INTRODUCTION

Flavor enhancers, both natural and synthetic, are widely used in food products. In addition to monosodium L-glutamate (2-amino pentanedioate or 2-amino glutaric acid), disodium 5’-guanylate (DSG) and disodium 5’-inosinate (DSI) are frequently used in traditional Asian foods [1]. The PERMENKES 033 of 2012 guidelines has approved the use of L-glutamic acid and its salts, guanylic acid and its salts, inosinic acid and its salts, and salts from disodium 5’-ribonucleotides as flavor enhancers [2].

The salt forms of inosine monophosphate and guanosine monophosphate are potent flavor enhancers that are frequently used as additives along with monosodium L-glutamate [3]. DSG and DSI are purine nucleotides that are synthesized in vivo at rates consistent with physiological needs. Purine biosynthesis involves three important components: An amphibolic intermediate, purine phosphorylase, and purine nucleoside phosphorylase [4].

The amount of a food additive consumed per day should not exceed the recommended acceptable daily intake (ADI), which is the maximum amount of a food additive measured in milligrams per kilogram of body weight that can be consumed daily for life without causing adverse health effects [5]. According to Fenaroli’s Handbook of Flavor Ingredients, the ADI values of DSG and DSI are about 0.07768 and 0.09053 mg/kg/day, respectively.

Purine nucleotide intake that exceeds the ADI may lead to metabolic disorders, such as gout, Lesch–Nyhan syndrome, adenosine deaminase deficiency, and purine nucleoside phosphorylase deficiency [4]. Of these, gouty arthritis is an inflammatory disorder caused by excessive consumption of DSG and DSI, which results in increased uric acid levels in the blood and subsequent inflammation due to the crystallization of sodium urate in the soft tissues and joints [6].

The daily use of flavor enhancers poses a potential danger to the amount of non-essential purine nucleotides that enter the body. Therefore, research is needed to optimize and validate analytical methods to determine the levels of DSG and DSI in spices, such as thin-layer chromatography (TLC)-densitometry. In addition, a previous study identified 10 types of nucleotides in shrimp using high-performance liquid chromatography [7]. However, that study did not report the levels of DSG and DSI in the spice samples that are widely used in Asian communities. In the present study, TLC-densitometry was chosen to determine the levels of DSG and DSI in food spices by thin-layer chromatography-densitometry.
The 2 concentration was carried out using a linear regression equation and 3 times. The calculation of the sample with aquadest in a 100-mL flask to a concentration of 30,000 ppm.

About 3 g of each of the six samples tested in this study was dissolved to a total volume of 25 mL. A 1-μl aliquot of the test solution at each concentration of 1000 μg/mL. A calibration curve was determined from a dilution of the main solution with a correlation coefficient of >0.999 [10]. The solution for the calibration curve was obtained from the calibration curves of DSG and DSI, was used to calculate the factors of linearity of the line, which had a correlation coefficient of >0.999 [10]. The solution for the calibration curve was obtained from a dilution of the main solution with a concentration of 1000 μg/mL. A calibration curve was determined using concentrations of 150, 200, 250, 300, 450, and 500 μg/mL for DSG and 150, 200, 250, 300, 350, 400, and 450 μg/mL for DSI. The limits of detection and quantitation were determined with the winCATS program by entering a wavelength range of 200–780 nm using deuterium and a tungsten lamp on the “Spectral-Scanner 3” menu.

A linearity equation, obtained from the calibration curves of DSG and DSI, was used to calculate the factors of linearity of the line, which had a correlation coefficient of >0.999 [10]. The solution for the calibration curve was obtained from a dilution of the main solution with a concentration of 1000 μg/mL. A calibration curve was determined using concentrations of 150, 200, 250, 300, 450, and 500 μg/mL for DSG and 150, 200, 250, 300, 350, 400, and 450 μg/mL for DSI. The limits of detection and quantitation were determined from the calibration curve equation [10]. Selectivity was determined by comparing the analytical results of the samples containing contaminants, one kind of compound, other foreign compounds, or placebo carriers without no other compounds [11].

For accuracy and precision testing, 750 mg of the sample powder was weighed and transferred to three 25-ml flasks (flasks 1, 2, and 3). Three different concentrations of standard solution (150, 300, and 450 ppm) were made. Flask 1 contained 3.75 mL of 150 ppm standard solution, flask 2 contained 7.5 mL of 300 ppm standard solution, and flask 3 contained 11.25 mL of 450 ppm standard solution. Standard solutions of low, medium, and high concentrations were obtained from the calibration curves. The standard solutions were mixtures of the standardized DSG and DSI. Afterward, aqueous test was added to each flask to a total volume of 25 mL. A 1-μl aliquot of the test solution at each concentration was analyzed 6 times.

