Heat-Induced Compounds in Strawberries

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Dimethyl sulfide, acetaldehyde, isobutyraldehyde, furan, 2-furaldehyde, 2-acetyl furan, and ethyl furoate were produced when pureed strawberries were heated to 120° C. for 30 minutes. Shorter heating periods were sufficient to produce the first three compounds. With the exception of acetaldehyde, none of the heat-induced compounds were detected in unheated, pureed strawberries. Compound identification was based on gas chromatographic retention time, reactivity with specific reagents, and mass spectrometry. Low-boiling components were separated and quantitated directly from a sample of puree by an on-column trapping gas chromatographic procedure. High-boiling components were isolated from the puree by low-temperature (<30° C.) vacuum steam distillation. The aqueous distillate was extracted, and the extract concentrated prior to gas chromatographic-mass spectral analysis. The concentrations of dimethyl sulfide, acetaldehyde, and isobutyraldehyde determined in heated strawberry puree are in excess of published flavor threshold values.

Investigation of strawberry flavor has been the subject of a number of research efforts for the past 30 years. Teranishi and McFadden employed a number of instrumental separation and identification techniques to investigate the strawberry condensate from a jam-making process (McFadden *et al.*, 1965; Teranishi *et al.*, 1963). Winter and Willhalm (1964) utilized paper chromatography of chemical derivatives to investigate the flavor components of a fresh strawberry steam distillate. Nursten and Williams (1967) have contributed a comprehensive review of the components identified in various fruit aromas.

Organoleptic comparison of raw and heated strawberries demonstrates that heating causes a manifest alteration in strawberry flavor. Since past investigations utilized a variety of techniques involving different methods of sample preparation (some involving concentration by heat and others not), this study was made to determine the volatile compounds altered or produced by the heating process.

EXPERIMENTAL

Washed and stemmed fresh Northwest strawberries, obtained from a local fruit processor, were individually quick frozen (IQF) at −32° C. and stored in 20-lb. fruit tins at −15° C. until analyzed. Gas chromatographic (GLC) analysis of fresh and frozen strawberries by the method of Morgan and Day (1965) failed to reveal any significant qualitative or quantitative changes in volatile compounds detectable by this means during the storage period.

Low-Boiling Components. Frozen strawberries were thawed until they could be pureed in a Waring blender. Thirty-gram aliquots of the cold puree were placed in 50-ml. culture tubes and sealed with Teflon-lined screw caps. A portion of the tubes was refrozen as raw controls, while the other portion of the filled tubes was autoclaved at 120° C. for selected time periods designated in Table II. Retention of volatile components in such tubes has been demonstrated by Bills and Keenan (1967). Quantitative and qualitative analyses of the raw and heat-treated strawberry samples were achieved by the on-column trapping, GLC technique developed by Morgan and Day (1965). The following constituents were added to the sample vials: 4 grams of strawberry puree, 5 grams of anhydrous sodium sulfate, and 4 ml. of distilled water containing 0.5 p.p.m. of 2-butanone as an internal standard. The internal standard was added to permit compensation for fluctuation in purging efficiency and GLC response. Concentration determinations were accomplished by comparing peak area values with standard curves (peak area value *vs.* known concentrations) of the compound being measured. Standard curves were prepared with known concentrations of authentic compounds added to raw strawberry puree. Peak areas were calculated as the products of peak height and retention time, as described by Carroll (1961). Retention time data were obtained from known concentrations of authentic compounds and 2-butanone in distilled water. A collection period of 10 minutes with the sample immersed in a 60° C. water bath and a nitrogen purge rate of 14 ml. per minute was used for preparation of standard curves and for the analyses of the strawberry samples.

Analyses were accomplished with an Aerograph Model 1200 gas chromatograph fitted with a 0.085-inch I.D. by 12-foot stainless steel column packed with 20% 1,2,3-tris(2-cyanoethoxy) propane on 60 to 80-mesh Celite 545. The column temperature was maintained at 60° C. with a nitrogen carrier gas flow rate of 24 ml. per minute.

Confirmatory gas chromatographic retention time data for acetaldehyde and isobutyraldehyde were also obtained using a 0.085-inch I.D. by 3-foot column packed with Poropak Q. The dimethyl sulfide peak, however, was not resolved from a larger peak with the same retention time on the Poropak column.

The method proposed by Bassette and Whitnah (1960) for removal of specific compounds by chemical reaction prior to GLC analysis was used. To confirm the GLC retention time identification of dimethyl sulfide, 2 grams of mercuric chloride was added to the sample vial and allowed to react for 10 minutes with frequent agitation. Nearly complete removal of the peak corresponding to dimethyl sulfide confirmed this identification. Aldehyde identities were confirmed by the addition of sodium bisulfite and subsequent removal of the corresponding GLC peaks.

