

Extraction Efficiency for the Analysis of Non-Nutritive Sweeteners, Stevioside and Sucralose in Different Types of Food Models

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Abstract— Extraction efficiency (%R) of stevioside and sucralose in food models spiked by different addition levels were observed. The measurement of %R value was finalized by spectrophotometry after a solvent extraction procedures. Results showed that extraction efficiency of stevioside in soft drink was significantly different from each addition levels. In pudding and steamed-bread, the %R's of stevioside extracted from low level were higher than middle and high level, whereas %R's between those both levels were not different. Extraction efficiencies of sucralose in all food models exhibit no certain pattern in different addition levels; however the range of %R's of sucralose was accepted considerably. Overall, steamed-bread was shown to be best food model in determination recovery rate of stevioside and sucralose. It produced smallest range of %R value closest to initial-spiked (95.82–106.46% compare to 100%).

Keywords: extraction efficiency, stevioside, sucralose, food models, recovery rate

I. INTRODUCTION

Stevioside and sucralose are among of non-nutritive sweeteners which used widespread to food product. Stevioside, chemically named steviol-glycosides extracted from *Stevia rebaudiana* Bertoni (Stevia), commonly acted as natural sweetener in beverages and food. It is being preferred over other non-caloric sucrose substances since it is heat-stable, resistant to acid hydrolysis, and non-fermentable (Ahmed & Smith, 2002). Besides as substitution for sucrose, for treatment of diabetes mellitus, obesity, hypertension, and for the prevention of caries, stevioside also exhibits bactericidal activity and inhibits the growth of *Escherichia coli* (Pol et al., 2007). Nowadays, stevioside is commercially available and used in many countries including South America, North America, Asia and some European countries (Kroyer, 2010; Cacciola et al., 2011).

Sucralose is a chlorinated carbohydrate nonnutritive sweetener of food and beverage products. Since 1998 it has got approval over 80 countries for use in thousands of food, consumer and pharmaceutical products (Hanko & Rohrer, 2004; Morlock & Vega-Herrera, 2007; Qiu et al., 2007; Morlock et al., 2011). Essentially, sucralose is a trichlorosucrose produced via selective chlorination of sucrose making it almost non-absorbable by the human

intestines. Despite being a chlorinated product, the toxic relevance of sucralose to humans is judged to be low. A daily intake of no more than 15 mg/kg bodyweight is recommended by the European Union Scientific Committee.

Due to concern on consumer safety, a legislation which limits the content of non-nutritive sweeteners in foodstuffs has been introduced (USFDA, 2006; EFSA, 2009; KFDA, 2009), in order to keep pace with technological developments and to indicate the maximum level of its use in specific food category. The maximum level at which sweeteners may be added to food is called maximum usable dose (MUD).

The study of determination recovery rate of non-nutritive sweeteners in food mostly employ their pure-analytical substance as internal standard for referring measurement in either qualitative or quantitative analysis. But in reality, utilization of non-nutritive sweeteners in foodstuff might possess complex dynamic interactions. In commercial food products which can be formed as liquid, semisolid, and solid state; these matrix differences could be affected in physicochemical reaction among additives contained in a particular food. Yet, it does not much take into account in many analytical measurement of food additives. Hence, a shape of food model need to be more explored as an alternative referring-material in determination of recovery rate of non-nutritive sweeteners.

Recently, utilization of food models in study of ingredients activity have been introduced. Gutierrez et al. (2009) observes antimicrobial efficacy of plant essential oils (EOs) for control of *Listeria* spp. and spoilage bacteria using food model media based on lettuce, meat and milk. Chen et al. (2009) investigate degradation kinetics and isomerization of lycopene in water- and oil-based tomato model systems as a function of thermal treatments and light irradiation.

To approach with the real-dynamic content in commercial food product, it will examine a food model capabilities for determination of recovery rate of two carbohydrate-based sweeteners. The objectives are to measure extraction efficiency of stevioside and sucralose contents in soft drink, pudding, steam-bread; and to investigate potential usefulness of food models for

achieving accurate qualitative and quantitative analysis of non-nutritive sweeteners in various foodstuffs.

