

6-*trans*-Nonenal: An Off-flavor Component of Foam-Spray-Dried Milks

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Abstract

6-*trans*-Nonenal has been identified as the compound responsible for the off-flavor which frequently appears in fresh, foam-spray-dried milks manufactured during the warm summer months in urban areas. The flavor threshold of this compound was found to be less than 0.07 part per billion in fresh, whole milk. Evidence suggests that 6-*trans*-nonenal originates by trace ozonolysis of minor lipid components on the surface of the dried product.

It has been observed for several years that foam-spray-dried milk, manufactured during the warm weather months in Washington, D.C., frequently had a flavor atypical of freshly prepared dry milks. Recent evidence (10) indicates that the off flavor arises as the result of chemical reactions involving ozone in the dryer air and can be controlled by filtering the dryer air through charcoal (9). This report summarizes our efforts in establishing 6-*trans*-nonenal as the major contributor to this unique off flavor.

Experimental Procedure¹

Foam-spray-dried whole milk was prepared according to the procedure reported by Bell et al. (1) and Hanrahan et al. (6). To ensure sufficient development of the off flavor for identification purposes, generated ozone (U.S. Ozone Co. of America, Scottsdale, Pennsylvania) was forced into the air intake of the dryer at a point beyond a charcoal filter at levels approximating 50 to 70 ppb of the air flow. Ozone concentrations of the dryer air were determined with a Mast Ozone Meter as previously described (10). The dried product, having the typical ozone induced off flavor, was cooled immediately with vaporizing liquid nitrogen and stored at -18 C in plastic bags until extracted.

Nitrogen extraction of milk powder. The

off flavor was extracted from 4.5 kg of dry whole milk in an 18.9-liter polyethylene carboy by passing ultra-high purity nitrogen gas through the rubber-stoppered neck of the inverted carboy and out the spigot at a rate of approximately 0.28 m³ per hour. The volatiles from the milk powder were condensed in an 80 by 1.25 cm (id) U-shaped glass tubing, containing glass helices, attached to the spigot of the carboy and cooled in a dry ice-ethanol bath. Approximately 7 m³ of nitrogen passed through the powder before water (about 5 ml) caused freeze-up of the U-tube. Two such nitrogen extractions of each carboy of powder were performed. Nine kilograms (two carboys) of powder were extracted for the results reported in these studies. The contents of the U-tube from each nitrogen extraction were recovered by addition of 75 ml of carbonyl-free hexane (13) to the U-tube. Following drying with sodium sulfate, the combined hexane solutions of volatiles were passed through a 4-g column of Analytical Grade Celite to which 2.4 ml of a saturated aqueous solution of sodium metabisulfite had been ground on to before packing the column. The off flavor was recovered by extruding the packing and decomposing the bisulfite with 100 ml of 10% Na₂CO₃, followed by extraction with two 50-ml portions of carbonyl-free hexane or ethyl ether.

Gas-liquid chromatography (GLC). Gas chromatographic studies were conducted with both a Barber-Colman Model 5000 Gas Chromatograph and the LKB Model 9000 Combination Gas Chromatograph-Mass Spectrometer employing 1.83-m by 0.64-cm and 3.04-m by 0.64-cm columns, respectively, and helium as the carrier gas. Column packings included 7.5% ethylene glycol adipate with 2% phosphoric acid on Anakrom ABS (Analabs, Incorporated, Hamden, Conn.) and 10% Apiezon L on Anakrom ABS. The Barber-Colman instrument was equipped with a flame ionization detector with one-third of the helium stream split to the exit port. The LKB 9000 employs as the GLC detection system a total ion current recording of the ionized components (electron energy of 20 ev) eluting from the gas chromatograph.

Mass spectrometry (MS). The LKB 9000 GLC-MS was used with the 3.04-m by 0.64-cm

Received for publication December 30, 1968.

¹ Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

Apiezon L column at 100 C and a helium flow rate of 20 ml per minute. The temperatures of the flash heater and molecular separator were maintained at 200 C. The mass spectra were obtained at a constant accelerating voltage of 3,500 v, with an electron energy of 70 ev and a scanning time of 2 sec over a M/e range of 15 to 150.

