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Influence of Hypoxia, Storage Period, or Temperature on the Taste of Broccoli Florets

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Abstract

In the present study, the paper aimed to maintain sulforaphane concentration by cold storage or by freezing, either of which could be expected to depress the reactions to reduce causing sulforaphane loss and off-odors such as ethanol after 2 days storage. Also, changes in the taste of broccoli florets over time at different storage temperatures were investigated by objective measurement. This experiment was conducted to confirm the influence of hypoxia, storage period, or temperature on the taste of broccoli florets. Each floret was sealed in a separate pouch. All pouches were stored in incubators at 20°C, 1°C and -20°C. The sulforaphane concentration and objective taste values were measured in four and three pouches, respectively, from each treatment group at 0 day, 2 days, and 6 days of storage. The results showed that sulforaphane concentration of broccoli florets kept for 2 days under hypoxia increased and investigated what effect subsequent storage time and temperature over 4 days had on the sulforaphane concentration and taste of the broccoli florets. After storage at -20°C (frozen) for 4 days, the sulforaphane concentration was not significantly reduced compared with the maximum level on day 2. Meanwhile, this concentration was not significantly higher than those at 20°C or 1°C on the same day. However, even when stored at -20°C, changes in taste (objectively measured using an electronic tongue) were not prevented, as demonstrated by PC analysis. Freezing may be a desirable method for maintaining sulforaphane levels in broccoli florets, and that future research could focus on an effective method for preventing changes in taste during storage.

Keywords

Phytochemical; Cruciferous vegetables; Hypoxia; Postharvest; Electronic tongue.

Introduction

Sulforaphane, an organosulfur compound obtained from cruciferous vegetables, is known to suppress gastric cancer, and food supplements containing high concentrations are currently being marketed (e.g., Super Sprout, vegetable cotyledons). Sulforaphane is produced via catalysis by myrosinase of the precursor glucoraphanin. However, it has been reported that the enzyme and its substrate do not coexist in the same cell, and so some studies have attempted to bring them into contact using physical methods to injure the vegetable tissues (Fan and Mattheis, 2000; Funamoto et al., 2002; Lemoine et al., 2010; Pellegrini et al., 2010).

Van Eylen et al. reported that high-pressure treatment of broccoli (*Brassica oleracea* var. *italica*) heads at 300 MPa for 35 minutes caused glucoraphanin to be con verted into sulforaphane, while Matusheski et al. found that mild heating of fresh broccoli sprouts or florets to 60°C prior to homogenization accelerated this conversion process. Pérez et al. proposed an optimized process consisting of blanching at 57°C for 13 minutes.

Furthermore, Ezaki and Onozaki reported that grating caused isothiocyanates, including sulforaphane, to be produced in radish (*Raphanus sativus* L.).

Makino et al. reported that sulforaphane con centration was increased between 1.6 and 2.3 times in broccoli florets kept in a hypoxic atmosphere at 20°C for 2 days compared to florets in a normoxic atmosphere, demonstrating for the first time a method that increased sulforaphane without the need for physical injury to the plant tissue (Murcia et al., 1999). However, the concentration began to decrease after 2 days even under hypoxic conditions (Eason et al., 2007). Also, it appears that hypoxia may cause an off-odor containing ethanol due to fermentation. In the previous study, in-package ethanol concentration was significantly increased after 2 days (Kasim and Kasim, 2007; Schouten et al., 2009).

In the present study, the aim was to maintain sulforaphane concentration by cold storage or by freezing, either of which could be expected to depress the reactions to reduce causing sulforaphane loss and off-odors such as ethanol after 2 days storage (Sanz et al., 2008). Also, changes in the taste of broccoli florets over time at different storage temperatures were investigated by objective measurement. This experiment was conducted to confirm the influence of hypoxia, storage period, or temperature on the taste of broccoli florets (Esturk et al., 2014).

