

Which Fruits and Vegetables Should Be Excluded from a Low-Salicylate Diet? An Analysis of Salicylic Acid in Foodstuffs in Taiwan

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Keywords

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Abstract

Background: Eliminating pseudoallergens is an important element of managing chronic spontaneous urticaria (CSU). Salicylic acid (SA) is a primary pseudoallergen in plant-based foodstuffs. Current dietary recommendations are not applicable in East Asia because data on the SA content of many vegetables and fruits commonly consumed in this region are lacking. **Methods:** We therefore determined the concentration of free SA in 79 popular vegetables and fruits frequently consumed in Taiwan using gas chromatography-mass spectrometry. **Results:** The SA content ranged from 0.09 to 2.3 mg/kg in the fresh vegetables examined, and from 0.01 to 0.48 mg/kg in the fruits. **Conclusions:** Data regarding the SA content of East Asian vegetables and fruits could help CSU patients limit their pseudoallergen consumption.

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Introduction

Chronic urticaria is a skin disorder that manifests as wheals or angioedema on an almost daily basis over a period ranging from several months to years. Based on the presence or absence of a specific trigger, the disease can be classified as either chronic inducible urticaria or chronic spontaneous urticaria (CSU), respectively. However, with the absence of a specific trigger, the management of CSU typically involves only symptom control. Some studies have reported an association between CSU and food intolerance. Results of several intervention trials showed that the elimination of “pseudoallergens” from a patient's diet can reduce the severity of CSU [1, 2]. Guidelines released by the European Academy of Allergy and Clinical Immunology (EAACI), the Global Allergy and Asthma European Network (GA²LEN), the European Dermatology Forum (EDF), and the World Allergy Organization (WAO) recom-

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mend that CSU patients consume a pseudoallergen-free diet for 3 weeks [3]. Even though data from some regions outside Europe dispute the therapeutic efficacy of dietary management in controlling CSU, in our practice, we have observed a reduction in the need for medication among Taiwanese CSU patients who consume a low-pseudoallergen diet.

Salicylic acid (SA) is widely recognized as a pseudoallergen that is important in the pathophysiology of CSU. SA is ubiquitous in foodstuffs derived from plants, including fruits, vegetables, and spices. Although the clinical significance of SA is still being debated, serum SA levels reportedly increase within hours after consuming fruits and vegetables [4]. Serum concentrations of SA in some vegetarians are reportedly comparable to those in subjects regularly taking low doses of aspirin [5]. Compared with other pseudoallergens, elimination of SA from the diet is particularly difficult to achieve. Fruits and vegetables are important sources not only of SA but also of fiber, potassium, and vitamin C. Therefore, strict avoidance of fruits and vegetables can adversely impact health. To determine which foodstuffs should be excluded from a low-salicylate diet, it is necessary to know the amount of SA in different fruits and vegetables.

Several studies have reported the SA concentrations of various fruits and vegetables. Swain et al. [6] determined the SA concentrations in 333 foodstuffs in Australia. More recently, a database of dietary SA concentrations was developed by Wood et al. [7] through systemic examination of the literature. However, data regarding SA levels in many fruits and vegetables frequently consumed in East Asia are generally lacking. Due to cultural diversity around foods, the foodstuffs commonly used in Asian recipes often differ substantially from those used in Western recipes. In addition, the concentrations of SA reported in previous studies vary widely, and can even vary in the same food, depending on its origin. Taken together, the results of previous studies do not provide particularly useful information for CSU patients in Asia. Today, many East Asian foodstuffs are not restricted to that region, but are also available in European and American groceries. It is thus essential to establish a database of the concentration of SA in East Asian foodstuffs.

The objective of the present study, therefore, was to determine which fruits and vegetables commonly used in East Asia are high in SA. The analytic method used in this study was gas chromatography-mass spectrometry (GC-MS), which has been reported to be sensitive and specific for detecting SA [8, 9].

Materials and Methods

Standards and Materials

A total of 95 food items were examined in this study. The SA concentration of each food item was analyzed using GC-MS. Standard reference materials (SA and ASA; acetylsalicylic acid, internal standard) were certified reference materials (certified purity 99.99%) and were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dichloromethane and N,O-Bis(trimethylsilyl)acetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) were also purchased from Sigma-Aldrich. Glacial acetic acid was obtained from Avantor (Center Valley, PA, USA), and acetonitrile (HPLC grade) was obtained from ThermoFisher Scientific Inc. (Pittsburgh, PA, USA). All water used in the present study was double deionized.

