Non-Commercial License(http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution,and reproduction in any medium, provided the original work is properly cited.

for the analysis of laccaic acid, one of which is the high-performance liquid chromatography coupled with ultraviolet-visible detection and mass spectrometry (HPLC-UV-VIS-MS)[10] technique. In the study by Katarzyna Lech et al.[11], high-performance liquid chromatography coupled with ultraviolet-visible detection and electrospray ionization tandem mass spectrometry (HPLC-UV-Vis-ESI MS/MS) was utilized, enabling the qualitative analysis of natural dyes, including laccaic acid. In a study by Santos et al.[12], high-performance liquid chromatography-diode array detector (HPLC-DAD) and MS were combined to analyze lac dyes.

The aim of this study was to determine whether quantitative analysis of laccaic acid is feasible using the relatively simple and commonly employed HPLC-DAD technique. Furthermore, DAD allows for the identification of the constituent peaks of laccaic acid in a sample by detecting the unique absorbance pattern of the target component. In this study, the method was validated using HPLC-DAD to quantify laccaic acid in various food matrices. The validation of the analytical method and the reliability of the results were confirmed through tests for specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), method detection limit (MDL), method quantification limit (MQL), precision, accuracy, recovery, and cross-validation between laboratories. Additionally, 101 laccaic acid-containing products sold in Korea were monitored to assess intake exposure. The findings of this study can serve as a reference for the toxicity evaluation of laccaic acid, the establishment of standards and specifications for its use as a food additive.

Materials and Methods

Chemicals and reagents

The laccaic acid standard (Natural Red 25, CAS No. 60687-93-6, 78.80%) was sourced from Toronto Research Chemicals (North York, ON, Canada). The mobile phase solvents and other chemicals used for extraction, including trifluoroacetic acid (TFA) and ammonium phosphate dibasic, were also procured from Sigma-Aldrich (St. Louis, MO, USA). Water, and methyl alcohol, all of HPLC grade, were obtained from J.T. Baker Chemical Co. (Radnor, PA, USA).

Sample collection

To evaluate the suitability of the suggested test method in this research, experiments were carried out on various food products available in Korea. The number of samples containing lac color collected for each food category was determined based on the 2020 Domestic Food Item Manufacturing Declarations and the 2021 Import Declarations from the Ministry of Food and Drug Safety (MFDS). As a result, the use of lac color was confirmed in seven food categories. A total of 101 food products were obtained from local and online markets, including candy (10), fruit and vegetable juices (16), mixed drinks (10), sausages (20), ham (25), bacon (10), and salted seafood products (10). All samples, which contained laccaic acid as a food additive, were homogenized and then frozen at -18°C. Each sample underwent the same pretreatment process three times.

Preparation of standard solution

Standard solutions (1,000 mg/L) were prepared by accurately weighing 12.69 mg of the laccaic acid standard and dissolving it in a mobile phase consisting of 0.3% TFA in water (A) and 0.3% TFA in methanol (B) at a 1:1 ratio, to make a final volume of 10 mL. Serial dilutions using mobile phase (A:B=1:1) were prepared to concentrations of 10, 25, 50, 100, 250, 500, 1,000 mg/L. The prepared standard solutions were refrigerated and freshly prepared as needed.

Preparation of samples

The pretreatment method for laccaic acid was applied by comparing the methods described by Shin et al.[9] and Santos et al.[12] For each homogenized food sample (approximately 5 g), a 50 mL conical tube was used, into which 20 mL of methanol and 5 mL of 0.3% ammonium phosphate were added, and thoroughly stirred. The solution was extracted with ultrasonic waves for approximately 5 minutes, centrifuged at 3,075 ×g for 5 minutes, and 12.5 mL of the supernatant was taken into a vacuum concentration vessel and evaporated to dryness using a rotary vacuum evaporator. After vacuum evaporation, the HPLC mobile phase (A:B=1:1, A: 0.3% TFA in water, B: 0.3% TFA in MeOH) was prepared to a total volume of 1 mL, filtered through a 0.45 μm syringe filter and then employed as the test solution.

HPLC condition

The analysis of laccaic acid was performed with an Agilent 1260 Series HPLC system. (Agilent Technologies, lnc., Santa Clara, CA, USA). A Unison UK-C8 (4.6 mm×250 mm, 3 μm, Imtakt Corporation, Kyoto, Japan) column set at 40°C was used for separation at a wavelength of 495 nm of a DAD. The mobile phase consisted of 0.3% TFA in water (A) and 0.3% TFA in methanol (B). The gradient condition was set as A 70%, B 30% for 30 min to A 64%, B 36%, A 62%, B 38% for 40 min, and A 70%, B 30% for 40.1 to 50 min. The volume of injection was 5 μL, and the flow rate was adjusted to 0.6 mL/min.