Materials and Methods

Samples

Seven broccoli (*B. oleracea var. italica* "Ohayo") heads were harvested at a farm in Aichi prefecture, Japan, one day before starting experiments. Immediately upon arrival at the laboratory, 42 florets (ca. 15 g each) were sampled from the seven heads. Seven of these florets were selected as 0-day storage samples, while the remaining 35 florets were randomly assigned as 2-or 6-day storage samples.

Storage Methods and Treatments

Each floret was sealed in a separate pouch. Properties of pouches used in the present study and treatments (storage temperature and period) had been assigned as presented in Table 1. All pouches were stored in incubators at 20℃, 1℃ and -20℃. The sulforaphane concentration and objective taste values were measured in four and three pouches, respectively, from each treatment group at 0 day, 2 days, and 6 days of storage.

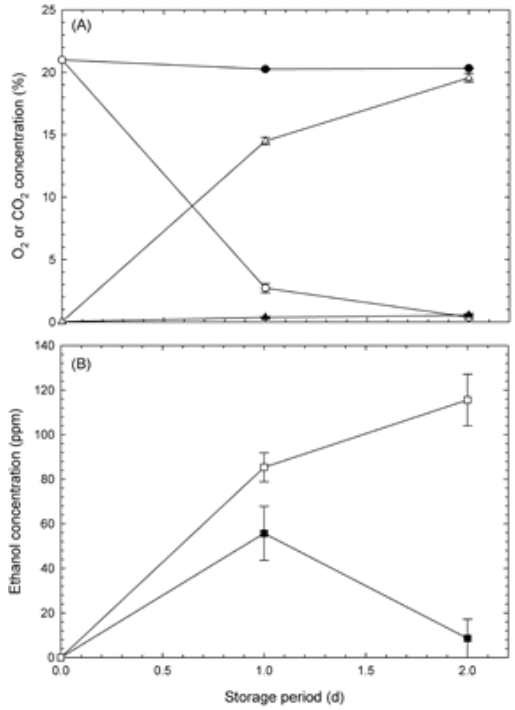

Figure 1: Temporal changes in (A) O_2 or CO_2 concentration and in (B) ethanol concentration inside plastic pouches containing broccoli florets and maintained at 20℃. Circles: O_2; triangles: CO_2; squares: ethanol; closed symbols: micro perforated pouches with O_2 transmission rates of 1.66 × 106 $mL \cdot m^{-2} \cdot d^{-1} \cdot atm^{-1}$ (normoxia); open symbols: high barrier pouches with O_2 transmission rates of 8.0 $mL \cdot m^{-2} \cdot d^{-1} \cdot atm^{-1}$ (hypoxia). Values are means ± SE of (closed symbols) seven or (open symbols) 28 observations.

Gas and Sulforaphane Measurements

The O_2 and CO_2 concentrations inside each pouch on 1 and 2 days were measured by the same method as that of Wang et al. using a CheckMate3 headspace gas analyzer (Dansensor A/S, Ringsted, Denmark). The ethanol concentration inside each pouch on 1 and 2 days was measured by the same method as that of Makino et al. using a XP-3160 gas detector (New Cosmos Electric Co., Ltd., Osaka, Japan).

Sulforaphane concentration in the broccoli florets was measured by the same method as that of Makino et al., and the concentration was converted to a ratio versus the initial (0 day) value.

Immediately after the floret had been removed from the pouch, 1 g of bud was homogenized in 10 mL dichloromethane, which had been dehydrated using anhydrous sodium sulfate. The homogenate was then centrifuged at 12,000×*g* for 20 minutes at 4℃, and the obtained supernatant was evaporated to dryness. The residue was dis solved in 2 mL acetonitrile and centrifuged for a further 10 minutes, following which 1 mL of the supernatant was sampled through a syringe filter (pore size, 0.22 μmØ), stored in a glass vial, and subjected to sulforaphane analysis.