Most of the vegetables and fruits analyzed in the study originated in Taiwan, with the exception of durian (Thailand), kiwi fruit (New Zealand), and Japanese yam (Japan; Table 1). All of the vegetables and fruits used in the study were fresh.

Sample Preparation

Only the edible portions of the fruits and vegetables were analyzed, and 3 samples per product were made into a composition sample by mixing equal amounts. Fresh fruits were homogenized within 24 h of purchase, and the resulting homogenates were kept frozen at -30°C until extraction. Vegetable material (25 g) was added to 25 mL of water and boiled for 20 min prior to blending. The blended material was allowed to cool to room temperature and was then kept frozen at -30°C until extraction. Prior to extraction, boiling water was added to tea or coffee samples at a ratio of 1:9 (w/v) as infusions (5 min). The resulting liquid was allowed to cool prior to extraction as a liquid sample.

Extraction and Derivation

SA was extracted by liquid-liquid extraction as previously described, with some modifications [9, 10]. Duplicate portions ($2-5 \pm 0.01$ g) were weighed into 50-mL polytetrafluoroethylene centrifuge tubes with lids, after which 20 mL of extraction solvent (acetonitrile:water:acetic acid, 25:75:5 [v/v/v]) was added. Following the addition of internal standard (200 μL , 100 $\mu\text{g}/\text{mL}$ in anhydrous ethyl acetate), the samples were mixed for 30 min on a rotary shaker and then centrifuged at 1,710 g (3,000 rpm) for 5 min. A 1-mL aliquot of the supernatant was transferred to a 2-mL glass vial, and the solvent was evaporated to dryness under vacuum at 35°C using a thermostatically controlled Thermo SavantTM SPD111 SpeedVacTM kit (ThermoFisher Scientific, Waltham, MA, USA). For dissolution and derivation, the dry residue was dissolved in 200 μL of dichloromethane with vigorous shaking for 5 min, after which 200 μL of BSTFA/TMCS was added for derivation at 60°C for 1 h.

Analytical Apparatus

GC-MS analyses were performed using an Agilent 6890TM series gas chromatograph configured with an Agilent 5973TM network mass-selective detector (Agilent Technologies Inc., Wilmington, DE, USA). Electron-impact ionization was performed at an electron energy level of 70 electron volts (eV). Chromatographic separation was performed using an HP-1TM (cross-linked methyl siloxane) high-performance capillary column (30 m $\times$ 0.32 mm $\times$ 0.1 μm film thickness; Hewlett-Packard, Palo Alto, CA, USA); helium served as the carrier gas, at a flow rate of 1.3 mL/min. The injector temperature was kept at 250°C , and the sample injection