II. RESEARCH METHOD

Materials and Reagents

The two carbohydrate-based sweeteners were obtained from different sources: Stevioside (purity was higher than 97%) from Daepyung Co., Ltd (Seongnam-si, Gyeonggi-do, Korea) and Sucralose from JK Sucralose Inc. (Sheyang County, Jiangsu, China). Materials comprising food models such as sugar, pudding premix, eggs, flour, and soda water were purchased from local-commercial market; except benzoic acid and ascorbic acid were provided from Sigma-Aldrich (St. Louis, Missouri, USA) and Junsei Chemical, Co., Ltd (Chuo-ku, Tokyo, Japan), respectively. Chemicals at recognized analytical grade were methanol (Fisher Scientific Korea Ltd, Kangnam-gu, Seoul, Korea), ethanol (SK Chemicals, Ulsan, Korea), and concentrated sulfuric acid, >98% (Daejung Chemical & Metals Co., Ltd, Siheung-si, Gyeonggi-do, Korea). Deionized water was produced in our laboratory using Water Still electrically heater (Model C-DIS1, Chang Sin Science, Jongno-gu, Seoul, Korea).

Anthrone reagent was prepared by dissolving 400 mg of crystalline anthrone, 97% (Sigma-Aldrich, St. Louis, Missouri, USA) in 16 mL of ethanol and 60 mL of deionized water. Amount of 200 mL concentrated sulfuric acid was then added slowly into the solution while immersed in an ice bath, and subsequently mixed until the anthrone crystals were completely dissolved in approximately 3–5 min (Guzman, 2010). Similarly, the ADOPA (aniline diphenylamine orthophosphoric acid) reagent was prepared by dissolving 1.2 g aniline, 99% (Sigma-Aldrich, St. Louis, Missouri, USA) and 1.2 g diphenylamine (Junsei Chemical, Chuo-ku, Tokyo, Japan) in 100 mL methanol and then added with 10 mL orthophosphoric acid. The reagent was stable for at least one month if stored at 5°C in the dark (Morlock & Vega-Herrera, 2007). Standard solution of stevioside was made by dissolving appropriate amount of stevioside in 25 mL deionized water, meanwhile for those of sucralose was dissolved in 25 mL methanol.

Designing Food Models

Commercial food model were prepared as described by Wibowotomo (2008) with some modifications (Table 1).

Table 1. Recipes composition of different food models

Materials	Food model		
	Soft drink	Pudding	Steamed-bread
1. Water (mL)	100	100	20
2. Sugar (g)	10*	12*	30
3. Flour (g)	-	-	30
4. Egg (g)	-	-	20
5. Benzoic acid (mg)	100*	-	-
6. Citric acid (mg)	100*	-	-
7. Premix (g)	-	0.7*	-
Total recipe weight (g)	100	100	100

*Assumed to dissolve in solution/water

1. Soft drink. A 10 g of sugar, 100 mg of benzoic acid, and 100 mg of ascorbic acid is put into 100 ml beaker glass; add 100 ml water and stirred thoroughly. After heating at 80°C for 10 min, filter the solution with 450 ng filter paper and keep in dark, vacuum bottle when reach ambient temperature.
2. Pudding. Amount of 12 g sugar is diluted in 100 ml of water. Add 700 mg of pudding premix, then mix until the pudding well blended. Boil for 1 min over medium heat while stirring regularly. Pour into bowl or individual serving cups. Chill until completely cool.
3. Steamed-bread. Mix 20 g of egg, 25 g of sugar and flavor until they make a smooth batter. Then put in turns 30 g of flour and 20 g water/soda, mix well. Divide the batter into individual cups evenly, then steamed for around 25 min.

Spiking and Preparation of Fortified Test Materials

Soft drink were degassed by sonication for 15 min, whereas pudding and steamed-bread were homogenized using a food blender (Model SMX-75CM). In order to investigate the extraction efficiency, appropriate weights (mg's) of individual sweetener were added to each types of food model as follows: 1) soft drink, mix well after heating 80°C, 10 min; 2) pudding, add straightly to the water together with 700 mg of pudding premix; and 3) steamed-bread, dissolved in 20 g of water or soda, before put in turns with 30 g of flour. Three concentration levels prepared following the method described by Zyglar et al. (2011) were low level, middle level, and high level; corresponding to 75%, 100% and 125% of a MUD value (Table 2). Initial addition was underlain to 100% MUD from KFSA (2009) for each respective food models.

Table 2. Calculated-concentration of sweeteners in 3 type of food models with different addition levels

Sweeteners	Addition level	Concentration in food model (mg L ⁻¹)		
		Soft drink	Pudding	Steamed-bread
Stevioside	Low (75%)	75	150	225
	Middle (100%)	100	200	300
	High (125%)	125	250	375
Sucralose	Low (75%)	300	750	375
	Middle (100%)	400	1,000	500
	High (125%)	500	1,250	625

Extraction Procedures

- a) Stevioside: A thousand-milligrams of sample was sonicated with 20 ml of methanol for 10 min. The mixture was centrifuged at 1500×g for 5 min, and the supernatant was transferred into a 25 ml flask, while the solid residue was extracted twice as described above. The volume obtained for every extract was set up by methanol, diluted with methanol, and centrifuged at 4000×g for 1 min. The alcoholic extract (1 ml) was diluted with water (2 ml), and then adjusted to 10 ml by

methanol (modified from Gardana et al., 2010).