The sulforaphane concentration in the prepared sample was measured using ultra-performance liquid chromatography with a tandem mass spectrometer (XevoTQMS; Waters Co., Milford, MA, USA) using the electrospray ionization method under the following conditions: capillary voltage, 2.93 V; cone voltage, 21 V; desolvation temperature, 650℃; desolvation gas flow, 900 $L \cdot h^{-1}$; mode, positive; MS/MS condition, 174 > 114; collision voltage, 10 V; and dwell time, 0.6 secons. Separation was achieved using a C18 column (Kinetex 2.6 mm Polar C18 100 Å, LC Column 100×2.1 mm; Phenomenex Inc., Torrance, CA, USA) with a 1 mL injection volume and a solvent flow rate of 0.35 $ml \cdot min^{-1}$. The mixing ratio of liquid A (water with 1.1% formic acid) to liquid B (acetonitrile with 0.1% formic acid) was as follows: 0-30 seconds, 90% A+10% B; 30-210 seconds, 90-60% A+10-40% B; and 210-2,800 seconds, 60-90% A+40-10% B.

Standard curve was prepared according to the method by Liang et al. Quantification was based on the external standard method. Pure sulforaphane (CAS No:4478-93-7) purchased from Funakoshi Co., Ltd. (Tokyo) was dissolved in acetonitrile and the final concentration were in the range of 0.0625-2.0 ppm. Peak areas were recorded for all the solutions.

Objective Measurement of Taste Using Electronic Tongue

A floret sample (15 g) was homogenized with an 80 mL of 17 MW ultrapure water at 100 rpm for 1 minute using a TML-200 food processer (Tescom & Co., Ltd., Tokyo). After filtration using gauze, the filtrate obtained was adjusted to 100 mL and used for measuring objective taste values using a SA402B taste sensing system (Intelligent Sensor Technology, Inc., Atsugi, Japan). Eight taste factors (sourness, bitterness, miscellaneous bitterness, astringency, basic astringency, umami, rich umami, and saltiness) were evaluated as relative values against control (ultrapure water). Where umami means the taste derived from amino acids as

glutamate or nucleic acids as inosinic acid, and rich umami means the sustainable umami taste. All values were normalized to a Gaussian distribution (mean 0, variance 1) for each taste using Equation 1:

$$z = \frac{x - \mu}{\sigma} \quad (1)$$

where x is the relative taste value against control, z the normalized value, μ the mean value calculated for a taste fac tor, and σ the variance calculated for a taste factor.

Statistical Analysis

The mean values were calculated for each treatment group, and the statistical significance of differences among treatments was tested using Tukey's honestly significant difference test in JMP® Pro ver. 12.2.0 (SAS Institute Inc., Cary, NC) with a significance level of $p<0.05$.

Results and Discussion

Atmosphere Inside the Pouches

Temporal changes in the inorganic gases inside the pouches containing broccoli florets are shown in Figure 1(A). The O_2 concentration inside the high-barrier pouches decreased from 21% to almost zero by day 2, while over the same period the CO_2 concentration inside the same pouches increased from 0.03% to approximately 20%. The O_2 and CO_2 concentrations inside the micro perforated pouches with a transmission rate of 1.66×10^6 mL·m^{-2}·d^{-1}·atm^{-1} O_2 were maintained at the same levels as in the air outside. The different atmospheres that were thus achieved were considered suitable for investigating the effects of atmospheric conditions on the sulforaphane con centration and taste in broccoli florets.

Volatile compounds that result from ethanol fermentation are known to unfavorably affect the flavor of fruits and vegetables, making it important that we also consider the effect of the atmosphere on ethanol production. Changes in the ethanol concentration over time as a result of fermentation of the broccoli florets in the pouches are shown in Figure 1(B).