Method validation

In this research, specificity, LOD, LOQ, precision, and

accuracy were assessed following the guidelines established by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH)[13] and the Association of Official Analytical Chemists (AOAC)[14]. This approach allowed for the validation of the analytical method.

Specificity refers to the ability to accurately measure the target compound without interference from other substances. The specificity of the analytical method for the selective measurement of laccaic acid was evaluated by comparing the spectra and chromatograms of blank and standard solutions.

Standard solutions prepared at six concentrations by preparing dilutions of the standard solution were analyzed in seven replicates using HPLC-DAD. A calibration line was drawn from the peak areas of the six concentrations, and the coefficient of determination (R^2) was obtained to evaluate the linearity. The LOD and LOQ were determined by multiplying the Y-intercept values of the calibration curves of the seven replicate analyses by 3.3 and 10, respectively, and dividing the results by the average slope values.

The accuracy was validated through a recovery experiment, and the precision was validated by calculating the Horwitz ratio (HorRat) based on repeated measurements. Beverage, candies, and sausages were selected as the types of food products that are commonly used in Korea. The HorRat value, which is an indicator used to assess the repeatability of an analytical method, was determined by calculating the actual relative standard deviation (RSD) of recovery results and comparing it to the predicted RSD. The predicted RSD is calculated using the formula $2C^{-0.15}$ [15], where C is the average concentration, expressed as a mass fraction.

Both intra-day (6 replicates of 3 concentrations within 1 day) and inter-day (9 replicates of 3 concentrations over 3 days) experiments were performed at short intervals under the same experimental conditions. For the recovery experiments, low, medium, and high concentrations (10, 20, and 40 mg/kg) of laccaic acid standards were added to beverage, candy, and sausage samples that did not contain laccaic acid.

Matrix effects refer to the influence of other components in the sample on the analyte's signal, which can manifest as either signal enhancement or suppression[16]. The ME was assessed by comparing the slope of the calibration curve for the standard solution with that of the curve generated from samples with varying concentrations of standard substances to samples such as beverages, candies, and sausages, and was calculated using the following equation[17].

$$\mathrm{ME}(\%) = \left[\frac{\mathrm{Slope}_{\mathrm{standard}}}{\mathrm{Slope}_{\mathrm{spiked}}} - 1\right] \times 100$$

Table 1. Calibration parameter results of laccaic acid

Parameters		laccaic acid[5]
Range (mg/L)		10-1000
Coefficient of determination (R^2)		0.9999
Calibration curve		y=8.04x-25.02
LOD[1] (mg/L)		9.67
LOQ[2] (mg/L)		29.31
MDL[3] (mg/kg)		1.93
MQL[4] (mg/kg)		5.86
Matrix effect (%)	Beverage	2.96%
	Candy	-1.35%
	Sausage	-2.24%

[1] Limit of detection.
[2] Limit of quantification.
[3] Method detection limit.
[4] Method quantification limit.

Inter-laboratory validation

To evaluate accuracy and precision, an interlaboratory validation was conducted by contrasting the analytical measurements of the same sample, analyzed with the same method across three different laboratories (Laboratories A, B, and C). The samples were taken from the same beverage, and for the recovery experiments, laccaic acid standards at concentrations of 10, 20, and 40 mg/kg were added. The experiment was performed three times, and recovery rates along with RSD% were calculated to verify accuracy and precision. Lab A used an Agilent 1260 HPLC system, while Labs B and C used Agilent 1200 systems (Agilent Technologies). All labs used the same column: Unison UK-C8 (4.6 mm×250 mm, 3 μm; Imtakt Corporation).

Estimated daily intake (EDI) of laccaic acid

The EDI of laccaic acid for different age groups and genders was calculated for both the population as a whole and the intake groups using the EDI equation below[18].

$$\mathrm{EDI} = \frac{\Sigma_i\left(\text{Daily laccaic acid consumption}\left(\frac{\mathrm{g}}{\mathrm{day}}\right)\right)_i}{\text{Average b.w. (kg)}} \times \frac{\left(\text{laccaic acid concentration}\left(\frac{\mathrm{mg}}{\mathrm{kg}}\right)\right)_i}{\text{Average b.w. (kg)}}$$

The daily intake of each food surveyed in the Korea National Health and Nutrition Examination Survey was multiplied by the laccaic acid content of the samples of different types of food, and the sum was divided by the average body weight to obtain the EDI of laccaic acid[19].

