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Determination of Sodium Alginate in Processed Food Products Distributed in Korea

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ABSTRACT - Sodium alginate is the sodium salt of alginic acid, commonly used as a food additive for stabilizing, thickening, and emulsifying properties. A relatively simple and universal analysis method is used to study sodium alginate due to the complex pretreatment process and extended analysis time required during the quantitative method. As for the equipment, HPLC-UVD and Unison US-Phenyl column were used for analysis. For the pretreatment condition, a shaking apparatus was used for extraction at 150 rpm for 180 minutes at room temperature. The calibration curve made from the standard sodium alginate solution in 5 concentration ranges showed that the linearity (R^2) is 0.9999 on average. LOD and LOQ showed 3.96 mg/kg and 12.0 mg/kg, respectively. Furthermore, the average intra-day and inter-day accuracy (%) and precision (RSD%) were 98.47-103.74% and 1.69-3.08% for seaweed jelly noodle samples and 99.95-105.76% and 0.59-3.63% for sherbet samples, respectively. The relative uncertainty value was appropriate for the CODEX standard with 1.5-7.9%. To evaluate the applicability of the method developed in this study, the sodium alginate concentrations of 103 products were quantified. The result showed that the detection rate is highest from starch vermicelli and instant fried noodles to sugar processed products.

Key words: Sodium alginate, High-performance liquid chromatography (HPLC), Food additive, Method validation

Recently, the domestic processed food industry has developed and the food additive industry has been steadily developing. As a result, consumers' preferences have diversified, increasing demand for new types of processed foods[1)]. Additionally, food additives are being used in various processes according to the need to develop products that could obtain functions of food such as nutrition and convenience, etc. Food additives are used for long-term preservation and storage, long-distance transportation, nutritional control, and to improve flavor, aroma, and color[2,3)]. There are two types of thickeners added to foods that require adhesion and viscosity, which require binding between food ingredients or only increase viscosity to give food stability. Recently, it has been used to induce stabilization of food using hydrophilic polymers and emulsifiers[4)]. These thickeners increase adhesiveness in food and improve emulsification stability. It is also added to maintain freshness and increase the texture by engaging in changes in heating or preservation during processing[5)]. Sodium alginate is used as an additive for stabilizers, thickeners and emulsifiers[6)].

Sodium alginate is a natural polysaccharide extracted from brown seaweeds[7)]. It primarily consists of the sodium salt of alginic acid, which is composed of 1,4-linked β-D-mannuronic acid (M), (1–4)-α-L-guluronic acid (G), and alternating (MG) blocks[8,9)]. Alginic acid has limited water stability and solubility, so it is used commercially by transforming it into propylene glycol or various salts, such as sodium, ammonium, potassium, and calcium alginate[10)]. Sodium alginate is widely used as a food additive to stabilize, thicken, and emulsify food products[11,12)]. Alginic acid and its derivatives are widely used in the food and pharmaceutical industries because of their gelation capacity, stabilization properties, and high viscosity[13)]. An aqueous solution of sodium alginate is somewhat viscous[14,15)]. Gelation of the aqueous solution of sodium alginate occurs with the addition of calcium ions, which form the intermolecular crosslinks with the polymer carboxyl groups[16)]. The mechanical properties of alginate can be further improved by chemical modification to promote crosslinking[17)].

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The quantitative method of sodium alginate is complex and time-consuming, so a simple quantitative analysis method, such as high-performance liquid chromatography (HPLC), is required[18]. To compensate for these shortcomings, this study sought to establish an analytical method based on high-performance liquid chromatography-ultraviolet-visible detector (HPLC-UVD), a relatively simple and universal analysis equipment. The method was validated and applied to determine the sodium alginate concentrations in various food products distributed in Korea. In addition, the measurement uncertainty that occurs during analytical measurements was calculated using mathematical treatment and statistical methods to quantify and evaluate the reliability of the results.

