Relative sweetness and sweetness quality of phyllodulcin [(3R)-8-Hydroxy-3-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-1H-isochromen-1-one]

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Abstract Phyllodulcin, a sweet-tasting compound extracted from *Hydrangea macrophylla* var. *thunbergii*, has recently received attention as a natural intense alternative sweetener with beneficial health effects. This study was conducted to determine the sweetness potency of phyllodulcin, relative to that of sucrose and to elucidate its sensory profile. Phyllodulcin solution was prepared by dissolving it in 1% ethanol (v/v) at 50°C. Relative sweetness to that of 3% sucrose in 1% ethanol solution was determined using the two-alternative forced-choice test. The sensory profile was obtained based on the descriptive analysis of 10 trained panelists. The relative sweetness of phyllodulcin was 434.8. Phyllodulcin was characterized by its bitterness, alcohol flavor, cooling sensation, and astringent aftertaste, but the persistence and onset of the sweetness of phyllodulcin were similar to those of sucrose. Further studies are required to improve the sweetness quality of phyllodulcin for facilitating its use in industrial applications by reducing its unfavorable flavor attributes.

Keywords: relative sweetness, descriptive analysis, phyllodulcin, intense sweetener, sweetness profile

Introduction

Increases in the incidences of obesity and chronic metabolic syndrome have spurred interest in strategies to reduce sugar consumption (1,2). Intense sweeteners have been widely used as one such strategy. Intense sweeteners refer to a group of compounds that generate intense sweetness in a very small quantity, thus replacing a significant amount of sugar without decreasing the sweetness (3). To date, synthetic intense sweeteners such as saccharin, aspartame, sucralose, and acesulfame-K have been used successfully in a wide range of food systems (4). However, because debates on potential adverse health effects are raising concerns about the use of synthetic sweeteners, consumers are increasingly demanding intense sweeteners made out of natural sources (5).

Over decades, various sweet-tasting compounds have been identified in and isolated from natural sources, but only a limited number of compounds have been launched as commercial sweeteners. One of the most commonly used natural intense sweeteners is steviol glycoside, which comprises several diterpene tricyclic glycosides extracted from the leaves of *Stevia rebaudiana* Bertoni. The major sweet glycosides are stevioside and rebaudioside A (RA) (6). Although stevioside is 100-300 times sweeter than sucrose, it elicits an unpleasant aftertaste and bitterness (3,6,7). To improve its

sensory quality, the molecular structure of stevioside was modified by transglucosylation using a bacterial enzyme, resulting in the production of enzymatically-modified stevia (EMST, glucosyl stevia) (7). In comparison with the sweetness of stevioside, that of RA is reported to be more potent and pleasant (8). Thus, the sweetness potency and quality of stevia sweeteners depend on their composition of glycosides and purity. Stevia sweeteners have shown various beneficial effects on health, including anti-inflammatory and antimicrobial activities, controlling blood sugar and cholesterol, and improving cell generation (9).

Luo han guo extract (LHGE) is another potential candidate for a commercial natural intense sweetener. LHGE is the fruit of *Siraitia grosvenorii* Swingle. Its extract has been used as a natural sweetener in drinks, tea, or traditional medicines in China (10). The major sweet compounds in LHGE are mogrosides, a group of triterpene glycosides found at 1% in a fresh LHGE fruit (6,10,11). Mogroside V, the most abundant mogroside, is 250-400 times sweeter than sucrose (12). LHGE has demonstrated an antioxidant capacity that may contribute to its anticancer, antiviral, and anti-inflammatory activities (6,12,13). LHGE has been recently approved by the US FDA as "generally recognized as safe" (14) and is used in drinks, dairy products, and confectionaries (15).

In Korea, the interest in *Hydrangea macrophylla* var. *thunbergii*

Makino, the leaves of which are dried and consumed as a sweet tea-like diffusion, has been increasing. The sweetness of *Hydrangea* leaves is elicited mainly by phyllodulcin, a dihydroisocoumarin derivative. Phyllodulcin is present in leaves as a glycoside, but it is hydrolyzed to aglycones by naturally occurring β -glucosidase, generating sweetness that is 400–800 times stronger than that generated by sucrose (11). No adverse health effects and several beneficial biological effects, including antiallergic and antiulcer effects (16) and inhibition of lipid peroxidation (17), have been reported for phyllodulcin.