Table 1. Free SA concentrations of 95 foodstuffs commonly consumed in Taiwan

Food	Free SA, mg/kg	Food	Free SA, mg/kg	Free SA, mg/100 mL
Fruits (fresh)		Vegetables (cooked)		
Lemon	0.01	Spring onion	0.09	
Bell fruit	0.03	Snap peas	0.14	
Kiwi (golden)	0.03	Sweet potato	0.20	
Plum	0.04	Green pepper	0.20	
Grape	0.04	Tomato	0.25	
Apple (megumi)	0.05	Burdock root	0.25	
Passion fruit	0.06	Cabbage	0.26	
Jujube	0.06	Carrot	0.27	
Pear (oriental)	0.07	Broccoli	0.28	
Sugar apple	0.07	Aubergine	0.28	
Dragon fruit (white)	0.08	Lettuce	0.28	
Kiwi fruit (green)	0.09	Onion	0.29	
Dragon fruit (red)	0.10	Japanese yam	0.32	
Persimmon	0.10	Cucumber	0.33	
Watermelon	0.12	Lotus root	0.34	
Star fruit	0.13	Asparagus	0.35	
Ponkan	0.14	Pumpkin	0.42	
Pomelo	0.15	Yam leaves	0.43	
Cantaloupe	0.15	Chinese chives	0.43	
Melon (oriental)	0.15	Bottle gourd	0.46	
Durian	0.15	Water bamboo shoot	0.48	
Lychee	0.15	Potato	0.49	
Mango	0.17	Bamboo shoot	0.50	
Papaya	0.18	Bean sprouts	0.53	
Watermelon (yellow)	0.18	Taro	0.54	
Strawberry	0.18	Rape	0.55	
Pineapple	0.19	Loofah	0.57	
Guava	0.19	Green bean	0.58	
Longan	0.21	Bitter melon	0.62	
Banana	0.22	Corn	0.64	
Avocado	0.40	Spinach	0.65	
Orange	0.48	Crown daisy	0.66	
		Celery	0.68	
Beans (dried)		Water convolvulus	0.70	
Soya bean	5.05	Enokitake mushroom	0.70	
Red bean	2.54	King oyster mushroom	0.74	
Mung bean	1.62	Baby corn	0.76	
Black bean	1.60	Shiitake mushroom	0.79	
Spices (fresh)		Cauliflower	0.82	
Chili	0.55	Alfalfa sprouts	0.91	
Basil	0.62	Bird's nest fern	1.02	
Ginger	0.69	Chinese kale	1.17	
Wasabi	0.94	Chinese mustard	1.29	
Goji (dried)	3.37	White button mushroom	1.41	
Seaweeds (dried)		Bok choy	1.86	
Kelp sprouts	0.66	Chayote leaves	2.05	
Kombu	23.29	Okra	2.30	
		Beverages		
		Soy milk		0.02
		Green tea		0.16
		Oolong tea		0.16
		Black tea		0.28
		Ground coffee		0.36

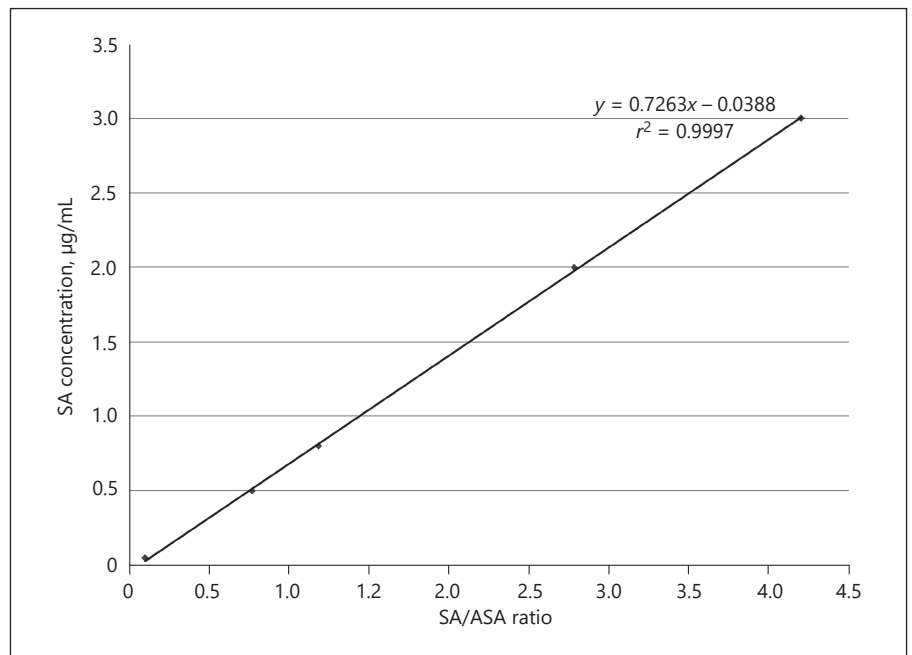


Fig. 1. Calibration curve for SA standards.

Table 2. Validation procedure for samples fortified with various concentrations of SA standard

Validation parameter	Replicates, <i>n</i>	Spiked concentration, µg/mL	Determined concentration, µg/mL	Mean of recovery, %	Coefficient of variation, %
Recovery	3	0.2	0.205	102.39	5.12
	3	0.8	0.731	91.39	4.30
	3	2	1.850	92.49	2.98
	3	4	4.359	108.97	4.31

volume was 1 µL at a split ratio of 1:10. The oven temperature was maintained at 40 °C for 1 min, then subsequently increased to 140 °C at 20 °C/min and held for 2 min, then increased to 300 °C at 20 °C/min and held for 5 min. A scan range of 50–450 atomic mass units was used for qualitative analyses, and the selected ion monitoring (SIM) mode was used for quantitative analyses. The silylated derivatives of SA ([M-CH₃]⁺) and ASA ([M-CH₃O]⁺) produce selected ions at *m/z* 267 and 195, respectively.