The ethanol concentration increased over time in the high-barrier pouches. In contrast, the ethanol concentration on 2 days in the micro-perforated pouches was almost the same as the initial level, although it was elevated temporarily on 1 day. These results match others previously reported was reproduced in the present study. Fermentation does not usually occur under normoxia. However, low concentration of ethanol was detected from the broccoli florets sealed in the pouch of 1.66×10^6 mL·m^{-2}·d^{-1}·atm^{-1} of O_2 transmission rate. Broccoli heads are often transported from a shipping field to a wholesale market in a box of styrene foam which has no gas transmission. This method may be a cause of fermentation in a head and the ethanol accumulated inside due to temporary fermentation during transportation was released into the sealed bag occasionally in the initial stage of storage.

Influence of Storage Atmosphere and Temperature on Sulforaphane Concentration in Broccoli Florets

Changes in the relative sulforaphane concentrations in broccoli florets that had been sealed in plastic pouches (Table 1) for 2 days are shown in Figure 2.

Table 1: Pouch properties and treatments.

Storage period (d)	O_2 transmission rate of pouch [mL·m^{-2}·d^{-1}·atm^{-1}]	Storage temperature
0	-	-
2	$1.66\times10^{6*}$	20℃
2	8.0^{**}	20℃
6	8.0^{**}	20℃, 2d+20℃, 4d
6	8.0^{**}	20℃, 2d+1℃, 4d
6	8.0^{**}	20℃, 2d+-20℃, 4d

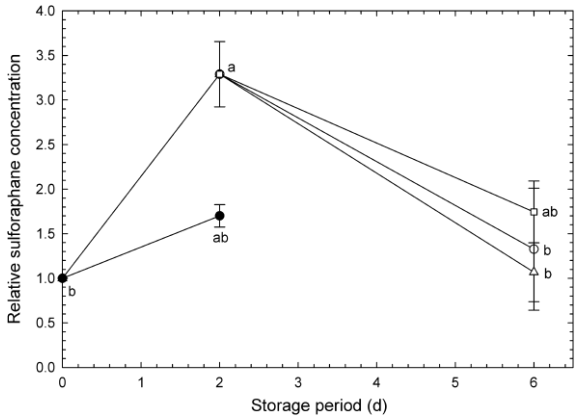

Figure 2: Changes in the relative sulforaphane concentrations in broccoli florets that had been sealed in plastic pouches for 2 or 6 days and maintained at various temperatures. All sulforaphane concentrations were calculated as relative values, taking the value on 0 d as unity. Closed symbols, stored in micro-perforated pouches with O_2 transmission rates of 1.66×10^6 mL·m^{-2}·d^{-1}·atm^{-1} (normoxia) for 2 days; open symbols, stored in high barrier pouches with O_2 transmission rates of 8.0 mL·m^{-2}·d^{-1}·atm^{-1} (hypoxia) for 2 days. After 2 days storage under hypoxia, florets were stored at (open circle) 20℃, (triangle) 1℃, or (square) -20℃. Values are means ± SE of four observations. Symbols followed by the same letter indicate that there were no significant differences ($p<0.05$, with Tukey's honestly significant difference test)

The sulforaphane concentration was significantly (3.3-fold) higher in broccoli florets that had been sealed in high barrier pouches stored for 2 days at 20℃ than in the initial samples. This concentration was 1.9-fold higher than the value in the florets stored in micro-perforated pouches with 1.66×10^6 mL·m^{-2}·d^{-1}·atm^{-1} of O_2 transmission rate. The effectiveness of storage under low O_2 and high CO_2 for 2 days at 20℃ on increasing sulforaphane concentration in broccoli florets confirms the results of Makino et al. In contrast, the sulforaphane

concentration in broccoli florets that had been sealed in micro-perforated pouches did not increase, again confirming the results of previous research.