Sample preparation
About 3 g of each of the six samples tested in this study was dissolved with aquadest in a 100-ml flask to a concentration of 30,000 ppm. The samples were then sonicated for 15 min and filtered through Whatman’s no. 41 filter paper.

Determination of sample level
All samples were prepared in the same manner. Briefly, 1 μl of each sample was analyzed under optimal analytical conditions. The experiment was repeated 3 times. The calculation of the sample concentration was carried out using a linear regression equation obtained from the obtained calibration curve.

Validation of analytical methods
A linearity equation, obtained from the calibration curves of DSG and DSI, was used to calculate the factors of linearity of the line, which had a correlation coefficient of >0.999 [10]. The solution for the calibration curve was obtained from a dilution of the main solution with a concentration of 1000 μg/mL. A calibration curve was determined using concentrations of 150, 200, 250, 300, 450, and 500 μg/mL for DSG and 150, 200, 250, 300, 350, 400, and 450 μg/mL for DSI. The limits of detection and quantitation were determined from the calibration curve equation [10]. Selectivity was determined by comparing the analytical results of the samples containing contaminants, one kind of compound, other foreign compounds, or placebo carriers without no other compounds [11].

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Determination of sample level
All samples were prepared in the same manner. Briefly, 1 μl of each sample was analyzed under optimal analytical conditions. The experiment was repeated 3 times. The calculation of the sample concentration was carried out using a linear regression equation obtained from the obtained calibration curve.
The selectivity test results had an α-value of 1.86, which met the requirement of α>1.0. Thus, the analytical conditions of both test substances were considered as good with selective separation potency.

The results of accuracy and precision testing showed that the methods had met the accuracy and precision requirements (% UPK, 98–102%, and % KV, ≤2.0%) [10,12,13].

| Concentration (µg/mL) | Measured area* (mm²) | Actual area** (mm²) | Measured concentration (µg/mL) | % UPK | % Average of UPK | SD | % KV |
|----------------------|----------------------|---------------------|---------------------------------|-------|------------------|----|------|
| 152.1               | 496.9                | 2372.2              | 153.15                          | 100.69|                  |    |      |
| 150.9               | 695.7                | 294.5               | 149.38                          | 98.99 |                  |    |      |
| 301.8               | 782.7                | 381.5               | 298.05                          | 98.76 |                  |    |      |
| 452.7               | 875.4                | 474.2               | 459.38                          | 101.48|                  |    |      |

DSG: Disodium 5’-guanylate, SD: Standard deviation, response detector (AU)

Table 2: Accuracy and precision test result of DSI

| Concentration (µg/mL) | Measured area* (mm²) | Actual area** (mm²) | Measured concentration (µg/mL) | % UPK | % Average of UPK | SD | % KV |
|----------------------|----------------------|---------------------|---------------------------------|-------|------------------|----|------|
| 150.9               | 695.7                | 294.5               | 149.38                          | 98.99 |                  |    |      |
| 301.8               | 782.7                | 381.5               | 298.05                          | 98.76 |                  |    |      |
| 452.7               | 875.4                | 474.2               | 459.38                          | 101.48|                  |    |      |

DSI: Disodium 5’-inosinate, SD: Standard deviation, response detector (AU)

*Measured area=Standard addition of the sample, **Actual area=Standard area of the addition, DSI: Disodium 5’-inosinate, SD: Standard deviation, Response Detector (AU)

The selectivity test results had an α-value of 1.86, which met the requirement of α>1.0. Thus, the analytical conditions of both test substances were considered as good with selective separation potency.

The results of accuracy and precision testing showed that the methods had met the accuracy and precision requirements (% UPK, 98–102%, and % KV, ≤2.0%) [10,12,13].

Fig. 1: A chromatogram from the separation analysis of both compounds using silica gel 60F 254 plate with a mobile phase of (6:3:1) isopropanol:water:25% ammonia and analyzed at a wavelength of 260 nm
The determined levels of DSG and DSI in the six samples were not in agreement with the recommended levels in Fenaroli’s Handbook of Flavor Ingredients of 0.07768 and 0.09053mg/kg/day, respectively.