Analytical Quality Assurance

Stock solutions of SA and ASA were prepared at a concentration of 1 mg/mL (0.02 g dissolved in 20 mL of anhydrous ethyl acetate) and stored below –30 °C. Prior to analyses, working standard solutions (100 µg/mL) were prepared from the stock standard solutions by appropriate dilution with anhydrous ethyl acetate. An SA calibration curve was constructed using SA samples at concentrations of 0.05, 0.50, 0.80, 2.0, and 3.0 µg/mL prepared by accurate dilution of the working standard solution. ASA working solution (8 µL, 100 µg/mL) was added to each vial to a final concentration of 0.8 µg/mL as an internal standard. After evaporation of the sol-

vent from each vial, the previously described procedures for dissolution and derivation were followed. Relative standard deviation (RSD) values were used to represent within-run variation. For recovery analyses, SA as a solution in anhydrous ethyl acetate was added to triplicate samples at final concentrations of 0.20, 0.80, 2.0, and 4.0 µg/mL.

Statistical Analysis

The median SA content between the vegetable group and the fruit group was compared using the nonparametric Mann-Whitney test.

Results

We determined the concentration of SA in 95 food-stuffs frequently consumed in Taiwan, including 47 vegetables, 32 fruits, 4 beans, 2 seaweeds, 5 spices, and 5 beverages (Table 1). The calibration curves of SA were con-