At this point (after 2 days sealed in high-barrier pouches at 20℃), the florets were subsequently stored for a further 4 days at either 20℃, 1℃ or -20℃. By 6 days, at both 20℃ and 1℃, sulforaphane concentration was significantly decreased. The result at 20℃ replicated results by Makino et al. Egner et al. similarly reported that in broccoli sprout beverages, sulforaphane is passively absorbed and rapidly conjugates with glutathione via glutathione S-transferases, following which it is sequentially metabolized by γ-glutamyl-transpeptidase, cysteinyl-glycinease, and N-acetyltransferase. Cold storage is considered to be effective for maintaining levels of active compounds, such as L-ascorbic acid, in perishables. However, in the present study, sulforaphane concentration decreased significantly even in cold storage at 1℃. Lim et al. reported that sulforaphane concentration in radish root was not maintained during storage for 3 months at 0℃. All these findings suggest that cold storage is not effective for maintaining levels of sulforaphane.

In contrast, sulforaphane concentration was not significantly decreased after storage at -20℃ for 4 days though the levels were reduced. No reports have been found concerning the influence of frozen storage on sulforaphane concentration in perishables. Meanwhile, no significant difference of sulforaphane concentrations among three temperature levels on 6 days was not found though mean value of the concentration at -20℃ was the highest.

Freezing is used for long-term storage of many kinds of perishables, including vegetables. Therefore, this treatment may be effective for keeping active compounds in perishables.

Influence of Storage Atmosphere, Period, and Temperature on the Taste of Broccoli Florets

Sensory tests, in which experienced evaluators called sensory panelists to actually taste samples to evaluate them, are the main method of evaluating taste in the food industry; however, this method has shortcomings, such as low objectivity and reproducibility as well as the great stress imposed on the panelists. To resolve this problem, a sensing technology for objectively discriminating and quantifying the taste of foods has been developed, named an “electronic tongue” after the human taste sense organ.

Since the year 2000, various reports involving application of an electronic tongue to evaluate fruit and vegetable juice have been published. Bleibaum et al. reported that tasting results using an electronic tongue on apple juice corresponded with those of consumers. Han et al., testing apple, black currant, and grape juice, successfully discriminated between commercial products. Yu et al. clarified the signal balance from seven kinds of electronic tongue using bayberry juice. In the present study, objective values measured using an electronic tongue were used for evaluating the taste of broccoli florets.

Changes in eight taste factors in broccoli florets over time and at varying temperature are shown in Figure 3. Bitter ness, astringency, and basic astringency were stable and independent of storage atmosphere, period, and temperature, while the other five tastes were affected by the storage conditions. Sourness, miscellaneous bitterness, and umami were clearly affected by the storage period, especially in the later 4 days storage period, although no influence of storage temperature on these three tastes was observed. In contrast, storage temperature affected rich umami, with the value at -20℃ significantly higher than that at 20℃ in particular. This finding agrees with the report by Qiu et al. that freezing affected the taste of strawberry juice.