In comparison with the number of studies about the extraction, identification, and biological activities of phyllodulcin (16–18), that of those about its sensory characteristics has not been much. The sensory profile of a sweetening compound is a critical factor for determining whether it can substitute sugar in food and beverage systems because consumers expect the same intensity, temporal profile, and pleasantness of sweetness as those of sucrose (11). Bassoli *et al.* (19) investigated the relative sweetness and temporal sensory profile of phyllodulcin and its derivative, in which the heterocyclic ring was modified by the addition of a sulfur and an oxygen atom [2-(3-hydroxy-4-methoxyphenyl)-4H-3]. They reported a persistent licorice- or anise-like flavor and a cooling sensation from both phyllodulcin and its derivative. These findings were consistent with a previous observation (11). However, phyllodulcin and its derivative were 2,500 and 10,000 times, respectively, sweeter than sucrose; these values were much higher than the previously reported values (400–800) (11). Therefore, to gain a clearer understanding of the sensory characteristics of phyllodulcin, a study using a systematic sensory approach is necessary.

The present study focused on the sensory characterization of phyllodulcin in an aqueous solution system in comparison with other commercially available intense sweeteners. More specifically, this study was conducted to assess the relative sweetness of phyllodulcin to that of 3% sucrose using the two-alternative forced-choice (2-AFC) test, and to determine the sweetness profile using a descriptive analysis protocol.

Materials and Methods

Stimuli Phyllodulcin was extracted from the leaves of *H. macrophylla* var. *thunbergii* Makino (Bubudawon, Daegu, Korea) using ultrasonic extraction. The leaves were lyophilized and pulverized into powder immediately after harvesting. Extraction was conducted in an ultrasonic bath (Model 5210; Branson, Danbury, CT, USA) at 35°C for 30 min using 70% ethanol. The leaf extracts were passed through a column of mixed-bed ion-exchange resins (Amberlite® IRA-67 and Amberlite® 200C NA+; Sigma-Aldrich, St. Louis, MO, USA) to eliminate colorants and massive residues. The resulting effluent was collected and filtered through an Oasis HLB 20cc LP extraction cartridge (Waters, Milford, MA, USA). The filtered extract was further

concentrated and phyllodulcin was isolated using recycling preparative high-performance liquid chromatography (HPLC) (LC-9104; Jai, Tokyo, Japan) equipped with two consecutive ODS-AP preparative columns (20 × 500 mm, Jai). Finally, the purified eluent was lyophilized. The purity of phyllodulcin in the lyophilized powder was 99% when validated using reverse-phase HPLC.

Other sweeteners were also tested to understand the sensory characteristics of phyllodulcin from a wider perspective. Sucrose (food-grade refined white sugar, TS Corporation, Seoul, Korea) was included as a control. Sucralose (95% powder, Tate & Lyle, McIntosh, AL, USA) and aspartame (98.5%, Ajou Food Additive, Incheon, Korea) were chosen as representative commercial alternative sweeteners (4). Natural intense sweeteners such as EMST (Steviol Glycosides 83.0%, Daepyeong, Sunghnam, Korea), RA (greater than 98.8%, Ingredion Korea, Icheon, Korea), and LHGE (mogrosides 80.2%, Guilin Layn Natural Ingredients, Guangxi, China) were chosen based on their commercial availability.

Determination of relative sweetness using the 2-AFC test

Sample preparation: Because of its low solubility in water, phyllodulcin was prepared as a stock solution by dissolving in ethanol (95%, food grade, Daehan Ethanol Life, Seoul, Korea) at concentrations 100 times higher than the final concentrations of tasting samples. The stock solutions were added gradually to hot filtered water (70°C, H-300; Everpure, Milwaukee, WI, USA) with stirring on a hot plate. The final concentration of ethanol was 1% (v/v) in the samples. The samples were then transferred to a hot water bath (345 × 710 mm, NTT-2200; Rikakikai, Tokyo, Japan) and maintained at 50°C until the test to prevent the precipitation of phyllodulcin. The phyllodulcin samples were tested within 2 h of preparation. Other sweeteners were also dissolved in 1% ethanol solution and maintained at 50°C until the test.