### Table 3: DSG Levels of the six spice samples

| Sample | Sample concentration (µg/mL) | Area (mm²) | Measured concentration (µg/mL) | Level (%) | Amount per pack (mg) | Amount of daily consumption based on ADI (mg/60 kg body weight/day) |
|--------|-------------------------------|------------|---------------------------------|-----------|----------------------|---------------------------------------------------------------|
| A      | 30126 2206.6 2281.5 2183.5   | 207.05     | 0.70                            | 63/9 g    | 1.05                 |
|        | 211.19 | 211.43                |                                  |           |                      |
| B      | 30090 2327.2 2307.6 2365.6   | 237.75     | 0.79                            | 86.9/11 g | 1.45                 |
|        | 231.14 | 247.36                |                                  |           |                      |
| C      | 30160 2368.6 2349.6 2244.2   | 248.12     | 0.78                            | 156/20 g  | 0.29                 |
|        | 243.31 | 216.58                |                                  |           |                      |
|        | Average 305.66                    | 236        |                                 |           |                      |
| D      | 30762 2594.7 2599.4 2592.5   | 305.45     | 0.99                            | 247.5/25g | 4.14                 |
|        | 306.64 | 304.89                |                                  |           |                      |
|        | Average 305.66                    | 305.66     |                                 |           |                      |
| E      | 30175 2859 2661.9 2506       | 372.46     | 1.08                            | 864/80g   | 14.4                 |
|        | 322.49 | 282.96                |                                  |           |                      |
|        | Average 305.97                    | 325.97     |                                 |           |                      |
| F      | 30226 2653 2690.7 2703.1    | 320.23     | 1.08                            | 10800/kg  | 180                  |
|        | 329.79 | 332.91                |                                  |           |                      |
|        | Average 327.64                    | 327.64     |                                 |           |                      |

DSG: Disodium 5'-guanylate, ADI: Acceptable daily intake

### Table 4: Results of DSI level on six samples of food spices

| Sample | Sample concentration (µg/mL) | Area (mm²) | Measured concentration (µg/mL) | Level (%) | Amount per pack (mg) | Amount of daily consumption based on ADI (mg/60 kg body weight/day) |
|--------|-------------------------------|------------|---------------------------------|-----------|----------------------|---------------------------------------------------------------|
| A      | 30126 886.9 887.1 881.2     | 198.02     | 0.66                            | 59.4/9 g  | 0.99                 |
|        | 200.38 | 198.78                |                                  |           |                      |
| B      | 30090 887.5 800.7 803.2    | 197.77     | 0.74                            | 81.4/11 g | 1.36                 |
|        | 233.72 | 232.69                |                                  |           |                      |
| C      | 30160 832.6 814 892.5     | 220.51     | 0.71                            | 142/20 g  | 2.37                 |
|        | 228.21 | 195.7                 |                                  |           |                      |
| D      | 30762 892.7 835.8 890.1   | 195.62     | 0.66                            | 165/25 g  | 2.75                 |
|        | 196.69 | 203.83                |                                  |           |                      |
| E      | 30175 978.3 973.2 961.7   | 160.16     | 0.54                            | 432/80g   | 7.2                  |
|        | 162.28 | 167.04                |                                  |           |                      |
| F      | 30226 820.6 880.6 927.8 | 163.16     | 0.67                            | 6700/kg   | 11.67                |
|        | 225.48 | 200.63                |                                  |           |                      |
|        | Average 202.49                    | 202.49     |                                 |           |                      |

DSG: Disodium 5'-guanylate, ADI: Acceptable daily intake

The determined levels of DSG and DSI in the six samples were not in agreement with the recommended levels in Fenaroli’s Handbook of Flavor Ingredients of 0.07768 and 0.09053mg/kg/day, respectively.

**CONCLUSION**

On the basis of these results, it can be concluded that (a) the optimum conditions of DSG and DSI for TLC-densitrometry were obtained with
silica gel 60 F\textsubscript{254} as the stationary phase, isopropanol:water:25% ammonia (6:3:1) as the mobile phase, and a maximum wavelength of about 260 nm. (b) Validation results indicated that the accuracy of the analytical method for DSG was about 99.11–99.96% with a coefficient variation (precision) of 0.70–1.41%, while that for DSI was 98.56–101.05% with a coefficient variation of 0.23–0.75%. The correlation coefficients for the analytical method for DSG and DSI were 0.9909 and 0.9976, respectively. (c) The results determined that the levels of DSG and DSI in samples A, B, C, D, E, and F were 0.70%/0.60%, 0.79%/0.74%, 0.78%/0.71%, 0.99%/0.66%; 1.08%/0.54%, and 1.08%/0.67%, respectively.

CONFLICTS OF INTEREST
All authors have none to declare.

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