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Off-flavor production in frozen strawberries

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Abstract

The development of off-flavor in frozen-thawed strawberries was attributed to the production of H_2S. The identity of H_2S was verified both chemically, through its reaction with lead acetate, and using gas chromatography-mass spectroscopy analyses. The duration of the production of H_2S was longer in berries stored at −40°C and −80°C than in those stored at −20°C. Because the amount of sulfide ion in frozen strawberries was lower than that in fresh berries, and because K_2S addition resulted in further increases in the production of H_2S, it is likely that H_2S evolution is due to the decrease in pH of the cytosol caused by the disruption of cells by freezing, resulting in the release of sulfide ion as H_2S. Vigorous crushing of fresh berries also gave rise to the production of H_2S.

Keywords: Strawberry; Fragaria × ananassa; Freezing; Off-flavor; Hydrogen sulfide; Sulfide ion

1. Introduction

The freezing of strawberries is usually associated with a reduction in aroma and the development of off-flavors. The decrease in aroma is due to a rapid decomposition and diffusion of esters (Deng and Ueda, 1993). Douillard and Guichard (1990) also reported a sharp decline in the levels of esters, whereas the concentrations of franeol and mesifurane, compounds that are linked to strawberry flavor, were not affected by freezing. The off-flavor of frozen strawberries (Kaneko et al., 1988; Masuda et al., 1988; Ueda and Iwata, 1982) differs from that usually noticed in frozen vegetables (e.g. beans) which have been subjected to insufficient blanching (Burnette, 1977; Sapers and Nickerson, 1962). Up to now the nature of the compounds involved in the off-flavor of frozen strawberries has not been elucidated. It is important to identify and characterize the compounds which contribute to this off-flavor.

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2. Materials and methods

Preparation and storage conditions of strawberries

'Toyonoka' and 'Nyoho' strawberries were purchased from retail stores, and 'Hokowase' berries were obtained from the greenhouse production unit at the experimental farm of the Osaka Prefecture University. After the calyxes had been removed, the berries were washed with tap water. They were frozen with dry ice and then with liquid nitrogen to speed up freezing and also to avoid cracking of berries. The frozen berries were wrapped in aluminum foil, sealed in polyethylene bags and stored at −20°C, −40°C and −80°C.

Monitoring of volatile compounds and sensory test of the off-flavor

Five frozen berries were crushed slightly into about five to eight pieces per berry under liquid nitrogen, and 10 g of the resultant berry pieces were put into 100-ml conical flasks which were then sealed with a silicon cap. The flasks were incubated at 30°C for 30 min after which 2 ml of the head-space gas was withdrawn by means of an air-tight syringe and injected into a gas chromatograph (GC) equipped with a flame photometric detector (FPD). The sulfur compounds were separated by two glass columns connected in series. The first column (10 cm in length, 3 mm i.d.) was packed with Porapack QN, the second (3 m in length, 3 mm i.d.) was packed with polyphenyl ether 5-rings (*m-bis*(*m*-phenoxyphenoxy)benzene, 5%). The oven temperature was 90°C.

Strawberries (cv. 'Toyonoka') which had been stored at −20°C and −40°C for 1 month were assessed for off-flavor. Berries stored at different temperatures were broken into about five to eight pieces per berry under liquid nitrogen and then used for sensory tests when in a halfthawed condition. Five persons from the staff and students in our laboratory were selected as panelists. They were familiar with off-flavor in frozen strawberries. They were asked to rank a pair of samples from two groups of berries on a hedonic scale shown in Table 2. Each panelist assessed the off-flavor twice with an interval of 2 h or longer. Statistical analysis was done in the paired comparison test by counting the samples which had more off-flavor.

Chemical and GC-MS detection of a sulfur compound

Strips of filter paper were dipped in a 10% solution of lead acetate and dried. These strips were then sealed in the conical flasks containing frozen berries. The flasks were incubated at 30°C for 30 min and the changes in the color of the paper strips were observed. At the same time the volatiles from the head space were analyzed by GC.