- b) Sucralose: Sample of soft drink, pudding, or steamed-bread (± 5.0 g) was mixed with 20 mL of methanol-water (1:1). The mixture was shaken, treated for 10 min in an ultrasonic bath (Bandelin, DE/RK510H) to extract sucralose. The obtained slurry was filtered, and then filled with the solvent mixture to 50 mL (modified from Spangenberg et al., 2003).

Measurement of Extraction Efficiency

1. Stevioside: A one-milliliter aliquot of sample or standard solution was mixed with 5 mL of the anthrone reagent in a 15 mL test tube. After vortex mixing, test tubes were covered with foil and immersed in a boiling water bath (JSSB-50T) for 8 min. Solutions were then quickly cooled to room temperature by placing in a water bath. Tubes were given a final vortex mix before dispensing to a 1 mL cuvette, with absorbance assessed at 630 nm relative to the anthrone reagent alone using UV-Vis spectrophotometer (Optizen 2120 UV).
2. Sucralose: In a 25 mL Erlenmeyer, 10 mL volume of ADOPA reagent was entered and subsequently added with 3 mL of each extract or standard solution. After 20 min heating by water bath (100°C), the gradation gray-bluish of final solution were measured by UV-Vis spectrophotometer at 405 nm against water as blank.

Data Analysis

The determination of extraction efficiency is calculated as

$$\%R = \frac{F-I}{A} \times 100\%$$

Where:

F is absorbance of the spiked portions

I is absorbance of non-spiked portions

A is absorbance of standard solution correspond to respective MUD value of each sweeteners

All experiments were performed in duplicate (n=2) along with two repetitive measurements for each sample. The results are expressed as the mean \pm standard deviation, and statistical significance was analyzed by analysis of variance (ANOVA) using SPSS 20.0 statistical software. LSD tests were used for comparisons amongst value. Differences were considered to be significantly different at $P < 0.05$.

III. RESULT

Effect of food models containing different addition levels on extraction efficiency of stevioside

Extraction efficiency of stevioside extracted from food models containing different addition levels are illustrated in Table 3. Extraction efficiency in soft drink were significantly different among different addition levels.

The %R value produced by low level concentration was highest and tend to be closest to initial-spiked value (73.29 mg L⁻¹ accounted from 75 mg L⁻¹), whereas middle level was lowest and most far from initial-spiked value (81.50 mg L⁻¹ accounted from 100 mg L⁻¹).

Table 3 Extraction efficiency of Stevioside extracted from various food models containing different addition levels (%)

Addition levels	Food models		
	Soft drink	Pudding	Steamed-bread
Low level (75 ppm)	97.72 \pm 0.04 ^A	-	-
Middle level (100 ppm)	81.50 \pm 0.33 ^C	-	-
High level (125 ppm)	93.19 \pm 0.24 ^B	-	-
Low level (150 ppm)	-	129.59 \pm 3.31 ^A	-
Middle level (200 ppm)	-	105.36 \pm 0.61 ^B	-
High level (250 ppm)	-	104.67 \pm 0.24 ^B	-
Low level (225 ppm)	-	-	102.49 \pm 1.09 ^A
Middle level (300 ppm)	-	-	95.82 \pm 1.06 ^B
High level (375 ppm)	-	-	97.33 \pm 0.75 ^B

*Same superscript in each column are not significantly different ($P < 0.05$)

In pudding and steamed-bread, recovery rate produced by low level was significantly higher than middle and high level, meanwhile middle and high level were not different. Extraction efficiency by low level in pudding was even though highest and had differences significantly, it was more considered out from acceptable %R range which is 70–120% (EC, 2010). Similarly, the best %R value of steamed-bread which came from low level concentration showed closest to initial-spiked value (100%) and made significant difference with those of middle and high level. Regarding to average %R value closest to initial spiked-value among food models, steamed-bread showed highest correlation (98.55%) compare to soft drink (90.80%) and pudding (113.21%).