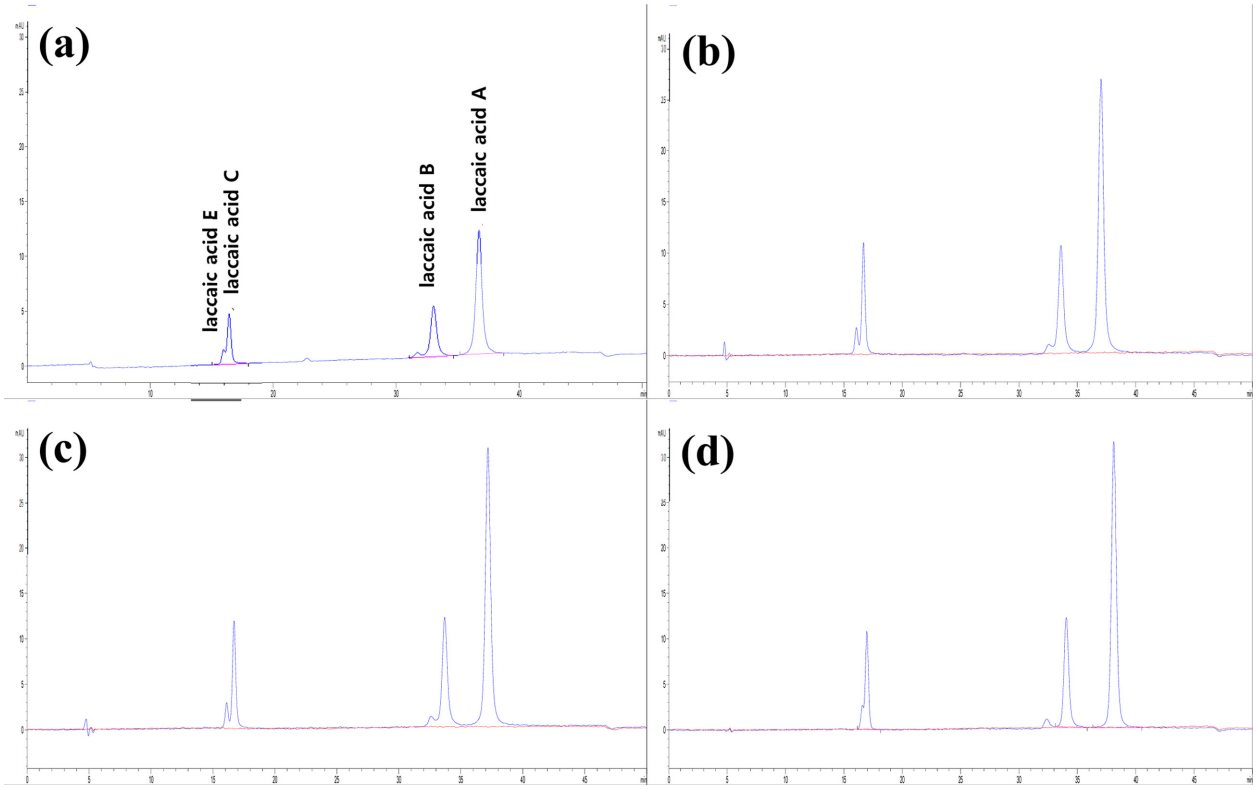

Fig. 1. Chromatograms using HPLC-DAD of the (a) 20 mg/kg laccaic acid standard solution, (b) Beverages of spiking 40 mg/kg, (c) Candies of spiking 40 mg/kg, (d) Sausages of spiking 40 mg/kg.

Results and Discussion

Method validation

Specificity

In Fig. 1, the chromatograms of the standard laccaic acid solution (20 mg/kg) and the standard laccaic acid solution (40 mg/kg) spiked into beverage, candy, and sausage samples are compared. The retention times of laccaic acid were identified as 16 min for laccaic acid E and C, 32.5 min for B, and 36 min for A. The retention times were further confirmed by ensuring consistency across different sample matrices and the absence of interfering substances.

Linearity, LOD, LOQ, MDL, and MQL

A standard solution of laccaic acid was diluted across six concentration ranges from 10 to 1,000 mg/L and analyzed in duplicate for seven repetitions to construct a calibration curve. The coefficient of determination (R^2) was 0.9999, demonstrating a high level of linearity that exceeded the minimum standard of ≥0.995 set by the FDA[20]. The LOD and LOQ were found to be 9.67 and 29.31 mg/L, respectively. Following sample preparation involving a fivefold concentration by vacuum evaporation, the MDL, and MQL were significantly reduced to 1.93 and 5.86 mg/kg, respectively, which is more than five times lower than the original LOD and LOQ.

