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Flavor Enhancement of Chicken Broth from Boiled Celery Constituents

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The flavor-enhancing effects of the volatile constituents in celery were investigated. The test samples were prepared by adding celery fractions to chicken broth at a concentration that distinct odors of them were not detected, and the samples were sensorially evaluated for the perceived intensities of 8 terms such as "thick," "impactful," "mild," "lasting," "satisfied," "complex," "refined," and "clarified," which are considered to be the elements of the complex flavor and for 3 terms such as "sweet," "salty," and "umami" taste. A comparison of effects between the volatile and nonvolatile fractions of celery revealed that the volatile compounds in celery enhanced the complex flavor of chicken broth more than the nonvolatile compounds. Among the characteristic odorants of celery, three phthalides, namely, sedanenolide, 3-*n*-butylphthalide, and sedanolide, were shown to contribute to the complex flavor of chicken broth, and sedanenolide was most effective. The three phthalides enhanced perceived intensities of "umami" and "sweet" despite their no taste properties in addition to the complex flavor.

KEYWORDS: Complex flavor; celery; chicken broth; 3-*n*-butylphthalide; sedanenolide; sedanolide

INTRODUCTION

Palatability is one of the most important factors in food ingestion because it promotes appetite, digestion, and the feeling of satisfaction. It is influenced by various conditions, such as chemical senses, physical senses, or psychological state. Above these, odor and taste are considered to play an important role in food character, and their mutual effects sometimes produce various complex flavors beyond the five basic tastes. For instance, the rich complex flavor of well-cooked bouillon is difficult to express using the five basic tastes like salty, sweet, sour, bitter, and umami, and the objective terms of odor like meaty, fatty, and/or vegetable like. "Mouthfulness" is sometimes used to express the character of the flavor. Yamaguchi has reported that flavor characters such as "continuity," "mouthfulness", "full-bodied", "impact", "mildness", and "thickness" of beef broth were enhanced by monosodium glutamate (MSG), and salt and sucrose, when used in appropriate amounts, improved the overall palatability of beef broth (*1–3*). Shima et al. have identified *N*-(1-methyl-4-hydroxy-3-imidazolin-2,2-ylidene) alanine in beef broth as a "brothy taste" modifier (*4*), whereas Ueda et al. have reported that alliin, cycloalliin, S-methylcysteine sulfoxide, and glutathione extracted from garlic resulted in flavor complexity when they were added to an umami solution (*5*). Ogasawara has reported that pyrazines and some peptides generated together in certain food during the process of boiling or aging for a long period brought "koku" (*6*). In most of these cases, nonvolatile compounds have been proposed as the main contributors to the complex flavor.

Moreover, it was reported that flavor is defined as a combined sense of taste and odor in the brain and that odor sense often affects the intensity of taste sense (*7*). Many studies have been made on the mutual effects of odor and taste senses and reveal that odor substances affect the perceived intensity of flavor, although, in most cases, examinations have been conducted in a simple matrix such as sweetness, sourness, and saltiness as a tastant with an odor like strawberry, lemon, and vanilla (*8–10*). We assumed that certain odor compounds could possibly affect the perceived intensity of flavor in a more complex matrix.

It is empirically known that celery has a remarkable effect of enhancing the complex flavor of and is considered to be important in making bouillon. It has a characteristic spicy odor that turns to a sweet spicy note after boiling; therefore, it is used to enhance the complex flavor of bouillon.

In our previous paper, we studied the potent odor compounds identified in raw and boiled celery, and we reported that 3-*n*-butylphthalide, sedanenolide, *trans*- and *cis*-sedanolide, (*E*)-2-nonenal, myrcene, and (3*E*,5*Z*)-1,3,5-undecatriene contributed to both raw and boiled celery odor, (*Z*)-3-hexenal and (*Z*)-3-hexenol contributed to raw, and β-damascenone, β-ionone, and sotolon were important to the character of boiled celery flavor. Among these constituents, 3-*n*-butylphthalide (**1**), sedanenolide

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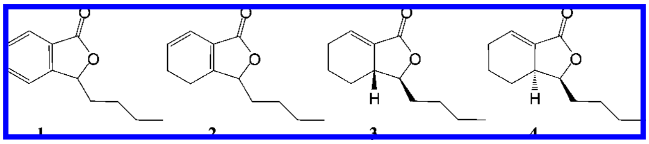

Figure 1. Structures of phthalides identified in celery volatiles. **1**: 3-*n*-butylphthalide, **2**: sedanenolide, **3**: *trans*-sedanolide, and **4**: *cis*-sedanolide.