Effect of food models containing different addition levels on extraction efficiency of sucralose

Extraction efficiency of sucralose extracted from food models containing different addition levels are described in Table 4. There was no certain pattern on differences of extraction efficiency amongst addition level in soft drink. Extraction efficiency of middle level reached highest rate, whereas %R value of high level showed smallest value and even closes to lowest range of recovery rate (75.51%). Interestingly, the %R value from low level (97.03%) showed closest value to initial-spiked value (100%). These %R value was relatively better than Zyglar (2011) which had %R value ranged 93.5–96.6% produced from cola drink spiked with 300 mg/g of sweeteners and extracted by SPE method.

Table 4 Extraction efficiency of Sucralose extracted from various food models containing different addition levels (%)

Addition levels	Food models		
	Soft drink	Pudding	Steamed-bread
Low level (300 ppm)	97.03 ± 0.80 ^B	-	-
Middle level (400 ppm)	109.22 ± 4.00 ^A	-	-
High level (500 ppm)	75.51 ± 1.20 ^C	-	-
Low level (750 ppm)	-	98.18 ± 1.82 ^A	-
Middle level (1,000 ppm)	-	93.09 ± 4.20 ^{AB}	-
High level (1,250 ppm)	-	87.65 ± 4.25 ^B	-
Low level (375 ppm)	-	-	106.46 ± 4.01 ^A
Middle level (500 ppm)	-	-	105.16 ± 0.06 ^A
High level (625 ppm)	-	-	99.61 ± 2.51 ^B

*Same superscript in each column are not significantly different ($P < 0.05$).

The ordered %R value in pudding exhibit decreasing pattern as increasing level of spiking, in which the %R of low level reached highest value and closest to initial-spiked value (100%). Similar tendency was also happened in steamed-bread regarding with lowering %R as increasing level of spiking, but opposite on closest %R to initial-spiked value (100%), which was produced by high level concentration. Among proposed matrices, steamed-bread achieved best average %R as well regarding closest value to initial spiked-value (103.74%) compare to soft drink (93.92%) and pudding (93.64%).

IV. DISCUSSION

Designing Food Models

The choice of food models designing is based on food matrix classification comprise of liquid food, semisolid food, and solid food (Rao, 2013). In this experiments, the liquid food was represented by soft drink type food model, whereas pudding and steamed-bread was purposed to be semisolid and solid food model, respectively. The composition of food model was comprised of basic materials that characterize specific matrix while minimizing addition of food additives, mainly flavoring and coloring. Hence, soft drink is made from water, sugar, benzoate, and citric acid (Mitchell, 1990) and steamed-bread is consisted of flour, sugar, white-egg, and baking soda (Wibowotomo, 2008).

Total recipe weight was obtained by counting up all materials for each food model. Some ingredients in Table 1 need to be assumed for dissolving in water, in order to reach constant total weight, even though they were essentially formed in powder. The calculated weight aggregate was then confirmed by real weighing of finished products (data not shown). Before adding to whole batter, dry powder of sweeteners are dissolved firstly in solvent/water, but its calculated-amounts always referred to the total recipe weight.

By composition, the proposed food models have similarity with recent works in determination of food additives. Zygler et al. (2011) used 200 g of blank yoghurt, home-made fish product and 200 mL of sugar sweetened cola drink to explore SPE-HPLC/MS procedure for analysis of a group of high-intensity sweeteners in various foodstuffs. Gutierrez et al. (2009) made three types of food systems to optimize antimicrobial efficacy of plant essential oils (EOs) for control of *Listeria* spp. and spoilage bacteria. They are (i) lettuce leaf media, suspension obtained from iceberg

lettuce (*Lactuca sativa* sp.) in 100 ml of deionized water with pH of 7.2; (ii) meat-based media, performed with beef extract; and (iii) milk media, made from mixing skimmed milk powder with agar solution to obtain a final solid media.

Spiking and Preparation of Fortified Test Materials

The addition level of sweeteners to obtain certain concentration was based on maximum usage doses for specific group of foodstuff (KFDA, 2009). Basically, the permitted use level in regulated standard was used as middle level (100%), and then it spread to low and high level by taking 75 and 125%, respectively. This range of concentration level was slightly different with Zygler (2011) which used 50 and 125% as low and high level, respectively.

Extraction Procedures

Application of solvent/liquid extraction method in recovery rate determination of stevioside and sucralose have been studied. Pól et al. (2007) observed comparison of methanol versus water solvents employed for pressurized fluid extraction of stevioside from *Stevia rebaudiana*. Methanol showed better extraction ability for isolation of stevioside from *Stevia rebaudiana* leaves than water within the range 110–160°C. In line with this, Kishi et al. (2001) developed simple and rapid methods for the determination of sucralose in foods using anion-exchange chromatography (AEC) and reverse-phase HPLC. Sucralose concentrate injected to AEC and HPLC was obtained from liquid-extraction with water or methanol, followed by cleaning-up on SPE cartridge. The final determination results of recovery rates of sucralose from foods were 80.6–102.0%.