Because of the limited solubility of phyllodulcin in water, sweetness equivalent to 3% sucrose in 1% ethanol solution was determined. Thus, for the 2-AFC test, five concentration levels were selected for each sweetener based on a previous study (20): 0.005–0.04% for RA and aspartame, 0.001–0.008% for sucralose, 0.02–0.06% for LHGE and EMST, and 0.0024–0.012% for phyllodulcin.

An aliquot of 30 mL of each sample was poured in a disposable cup (75 mL, Easepack, Namyangju, Korea) coded with a three-digit random number and presented to panelists immediately before the test. Filtered warm water was provided as a rinsing agent. The temperature of the water was set at 40 ± 1°C throughout the preliminary tests to prevent panelists from being biased with respect to oral temperature fluctuation as well as perceiving rinsing water as too hot.

Panel: Forty-five panelists (17 males and 28 females, 19–49 years of age) were recruited from the Kookmin University (Seoul, Korea) campus and the surrounding local community. They reported no health problems that may influence their sensory perception or be associated with sucrose and artificial sweeteners. Those who were,

or may be, pregnant were excluded. All procedures for the 2-AFC test and descriptive analysis were approved by the Institutional Review Board of the Kookmin University (KMU-201410-HR-037-P1-C1), and informed consent forms were obtained from all panelists.

Evaluation procedure: Relative sweetness was determined using the 2-AFC test protocol (20,21), which is also known as the directional paired comparison test. Panelists were asked to taste a sweetener sample and the 3% sucrose in 1% ethanol solution and choose the sweeter one. Each sweetener, except phyllodulcin, was tested on separate sessions: one session per day. In a session, panelists conducted five series of 2-AFC test, each testing one of the sweetener samples at five concentration levels. The serving order of pairs was randomized and counterbalanced within and between sample pairs as well as across panelists. The session order was randomized across days. In preliminary tests, some panelists reported desensitization after tasting the phyllodulcin solution. Thus, for the 2-AFC test of phyllodulcin, only one sample pair was tested per session per day to prevent potential desensitization.

The sample tasting protocol was identical to the protocol developed by Kim *et al.* (20). Panelists swirled approximately 10 mL of a sample around in the oral cavity for 5 s, expectorated, and waited for another 5 s to perceive residual sensations before rinsing the mouth. They evaluated the overall sweetness of the sample perceived for 10 s (by holding it in the mouth for 5 s before and after expectoration). Panelists were instructed not to swallow the samples to prevent any potential health risk associated with alternative sweeteners. They were asked to rinse their mouth with warm water when they took a 1-3-min mandatory break between samples. The test was conducted under dim red light to mask color differences.

Data analysis: The relative sweetness values were determined following the method suggested by Kim *et al.* (20) and Heikel *et al.* (21). Concentration-response (C-R) curves were constructed by plotting the ratio of responses that a sample was sweeter than 3% sucrose against the concentration levels. The concentration that produced 50% of the responses was calculated from the regression equation of the C-R curves. The relative sweetness values were obtained by dividing the sucrose concentration (3%) by the sweetener concentration that yielded 50% of the responses.

Characterization of the sensory attributes of phyllodulcin using descriptive analysis

Sample preparation: The sweetener samples were formulated to have equal sweetness intensity as that of 3% sucrose based on the results of the 2-AFC test. The concentrations of the samples were finalized through the validation process suggested by Gwak *et al.* (22), wherein the concentration was readjusted if the mean sweetness intensity of a sample rated by descriptive panelists was found to exceed the 95% confidence interval of the 3% sucrose sample. The final concentrations of the samples were as follows: 3% for sucrose, 0.01495% for RA, 0.01939% for aspartame, 0.00439% for sucralose, 0.03446% for LHGE, 0.045% for EMST, and 0.006% for

phyllodulcin.

The samples were prepared and presented following the protocol identical to that used in the 2-AFC test. The samples for the evaluation of appearance were prepared separately from those for the evaluation of other sensory characteristics. They were filled in a 50 mL screw-capped glass vial coded with different random numbers from those used for other attributes to prevent stimulus bias resulting from association between the color and flavor (23).