A 5971MSD quadruple-type (electron accelerating voltage 70 eV) Hewlett-Packard mass spectrometer (MS) was used. The volatiles were separated on a cross-linked methyl silicone capillary column DB1 (60 m in length, 0.25 mm i.d. 1 μm thick coating), at 40°C. One ml of head-space gas was withdrawn from the conical flasks and injected into the GC-MS. The gas flow was split up in ratio of 1 : 6. The emerging volatiles from the GC were tracked by an MS in the selective ion monitoring (SIM) mode. Two ions of H_2S with a mass/charge (m/z) ratio of 34 and 33, representing the molecular ion, M^+ and the fragment ion M_1^+, that had lost a hydrogen atom from M^+ respectively, were selected. The mass spectra of the strawberry volatiles were compared to those of pure H_2S.

Sulfide ion contents in the berries

A modification of the methylene blue method (Gustafsson, 1960) was used for sulfide ion measurement. The strawberries were homogenized in two volumes of sodium acetate (0.5 M) and adjusted to pH 8.5 with NaOH. An aliquot (10 ml) was mixed with 4 ml of a solution containing 0.1 M sodium acetate and 0.25 M lead acetate, 2 ml of aminodimethylaniline reagent (93 mg% in 3 M sulfuric acid solution) together with 0.4 ml of 0.25 mM ammonium iron(III) sulfate $NH_4SO_4 \cdot FeSO_4$. A blue color was formed after standing for 15 min in the light. After centrifugation and filtration with membrane filter, the blue and the natural pink pigments of the strawberries were separated using a Sep-pak C18 column. The column was first eluted with 50% aqueous methanol solution which removed the pink pigments, and then with pure methanol to remove the blue pigment. The concentration of the latter was determined by measuring its absorption at 565 nm and calculated from a calibration curve using K_2S.

3. Results and discussion

Identification of off-flavor compounds

In preliminary experiments, we attempted to identify the off-flavor volatile by sniffing at the outlet from the GC column. These initial investigations indicated that the volatile in question had a short retention time which varied with the speed of the injection. It was found that the combination of two columns in series, i.e. a 10-cm column packed with Porapak QN and a 3-m column packed with polyphenyl ether 5-ring (5%), resulted in a better separation and reproducible retention time (65 s) for the low-boiling volatile which was responsible for the off-flavor.

The olfactory properties indicated the presence of sulfurous compounds. Based on this evidence, the sulfur-specific detector (FPD) was used for the subsequent analysis of the off-flavor volatile.

The injection of head-space gas from the flasks containing frozen strawberries resulted in a large peak with a 65-s retention time (Table 1). This peak was not present in the head-space gases of fresh strawberries. Further injection of a mixture of head-space gas from both frozen strawberries and pure H_2S resulted in a single peak with the same retention time (data not shown). It was also observed that 'Hoko-wase' berries produced smaller amounts of sulfur volatile than did the other two cultivars used in these experiments (Table 1). These preliminary results indicate that the amount of off-flavor compounds from frozen strawberries is cultivar-dependent.

The inclusion of strip of papers impregnated with a 10%-solution of lead acetate in the sealed conical flasks resulted in the development of grayish-brown color only in the frozen-thawed strawberries but not in the fresh ones (data not shown). Furthermore, the inclusion of the paper strips eliminated the appearance of the peak corresponding to the one in Table 1.

Identification of the compound was carried out using GC-MS. One ml of the head space gas in the flask containing frozen-thawed strawberries was injected into the GC-MS. The SIM mode (m/z = 34 (Fig. 1A) or 33 (Fig. 1B)) analysis of the head-space gas by GC-MS revealed that the response, labeled peak 1, appeared at identical retention times of 6.19 min.

Table 1
Production of a volatile sulfur compound[a] in frozen-thawed strawberries

Year	Cultivar			Mean[b] of the year
	'Hoko-wase'	'Toyonoka'	'Nyoho'	
1993	2.46[c]	3.89	3.53	3.09 c
	2.59	2.66	3.53	
	2.52	3.35	3.31	
1994	2.31	3.35	3.51	3.27 c
	2.46	3.92	3.57	
	2.60	4.41	3.33	
Mean[b] of the cultivar	2.49 a	3.60 b	3.46 b	

[a] The peak of the compound has a retention time of 65 s using columns of Porapack QN and polyphenyl ether 5-ring.
[b] Values within a column or line followed by different letters represent significant differences at $P < 0.05$ (Duncan's multiple range test).
[c] μmol/kg h^{-1} as H_2S.