Accuracy and precision

Table 2 presents the accuracy and precision results for both intra-day and inter-day. The accuracy of intra- and inter-day evaluation of the recovery experiment results obtained by adding 10, 20, and 40 mg/kg of laccaic acid to beverage, candy, and sausage was 92.7-95.8%, 91.5-96.2% for beverage, 95.9-99.7%, 98.1-100.9% for candy, and 96.1-100.8%, 96.5-100.4% for sausage. The inter- and intra-day %RSD values were 0.8-1.7% and 0.6-1.5% for beverage, 1.0-1.8% and 1.2-2.6% for candy, and 1.1-1.8% and 0.9-1.6% for sausage. These results fell within the appropriate thresholds outlined by the AOAC validation guidelines[15]. Moreover, the intra-day HorRat values ranged from 0.92 to 1.01, while the inter-day HorRat values ranged from 0.93 to 1.01. These calculated HorRat values fall within the acceptable range of $0.5 \leq \text{HorRat} \leq 2$, indicating that this analytical method is reliable and reproducible for the quantification of the target analytes across various concentration levels[15].

Matrix effect (ME)

The ME values were calculated and found to be 2.96% for

Table 2. Validation results of accuracy, precision, HorRat value of laccaic acid

Matrix	Added standard (mg/ kg)	Intra-day[1]		HorRat value[4] (%)	Inter-day[2]		HorRat value (%)
		Accuracy (%)[3]	Precision (%RSD)		Accuracy (%)	Precision (%RSD)	
Beverage	10	92.7±0.8	0.8	0.92	91.5±1.3	1.5	0.93
	20	94.3±1.6	1.7	0.95	94.5±1.2	1.3	0.94
	40	95.8±0.8	0.9	0.96	96.2±0.6	0.6	0.96
Candy	10	95.9±1.3	1.4	0.98	98.1±2.5	2.6	0.96
	20	98.6±1.0	1.0	1.00	99.5±1.4	1.4	0.99
	40	99.7±1.8	1.8	1.01	100.9±1.2	1.2	1.00
Sausage	10	98.7±1.0	1.1	0.98	97.8±0.9	0.9	0.99
	20	96.1±1.3	1.3	0.97	96.5±1.6	1.6	0.99
	40	100.8±1.8	1.8	1.00	100.4±1.4	1.4	1.01

[1] Analysis was conducted six time/day.
[2] Analysis was conducted three times on three days.
[3] Average±SD.
[4] RSD observed / RSD expected.
[5] Laccaic acid: sum of laccaic acid A, B, C and E.

Table 3. Inter-laboratory reproducibility results of laccaic acid

Sample	Added standard (mg/kg)	Recovery±SD (%)			Average±SD (%)	%RSD
		Lab. A	Lab. B	Lab. C		
Beverage	10	94.49±0.62	97.54±0.87	96.97±0.62	96.45±1.52	1.58
	20	98.45±1.52	96.95±0.43	95.05±0.27	96.83±1.59	1.64
	40	100.87±0.75	100.49±0.51	98.20±0.34	99.92±1.29	1.30

beverage, -1.35% for candy, and -2.24% for sausage. According to the European Commission's criteria, when the ME value is within 20%, ME calibration is not required for determination, and the analytical results are considered reliable[16].

In the study by Kim et al.[21], the matrix effects of phloroglucinol and dieckol were 1.02% and 1.07%, respectively, which were within the acceptable range of ±20%. Therefore, it was concluded that the matrix did not significantly affect the analysis. In contrast, Cho et al.[22] reported that certain matrix effects exceeded the ±20% threshold, necessitating the application of matrix-matched calibration to improve the accuracy and precision of the analytical results. Similarly, Park et al.[23] observed ME values of -21.53% for soju and -19.69% for beverages, which were close to or exceeded the ±20% criterion. As a result, matrix-matched calibration was employed to ensure reliable quantification. In the case of candy, however, the matrix effect was considered negligible at -1.10%.

Consequently, the matrix effect did not influence the analysis of beverage, candy, and sausage samples. In this study, a matrix-matched calibration curve was applied and validated to ensure accurate and precise analysis.

Inter-laboratory validation

Recovery experiments were performed in three laboratories by spiking laccaic acid into beverage samples, and the results were analyzed for comparison. The findings are reported as recovery±standard deviation (%) for each laboratory, with the average and variability (%RSD) summarized in Table 3. The recoveries in Lab A ranged from 94.49-100.87%, in Lab B from 96.95-100.49%, and in Lab C from 95.05-98.20%. The corresponding precision was in the range of 1.30-1.64% RSD. These results met the criteria set forth in the AOAC guidelines[14].