Evaluation procedure: Nine panelists (all women, age 30-49 years) who had previous experiences of sensory evaluation participated in descriptive analysis. Sensory profiles of the samples were obtained using the generic descriptive analysis procedure (23). Panel training was conducted 3 times per week for 3 months. Each training session took approximately 1 h. At the first session, the panelists were introduced to the purpose of the study and educated for the principles of descriptive analysis. Over the next 16 sessions, the panelists developed a lexicon and reference standards (Table 1) by consensus. Practice tests were conducted in quadruplicates, and the result was analyzed using analysis of variance (ANOVA) to check panel performances. Additional trainings were given to those who showed a poor reproducibility or inconsistent rating pattern based on the group results.

Samples were presented in monadic sequence following a William Latin square design to minimize first-sample effect. Panelists tasted the samples in the same manner as for the 2-AFC test and rated the intensity of each attribute on a 15-cm line scale (1.25 cm: very weak/very fast, 13.75 cm: very strong/very slow). Panelists cleaned the palates with warm filtered water (40±1°C) when they took a 3 min break between samples. Appearance was evaluated under the daylight setting (North Sky Day Light, CIE-F7, 6500K, Multi Light Booth, Super Light VI; Bowoo Engineering, Gunpo, Korea), whereas other attributes were rated under dim red light to prevent color bias. The tests were repeated 3 times.

Data analysis: ANOVA was performed to determine if there were significant sensory differences among the sweeteners. Sample, panel, replication, and their two-way interactions were included in the ANOVA model. Duncan's multiple range test was conducted as a post-hoc comparison ($p < 0.05$). The multivariate relationship between samples and sensory attributes was investigated by conducting principal component analysis (PCA) on the mean intensity ratings. Data were centered by panelist to remove a scale effect (24) for PCA. ANOVA was performed using SPSS for Windows version 18.0 (IBM, Chicago, IL, USA). PCA was conducted using the PCA function in the SensoMineR package in R (version 2.15.0; R Development Core Team).

Results and Discussion

Relative sweetness Table 2 shows that the coefficient of regression equation (R^2) for each sample ranged from 0.84 to 0.99. This

Table 1. Definitions of and reference materials for the descriptive attributes of sweetener samples

Attributes		Definition	Reference materials	Scale value (0 to 15)
Appearance	Yellow	Yellow color	NCSS1015-Y10R (NCS color index, Scandinavian Colour Institute AB, Stockholm, Sweden).	7.5
Odor	Honey	The smell associated with honey	3% (w/v) honey (Dong suh honey, Dong suh Co., Jinchun, Korea) and 1% (v/v) ethyl alcohol in water.	6.9
Taste	Sweet	Fundamental taste sensation of which sucrose is typical	3%, 5% (w/v) sucrose (Foodream refined white sugar, TS Co.) in 1% (v/v) ethyl alcohol in water.	5.6 and 11
	Bitter	Fundamental taste sensation of which caffeine and quinine is typical	0.05% (w/v) caffeine (Sigma-Aldrich) in 1% (v/v) ethyl alcohol in water.	10.4
Flavor	Alcohol	Aromatics associated with alcohol flavor	3% (w/v) sucrose (Foodream refined white sugar, TS Co.) and 1% (v/v) ethyl alcohol in water.	7.5
	Nurungji candy	Aromatics associated with Nurungji (scorched rice) candy	Nurungji candy (Nurungji on iron pot, Haitai Co., Gwangju, Korea).	11.5
	Artificial	Aromatics associated with an artificial sweetener such as saccharin and plastic flavor	Abstraction and generalization using experience with artificial sweetener such as saccharin.	N/A ¹⁾
Trigeminal senses	Cooling	Cooling sensation associated with menthol	0.3% (w/v) peppermint (peppermint powder, ESfood, Gunpo, Korea) and 1% (v/v) ethyl alcohol in water.	13.75
Aftertaste	Astringent	The feeling which shrivels the tongue associated with aluminum potassium sulfate after expectoration	0.3% (w/v) aluminum potassium sulfate (aluminum potassium sulfate, Daejung chemicals & metals Co., Shiheung, Korea) and 1% (v/v) ethyl alcohol in water.	11.25
Persistence	Sweet	The persistence of sweet taste after expectoration	See reference material for sweet taste.	
Onset	Sweet	The time when maximum sweetness was first perceived	within 1-2 s	1.25
			within 5-6 s	13.75

¹⁾N/A means that the reference standard of a sensory attribute did not have a specific scale value because the reference sample was only used for assessors' concept alignment.