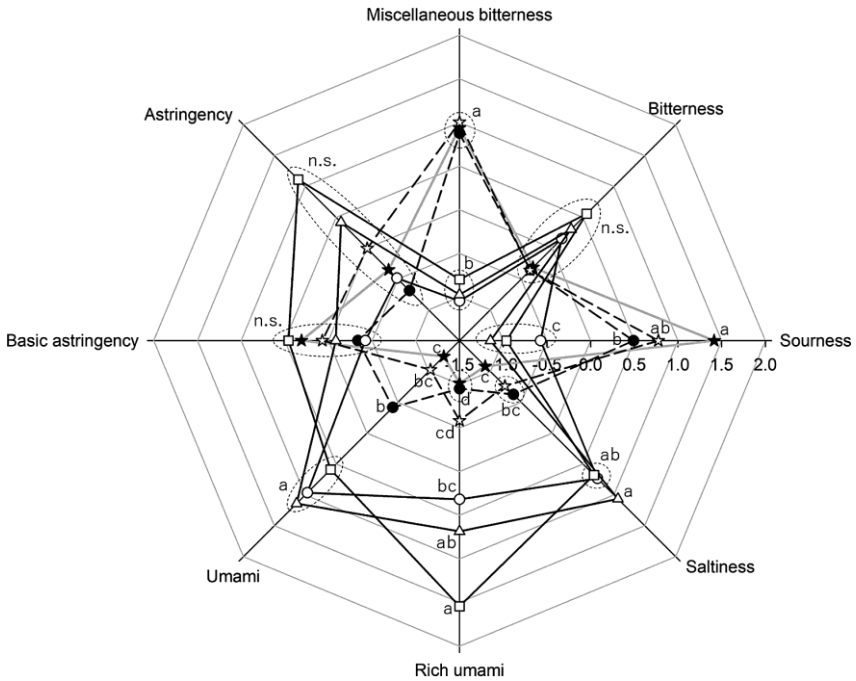

Figure 3: Changes in taste factors of broccoli florets that had been sealed in plastic pouches for 2 or 6 days and

maintained at various temperatures, measured using electrode sensors (electronic tongue). All values were normalized to a Gaussian distribution (mean 0, variance 1 for each taste. Closed star, 0 day; closed circle, stored in micro-perforated pouches with O_2 transmission rates of 1.66×10^6 mL·m^{-2}·d^{-1}·atm^{-1} (normoxia) for 2 days; open star, stored in high-barrier pouches with O_2 transmission rates of 8.0 mL·m^{-2}·d^{-1}·atm^{-1} (hypoxia) for 2 days. After 2 days storage under hypoxia, florets were stored at (open circle) 20℃, (triangle) 1℃, or (square) -20℃. Values are means ± SE of three observations. Symbols followed by the same letter indicate that there were no significant differences (p<0.05, with Tukey's honestly significant difference test) within the same taste factor.

Results of principal component (PC) analysis using the eight taste factors as input variables are shown in Figure 4. The effectiveness of PC analysis for evaluating the taste of pomegranate juice using an electronic tongue has been demonstrated by Bett-Garber et al. As expected, the storage period was the component exhibiting the maximum variance (58%) and is taken as PC1 (Figure 4(A)). Hong and Wang, in a study on cherry tomatoes, also reported that the storage period was clearly discriminated using an electronic tongue combined with PC analysis. According to the loading plot of this variable [Figure 4(C), open diamonds], sourness, miscellaneous bitter ness, umami, rich umami, and saltiness affected the taste of broccoli florets most over time (absolute loading values-0.4). In contrast, the influence of bitterness, astringency, and basic astringency on taste over time was lower (absolute loading values 0.08-0.25). That is, taste of broccoli florets changed during storage over time (Figure 4(A)) which was especially affected by five kinds of values of electronic tongue (Figure 4(C)).

As the influence of temperature was unclear in the result covering the 6-day period [Figure 4(A)], PC analysis using only the data for the 4 days storage period was con ducted [Figure 4(B)]. The influence of temperature on the later 4 days storage was observed as PC2. According to the loading plot of this variable [Figure 4(C), closed diamonds], the influence of temperature on rich umami was greatest (see also Figure 3).

These results suggest that maintaining the taste of broccoli florets would be difficult even if cold storage or freezing were selected. Tahara and Toko reported that bitterness and sourness are the signals associated with toxic and rotten substances. However, bitterness and sour ness were not increased under normoxia or hypoxia over time; therefore, harmful compounds may not have been produced in the florets in the present study.

Makino et al. proposed storage under hypoxia for 2 days as a pre-treatment method to increase the sulfo raphane content and hence commercial value of broccoli, on the premise that consumers would obtain more com pounds beneficial for their health from the pre-treated broccoli (raw or cooked) than from untreated broccoli. As freezing at -20℃ was effective for maintaining sulforaphane concentration, this method may be useful for storing broccoli if it is not to be consumed promptly after harvesting. As a change in taste was not prevented by frozen storage at -20℃, future research into an effective method for preventing such change in broccoli is necessary.