High-Boiling Components. Ten pounds of IQF strawberries were thawed and vacuum sealed in size 303 cans lined with fruit enamel. The cans were retorted at 120° C. for 30 minutes. After cooling, the heated strawberries were pureed in a Waring blender and then steam distilled at 2 mm. Hg pressure with the sample temperature maintained below 30° C. Volatiles stripped from the sample were condensed in dry ice and liquid nitrogen-cooled traps. The distillation was continued for three hours and approximately 1500 ml of distillate was collected. The aqueous distillate was saturated

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with sodium chloride and extracted with diethyl ether for 24 hours in a continuous liquid-liquid extractor. The ethereal flavor extract was dried over anhydrous sodium sulfate and concentrated by fractional distillation to remove the excess ether using a 1.0- × 60-cm. fractionation column packed with glass helices. The rate of distillation was controlled electronically at a reflux rate of 1 to 2 (collect to return). The concentrate was transferred to a 2-ml. graduated conical sample tube and further concentrated under a stream of nitrogen until a final volume of 20 μl. was obtained. A raw control strawberry concentrate was prepared in precisely the same manner, with the exception of the retorting procedure.

Three microliters of each strawberry concentrate was then analyzed with a F and M Model 810 gas chromatograph fitted with a 0.01-inch I.D. by 300-foot butanediol succinate (BDS) capillary column. The GLC column was held at 60° C. for 5 minutes, then temperature-programmed at 2° C. per minute to 200° C. and maintained at this temperature. The injected samples were split 1 to 50 (1 on column, 50 vented) prior to entering the column. The GLC effluent was monitored by the 20-eV. (GLC trace) and the 70-eV. ion sources of an Atlas CH-4 mass spectrometer equipped with a dual ion source.

Heated and raw strawberry concentrates were also chromatographed on an Aerograph Model 1200 gas chromatograph equipped with a hydrogen flame detector and fitted with a 0.02-inch I.D. by 50-foot BDS support-coated open tubular column. Retention time data for the raw and heated extracts were compared with retention time data for authentic compounds.

RESULTS AND DISCUSSION

Tentative identifications for the low-boiling components detected in the raw and autoclaved strawberry purees are listed in Table I. Although most of these compounds have been previously reported as components of strawberry material, this listing indicates the detection capabilities of the on-column trapping method used and further substantiates previous identification. Dimethyl sulfide and isobutyraldehyde were present in the autoclaved strawberry puree, but were not detectable in the raw strawberry puree. These two compounds have not been previously reported as components of strawberries, although Winter and Willhalm (1964) have reported other sulfur compounds and hypothesized the enzymatic oxidative decarboxylation of alanine and valine to acetaldehyde and isobutyraldehyde, respectively. Acetaldehyde was present in the raw sample and increased in concentration upon heating. The concentration of isobutyraldehyde was significantly increased by the addition of 1.0 mg. of valine per gram of strawberry puree prior to the autoclave treatment. This observation substantiates the probable origin of isobutyraldehyde in heated strawberries *via* Strecker degradation of valine. Concentrations of heat-induced compounds at selected autoclaving periods are presented in Table II. The maximum concentration of dimethyl sulfide was produced after 10 minutes of heating and was not significantly altered by extending the heat treatment. The acetaldehyde concentration was increased by the autoclave treatment, but a relation between concentration and length of heating period could not be concluded from the data obtained. The concentration of isobutyraldehyde appeared to increase linearly with the length of heat treatment.

The mass spectral analysis of the ether extracts obtained from the distillates of raw and retorted strawberries indicated the following additional compounds were present in the retorted extract but absent in the raw extract: furan, 2-furaldehyde, 2-acetyl furan, and ethyl furoate. The spectra of the first three compounds agreed with the reference spectra tabulated by Cornu and Massot (1966). Gas chromatographic retention time confirmation of heat-induced components of the extract was obtained with authentic compounds for 2-furaldehyde, 2-acetyl furan, and ethyl furoate. Confirmation of the furan mass spectra by GLC was inconclusive because the authentic furan retention time did not coincide with detectable GLC unknown peaks in the retorted strawberry extract. McFadden *et al.* (1965) have previously found 2-furaldehyde and 2-acetyl furan in condensate from the strawberry jam-making process. An extensive literature search indicated that ethyl furoate had not been reported previously. Also, a reference spectrum for ethyl furoate was not found; therefore, a mass spectrum of authentic ethyl furoate (Aldrich Chemical Co.) was obtained in this laboratory and the results are presented in Table III.