Table 2. Relative sweetness of six intense sweeteners calculated from the C-R curves

Sample ¹⁾	Regression equation	R ²	Relative sweetness obtained by 2-AFC	Relative sweetness from literature data (References)
RA	y=25.122x + 0.1024	0.8425	189.9	100-300 (2,13)
Aspartame	y=31.87x – 0.1182	0.8619	154.6	166-200 (6,21)
Sucralose	y=162.93x – 0.2145	0.9313	681.8	500-750 (14,39,40)
LHGE	y=17.209x – 0.093	0.9621	87.0	150-500 (14,15)
EMST	y=19.07x – 0.1674	0.9756	85.7	100-300 (5)
Phyllodulcin	y=72.491x + 0.0011	0.9457	434.8	350-800 (30-33)

¹⁾RA, rebaudioside A; LHGE, luohan guo extract; EMST, enzymatically-modified stevia

indicated that our data could be explained well by the regression model. The relative sweetness of phyllodulcin to that of 3% sucrose was identified to be 434.8, which was close to the relative sweetness values previously reported (350-800) (30-33). However, relative sweetness in the present study was much lower than the value 2,500 determined by Bassoli *et al.* (19). In their study, the relative sweetness of phyllodulcin was estimated by calculating the ratio of the sweetness recognition threshold of phyllodulcin to that of sucrose. Because the recognition threshold was assessed at low concentration levels (1.62×10^{-4} – 5.20×10^{-3} g/L), other sensory attributes of phyllodulcin

such as bitterness and a cooling sensation may not be elicited. Bitterness of alternative sweeteners increases as a function of concentration (25). Thus, sweetness perception may not be influenced by these attributes, resulting in a high relative sweetness value. Overall, this conclusion is consistent with the previous findings that the relative sweetness of a sweetener can vary based on the protocol and concentration range tested (26,27). This suggests that the relative sweetness of phyllodulcin is system dependent, exhibiting different sweetness potencies at different concentration ranges.

The relative sweetness value of sucralose identified in the present

study was 681.7, which was similar to that reported in previous studies (11,28; Table 2). LHGE showed a relative sweetness value of 87.0, which was slightly higher than the value 75.8 reported by Kim *et al.* (20). The relative sweetness values of aspartame, RA, and EMST were 154.6, 189.9, and 85.7, respectively. These values were slightly lower than those previously reported (20,22,26; Table 3). These differences could be attributed to the effect of the concentration range tested and testing protocol, as previously mentioned, or the effect of the matrices at which a sweetener was delivered, including the serving temperature and composition (29,30). Calvino (29) reported that a 3% sucrose solution was perceived sweeter at 37°C and 50°C than at 7°C. Schiffman *et al.* (30) also found that the sweetness of intense sweeteners such as aspartame, neohesperidin, dyhydrochalcone, and RA was perceived stronger at 50°C and that the enhancing effect of temperature on perceived sweetness was more dominant at lower concentration ranges. In addition, it is assumed that ethanol added to the sample matrices affected the sweetness perception. Scinska *et al.* (31) observed that ethanol was most frequently described as bitter and sweet, and quinine solution that did not contain ethanol evoked an ethanol flavor for some panelists. Moreover, the addition of sucrose to a quinine solution increased the perception of ethanol flavor. These results imply that flavor association among bitterness, sweetness, and ethanol may have influenced the perception of sweetness when the sweeteners

were dissolved in an ethanol solution.

Sensory profiles There were significant differences in all sensory attributes among the samples ($p < 0.001$), except sweetness (Table 3). This indicated that all samples were formulated successfully to have equal sweetness intensity through the validation process. Figures 1 and 2 summarize the sensory representation of samples and attributes obtained using PCA. Dim 1, Dim 2, and Dim 3 explained 43.9, 27.2, and 17.5% of the total variance, respectively.