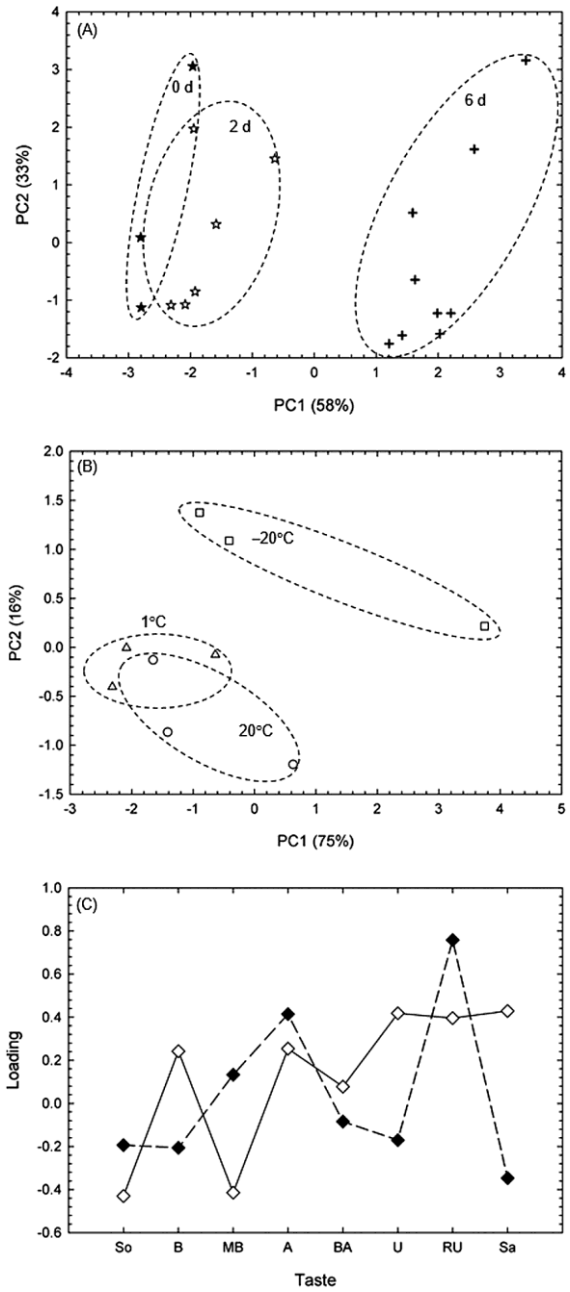

Figure 4: Principal component (PC) analysis of the taste of broccoli florets that had been sealed in plastic pouches for 2 or 6 days and maintained at several temperatures measured using electrode sensors using (A) all data or (B) only 6 days storage data; (C) loading plots calculated from the analysis in figures (A) and (B) were shown. All the analyses were conducted using normalized values in Gaussian distribution (mean 0, variance 1). Values in parentheses on

vertical and horizontal axes in (A) and (B) denote the contribution rate of PCs. Closed star, 0 day; open star, 2 days; cross hair, 6 days; circle, 20, (triangle) 1, or (square) -20℃; open diamond: PC1 in (A); closed diamond: PC2 in (B). Abbreviations for tastes; So, sourness; B, bitterness; MB, miscellaneous bitter ness; A, astringency; BA, basic astringency; U, umami; RU, rich umami; Sa, saltiness.

Conclusions

We confirmed that sulforaphane concentration of broccoli florets kept for 2 days under hypoxia increased and investigated what effect subsequent storage time and temperature over 4 days had on the sulforaphane concentration and taste of the broccoli florets. After storage at -20℃ (frozen) for 4 days, the sulforaphane concentration was not significantly reduced compared with the maximum level on day 2. Meanwhile, this concentration was not significantly higher than those at 20℃ or 1℃ on the same day. However, even when stored at -20℃, changes in taste (objectively measured using an electronic tongue) were not prevented, as demonstrated by PC analysis. Freezing may be a desirable method for maintaining sulforaphane levels in broccoli florets, and that future research could focus on an effective method for preventing changes in taste during storage.

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