Materials and Methods

Chemicals and materials

Alginic acid sodium salt, the standard product of sodium alginate used in the experiment, and phosphoric acid, used for HPLC analysis, were purchased from Sigma-Aldrich (St. Louis, MO, USA). To apply the proposed method, food containing sodium alginate as a food additive was investigated, and samples were purchased from supermarkets, convenience stores, and online markets from March to June 2020. Ice creams and sherbets (n = 33), instant fried noodles (n = 19), starch vermicellis (n = 10), seaweed jelly noodles (n = 6), processed sugars (n = 18), and breads (n = 17) were applied for monitoring.

Standard solutions

The standard solution of sodium alginate was prepared by precisely taking 40.0 mg of the standard product (alginic acid sodium salt) in a 20-mL flask and adding distilled water to the solution. The prepared 2,000 mg/L stock solution was refrigerated and used in the experiment. The standard solution was prepared at working concentrations of 20, 50, 100, 200, and 500 mg/L by serial dilution of the stock solution with water.

Sample preparation

For sample extraction, 2 g of homogenized sample was placed in a 50-mL conical tube, and then 20 mL of water was added and the solution mixed for 180 min using a shaker (SI-600R, Jeiotech Co., Daejeon, Korea). To collect the supernatant, the mixture was centrifuged at 3,070 g-force for 10 min. Additionally, for samples containing fat, such as ice cream or instant fried noodles, the fat was removed by extraction with hexane using a separation funnel, and the aqueous layer was collected. Each solution was filtered through a 0.45-μm syringe filter (Sartorius Minisart® RC, Sartorius Co., Göttingen, Germany) and used as a sample solution.

Analytical instruments

The extraction and mobile phase solvents were HPLC-grade products purchased from J.T. Baker (Phillipsburg, NJ, USA). The HPLC analytical conditions were applied following previous study with some modifications[2]. The HPLC system was an UltiMate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a UV detector (200 nm) and a Unison US-Phenyl (5 mm, 4.6 mm ×250 mm; Imtakt, Kyoto, Japan) column (25°C). The flow rate and the injection volume were 0.7 mL/min and 20 μL, respectively.

Method validation

For validation, the linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy were calculated by referring to ICH guidelines[19]. A calibration curve was constructed from the working standards covering the range 20-500 mg/L by plotting the area of the peak against concentration obtained by seven repeated analyses by HPLC-UVD. Linearity was expressed as the coefficient of determination (R^2). Recovery rates, precision, LOD and LOQ were then measured. To obtain LOD and LOQ, the lowest three concentrations were selected and analyzed three times to prepare a calibration curve. The standard deviation (SD, σ) of the y-intercept value of the calibration curve and the average (S) of the slope value were used in the following equations: LOD = $3.3\times\sigma/S$, while the LOQ = $10\times\sigma/S$.

Accuracy refers to the degree to which the measured concentration of the analyte is close to the true value, and this was determined through a recovery rate experiment. Precision represents the degree of proximity between each measurement when a sample of the same concentration is repeatedly tested, which is expressed as a relative standard deviation (RSD) of the results of repeated measurements. To evaluate the accuracy, the test was carried out by adding low, medium, and high concentrations of sodium alginate (20, 50, and 100 mg/kg) to the seaweed jelly noodle and sherbet samples, respectively. For inter-day precision, three concentrations were repeated for three days, and a test was conducted for intra-day precision six times a day.

Measurement uncertainty estimation

The uncertainty of a measurement is defined as "a parameter, which is associated with the result of a measurement and characterizes the dispersion of the values that could reasonably be attributed to the measurand"[20]. The estimation of the measurement uncertainty for the methods was evaluated according to the EURACHEM method[21] and the "Guide to the expression of uncertainty in measurement" (GUM)[22]. In brief, possible sources of uncertainty the standard stock solution ($uSSS$), sample preparation (uSP), calibration curve (*uCal*), and repeatability for the determination of sodium alginate sample (*uRP*)—associated with the analysis of sodium alginate were identified. These measurement uncertainty sources were estimated and calculated as an expanded uncertainty (*Uc*) using a coverage factor (*k*) of 2 at the confidence level (CI) of 95%.