(**2**), and *trans*- and *ci*s-sedanolide (**3** and **4**, respectively), shown in **Figure 1**, were evaluated as the most characteristic of both raw and boiled celery odor because of their strong resemblance to the odor of celery (*11*).

In this study we investigated the flavor enhancing effects of volatile and nonvolatile compounds on chicken broth.

MATERIALS AND METHODS

Chemicals. 3-*n*-Butylphthalide (purity estimated by GC: >99.9%) was purchased from Givaudan (Vernier, Switzerland). Sedanenolide (85.3%) was synthesized from 3-*n*-butylphthalide according to the method of Li et al. (*12*). Sedanolide (>99.9%) was purchased from Wako Pure Chemical Industries (Osaka, Japan), and 3-propylidenephthalide (99%) was purchased from International Flavors and Fragrances (NJ, USA). Sotolon (79.6%) and (3*E*,5*Z*)-1,3,5-undecatriene (99.8% including geometric isomers) were obtained from T. Hsaegawa Co., Ltd. (Tokyo, Japan).

Preparation of Chicken Broth. Chicken bone and meat (1 kg) in 6 L of deionized water was boiled for 3 h down to 3 L, with the scum and fat being skimmed off. The resulting broth was filtered through gauze and stored at −20 °C until used. NaCl was added at a concentration of 0.3% before sensory evaluation.

Preparation of the Volatile and Nonvolatile Fractions of Boiled Celery. Celery stalks (500 g) grown in Nagano prefecture and purchased from a local market were cut into 1 square-cm, boiled in 2 L of distilled water for 20 min, and then distilled for 40 min under atmospheric pressure to yield 300 g of distillate as the volatile fraction. The 1700 g aqueous residue was filtered through gauze for subsequent use as a nonvolatile fraction.

Determination of Phthalides. The relative amounts of 3-*n*-butylphthalide, sedanenolide, and sedanolide in the volatile and nonvolatile fractions were next determined. 3-Propylidenephthalide was put into 100 g of each celery fraction at a concentration of 1 ppm as an internal standard (IS). To facilitate extraction of volatile components, 15 g NaCl was added, after which each fraction was extracted with diethyl ether, and ether extract was dried over anhydrous sodium sulfate and was concentrated under atmospheric pressure. An Agilent 6890 gas chromatograph combined with a 5973 mass selective detector equipped with a TC-1 capillary column (0.25 mm i.d. × 60 m; GL Sciences Co., Tokyo, Japan) was used. The injector was operated in the split mode (50:1) and was kept at 250 °C, and the oven temperature was kept at 40 °C for the initial 3 min and then increased to 300 °C at a rate of 3 °C/min. The ratios to IS were calculated using the GC peak areas.

Evaluation Panel and Environment. The panelists consisted of 6–10 females aged from 22 to 27 years old (average was 23.5 years old) who were the members of the Food Chemistry Laboratory in Ochanomizu University. In order to ensure the selected panelists had an accurate sense of smell, a screening test was conducted by sensory evaluation of T & T olfactometer reagents (Daiichi Yakuhin Sangyou, Tokyo, Japan). The sensory analysis was conducted in a quiet room kept at 23 °C and 40–50% humidity. Two booths were set in the room, and each panelist was separately assigned to a booth to avoid influencing each other.

Attributes. Prior to the evaluation of attributes to be used in this study, the panelists were oriented on the elements of complex flavor of good bouillon by letting them taste the sample. They were instructed to remember the taste of the bouillon. Afterward, all panelists were presented the chicken broth (control) and the chicken broth with 0.5% of the volatile fraction of celery added (test sample). They were provided with a list of 36 descriptors and had to attribute them to the flavor of the samples assessed (*13, 14*). After the first session, they collaboratively selected from 36 descriptors, 8 attributes to describe the complex flavor and 3 attributes for the taste sense (**Table 2**), and finally, they agreed to the attributes after several consultations. They repeated the test sessions using various types of chicken broth to confirm the scale of perceived intensity.

Table 1. Relative Amounts of Three Kinds of Phthalides in the Volatile and the Nonvolatile Fractions of Celery

	ratio to IS	
	volatile fraction	nonvolatile fraction
3-*n*-butylphthalide	0.90	0.03
sedanenolide	6.06	0.46
trans-sedanolide	0.19	0.01
cis-sedanolide	0.05	n.d.[a]

[a] Not detected.