Yellowness, honey aroma, and Nurungji candy flavor were highly loaded in the positive direction of Dim 1, whereas bitterness, cooling sensation, alcohol flavor, and astringent aftertaste were loaded in the negative direction. Dim 1 contrasted LHGE from phyllodulcin. Dim 2 separated LHGE and phyllodulcin from the other samples that had relatively little or weak flavor components. LHGE was highly and positively correlated with yellowness, honey aroma, and Nurungji candy flavor (Fig. 1, Table 3). This was consistent with the sensory profile of aqueous LHGE solutions whose sweetness was equivalent to a 5% sucrose solution (20). Phyllodulcin was characterized by significantly stronger bitterness, cooling sensation, alcohol flavor, and astringent aftertaste than the other samples (Fig. 1, Table 3). Bassoli *et al.* (19) reported that 0.0012% phyllodulcin in a 0.4% hydroalcoholic solution elicited a sweet taste, anise/licorice-like flavor, and a cooling sensation. This slight difference in the sensory

Table 3. Mean intensities of descriptive sensory attributes elicited by sucrose and six intense sweeteners¹⁾

	Sucrose	Sucralose	Aspartame	EMST	RA	LHGE	Phyllodulcin
Yellowness	0 ^{a2)} (0.00) ³⁾	0 ^a (0.00)	0 ^a (0.00)	0 ^a (0.00)	0 ^a (0.00)	4.49 ^b (0.96)	0 ^a (0.00)
Honey aroma	0.05 ^a (0.24)	0 ^a (0.00)	0 ^a (0.00)	0 ^a (0.00)	0 ^a (0.00)	3.80 ^b (0.68)	0 ^a (0.00)
Sweetness	5.44 (0.76)	5.55 (0.56)	5.72 (0.56)	5.42 (0.71)	5.59 (0.53)	5.63 (0.54)	5.50 (0.79)
Bitterness	0.09 ^a (0.33)	0.34 ^{ab} (0.67)	0.28 ^{ab} (0.53)	0.43 ^b (0.59)	0.44 ^b (0.70)	0.14 ^a (0.40)	3.60 ^c (0.93)
Alcohol flavor	1.11 ^a (0.37)	1.16 ^a (0.43)	1.11 ^a (0.37)	1.14 ^a (0.32)	1.20 ^a (0.49)	1.11 ^a (0.37)	3.46 ^b (0.71)
Nurungji candy flavor	0.05 ^a (0.24)	0.05 ^a (0.24)	0.05 ^a (0.24)	0.18 ^a (0.94)	0 ^a (0.00)	3.76 ^b (0.73)	0 ^a (0.00)
Artificial flavor	0.15 ^a (0.77)	0.23 ^{ab} (0.49)	0.56 ^{bc} (1.12)	3.82 ^d (1.16)	0.74 ^c (1.25)	0 ^a (0.00)	0 ^a (0.00)
Cooling sensation	0.18 ^a (0.37)	0.18 ^a (0.41)	0.23 ^a (0.46)	0.25 ^a (0.50)	0.33 ^a (0.52)	0.14 ^a (0.35)	3.56 ^b (1.02)
Astringent aftertaste	0.21 ^a (0.60)	0.60 ^{bc} (0.85)	0.63 ^c (0.71)	0.96 ^d (0.76)	1.01 ^d (0.74)	0.31 ^{ab} (0.53)	3.31 ^e (0.77)
Persistence of sweetness	1.89 ^a (1.21)	2.21 ^{ab} (1.49)	3.83 ^d (1.90)	3.57 ^{cd} (1.87)	3.28 ^c (1.94)	3.36 ^{cd} (1.52)	2.60 ^b (1.61)
Onset of sweetness	2.36 ^a (2.58)	2.51 ^a (2.57)	4.63 ^{bc} (4.24)	4.98 ^{bc} (4.57)	5.24 ^{bc} (4.47)	5.70 ^c (4.77)	3.67 ^{ab} (3.62)

¹⁾RA, rebaudioside A; LHGE, luo han guo extract; EMST, enzymatically-modified stevia

²⁾Different letters indicate a significant difference among the samples ($p < 0.05$).