Results and Discussion

Method validation

Chromatogram analysis of the sodium alginate standard solution found no interfering peaks at the retention time of the analyte. It confirmed that the selectivity was suitable for analyzing sodium alginate using HPLC-UVD. To construct the calibration curve, the standard stock solution was diluted at five concentrations between 20 and 500 mg/kg and each concentration was analyzed seven times. In this range, good linearity was achieved (average $R^2 \geq 0.9997$). The LOD, obtained by repeatedly analyzing three times, was 3.96 mg/kg, the LOQ was 12.0 mg/kg, and the range was 12-500 mg/kg. The values are shown in Table 1.

In the recovery experiment for evaluating accuracy and precision, HPLC analysis showed that for intra-day, the average recovery was 101.03-102.57% for seaweed jelly noodle samples and 101.2-104.83% for sherbet samples. The inter-day accuracy and precision, which was tested for 3 days, showed the average recovery was 98.47-103.74% for seaweed jelly noodle samples and 99.95-105.76% for sherbet samples. According to ICH Guideline Q2 (R1)[19], the average recovery rate was within the range of 80-120%, and it was confirmed to be acceptable. The RSD results for intra-day and inter-day precision were 2.00-3.08% and 1.69-2.87% for seaweed jelly noodle samples and 0.59-2.34% and 1.47-3.63% for sherbet, respectively. The validation results for accuracy and precision were similar to those found in the literature[18]. This demonstrates that established methods are suitable for quantification of sodium alginate and have acceptable repeatability and reproducibility.

Table 1. Calibration parameter results obtained for sodium alginate

Parameters	Sodium alginate
Range of calibration (mg/kg)	20-500
Slope (±SD)	1.43±0.01
Intercept (±SD)	1.56±0.64
Regression coefficient (R^2)	0.9999±0.00014
LOD[1)] (mg/kg)	3.96
LOQ[2)] (mg/kg)	12.0

[1)] LOD: Limit of detection, [2)] LOQ: Limit of quantitation

Measurement uncertainty

In this study, the validated method was applied to seaweed jelly noodle and sherbet spiked with the sodium alginate standard product at low, medium, and high three concentration levels (20, 50, and 100 mg/kg), and the uncertainty values for the developed method were calculated with these samples. The measurement uncertainty factors related to the analysis, such as sample weight, final volume, standard weight, purity, standard solution, calibration curve, and repeated measurement, were considered.

The calculated sodium alginate content of seaweed jelly noodle samples, considering dilution multiple of 10 at low, medium, and high concentrations, was 196±15, 511±16, and 1,029±22 mg/kg (95% CI, *k* = 2), respectively. The relative Uc was 7.9% (15/196% 100), 3.1% (16/511% 100), and 2.2% (22/1,029% 100), respectively. In the same way, the calculated sodium alginate content of sherbet samples at low, medium, and high concentrations were 199±15, 523±17, and 1,019±15 mg/kg (95% CI, *k* = 2), respectively. The relative Uc was 7.5% (15/199% 100), 3.2% (17/523% 100), and 1.5% (15/1,019% 100). The higher the concentration, the lower the relative Uc. These results meet the CODEX

Table 2. Validation data for accuracy, precision, and measurement uncertainty

Samples	Added standards (mg/kg)	Intra-day		Inter-day		Relative uncertainty (%)
		Accuracy (%)[1)]	Precision (RSD%)[2)]	Accuracy (%)	Precision (RSD%)	
Seaweed jelly noodle	20	101.03±3.10	3.08	98.47±2.83	2.87	7.9
	50	102.57±2.05	2.00	103.74±1.75	1.69	3.1
	100	102.24±2.04	2.00	102.29±1.73	1.69	2.2
Sherbet	20	102.77±2.35	2.28	99.95±3.63	3.63	7.5
	50	104.83±2.45	2.34	105.76±2.57	2.43	3.2
	100	101.20±0.60	0.59	101.07±1.49	1.47	1.5

[1)] Recovery±SD (%), [2)] Relative standard deviation %.