Table 2. Defined Attributes for Sensory Evaluation of Chicken Broth Flavor

attributes	definition
Attributes for Complex Flavor	
thick	generous, deep, rich
impactful	impressive, characteristic
mild	mellow, harmonious, balancing
lasting	mouthfulness, continuity, aftertaste
satisfied	impressive, mellow, full
complex	mixed
refined	sophisticated, elegant
clarified	reduced unfavorable meaty or fatty
Attributes for Taste Sense	
sweet	perceived as sweet taste
salty	perceived as salty taste
umami	perceived as umami taste

Sensory Analyses. *Evaluation of the Effects of Volatile Fraction Added To the Chicken Broth.* Three test samples were prepared (A) chicken broth (control), (B) chicken broth with the volatile fraction added, (C) chicken broth with the nonvolatile fraction added. A 20 ml portion of each sample was put in a disposable plastic cup covered with aluminum foil and was kept at 60 °C. The test samples were coded by a random three-digit number. A set of one control and two test samples was presented to the panelist at the same time. The order of the samples was randomized. The panelists were asked to sip each sample, keep it in the mouth for 10 s, simultaneously feel the odor retronasaly, and then expectorate. Between tasting, the panelist rinsed their mouth with water. The panelists were then requested to rate the perceived relative intensity of 11 attributes using a 14 cm-long line scale with 0 in the middle for control and −7 for weak on the left and +7 for strong on the right side (**Figure 2**). The scores were averaged and the differences between the control and samples were compared by the *t* test. The difference between samples was compared by the paired *t* test.

The evaluation was repeated twice using different samples following the same procedure in the first session.

Evaluation of the Taste Sensation of Phthalides Added To the Chicken Broth. This evaluation was carried out with the use of a nose-clip. A set of duplicated controls (chicken broth) and one test sample (chicken broth containing a phthalide) was presented to the panelists in the same manner as described above. Each panelist, without use of a nose-clip, was asked to taste the three samples and to identify the one containing the phthalide. In another session, the same panelist was asked to evaluate another set of the same samples with the nose-clip on. Each panelist used three sessions to evaluate the three kinds of

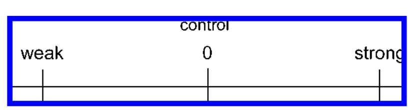

Figure 2. The 14 cm-long line scale used in the session.

Table 3. Evaluation of Chicken Broth Flavor Added with the Volatile and Nonvolatile Fractions of Celery

attribute[b]	average score[a]	
	volatile fraction[c]	non-volatile fraction[d]
thick**	*4.7	2.3
impactful**	*4.4	1.9
mild*	*3.2	−0.1
lasting*	*4.1	1.0
satisfied*	*4.4	1.0
complex*	*4.5	2.5
refined	4.1	−0.5
clarified*	*3.3	−0.6
sweet	1.9	1.7
salty	0.7	0.5
umami*	*4.0	2.2

[a] n = 7. Asterisks on the left side of the scores mean significant difference between the control and chicken broth added with volatile or non-volatile fraction (*:$P < 0.05$, **:$P < 0.01$). Values correspond to the attributes in Figure 3. [b] Asterisks mean significant difference between the volatile and non-volatile fractions (*:$P < 0.05$, **:$P < 0.01$). [c] concentration: 0.7%. [d] concentration: 7%.

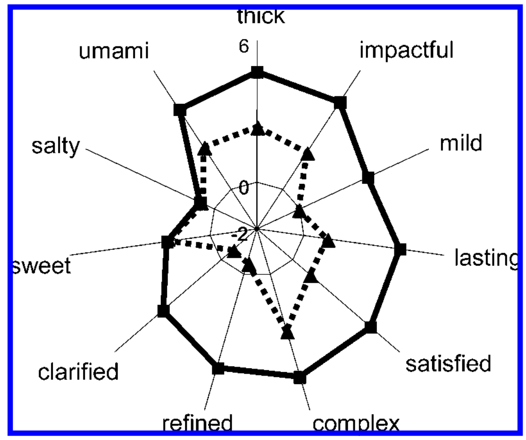

Figure 3. Flavor profile of chicken broth added with the volatile and nonvolatile fractions of celery, (—) chicken broth (control) with a score for control of 0, (—■—) chicken broth added with volatile fraction of celery (0.7%), (--▲--) chicken broth added with nonvolatile fraction of celery (7%).

phthalide, with an interval of at least one day between sessions. The number of correct answers in the triangle test was evaluated.

