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A study on the analysis of artificial sweeteners in processed foods including emulsified foods by HPLC-DAD

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Abstract: This study has been carried out to develop a standard method for quantifying of 3 permitted artificial sweeteners (including sodium saccharine, aspartame, acesulfame potassium) contained in foods by HPLC-DAD. A simple and rapid sample pretreatment method was used to remove fat and protein from the test solution with Carrez clearing regent precipitant know to be effective for protein and fat removal. The artificial sweeteners in the test solution purified through sample pretreatment were detected by high performance liquid chromatograph using a Reverse phase C18 column (5 μm, 4.6 × 250 mm). The simultaneous quantitative test of 3 kinds of artificial sweeteners can be effectively performed on the high fat emulsified foods containing a large amount of fat. Using the established simultaneous quantitative test method, artificial sweeteners were tested in foods such as dairy products, snacks and chocolate. The results calibration curve showed good linearity with high regression coefficients and the result of recovery test showed satisfactory recoveries within 80~110 %.

Key words: artificial sweeteners, simultaneous analysis, fat and emulsified food, high performance liquid chromatograph, food additive

1. Introduction

The Food Sanitation Act in Korea defines food additives as *substances used in foods for sweetening, coloring, bleaching, or antioxidation in the process of manufacturing, processing, cooking, or preserving foods*. Considering that a strong sweet flavor can result from the addition of just a small quantity of artificial sweetener, there has been a recent increase in the consumption of artificial sweeteners, as they have been added to a variety of instant and processed foods. Sweeteners with taste sweeter than sugar are being widely used as alternatives to sugar to resolve increased health concerns in consumers, which are caused by elevated sugar intake.[2] However, the overconsumption of artificial sweeteners has been known to induce the presence of various diseases, such as obesity, diabetes, and hyperlipidemia, as well as sweetness addiction. Sweeteners can be classified as artificial sweeteners and natural sweeteners, with

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the former containing a higher level of sweetness and, thus, is used frequently in the food industry. Artificial sweeteners are also known as synthetic sweeteners, as they are chemical synthetics. The sweeteners currently approved for use in Korea are sodium saccharin, acesulfame K, aspartame, sucralose, and neotame. Regarding sodium saccharin, acesulfame K, and aspartame, the Korea Food Standard Codex lists the methods of simultaneous analysis based on high-performance liquid chromatography (HPLC).[1] Testing methods applicable to various foods need to be developed; consequently, attempts have been made to develop standard testing methods based on food characteristics.

To quantitatively analyze artificial sweeteners, several analytical methods are available,[15-23] with the reported methods including gas chromatography (GC) and ion chromatography; meanwhile, for the simultaneous analysis of sodium saccharin, acesulfame K, and aspartame, an HPLC-UV detector (HPLC-UVD) can be used.[6,8] In addition, studies based on mass spectrometry or evaporative light scattering detection, which allows the simultaneous analysis of multiple artificial sweeteners, are ongoing. Such methods have also been reported as approved testing methods in Europe.[11] As sweeteners are currently being added to a variety of food types in Korea, a standard testing method enabling the pre-treatment and quantitative simultaneous analysis of sweeteners across all food products should be developed.

The approved testing methods of artificial sweeteners overseas include the Association of Official Analytical Chemists (AOAC) and EN methods (EUROPEAN STANDARD) through which three types of artificial sweeteners can be simultaneously analyzed.[11,12,13] The method performed by the International Dairy Federation is an approved method for the international testing of dairy products, as various pre-treatment methods are listed based on HPLC. The methods are categorized as those involving emulsified and high-fat foods, for which pre-treatment is difficult.[14]

The use of the Carrez reagent is currently applicable to the pre-treatment of high-protein foods and is listed among the methods of simultaneous analysis of three artificial sweeteners in the Korea Food Standards Codex.[1] The use of the reagent has also been reported in a study analyzing sweetener contents in 83 samples, including various beverages, canned fruits, corn, and chewing gum.[24] In addition to its use in artificial sweeteners, numerous studies have reported the use of the Carrez reagent in sample pre-treatment for various food additives.[25] Emulsified foods are characterized by the fact that they dissolve in both water and oil; thus, their pre-treatment requires either a strong acid or base to remove this emulsifying property prior to pre-treatment. High-fat foods also pose difficulties in pre-treatment and isolation during the analysis, as the removal of fat is challenging. To resolve such technical limitations, this study was aimed at developing a fast and simple standard testing method.