Application

To determine the suitability of the proposed HPLC method, various products labeled as 'lac color' and distributed in Korea were analyzed. The tested products included candy, fruit and vegetable juice, sausage, ham, salted seafood products (jeotgal), and bacon. Each sample was analyzed three times, and the average value was computed to determine the actual content. The findings are presented in Table 4.

Samples in which no laccaic acid was detected during the analysis were marked as not detected (N.D.). Following the

Table 4. Concentration (mg/kg) of laccaic 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 lac color labeled						
Candies	10	4	N.D.[3]	43.0±0.53	5.62±13.19	14.06±18.15
Fruit/Vegetable beverages	16	16	tr [4]	33.81±0.18	7.95±7.90	7.95±7.90
Other beverages	10	9	N.D.	9.67±0.32	3.80±3.35	4.23±3.26
Sausages	20	17	N.D.	12.88±0.18	2.32±2.99	2.74±3.07
Hams	25	21	N.D.	6.30±0.02	1.95±1.58	2.32±1.45
Salted seafood products	10	0	N.D.	N.D.	N.D.	N.D.
Bacons	10	4	N.D.	tr [4]	0.50±0.66	1.25±0.36

[1] Average and standard deviation of all samples.
[2] Average and standard deviation of detected samples.
[3] N.D.: Not detected (below MDL).
[4] tr: Between MDL and MQL.

quantitative analysis of laccaic acid content in a total of 101 food items across 7 food groups available in Korea, 71 out of 101 samples were detected, which is a high detection rate of 70.30%. Among the collected samples, the Fruit and Vegetable Drinks category had the highest detection rate as laccaic acid was detected in all samples, but the highest average detection content of 43 mg/kg was found in candy. On the other hand, the salted seafood products category had the lowest detection rate, with no laccaic acid detected in any of the samples, and the lowest average detection was found in bacon at 0.50 mg/kg.

Although the LOD and LOQ were determined to be 9.67 and 29.31 mg/L during method validation, the actual sample analysis was conducted using MDL (1.93 mg/kg) and MQL (5.86 mg/kg), reflecting the enhanced sensitivity achieved through vacuum evaporation. Based on the efficiency of the pretreatment process and the method's high sensitivity, this analytical approach is well suited for the quantification of laccaic acid in processed foods.

Table 5. Estimated daily intake based on monitoring results (mg/kg bw/day)

Food items	Laccaic acid intake-nationwide (*per capita*)									
	All	Male	Female	0-2	3-6	7-12	13-19	20-64	≥65	Contribution to intake (%)
Candies	0.0000	0.0000	0.0000	0.0003	0.0005	0.0001	0.0000	0.0000	0.0000	0.00
Fruit/Vegetable beverages	0.0027	0.0022	0.0032	0.0233	0.0167	0.0067	0.0034	0.0025	0.0009	75.00
Other beverages	0.0009	0.0011	0.0007	0.0029	0.0044	0.0019	0.0027	0.0007	0.0003	25.00
Sausages	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.00
Hams	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.00
Bacons	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00

Food items	Laccaic acid intake-consumer only									
	All	Male	Female	0-2	3-6	7-12	13-19	20-64	≥65	Contribution to intake (%)
Candies	0.0012	0.0011	0.0014	0.0042	0.0051	0.0021	0.0013	0.0011	0.001	2.19
Fruit/Vegetable beverages	0.0306	0.0287	0.0324	0.1036	0.0769	0.0476	0.0287	0.0286	0.0256	55.84
Other beverages	0.0202	0.0209	0.018	0.0754	0.0509	0.0294	0.0247	0.0184	0.0107	36.86
Sausages	0.0018	0.0015	0.002	0.0082	0.0047	0.0054	0.0013	0.0017	-	3.28
Hams	0.0009	0.001	0.0008	-	0.003	0.0006	0.0019	0.0007	0.0012	1.64
Bacons	0.0001	0.0001	0.0001	0.0002	0.0002	0.0002	0.0001	0.0001	0	0.18

Estimated daily intake (EDI) of laccaic acid

There are not many toxicity studies reported on laccaic acid, and a safety evaluation of laccaic acid conducted by the MFDS in 2021 reported negative genotoxicity tests and no allergic reactions were observed in immunotoxicity tests up to 4 mg/kg·bw[24]. Based on the results of the monitoring conducted in this study, Table 5 shows the results of the EDI assessment of laccaic acid among different age groups and genders for the population as a whole and for the average intake. The estimated intake of laccaic acid ranged from 0.0000 to 0.0233 mg/kg·bw/day for the nationwide population (*per capita*), and from 0.0001 to 0.0769 mg/kg·bw/day for consumers.