Production of the heat-induced compounds reported in this paper is significant, but not surprising. Dimethyl sulfide has been reported in a number of heated foods, as summarized by Bills and Keenan (1968). Casey *et al.* (1963) reported that dimethyl sulfide was formed when methionine was heated in the presence of pectin. Strawberries contain natural pectin which could serve as a methyl donor, although the presence of

Table I. Tentative Identification of Low-boiling Components in Strawberry Purees

Compounds	Relative Retention Time[a]		
	Authentic compounds	Strawberry Puree	
		Autoclaved	Raw
...		0.10	0.10
Dimethyl sulfide	0.22	0.22	...
Acetaldehyde	0.27	0.27	0.27
Propionaldehyde	0.44	0.43	[c]
Ethyl formate	0.46	[c]	0.46
Isobutyraldehyde	0.47	0.47	...
Methyl acetate	0.51	0.51	0.51
Acetone	0.65	0.65	0.65
Ethyl acetate	0.66	0.65	0.65
Methanol	0.66	[c]	[c]
Butyraldehyde	0.70	[c]	[c]
Methyl isobutyrate	0.72	[c]	[c]
Ethanol	0.76	0.76	0.76
Ethyl isobutyrate	0.85	[c]	[c]
Ethyl propionate	0.92	0.92	0.92
Butanone	1.00	1.00	1.00
Methyl butyrate	1.13	1.13	1.13
...	[b]	1.26	1.24
Ethyl butyrate	1.38	1.38	1.38
...	[b]	1.54	1.55
Diacetyl	1.66	1.63	1.65

[a] Column: 0.085-inch I.D. by 12-foot stainless steel coated with 20% 1,2,3-tris-(2-cyanoethoxy) propane on 60–80 mesh Celite 545.
[b] Trace components for which a tentative identification could not be assigned.
[c] Peaks incompletely resolved.

Table II. Concentration of Heat-Induced Compounds in Strawberries Heated at 120° C. for Selected Times

Time, Min.	Concentration of Compound (P.P.M.)		
	Me_2S	Acetaldehyde	Isobutyraldehyde
0	0.00	1.85	<0.05
10	0.44	4.95	0.195
30	0.30	3.65	0.35
60	0.41	5.15	0.65
120	0.25	6.20	1.08
30[a]	0.49	7.95	10.70

[a] 1.0 mg. of valine per 1.0 gram of strawberry puree added before heating.

Table III. Mass Spectral Confirmation of Ethyl Furoate in Heated Strawberry Extract

m/e	Relative Intensity: Compound isolated from heated strawberries	Relative Intensity: Authentic compound
38	17	11
39	41	29
68	16	12
95	100	100
96	19	19
112	39	39
140P	18	15

methionine in strawberries has not been documented as yet. Another possible precursor would be S-methyl methionine sulfonium salt, which was isolated by Bills and Keenan (1968) from unheated corn.

Acetaldehyde and isobutyraldehyde are well-known Strecker degradation products of alanine and valine, respectively. Hodge (1967) also reported that acetaldehyde can be derived from hydrolytic cleavage of a methyl α-dicarbonyl intermediate of the Maillard reaction.

Furan, 2-furaldehyde, and 2-acetyl furan were reported by Walter (1967) as products of the thermal degradation of glucose. Hodge (1967) reported that these compounds were products of carbohydrate caramelization and dehydration. At the pH of strawberries (3.4 to 4.2), sugars would have a tendency to form furfurals, as discussed by Reynolds (1965). The 2-furaldehyde GLC peak was much larger than the other furan compounds present in the retorted strawberry extract. Since 2-furoic acid is a product of caramelization and dehydration of carbohydrates, it might be speculated that ethyl furoate is formed by some secondary reactions of the Maillard reaction. Reynolds (1965) discussed the browning reactions of ascorbic acid and listed 2,5-dihydro-2-furoic acid as a product of the anaerobic decomposition of L-ascorbic acid. Since strawberries have a relatively high ascorbic acid content (Watt and Merrill, 1963), the browning reactions of ascorbic acid may also contribute to heat-induced furan compounds.

The heat-induced compounds isolated in this study could easily influence the flavor of heated strawberries. Patton *et al.* (1956) reported a flavor threshold for dimethyl sulfide of 0.012 p.p.m. in distilled water. Reynolds (1965) reported a burnt or malty flavor for acetaldehyde at 1.3 p.p.m. Sheldon (1968) found that the flavor threshold of isobutyraldehyde in water was 0.18 p.p.m. Hodge (1967) discussed the flavor properties of a number of caramelization and dehydration products; pungent, ethereal odors and burning, sweetish tastes are characteristic of many furan compounds.

A number of acetals were reported to be present in the strawberry condensates investigated by McFadden *et al.*, 1965. Since the emphasis of our work was to identify heat-induced compounds, no attempt was made to identify a number of components that were common to both raw and heated strawberries. Hence, lack of data for the acetals and other previously identified compounds should not be taken as evidence for their absence.

The heat-induced components discussed in this paper should not be considered as an exhaustive listing. Other Strecker aldehydes may have been unresolved, and some of the higher-boiling Maillard products may not have been separated with the techniques employed. The presence of heat-induced compounds in heated strawberries reaffirms the importance of avoiding or recognizing such artifacts during the course of a flavor chemistry study of a raw commodity.

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Received for review February 5, 1969. Accepted June 20, 1969. This investigation was supported by a National Defense Education Act Title IV Fellowship to the senior author. Technical Paper 2616, Oregon Agricultural Experiment Station.