RESULTS AND DISCUSSIONS

The effects of volatile constituents of celery on complex flavor of chicken broth were examined. We focused on the complex flavor that is reminiscent of well-cooked bouillon, and we intended to separate the complex flavor into 8 attributes, i.e., thick, impactful, mild, lasting, satisfied, complex, refined, and clarified. Although these attributes are closely related to each other, they were regarded to be elements of the complex flavor. The three taste intensities sweet, salty, and umami were also selected, because they were regarded as taste elements of chicken bouillon.

Effects of the Volatile and Nonvolatile Fractions. The effects of the volatile and nonvolatile fractions of celery were compared to determine which of them affected the flavor of chicken broth. Whereas the volatile fraction possessed a strong and characteristic celery-like odor with no taste, the nonvolatile fraction possessed a strong sweet and umami taste with a weak sweet boiled vegetable-like odor. The relative amount of the phthalides in the volatile fraction was 1–30 times more than the nonvolatile fraction, as shown in **Table 1**.

For the test solutions, the volatile and nonvolatile fractions were added to the chicken broth at a respective concentration of 0.7 and 7%. These concentrations were estimated based on the practical soup preparation, and at these concentrations, most of the panelists could not detect the distinct celery-like flavor in either sample.

The results of the evaluation of chicken broth flavor added with the volatile and nonvolatile fractions of celery are shown in **Table 3**, and the flavor profile is shown in **Figure 3** as a radar chart plotted with averaged scores. The differences of perceived intensity scores between the control and the chicken broth added with the volatile fraction were significant in all attributes except for refined, sweet, and salty ($P < 0.05$). Whereas those of the chicken broth with the nonvolatile fraction were not significant in all attributes. The differences between the chicken broth containing the volatile and nonvolatile fractions were similarly significant in the attributes of thick, impactful ($P < 0.01$), mild, lasting, satisfied, complex, clarified, and umami ($P < 0.05$). These results indicate that the volatile fraction containing only odor compounds of celery was more effective for enhancing the flavor complexity and perceived intensity of the umami taste than the nonvolatile fraction containing such taste compounds as sugars, amino acids, and organic acids.

Effect of the Characteristic Odor Compounds of Celery. To identify the most effective compounds in the volatile fraction of celery, the effects of sedanenolide, sotolon, and (3*E*,5*Z*)-1,3,5-undecatriene, as representative odor compounds in celery, were compared. In our previous examination, we reported that sedanenolide was one of the most important compounds in both raw and boiled celery because of its high flavor dilution (FD) factor and odor characters that were reminiscent of celery. Sotolon was found to be important to only boiled celery odor, and (3*E*,5*Z*)-1,3,5-undecatriene was also judged important to both raw and boiled celery odor. The odor character of sotolon itself is spicy and crude sugar-like (*15*), and that of (3*E*,5*Z*)-1,3,5-undecatriene is fragrant flowery (*16*), the respective odor threshold values being 0.001 ppb in water and 0.001 ppb in air, and both of them were thought to contribute to celery odor.

Four test solutions were prepared; (A) chicken broth (control), (B) broth with sedanenolide added (0.7 ppm), (C) broth with sotolon added (0.0007 ppm), and (D) broth with (3*E*,5*Z*)-1,3,5-undecatriene added (0.007 ppm), separately. The concentrations were estimated based on the amounts of practical use, and most of the panel did not perceive the distinct characteristic odor of the compound itself. The same evaluation was applied following the procedure used in the effects of the volatile and nonvolatile fractions part mentioned above. Statistical analyses were made using the *t* test to compare the control and the chicken broth added with these compounds, and Tukey's multiple comparison test was applied between test samples.

The results of the evaluation of chicken broth flavor added with sedanenolide, sotolon, and (3*E*,5*Z*)-1,3,5-undecatriene are shown in **Table 4**, and the flavor profile is shown in **Figure 4**. The differences of perceived intensities between the control and the test samples were as follows; (B) chicken broth containing sedanenolide was significant in all attributes ($P < 0.01$) except for salty ($P < 0.05$), (C) chicken broth containing sotolon was significant in the attributes of mild, lasting, satisfied, complex, refined, and umami ($P < 0.01$), and (D) chicken broth containing (3*E*,5*Z*)-1,3,5-undecatriene was significant only in the attribute of satisfied. In comparison with the sample containing sedanenolide, the score of the sample containing sotolon was lower in the attributes of thick and satisfied; the score of the sample containing (3*E*,5*Z*)-1,3,5-undecatriene was lower in the attributes