Therefore, consumers were found to consume significantly lower amounts of laccaic acid compared to the non-toxic threshold of 4 mg/kg·bw, which is considered to pose a low risk for adverse effects such as allergies. Additionally, since infants (0-2) and young children (3-6) have higher intake and exposure levels than other age groups, continuous monitoring is required. In Fig. 3, the analysis results on intake contribution by food type indicated that, for both the nationwide population (*per capita*) and consumers, fruit/vegetable beverages showed the highest exposure contribution at 75% and 55.84%, and other beverages showed the second highest

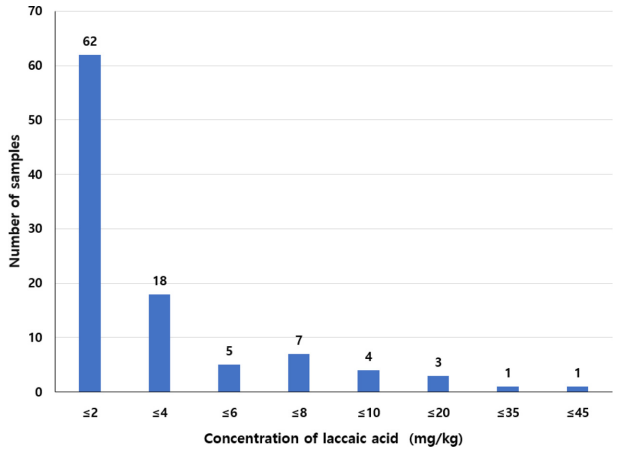

Fig. 2. Frequency of laccaic acid detected in foods by concentration.

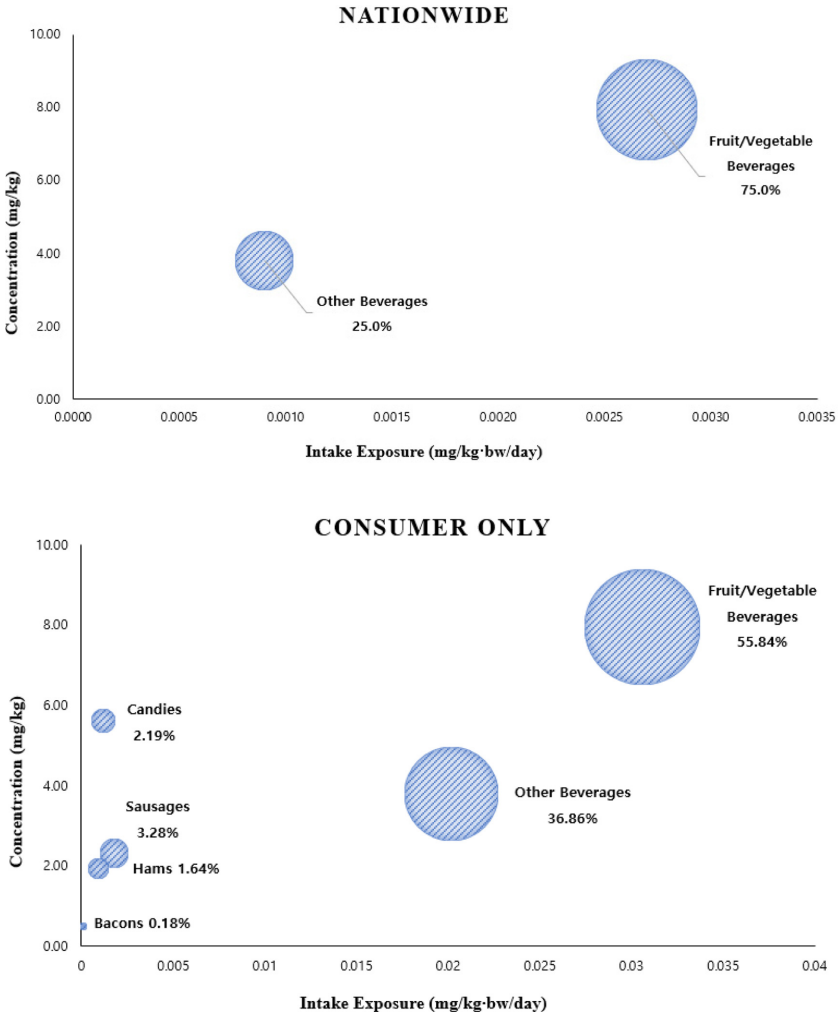

Fig. 3. Contribution of laccaic acid intake by food type a) Nationwide (*per capita*) and b) consumer only.

exposure contribution at 25% and 36.86%.