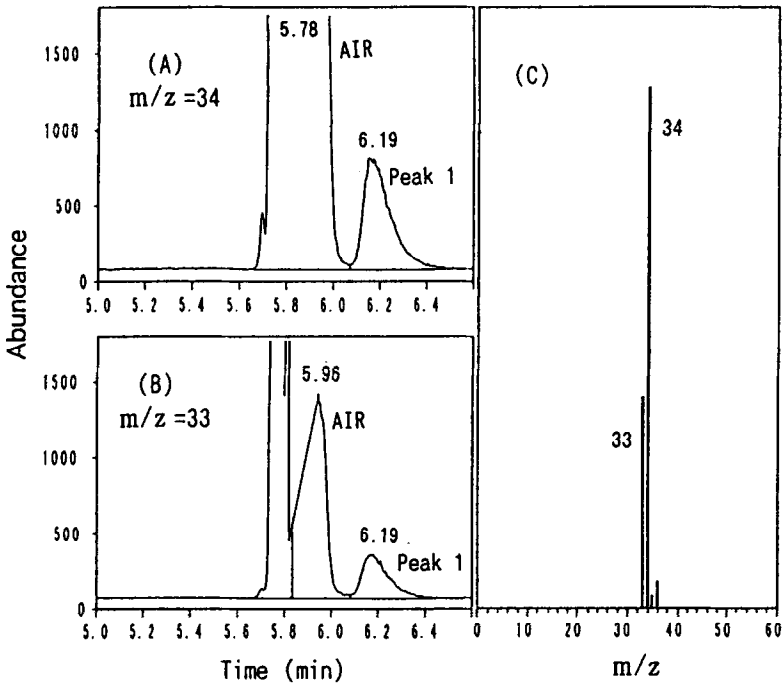

Fig. 1. Selective ion monitoring and GC-MS spectra of head-space gas from the flask containing frozen-thawed strawberries (A) Trace of m/z = 34, (B) Trace of m/z = 33 and (C) mass spectrum of peak 1.

The mass spectrum of H_2S from the data book (EPA/NIH mass spectral data base) shows a main peak at 34 m/z, and two peaks belonging to 33 m/z and 32 m/z fragments of H_2S which have about half the intensity of the main peak. The spectra of the

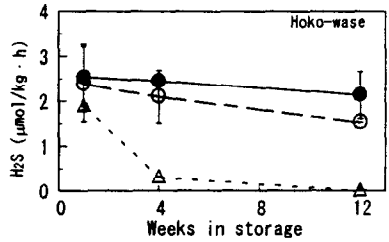

Fig. 2. Effects of storage temperatures, (•: −80°C; ○: −40°C; and △: −20°C), and storage period on H_2S production from frozen-thawed strawberries ('Hokowase'). Bars represent standard errors ($n = 3$).

head-space gas both from the flasks with frozen-thawed strawberries and from the dilute H_2S mixture showed two peaks of 34 m/z and 33 m/z with the same ratio as in the data books (Fig. 1C). The expected 32 m/z peak disappeared due to subtraction of the background peaks caused by air. The results thus indicate that the sulfur compound (peak-1) which came from the frozen-thawed strawberries was H_2S. The concentration of peak-1 in the flask was about 20 ppm. Show et al. (1980) reported that the odor threshold value of H_2S is between 0.18 to 130 ppb. This value strongly suggests that the off-flavor compound in frozen strawberries is H_2S.

Previous publications reported H_2S production from various other commodities, such as orange juice (Show et al., 1980), Satsuma mandarin juice (Imagawa et al., 1974) and fresh strawberries (Winter, 1963). In addition, the Satsuma mandarin juice contained dimethyl sulfide (Imagawa et al., 1974) and fresh strawberries contained small amounts of methanethiol (Winter, 1963). Dirinck et al. (1981) observed that sulfur volatile compounds other than H_2S contributed to the aroma of fresh strawberries. Our data is the first to show that relatively significant amounts of H_2S are produced by frozen strawberries.