Table 4. Evaluation of Chicken Broth Flavor Added with Sedanenolide, Sotolon, and (3*E*,3*Z*)-1,3,5-Undecatriene

	average scores[a]		
attributes	sedanenolide[b]	sotolon[c]	(3*E*,5*Z*)-1,3,5-undecatriene[d]
thick	**4.2 a	2.2 b	1.5 b
impactful	**4.3 a	2.0 ab	1.0 b
mild	**5.3 a	**3.8 a	1.2 b
lasting	**4.2	**2.4 a	1.3 b
satisfied	**4.2 a	**2.6b	*2.1 b
complex	**3.8 a	**3.1 a	2.0 a
refined	**4.1 a	**3.9 a	1.4 a
clarified	**4.5 a	2.7 a	1.5 b
sweet	**3.0 a	1.8 a	1.3 a
salty	*1.6 a	0.8 a	0.3 a
umami	**4.0 a	**2.3 a	0.7 b

[a] n = 6. Asterisks mean significant difference between the control and each test sample (*: $P < 0.05$, **: $P < 0.01$). Different letters (a and b) cited on the right side of the score mean significant difference by Tukey's multiple comparison test ($P < 0.05$). Values correspond to the attributes in Figure 4. [b] concentration: 0.7 ppm. [c] concentration: 0.0007 ppm. [d] concentration: 0.007 ppm.

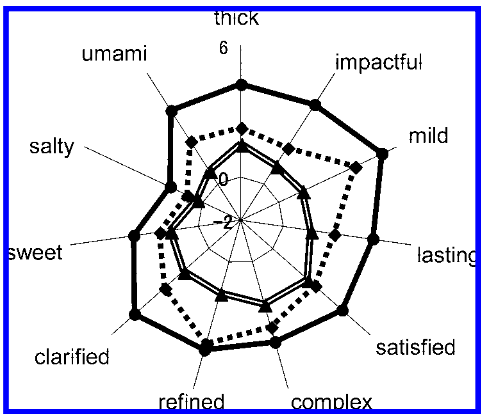

Figure 4. Flavor profile of chicken broth added with sedanenolide, sotolon and (3*E*,5*Z*)-1,3,5-undecatriene; (—) chicken broth (control) score of 0 for control, (–●–) chicken broth with sedanenolide added (0.7 ppm), (--◆--) chicken broth with sotolon added (0.0007 ppm), (=▲=) chicken broth with (3*E*,5*Z*)-1,3,5-undecatriene added (0.007 ppm).

of thick, impactful, mild, satisfied, clarified, and umami. In comparison with the sample containing sotolon, the score of the sample containing (3*E*,5*Z*)-1,3,5-undecatriene was lower in the attributes of impactful, mild, lasting, clarified, and umami. All three compounds were identified as potent odorants in celery odor, but their contributions to flavor enhancement differed. Sedanenolide has already been found to be the most potent odorant because of its odor intensity and impression, and the result in this study revealed that it is also most potent for flavor-enhancement.

Effect of Phthalides on Chicken Broth Flavor. In addition to sedanenolide, 3-*n*-butylphthalide, and *trans*- and *cis*-sedanolide were also found to contribute to the characteristic odor of celery. So the effects of these three kinds of phthalides were compared. Only *trans*-sedanolide was commercially available in pure form, and it was used in the sensory evaluation.

Four test solutions were prepared; (A) chicken broth (control), (B) broth with sedanenolide added (0.7 ppm), (C) broth with 3-*n*-butylphthalide added (0.2 ppm), and (D) broth with sedanolide added (0.2 ppm), separately. The concentrations were settled preliminarily at the levels where the characteristic odor was not sensorially recognized distinctly. The same evaluation was applied following the procedure used in the previous evaluation mentioned above.

Table 5. Evaluation of Chicken Broth Flavor Added with Sedanenolide, 3-*n*-Butylphthalide and Sedanolide

	average scores[a]		
attributes	sedanenolide[b]	3-*n*-butylphthalide[c]	sedanolide[d]
thick	**4.3 a	*2.2 b	**3.6 ab
impactful	**3.2> a	**1.8 a	*3.0 a
mild	**4.0 a	**3.2 a	**4.0 a
lasting	**3.6 ab	**1.8 b	**3.1 a
satisfied	**3.4 a	*2.3 a	*3.3 a
complex	**3.7 a	**2.3 a	**4.1 a
refined	*3.9 a	2.2 a	2.6 a
clarified	**3.6 a	1.5 a	*3.1 a
sweet	*2.9 a	*2.3 a	*3.1 a
salty	1.0 a	1.2> a	1.0 a
umami	*2.8 a	**2.2 a	*2.6 a

[a] n = 6. Asterisks mean significant difference between the control and each test sample (*: $P < 0.05$, **: $P < 0.01$). Different letters (a and b) cited on the right side of the score mean significant difference by Tukey's multiple comparison test ($P < 0.05$). Values correspond to the attributes in Figure 5. [b] concentration: 0.7 ppm. [c] concentration: 0.2 ppm. [d] concentration: 0.2 ppm.