Consequently, HPLC-DAD was used for qualitative and quantitative analysis of laccaic acid, and the method was validated in terms of specificity, linearity, accuracy, precision, HorRat and ME. It was confirmed to be a reliable method that meets the standard validation guidelines. The proposed method was shown to be applicable for quantifying the amount of laccaic acid used as an additive in various food matrices such as candy, vegetable juice, mixed beverage, sausage, ham, salted seafood products, and bacon. Therefore, the analytical results provided important information on the content of laccaic acid present in various commercial foods, and can be further utilized in the assessment of risk.

In this study, Natural Red 25 was used as a reference standard, and the peaks corresponding to laccaic acid A, B, C, and E were identified by comparison with the LC-MS/MS data reported by Shin[9]. Quantification of lac color was performed by summing the peak areas of these four components, a method that demonstrated excellent linearity and reproducibility. This approach was validated as a reliable and versatile analytical method, suitable for routine monitoring and quantitative determination of lac color across a wide range of processed food products and matrices.

Acknowledgements

This research was funded by a grant from the Ministry of Food and Drug Safety, Republic of Korea, in 2022.

국문요약

곤충 *Laccifer lacca*의 분비물인 stick lac에서 얻어지는 락색소는 물에 용해되는 붉은색 식용 색소로, 식품에 붉은 색을 더하는 착색제이다. 주요 성분은 안트라퀴논계 기반의 laccaic acid로 A, B, C, D, E로 구성된다. 본 연구의 목적은 HPLC-DAD를 활용하여 laccaic acid를 정량적으로 분석할 수 있는 방법을 검증하는 것과 섭취 노출량을 산출하는 것이다. 제안된 분석법은 특이성, 선형성, LOD, LOQ, 정확성, 정확도 등 다양한 검증 항목에서 평가되었으며, 매트릭스 효과 또한 충분히 고려되었다. 모든 매트릭스에서 검량선은 높은 선형성 (R^2≥0.999)을 보였고, 락색소의 MDL과 MQL은 각각 1.93 mg/kg와 5.86 mg/kg로 확인되었다. 일내 및 일간 회수율 시험에서 정확도는 91.5-100.9% 사이였고, 정밀도는 0.8-2.6%RSD로 AOAC 기준을 충족하였다. 한국에서 유통되는 다양한 식품들을 수집하여 락색소를 모니터링하여 식품 유형별 락색소 섭취 노출량을 평가하였다. 락색소 섭취 수준의 범위는 국민전체, 섭취자 각각 0.0000-0.0027 mg/kg·bw/day와 0.0000 -0.0306 mg/kg·bw/day였다. 개발된 분석법은 다양한 식품 유형에 적용 가능하여 락색소 추출물의 효과적인 모니터링을 가능하게 하며, 섭취량 평가를 통해 락색소의 안전성 평가에 추가적으로 활용될 수 있을 것이다.

Conflict of interests

The authors state that there is no potential conflict of interest.

ORCID

Ji-Eun Kang https://orcid.org/0009-0004-1476-0825
Ga-Yeong Lee https://orcid.org/0000-0002-9669-0135
Choong-In Yun https://orcid.org/0000-0002-4305-678X
Young-Jun Kim https://orcid.org/0000-0001-7232-6008