³⁾Standard deviation

Fig. 1. Principal component loading of the sensory attributes (A) and scores of the samples (B) from sucrose and six intense sweeteners on Dim 1 and Dim 2

Fig. 2. Principal component loading of the sensory attributes (A) and scores of the samples (B) from sucrose and six intense sweeteners on Dim 1 and Dim 3

descriptors may be because of either differences in sample formulation or cross-cultural differences in descriptor generation (32). It is worth to note that alcohol flavor of phyllodulcin was significantly stronger than that of the other sweetener samples, although all samples were dissolved in 1% ethanol solution. As previously mentioned, because it was observed that bitterness in solution drove the perception of ethanol in some subjects (31), strong bitterness and a cooling sensation of phyllodulcin may have enhanced the perception of alcohol flavor more significantly than those of the other samples.

The sensory differences among sucrose, sucralose, RA, EMST, and aspartame were more clearly illustrated on Dim 3 (Fig. 2). The artificial flavor, persistence of sweetness, and onset of sweetness were highly loaded in the positive direction of Dim 3 with EMST, aspartame, and RA. These sweeteners were perceived as significantly more artificial than sucrose and sucralose, and their sweetness developed slowly and lasted longer (Table 3). EMST was perceived as more artificial than RA, although they both originated from *S. rebaudiana* (Table 3). This difference likely resulted from the main

sweetening compound in each sweetener (6). Less artificial flavor of RA was consistent with previous reports that RA had better sensory quality than other steviol glycoside sweeteners (8). Sucrose and sucralose shared sensory similarities in that they did not have any specific flavor/aroma components. Sucralose has been widely reported to have a very similar sensory profile to that of sucrose (20,22,27). However, sucralose had a slightly, but significantly, stronger astringent aftertaste than sucrose (Table 3).

The onset and persistence of sweetness (Table 3) provides some clues to understand the temporal sweetness profile of sweeteners. The sweetness of aspartame, EMST, RA, and LHGE developed significantly more slowly, but persisted longer, than that of sucrose. Although not significant, LHGE had the slowest onset and lasted the longest among the samples tested. Phyllodulcin did not exhibit any significant difference in the onset and persistence of sweetness compared with sucrose and sucralose (Table 3), contradicting a previous observation that phyllodulcin had a delayed the onset of sweetness (11). In contrast, Bassoli *et al.* (19) reported results similar

to those observed in the present study. In their study, sweetness of a synthetic derivative of phyllodulcin reached maximum intensity more slowly than that of sucrose, but the temporal sweetness of the natural phyllodulcin compound was not significantly different from that of sucrose. Sucrose-like quick onset with little persistence can facilitate the application of phyllodulcin compounds as sugar substitutes in a variety of products. However, because the sweetness of an alternative sweetener lingers longer as the concentration increases (33), the temporal profile of phyllodulcin needs to be re-examined if it is to be used at higher concentrations than those used in this study.

In conclusion, phyllodulcin elicited equal sweetness intensity to that elicited by 3% sucrose at 0.006–0.007% when delivered in a 1% ethanol solution at 50°C. At this concentration, phyllodulcin was distinguished from sucrose because of its characteristic cooling sensation, bitterness, alcohol flavor, and astringent aftertaste. However, unlike other alternative sweeteners, phyllodulcin exhibited an onset and persistence of sweetness similar to those exhibited by sucrose.

Phyllodulcin remains an attractive candidate as a commercial alternative sweetener with a natural origin because of its high relative sweetness and various health-promoting effects such as its antiallergic, antiulcer, and antioxidative effects. However, at the same time, its low solubility in water and the presence of other sensory attributes limit its commercial applications (11). In case of steviol glycosides, their solubility, stability, and sweetness quality have been improved remarkably as technologies for extraction, purification, and structure modification have evolved. Therefore, efforts should be undertaken continuously to improve the sensory quality and solubility of phyllodulcin.

Besides solubility and sensory quality, the stability of sweetness in various conditions is important for the practical application of phyllodulcin. Some alternative sweeteners are unstable at low pH or high temperature that is commonly observed during food processing, resulting in decreased sweetness or generation of off-flavors. For example, aspartame is unstable at a high temperature and neutral pH (34) and steviol glycoside is chemically degraded in strong acidic conditions or temperatures above 140°C (35). Future studies should determine if processing conditions influence sweetness and other sensory qualities of phyllodulcin in real food systems.

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