Effect of storage temperature on H_2S production

The data presented in Fig. 2 shows the amount of H_2S produced by 'Hoko-wase' strawberries stored at either −2°C, −40°C or −80°C. The other two cultivars showed exactly similar tendency. It can be seen that the evolution of H_2S in strawberries stored at −20°C decreased considerably within 4 weeks, whereas berries stored at either −40°C

Table 2
Sensory test (Paired comparison) for off-flavor in frozen 'Toyonoka' strawberries stored at −20°C and −40°C for one month

Panelist: [a]	A		B		C		D		E	
Trial:	1	2	1	2	1	2	1	2	1	2
−20°C [b]	+ [c]	−	++	−	−	−	−	−	−	−
−40°C	+++	+++	+++	++	+++	+++	++	+	++	++

[a] Panelists were familiar with off-flavor in frozen berries (semitrained) and were given one pair of half-thawed samples to assess, and two hours later, another similar pair.
[b] Off-flavor estimates for berries stored at −20°C and −40°C were significantly different ($P < 0.05$).
[c] Score of off-flavor: −, undetectable; +, slight; ++, moderate; +++, strong; ++++, very strong.

or −80°C maintained relatively high levels of H_2S evolution for longer periods. The large differences in H_2S production between −20°C and −40°C storage temperatures may be due to the low boiling point of H_2S (−59.0°C).

A sensory test for off-flavor (paired comparisons) using fruits kept at −20°C and −40°C for one month was conducted. The fruits kept at −20°C had a significantly weaker off-flavor than those stored at −40°C (Table 2). The similarity between the sensory test and the H_2S production results indicate that the off-flavor compound of frozen berries was H_2S. In addition, some of the panelists noticed that the off-flavor from frozen thawed berries had a smell similar to dilute H_2S.

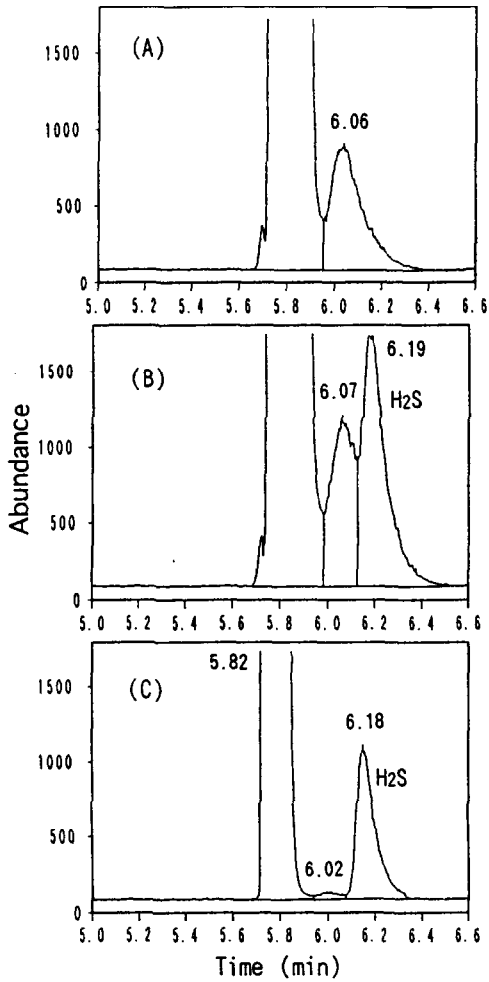

Fig. 3. Selective ion monitoring of a mass fragment (m/z = 34) of head space gas in the flasks with fresh berry slices (A), mechanically damaged fresh berries (B), and mechanically damaged fresh berries in which air was replaced with N_2 (C).

H_2S production in crushed fresh berries

Slices of fresh berries were put into conical flasks, which were subsequently securely sealed. Shaking the flasks vigorously by hand resulted in the production of significant amount of H_2S which was detected with GC-MS, SIM mode (m/z = 34) (Fig. 3B). Undamaged fresh slices gave no such peak (Fig. 3A). The separation of H_2S produced by shaking was enhanced when the incubation flasks were flushed with N_2 instead of air (Fig. 3C). The peak with a retention time of approximately 6.06 min may be either 0_2 or $C0_2$. The amount of H_2S emanating from crushed fresh berries is similar to that produced by frozen berries.