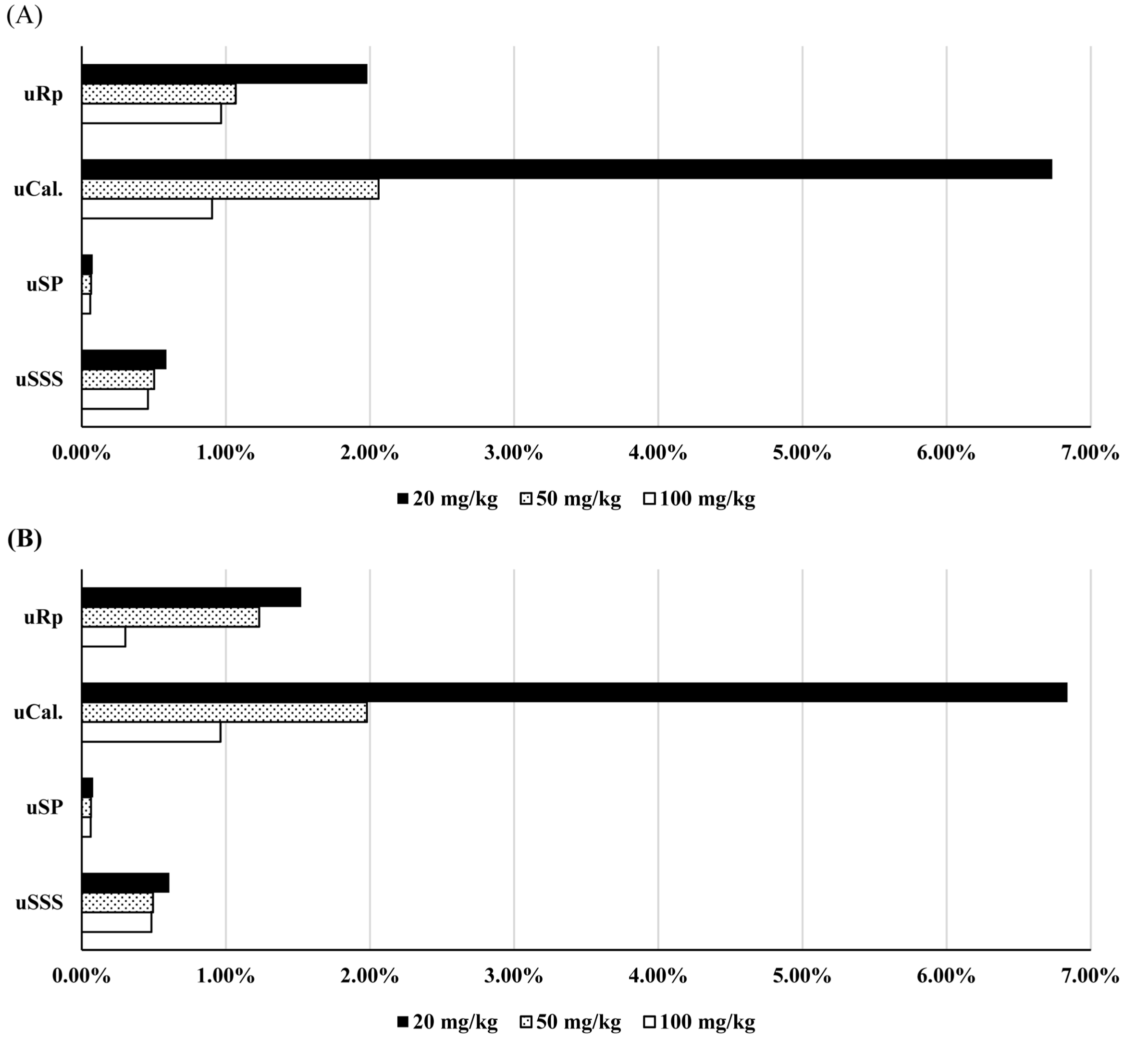

Fig. 1. Uncertainty contributions (*uRP*: Uncertainty of Repeatability, *uCal*: calibration curve, *uSP*: sample preparation, *uSSS*: standard stock solution) to the expanded uncertainty of spiked sodium alginate: seaweed jelly noodles (A) and sherbets (B)