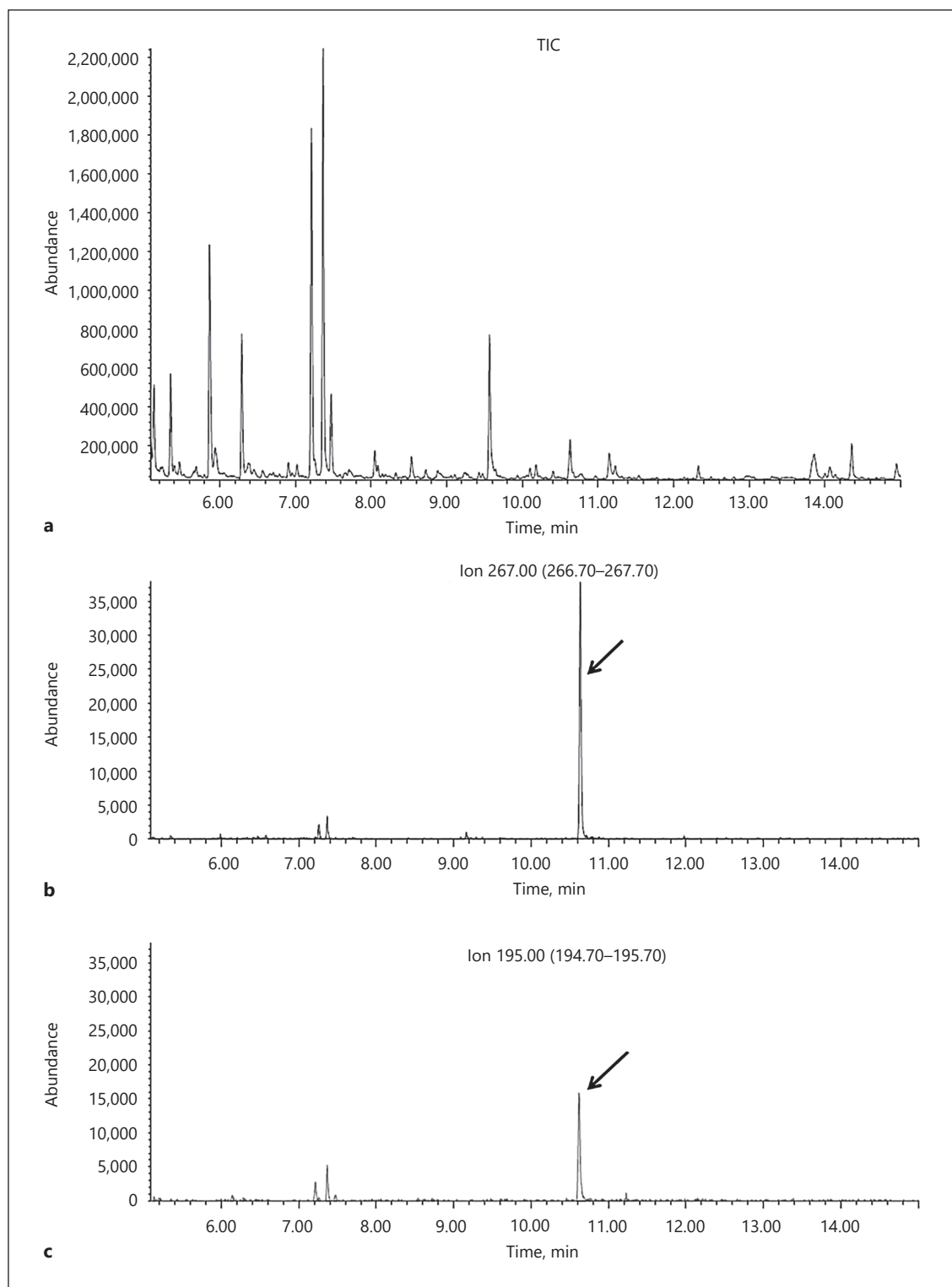


Fig. 2. The chromatographic results of the GC-MS analysis of SA and ASA standards. **a** Total ion chromatogram (TIC) of SA and ASA. **b** SIM chromatogram of SA. **c** SIM chromatogram of ASA. Arrows indicate retention times.

structured by GC-MS method in the range of 0.05–3.0 µg/mL in linear form, as shown in Figure 1. The linear regression equation for SA was $y = 0.7263x - 0.00388$, and the correlation coefficient was $r^2 = 0.9997$. The lower limit of detection for SA was 0.026 µg/mL. The validation experiments for the recovery results are shown in Table 2. The analytical recovery of SA varied from 91 to 108% (mean 98.81%, RSD < 5%), followed by a coefficient of variation lower than 6%.

The total ion chromatogram was obtained from SA and ASA after derivatization with BSTFA and detected by GC-MS (Fig. 2a). At the SIM mode, the silylated derivatives of SA and ASA produce fragment ions at m/z 267 (retention time of 10.640 min; Fig. 2b) and 195 (retention time of 10.623 min; Fig. 2c), respectively.

ASA was determined by isotope dilution GC-MS, using deuterated internal standard d4-SA. ASA contents were lower than the limit of detection (0.026 µg/mL) in all the foods studied (data not shown).

The results of the analyses are summarized in Table 2. The concentration of free SA is reported as mg/kg of edible portion for the solid foods and as mg/100 mL for the beverages. The concentration of SA in the analyzed vegetables and fruits ranged from 0.09 to 2.3 mg/kg and 0.01 to 0.48 mg/kg, respectively. The median SA content of the 47 vegetables was significantly higher than that of the 32 fruits tested ($p < 0.001$), as shown in Figure 3.

Discussion

To integrate the database of dietary SA concentrations that would facilitate determinations of vegetables and fruits that should be excluded from a low-pseudoallergen diet for CSU patients, we used GC-MS to analyze the SA concentration in 95 foodstuffs frequently consumed in Taiwan. Our results differed from those of previous studies. In this study, the SA concentration was higher in the vegetables compared with the fruits. Current pseudoallergen-free diet recommendations are based on the protocol developed by Zuberbier et al. [11]. According to that protocol, consumption of all fresh fruits is prohibited, whereas consumption of most vegetables is allowed. The results of our study, however, suggest that in East Asia it is just as important to consider dietary salicylate levels in vegetables as it is to consider these levels in fruits. Among the fruits and vegetables analyzed, okra and chayote leaves exhibited the highest SA concentrations (2.30 and 2.05 mg/kg, respectively). We observed relatively high SA levels in leafy vegetables, such as spinach, chayote leaves,