2. Experimental Design

2.1. Reagents and apparatus

In this study, samples were composed of commercially available foods purchased from major supermarkets and common grocery stores in Gyeonggi province between September 2016 and June 2017. The purchased foods included high-fat, emulsified, and non-fat foods. Among them, potato snacks and candies were representative of high-fat foods; chocolate milk, chocolate jam, and yogurt were characterized as emulsified foods; and, fruit and vegetable juices, energy drinks, and ketchup represented non-fat foods. Regarding standard artificial sweeteners, sodium saccharin, aspartame, and acesulfame potassium were obtained from Sigma (St. Louis, MO, USA), and the water used in the preparation of the mobile phase was the product of HPLC grade B&J (Germany). In addition, 1.0 M tetrapropyl ammonium hydroxide solution (TPA-OH) was purchased from Sigma (USA). An ultrapure water generator was used to prepare tertiary distilled water for the analyses.

2.2. Experimental procedures

2.2.1. Preparation of the standard solution

To prepare the standard solution of sodium saccharin, aspartame, and acesulfame K, a reference stock of

1000 mg/L concentration was prepared by dissolving 100 mg of standard artificial sweetener in water. Each reference stock was then mixed and diluted with water to prepare 1, 5, 10, 20, 50, and 100 mg/L solutions to be used as the standard solution for the construction of calibration curves.

2.2.2. Preparation of the test solution

1) Solid and semi-solid foods

Samples of 2–5 g were accurately weighed to 1 mg accuracy and placed in a 100 mL conical flask. Subsequently, 50 mL water was added and mixed well with the sample. Through the action of the ultrasonic extractor, the temperature of the mixture was decreased from 40 °C to room temperature for 20 min. High-fat foods, high-protein foods, and emulsified foods require a process of sample pre-treatment, in which the fat or the emulsifying property is removed. Thus, 2 mL of Carrez reagent I was added and mixed well; subsequently, 2 mL of Carrez reagent II was added and mixed well. The mixture was added to 100 mL of water until reaching the marked line, and the resulting mixture was left to stand at room temperature for 10 min. Subsequently, 100 mL water was added, and the mixture was filtered through a filter paper (the first 10 mL was discarded). A set amount of filtered, clear solution was filtered again using a 0.45 μm membrane filter, and the resulting solution was used as the test solution.

2) Liquid foods

Samples of 2–5 g were accurately weighed down to 1 mg accuracy and placed in a 100 mL conical flask. Subsequently, 50 mL water was added and mixed well with the sample. Using the ultrasonic extractor, the temperature of the mixture was maintained at 40 °C for 10 min (to remove the carbonated gas). For foods containing alcohol, the alcohol was evaporated by heating the food in a water bath at 70 °C. After the mixture was cooled at room temperature, 2 mL of Carrez reagent I was added and mixed well; subsequently, 2 mL of Carrez reagent II was added and mixed well. After the mixture was added to 100 mL of water until reaching the marked line, the mixture was left to stand at room temperature for 20 min. A set amount of the supernatant was filtered using a 0.45 μm membrane filter, and the resulting solution was used as the test solution.

2.2.3. Analysis device and conditions

The device used for the analysis was the HPLC-Photo Diode Array Detector (Shimadzu; Tokyo, Japan), and the Capcell-Pak C_{18} (4.6 mm × 250 mm, packed with 5 μm) was used for the simultaneous analysis. The mobile phase was prepared by adding 10 % TPA-OH (pH 4.0) to a mixture of water and methanol at 8:2, v/v. The optimum detection wavelength was 210 nm, as it allowed the isolation of three sweeteners used in the analysis.