criteria (<8%)[20] and are thus considered appropriate. Table 2 shows the precision, accuracy, and relative uncertainty values associated with the measurement parameters. Fig. 1 shows the effect of each uncertainty factor on the overall uncertainty and displays the percentage (%) of each uncertainty factor relative to the sum of the relative standard uncertainties. In addition, Table 2 shows the total validation results obtained from the recovery experiment. For the analysis of seaweed jelly noodle samples with low and medium concentrations (20 and 50 mg/kg), the *uCal* was the highest, followed by *uRP*, *uSSS*, and *uSP*. At the high concentration (100 mg/kg), the measurement uncertainty was contributed in the order of *uRP*, *uCa*l, *uSSS*, and *uSP*. For sherbet spiked with low and medium concentrations, the *uCal* was the highest, followed by *uRP*, *uSSS*, and *uSP*. For ice sherbet samples with a high concentration, the uncertainty was influenced most by *uCal*, followed by *uSSS*, *uRP*, and *uSP*.

Application

All the collected samples were analyzed for sodium alginate. The average of triplicate analyses was reported. A quantitative analysis of the sodium alginate content in 103 foods covering seven types of food products distributed in Korea showed that sodium alginate was detected in 83 samples, a detection rate of 80.6%. Samples with starch vermicelli were detected (100%) in all 10 samples collected, showing the highest detection rate, followed by instant fried noodles, sugar processed products, and ice creams. The lowest detection rate of 33% was found for seaweed jelly noodle samples. The average detection of the samples was the highest for one of the processed sugar (2,620.1 mg/kg), followed by starch vermicelli (1,555.8 mg/kg), instant fried noodle (1,314.3 mg/kg), and seaweed jelly noodle (874.5 mg/kg) (Table 3). Comparing the monitored sodium alginate content with other study[23]), this experiment detected about

Table 3. Concentrations and the range of sodium alginate in various foods

Food type	Number of samples	Concentration (mg/kg)		
		Range	Overall average concentration	Positive average concentration
Instant fried noodles	19	N.D.[1]-3,744.1	1,245.1	1,314.3
Starch vermicellis	10	146.7-2,739.6	1,555.8	1,555.8
Seaweed jelly noodles	6	N.D.-1,170.6	291.7	874.5
Sugar processed products	18	N.D.-11,893.8	2,474.5	2,620.1
Sherbets	20	N.D.-1,144.8	376.5	412.7
Ice creams	13	N.D.-1,637.4	366.1	680.0
Breads	17	N.D.-927.5	202.2	323.2

[1]N.D.: Not detected or less than LOQ

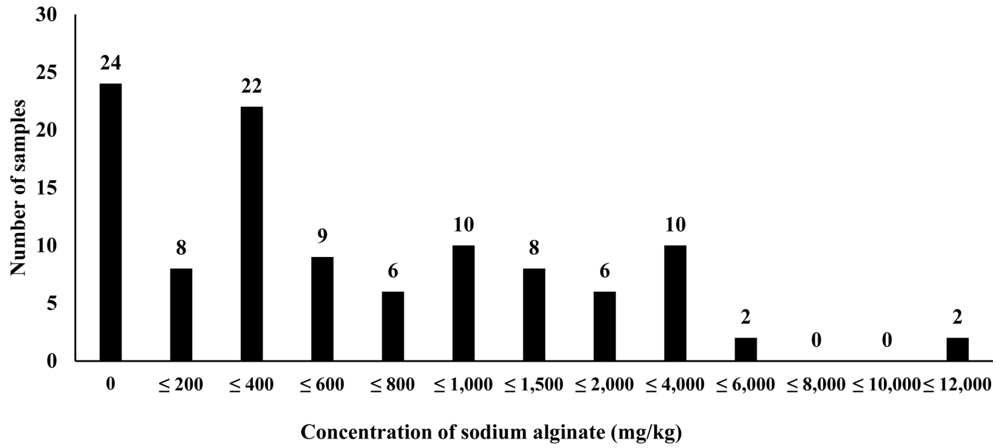

Fig. 2. Frequency distribution histogram of sodium alginate concentrations in foods