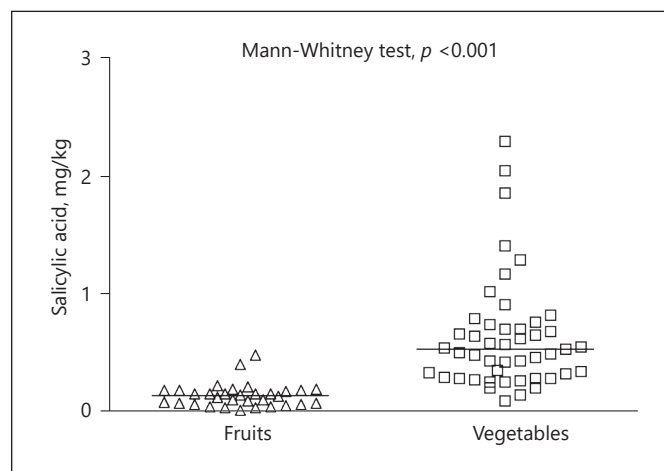


Fig. 3. Dot plot of SA concentration in fruits and vegetables. The horizontal bar represents the median.

bok choy, and Chinese kale (0.65, 2.05, 1.86, and 1.17 mg/kg, respectively). Root vegetables, such as sweet potato, burdock root, and carrot, tended to exhibit lower SA concentrations (0.20, 0.25, and 0.27 mg/kg, respectively). Swain et al. [6] reported that the peel of vegetables and fruits contain considerable amounts of salicylate. Most root vegetables are eaten without the peel, however, which might provide an explanation for their relatively low SA concentrations shown in the present study, which examined only the commonly consumed portions of plants.

We observed higher concentrations of SA in cooked tomatoes, pineapples, and bean sprouts (0.25, 0.22, and 0.53 mg/kg, respectively), compared with their fresh equivalents (0.16, 0.19, and 0.38 mg/kg, respectively), which contradicts previous findings reported by Scotter et al. [12]. To exclude the influence of individual variation, a single pineapple was used for both fresh and cooked sample analyses. One possible explanation for the difference between fresh and cooked samples is that SA bound as an ester or as a glycoside is liberated when heated, as hypothesized by Robertson [13]. An effect of the heating process could also explain the higher SA concentrations observed in the vegetables analyzed in the present study, which were cooked before analysis. Dried shiitake mushrooms would be expected to exhibit a higher concentration of SA in the fresh state versus cooked state due to the removal of water (25.28 and 0.79 mg/kg, respectively). When we calculated the total SA content for each shiitake mushroom examined, based on weight, the dried mushrooms exhibited a higher SA content than their fresh counterparts (0.047 vs. 0.012 mg, respectively).

Dried shiitakes are prepared at a temperature of around 60 °C; therefore, the liberated SA could be attributed to hours of heating.

Many investigators have reported high levels of SA in a variety of spices. The SA concentration in the spices analyzed in the present study (ranging from 0.55 to 0.94 mg/kg) tended to be lower than levels reported in previous studies (5.74 to 450 mg/kg) because we analyzed only fresh foodstuffs [7]. Wasabi exhibited the highest concentrations of SA among the fresh spices we analyzed (0.94 mg/kg). Instead of using chili powder, we analyzed fresh chili, which exhibited lower levels of SA (0.55 mg/kg) than most of the vegetables analyzed. Dried goji, which is used both as a spice and as a herbal medicine, also exhibited a high SA concentration (3.37 mg/kg), suggesting that herbal medicines may be sources of considerable amounts of SA.

Of the beverages analyzed, ground coffee exhibited the highest SA concentration (0.36 mg/100 mL). Black tea had a higher SA concentration than green tea or oolong tea (0.28 vs. 0.16 and 0.16 mg/100 mL, respectively). An important difference between green tea, oolong tea, and black tea is their degree of oxidation, often referred to as fermentation, during the manufacturing process. Generally, green tea is nonfermented or minimally fermented,

oolong tea is partially fermented, and black tea is fully fermented. This suggests that SA may be liberated during the fermentation process. Despite the relatively high concentration of SA in soybeans, the concentration in soy milk was low (0.02 mg/100 mL), indicating that soy milk should be safe for patients sensitive to salicylates.

In the present study, we determined the SA concentration in 95 foodstuffs frequently consumed in Taiwan, including many food items not analyzed in previous studies. Our results provide information that could be useful for CSU patients in East Asia who are attempting to implement a pseudoallergen-free diet. The differences of results among SA studies reflect the importance of local research in dietary advice.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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