3. Results and Discussion

3.1. Validation

In the case of the testing method described in the Korea Food Standards Codex and the AOAC method, the scope of the sample application is considerably wide such that sample pre-treatment may be unsuccessful for certain food groups. For emulsified foods, in particular, liquid–liquid partition is difficult in the process of extraction and isolation of artificial sweetener from the sample using a solvent. Likewise, for high-fat samples, the removal of fat through Soxhlet extraction is difficult, as the column could become contaminated or the yield could be substantially reduced, making analysis challenging in practice.

In the improved testing method, the Carrez reagent is used to lead to excellent liquid–liquid partition and fat removal in emulsified and high-fat foods, resulting in better yield than those of conventional testing methods, as confirmed by the results of the validation.

3.1.1. Selectivity and linearity

Selectivity was verified by applying the HPLC-UVD to three artificial sweeteners. The result of the isolation of the three standard solutions is shown in *Fig.* 1, and *Fig*. 2 shows the result of the isolation of the three high-fat foods. The order was as follows: acesulfame K, sodium saccharin, and aspartame. Standard solutions of the three artificial sweeteners

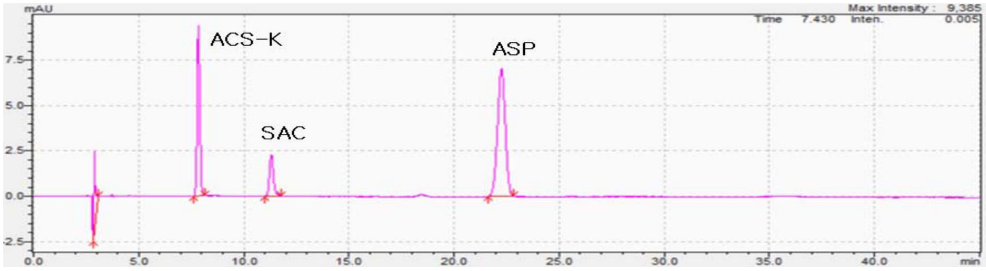

Fig. 1. HPLC chromatogram of acesulfame potassium, sodium saccharin, aspartame.

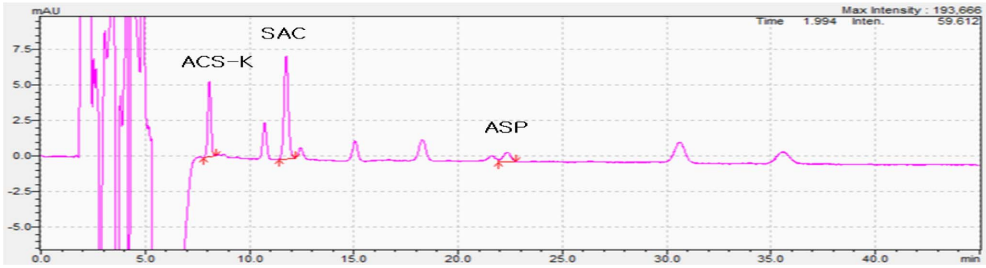

Fig. 2. HPLC chromatogram of HPLC chromatograms of sweetners in fatty food.

were prepared between 1-100 mg/L concentrations and were analyzed using the HPLC-UVD to obtain the calibration curve. The coefficients of determination (R2) for the calibration curves of acesulfame K, sodium saccharin, and aspartame were ≥ 0.9998, 0.9994, and 0.9995, respectively, which indicated an acceptable level of linearity.

3.1.2. Limits of quantification and detection

The limit of detection (LOD) and limit of quantification (LOQ) were estimated using the slope (S) and standard deviation of the y-intercept (σ) of the calibration curve, obtained through seven repeated analyses (*Table* 1). The serial dilution of the standard solution was based on a signal/noise ratio (S/N) ≥ 3 for the LOD and S/N ≥ 10 for the LOQ.