Mechanism of H_2S formation

The evolution of H_2S after crushing occurred at decreasing pH from 5.0 even down to pH 2. The production also occurred when the berries were crushed in 80% ethanol (data not shown), which is expected to eliminate enzymatic involvement in the emanation of H_2S.

Enzymatic production of aroma in response to mechanical damage in cabbage (Chin and Lindsay, 1993), onions (Schwimmer, 1971), and Shiitake mushrooms (Morita and Kobayashi, 1966) is a well-known phenomenon. The production of H_2S reported here, however, appears to be nonenzymatic in origin.

Usually H_2S is derived from the sulfur-containing amino acids, cysteine or methionine during food processing. We followed the changes in amino acid content during freezing and thawing of strawberries but found no detectable amounts of cystine or methionine in fresh, frozen and frozen-thawed strawberries (Table 3). These results agree with a previous report (Perez et al., 1992). The results thus tend to preclude the possibility of an amino acid being the main precursor of the off-flavor compounds.

In order to identify other possible precursors of H_2S, a number of chemicals such as methionine, cysteine, K_2SO_4, K_2SO_3, KSCN and K_2S were added singly into conical flasks along with the frozen strawberries. Only the addition of K_2S increased the production of H_2S (data not shown).

The data presented in Fig. 4 show that the content of sulfide ion and its salt in the fresh berries was higher than those in frozen-thawed ones. This therefore suggests that sulfide ion was released from the frozen-thawed strawberries as H_2S. One cultivar

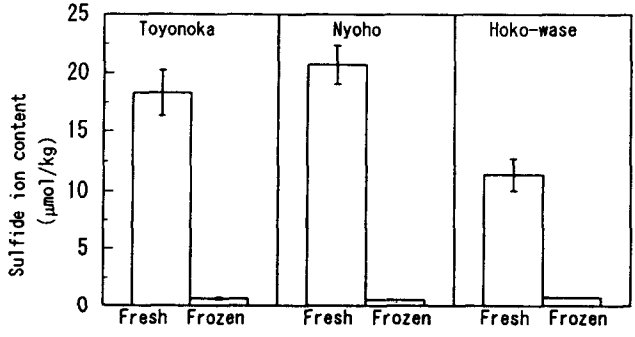

Fig. 4. Sulfide ion contents in fresh and frozen-thawed strawberries. Bars represent standard errors ($n = 3$).

Table 3
Amino acid content in 'Hoko-wase' strawberries (mg per 100 g fruit)

Amino acid	Fresh [a]	Frozen	Frozen-thawed
Asp	31.9	35.3	34.8
Thr	3.5	4.6	4.1
Ser	6.8	8.3	7.7
$AspNH_2$	81.8	77.2	84.5
Glu	17.0	24.6	23.4
$GluNH_2$	2.8	3.5	3.8
Pro	0.1	0.2	0.1
Gly	0.7	0.5	0.8
Ala	11.3	14.4	11.9
Cys	nd	nd	nd [b]
Met	nd	nd	nd
Ileu	0.4	0.5	0.6
Leu	0.3	0.3	0.3
Tyr	1.4	1.7	1.4
Phe	4.9	3.3	1.4
Lys	0.3	0.3	0.4
His	0.2	0.2	0.3
Arg	0.1	0.1	0.1
Total	163.5	175.0	175.6

[a] Berries of each treatment were analyzed twice. Only one representative is presented.
[b] nd = None detected.

('Hoko-wase') had a lower sulfide ion content than others indicating that the level was cultivar-dependent.

In conclusion, the above data indicate that the off-flavor development in frozen berries can be attributed to the breakdown of the cells by freezing, thereby decreasing the pH in the cytosol, which in turn leads to the emanation of sulfide ion as H_2S. Furthermore, its production depends on the concentration of sulfide ion in the berries. It is therefore advisable, when freezing strawberries, to avoid the use of cultivars with high concentrations of the sulfide ion. It is also recommendable that only cultivar of strawberry low in sulfide be grown.

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