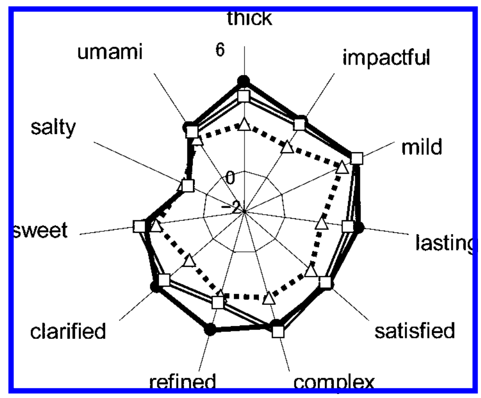

Figure 5. Flavor profile of chicken broth added with sedanenolide, 3-*n*-butylphthalide, and sedanolide; (—) chicken broth (control) with a score of 0 for control, (–●–) chicken broth with sedanenolide added (0.7 ppm), (--Δ--) chicken broth with 3-*n*-butylphthalide added (0.2 ppm), (=□=) chicken broth with sedanolide added (0.2 ppm).

The results of the evaluation of chicken broth flavor with added sedanenolide, 3-*n*-butylphthalide, and sedanolide are shown in **Table 5**, and the flavor profile is shown in **Figure 5**. In comparison with the control, all scores except for salty increased with sedanenolide, all scores except for salty and refined increased with sedanolide, and all scores except for salty, refined, and clarified increased with 3-*n*-butylphthalide addition. Among the three samples, no difference was observed in almost all attributes, but scores of 3-*n*-butylphthalide for thick and lasting tended to be lower than those of sedanenolide and sedanolide. These results indicate that all three phthalides were effective in enhancing the flavor of chicken broth.

Evaluation of the Effect of Phthalides on Taste Sensation. The results obtained from the foregoing two evaluations revealed that the three phthalides enhanced perceived intensities of umami and sweet. In this session, taste properties of these three phthalides were evaluated using a triangle test with and without a nose-clip that closed the nasal cavity to prevent the flow of retronasal odor to the olfactory epithelium.

Table 6 shows the number of correct responses in detecting the test samples with and without attaching the nose-clip in the triangle test. Although the panelists significantly detected the samples containing sedanenolide and sedanolide without attaching the nose-clip, they did not detect the samples containing these compounds with the nose-clip attached. Although the detection

Table 6. Number of Correct Responses for Detection of Phthalides in Chicken Broth with and without a Nose-Clip in the Triangle Test

	number of correct answers[a]	
	without nose-clip	with nose-clip
3-*n*-butylphthalide[b]	6	1
sedanenolide[c]	9*	1
sedanolide[d]	9*	1

[a] n = 10. Asterisks indicate P < 0.001. [b] Added to chicken broth at the concentration of 0.15 ppm. [c] Added to chicken broth at the concentration of 0.7 ppm. [d] Added to chicken broth at the concentration of 0.2 ppm.

of the sample containing 3-*n*-butylphthalide was not significant without the nose-clip attached, the number of correct responses was significantly lower ($P < 0.001$) with the nose-clip attached. These indicate that the changes induced by phthalides could not be detected without retronasal odor stimuli. In other words, the phthalides dissolved in chicken broth had no taste properties involving chemical and physicochemical stimuli. We considered that the increase of intensity of umami and sweet perceived in the samples containing phthalides were not actual values, but rather the increase of intensity of impression of umami and sweet, which was induced by olfactory sensations brought about by the retronasally delivered phthalides.

From these results, we conclude that the volatile compounds in celery, i.e., 3-*n*-butylphthalide, sedanenolide, and sedanolide enhanced the complex flavor of chicken broth. The three phthalides enhanced perceived intensities of umami and sweet despite of their no-taste properties, in addition to the complex flavor such as koku-mi. The mechanism is hard to understand and needs further study.

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