3.1.3. Accuracy and precision

After the spiking of the sample by the standard solution at 30 mg/L concentration, followed by the process of test solution preparation, the collection rate was evaluated. The artificial sweetener content in the purchased samples was as follows: 204 mg/kg aspartame in fruit and vegetable juices; 31 mg/kg sodium saccharin and 20 mg/kg acesulfame K in a chocolate jam; and, 33 mg/kg acesulfame K in ketchup. The results of the evaluation showed that the collection rate of acesulfame K, sodium saccharin, and aspartame were 80.2 %-118.2 %, 81.1 %-102.1 %, and 80.1 %-107.5 %, respectively, for candies, snacks, beverages, coffee, ketchup, jam, fruit and vegetable juices, and emulsified foods. The relative standard deviation (% RSD) obtained through three repeated analyses was 0.2 %-3.4 % for acesulfame K, 0.2 %-3.7 % for sodium saccharin, and 0.2 %-6.9 % for aspartame (*Table* 2).

Table 1. Linearity, LOD and LOQ determined in this study

Sweeteners	Correlation coefficient (R^2)	LOD[a] (Unit : mg/L)	LOQ[b] (Unit : mg/L)
Acesulfame K	$R^2 > 0.99$	0.02	0.06
Saccharin Na	$R^2 > 0.99$	0.01	0.04
Aspartame	$R^2 > 0.99$	0.11	0.33

a : Limit of detection (3.3 σ), mg/L
b : Limit of quantitation (10 σ), mg/L

4. Conclusions

In this study, the Carrez reagent was used in the preparation of the test solution for high-fat and emulsified foods, among the processed foods containing

Table 2. Result of recovery test for 3 artificial sweeteners by 3 institutes

Sample name	Recovery (%) ± RSD (%)		
	Acesulfame K	Saccharin Na	Aspartame
Candy	93.7 ± 2.5	90.0 ± 0.7	88.0 ± 0.5
Potato snack	100.3 ± 2.5	94.6 ± 3.1	107.5 ± 7.5
Energy drink	96.1 ± 3.4	87.2 ± 1.9	93.6 ± 2.3
Processed milk (chocolate)	102.1 ± 1.6	90.1 ± 1.1	85.9 ± 0.2
Ketchup	86.1 ± 1.8	102.1 ± 0.2	80.1 ± 2.5
Chocolate Jam	80.2 ± 0.2	80.7 ± 3.7	97.0 ± 4.2
Fruit juice	118.2 ± 1.2	81.1 ± 1.0	99.6 ± 6.9
Yogurt drink	117.8 ± 0.5	81.7 ± 1.5	81.3 ± 1.2

artificial sweeteners, to develop a simple and efficient pre-treatment for removing the high fat or protein content or emulsifying property within a short time. This effort included the isolation of three artificial sweeteners that were simultaneously analyzed using the HPLC-UVD under pre-established optimum device conditions. The test results for the three artificial sweeteners—acesulfame K, sodium saccharin, and aspartame—showed that the linearity was excellent at ≥ 0.99 for all three sweeteners. In the case of LOD and LOQ, aspartame exhibited the highest levels at 0.11 mg/L and 0.33 mg/L, respectively. The result of yield evaluation, after spiking at 30 mg/L during pre-treatment with the Carrez reagent, showed that the content of acesulfame K was slightly high in fruit and vegetable juices (mixed beverage), whereas ketchup had a slightly low aspartame content at 80.1 % (*Table* 2). The precision of three repeated analyses was shown to be the highest, with a standard deviation of 7.5 % for snacks, a type of high-fat food. The device conditions, as well as the pre-treatment conditions of the simultaneous analysis for artificial sweeteners developed in this study, were found to be applicable to the analyses in practice, allowing the fast and efficient sample pre-treatment of high-fat or emulsified foods in the quantification of sweeteners approved as food additives in Korea.

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