The main goal of sample preparation or clean-up is to achieve maximum recovery by suppressing interfering compounds, which can be formed in turbidity or emulsions (Zygler et al., 2009). For simple matrix (i.e. beverages, powdered drinks, syrups and juices), these procedures can be done simply by diluting or dissolving samples in deionized water, an appropriate buffer or a mixture of a buffer with methanol or ethanol. Whichever techniques are taken, samples must be filtered before final determination.

Regarding with our sweeteners sample, the selection of extraction method for was undertaken by considering simplicity of procedures, reagent exertion, and ability to produce clear final-solution. Hence, the extraction step of stevioside were merely consisted of sonication in 20 mL of methanol, twice centrifugation for 5 and 1 minutes, then adjusting volume to 10 mL with methanol. For sucralose acquisition, the sonication was also done, but differently, it was then followed by treating in water-bath for 10 minutes and set-up volume by mixture of methanol-water (1:1) to 50 mL after filtering. Both of these extraction techniques were proven to yielded clear aliquots without emulsion.

Effect of Food Models Containing different Addition Levels on Extraction Efficiency of Stevioside

The obtained extraction efficiency of stevioside ranged from 81.50 to 129.59%; meanwhile the average

%R of each food model were 90.80% for soft drink; 113.21% for pudding; and 98.55% for steamed-bread. These overall range of %R was closely similar with Gardana et al. (2010) who undertook assay of Steviolglycosides extracted by methanol, cleaned-up by SPE and separated by UHPLC; demonstrated good accuracy of 89–103%. However, in comparison with Bovanova et al. (1998) who resulted recovery rates from 92.8% to 97.8% in running HPLC for determination of stevioside in *Stevia rebaudiana* and in some beverages, these output was slightly less sensitive. The most recent accurate extraction efficiency of stevioside was perhaps by Bergs et al. (2012) who developed fast HPLC for analysis steviol glycosides from *Stevia rebaudiana* leaves; achieved percentage of mean recovery rate to 100.99 ± 2.01 %.

The ascending %R pattern of stevioside from soft drink and steamed-bread to pudding is actually quite out of the ordinary. Kroyer (2010) reported that stevioside in aqueous solutions is remarkable stable in a pH range 2–10 under thermal treatment up to 80°C. Moreover, in a stevioside-sweetened coffee and tea beverage practically, no significant changes neither in caffeine content nor in stevioside content could be noticed. FDA (2009) also suggested that stevioside is more suitable to use in drink and beverage products. These big gap of %R from initial-spiked value in soft drink and pudding is needed to be investigated further.

Effect of food models containing different addition levels on extraction efficiency of sucralose

The resulted extraction efficiency of sucralose ranged from 75.51 to 109.22%; whereas the median of %R in each food model were 93.92% for soft drink; 93.64% for pudding; and 103.74% for steamed-bread. These recovery rate was considerably consistent with recent works, but slightly wider in the %R range. Kishi and Kawana (2001) obtained recovery rates were 80.6–102.0% in developed simple and rapid methods for determination of sucralose in foods using anion-exchange chromatography (AEC) with pulsed amperometric detection (PAD) and reverse-phase HPLC with refractive index detection. Kobayashi (2001) developed a method for determination of sucralose in foods by RI-HPLC and ion chromatography with PAD. The recoveries of various foods spiked at 50 mg/g and 200 mg/g ranged from 88% to 100%.

V. CONCLUSION

Extraction efficiency of stevioside in soft drink was significantly different among addition levels. In both pudding and steamed-bread, the %R extracted from low level was significantly higher than both middle and high middle, whereas the %R between middle and high level were not different.

There was no certain pattern on differences of extraction efficiency of sucralose in soft drink amongst addition level. The %R in pudding and steamed-bread exhibited decreasing pattern as level of spiking increased. The %R in pudding produced by low level reached highest value and closest to initial-spiked value (100%), whereas the highest %R and closest to initial-spiked

(100%) in steamed-bread was achieved by high level concentration.

Overall, the steamed-bread was shown to be best food model in determination recovery rate of stevioside and sucralose. It produced smallest range of %R value closest to initial-spiked (95.82–106.46% compare to 100%)..

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