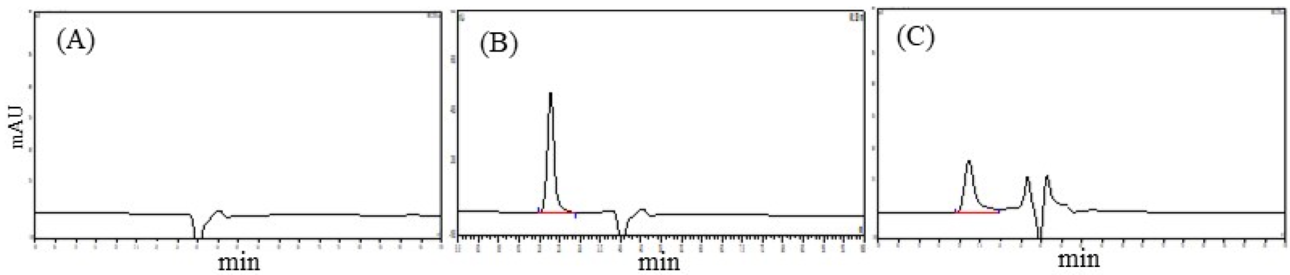

Fig. 3. HPLC chromatograms of blank (A), sodium alginate standard 200 mg/kg (B), and starch vermicelli sample (C).

0.33% of the sodium alginate content is seaweed jelly noodle compared to 0.16%, showing a lower result. It is expected that sodium alginate was added to ice cream as a food additive to smooth the structure of ice crystals and maintain viscosity as a frozen dessert stabilizer and emulsifier, and to prevent aging by using hygroscopicity in the case of noodles. The frequency histogram of food containing sodium alginate as a food additive is shown in Fig. 2. Also, HPLC chromatograms are shown in Fig. 3. Sodium alginate levels ranged from below LOQ (12 mg/kg) to 11,894 mg/kg, for a detection rate of 80.6% (83/103). As a result, the sodium alginate in food products distributed in Korea were within the recommended safe standard. Based on the monitoring results, the developed and validated HPLC-UVD method is expected to be useful for quantifying the levels of sodium alginate used as a food additive in various foods.

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국문요약

식품첨가물로 사용되는 알긴산나트륨은 알긴산염류로서 안정제, 증점제, 유화제 등의 기능을 한다. 알긴산나트륨의 정량법은 전처리가 복잡하고 분석시간이 많이 소요되어 상대적으로 간편하고 보편적인 분석법 연구가 요구되고 있다. 분석장비로는 HPLC-UVD 및 Unison US-Phenyl 컬럼을 사용하였으며, 전처리 조건으로 진탕기를 이용하여 실온에서 150 rpm으로 180분간 추출하였다. 알긴산나트륨의 표준용액을 5개 농도 범위에서 검량선을 작성한 결과 직선성(R^2)은 평균 0.9999로 측정되었으며 검출한계(LOD) 및 정량한계(LOQ)는 각각 3.96 mg/kg, 12.0 mg/kg이었다. 또한, 천사채를 이용해 얻은 일내 및 일간 평균 회수율과 정밀도는 각각 98.47-103.74%, 1.69-3.08 RSD%이고, 빙과류에 대한 일내 및 일간 평균 회수율과 정밀도는 각각 99.95-105.76%, 0.59-3.63 RSD%이다. 상대불확도%는 CODEX의 기준에 적합한 1.5-7.9%의 결과를 나타냈다. 본 연구에서 확립한 방법의 적용성 검토를 위해 총 103개 품목에 대한 알긴산나트륨의 함량을 정량한 결과 당면, 유탕면, 당류가공품 유형 순으로 높은 검출율을 보였다.

Conflict of interests

The authors declare no potential conflict of interest.

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