References

1. Srivastava, S., Ray, D.P., Pandey, S.K., Prasad, K.M., Prasad, M., Baboo, B., 2013. Pure lac dye: a potential natural food additive, Indian Institute of Natural Resins and Gums, Namkum Ranchi, National Institute of Research on Jute and Allied Fibre Technology, Kolkata, India.
2. Dey, S., Nagababu, B.H., Applications of food color and bio-preservatives in the food and its effect on the human health. *Food Chem. Adv.,* **1**, 100019 (2022).
3. Oka, H., Ito, Y., Yamada, S., Kagami, T., Hayakawa, J., Harada, K., Atsumi, E., Suzuki, M., Suzuki, M., Odani, H., Akahori, S., Maeda, K., Nakazawa, H., Ito, Y., Separation of lac dye components by high-speed counter-current chromatography. *J. Chromatogr. A.,* **813**, 71-77 (1998).
4. Ministry of Food and Drug Safety (MFDS), 2023. Standards and specifications for food additives, Cheongju, Korea.
5. Japan Food Chemical Research Foundation (FFCR), 2014. List of existing additives, Tokyo, Japan.
6. Park, J.S., Kim, S.H., Kim, Y.S., Kwon, E., Lim, H.J., Han, K.M., Choi, Y.K., Jung, C.W., Kang, B.C., Nonclinical safety evaluation of food colorant lac dye via systematic toxicity profiling with assessment of in vivo antigenic potential. *Front. Pharmacol.,* **13**, 1020379 (2022).
7. Lim, H.J., Han, K.M., Kim, S.H., Ryu, S.K., You, J.R., Yoon, J.H., Kwon, E., Kim, J.E., Kang, B.C., Antigenicity evaluation of lac color and exploratory study for identifying potential biomarkers of anaphylaxis. *Lab. Anim. Res.,* **40**, 40 (2024).
8. Hong, Z., Guigan, F., Hua, Z., Kun, L., Determination of anthraquinone content in lac dye through combined spectrophotometry and HPCE. *Procedia Eng.,* **18**, 86-94 (2011).
9. Shin, J.W., Lee, H.J., Lim, E., Kim, J.B., Analytical method for determination of laccaic acids in foods with HPLC-PDA and monitoring. *J. Food Hyg. Saf.*, **38**, 390-401 (2023).
10. Szostek, B., Orska-Gawrys, J., Surowiec, I., Trojanowicz,

M., Investigation of natural dyes occurring in historical Coptic textiles by high-performance liquid chromatography with UV–Vis and mass spectrometric detection. *J. Chromatogr. A*, **1012**, 179-192 (2003).
11. Lech, K., Fornal, E., A mass spectrometry-based approach for characterization of red, blue, and purple natural dyes. *Molecules,* **25**, 3223 (2020).
12. Santos, R., Hallett, J., Oliveira, M.C., Sousa, M.M., Sarraguça, J., Simmonds, M.S.J., Nesbitt, M., HPLC-DAD-MS analysis of colorant and resinous components of lac-dye: A comparison between *Kerria* and *Paratachardina genera. Dyes Pigm.,* **118**, 129-136 (2015).
13. International Conference on Harmonisation (ICH), 2005. ICH harmonised tripartite guideline. Validation of analytical procedures: text and methodology Q2 (R1), San Diego, CA, USA.
14. Association of Official Analytical Chemists (AOAC), 2016. Official methods of analysis of AOAC International, 20th ed, Rockville, MD, USA.
15. Horwitz, W., Albert, R., The Horwitz ratio (HorRat): a useful index of method performance with respect to precision. *J. AOAC Int.,* **89***,* 1095-1109 (2006).
16. European Commission, 2021. SANTE/2020/12830, Rev.1. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed, Brussels, Belgium.
17. González-González, R.M., Barragán-Mendoza, L., Peraza-Campos, A.L., Muñiz-Valencia, R., Ceballos-Magaña, S.G., Parra-Delgado, H., Validation of an HPLC-DAD method for the determination of plant phenolics. *Rev. Bras. Farmacogn*., **29**, 689-693 (2019).
18. Lee, J.Y., Kim, M., Kim, J., Im, H., Kim, Y.S., Monitoring, exposure and risk assessment of formaldehyde in alcoholic beverages in Korea. *Food Addit. Contam. Part A*, **41**, 237-248 (2024).
19. Korea Disease Control and Prevention Agency (KDCA), 2022. The 8th korea national health and nutrition examination survey (KNHANES VIII), Cheongju, Korea.
20. Food and Drug Administration (FDA), 2023. ORA Lab manual vol. II - methods, method verification and validation (ORALAB.5.4.5). FDA office of regulatory affairs quality, Silver Spring, MD, USA.
21. Kim, D.Y., Park, H.J., Yoon, C.I., Kim, Y.J., Method development and validation of phloroglucinol and dieckol in *Ecklonia cava* using HPLC-DAD*. Food Sci. Biotechnol.,* **34**, 621-628 (2024).
22. Cho, J., Kim, S.M., Yun, C.I., Kim, Y.J., Method validation and application of gas chromatography–mass spectrometry for eight organotin compounds in marine products. *Food Anal. Methods*, **18** (2025).
23. Park, H.J., Yun, C.I., Kim, D.Y., Kim, Y.J., Optimization, validation, and measurement uncertainty of HPLC-UVD method for quantification of thaumatin in food. *Food Sci. Biotechnol.,* **34**, 1849-1856 (2025).
24. Ministry of Food and Drug Safety (MFDS), 2021. A study on the safety evaluation of natural pigments (Lac pigments), Cheongju, Korea.