2,4-Dinitrophenylhydrazine chromatography. The 2,4-DNPhydrazone (DNP hereafter refers to dinitrophenyl) of the off flavor was prepared by passing the hexane extract of the decomposed bisulfite column through a 2,4-DNPhydrazone reaction column (13). The 2,4-DNPhydrzones were separated initially by column partition chromatography using a 25-g acetonitrile-hexane partition system according to the method of Corbin et al. (2). Thin-layer chromatography (TLC) of the 2,4-DNPhydrazone fraction from the partition column containing the off flavor was performed by a slight modification of the Meijboom and Jurriens method (11). Silica Gel G containing 8% AgNO₃ was used as the adsorbent and 10% hexane in benzene as the developing solvent.

Regeneration of the 2,4-DNPhydrzones. The 2,4-DNPhydrzones of the off-flavor and reference compounds were regenerated for GLC and MS studies employing the system of Wong and Schwartz (14). The 2,4-DNPhydrzones were adsorbed from hexane solutions on silica gel-packed capillaries, 25 μ liters of 8% aq phosphoric acid was added, and the samples regenerated at 200 C in the area of the flash heater of the gas chromatograph. Odor evaluations of the regenerated compounds were made on the Barber-Colman instrument at the predetermined retention times by smelling the exit port of the stream splitter.

Flavor studies. A 0.155- μ mole sample of 6-*trans*-nonenal 2,4-DNPhydrazone (determined by spectrophotometric methods) was regenerated in the inlet system of the gas chromatograph according to the procedure outlined above. The parent compound was trapped by bubbling the effluent of the stream splitter through 10 ml of fresh whole milk in a 25-ml glass test tube. The test tube was rinsed four times with 10-ml portions of fresh whole milk and a stock solution containing, theoretically, 7.23 μ g of 6-*trans*-nonenal in 100 ml of fresh whole milk prepared. Various dilutions of the stock solution were presented to a six-member taste panel and the flavor threshold determined by the method of Patton and Josephson (12). In addition to threshold studies, samples of fresh whole

milk containing, theoretically, 0.7 ppb of 6-*trans*-nonenal were presented to a 12-member taste panel for flavor characterizations.

Results and Discussion

Initial studies on the ozone-induced off flavor suggested that the off flavor was unique in that, unlike most off flavors of dry milk, it could be extracted without previously hydrating the powder. This was in keeping with other observations (9, 10) which indicated that the reactions involved in the development of the off flavor take place on the surface of the dried product; hence, the off flavor compound(s) is readily available to extraction procedures. The exceptionally small quantity of the significant compound in the off-flavored dry milk made steam distillation and solvent extraction procedures impractical and necessitated the nitrogen extraction procedure employed, despite its obvious limitations.

The visual detection of the off-flavored compound in preliminary studies employing GLC and MS was obscured by substantial quantities of aromatic and aliphatic hydrocarbons in the extract. Further studies revealed that the off flavor reacted with 2,4-DNPhydrazone and formed a bisulfite addition product, suggesting the presence of an aldehydic carbonyl group. Therefore, the parent off-flavor compound could be extracted free of the hydrocarbon contaminants, by formation and decomposition of the bisulfite addition products followed by solvent extraction.

TABLE 1. Relative retention times of ozone-induced off flavor and some isomeric nonenals.

Compound	R_t/R_t^a	
	Column 1 ^b	Column 2 ^c
Nonanal	1.00	1.00
2-Nonenal	1.54	2.14
3-Nonenal	0.87	1.30
4- <i>trans</i> -Nonenal	0.88	1.18
5- <i>trans</i> -Nonenal	0.88	1.10
6- <i>trans</i> -Nonenal	0.89	1.24
6- <i>cis</i> -Nonenal	0.91	1.34
8-Nonenal	1.41
Off flavor	0.89	1.31

^a R_t/R_t , Relative retention time based on nonanal is 1.00.

^b Packed column, 3.04-m by 0.64-cm od, 10% Apiezon L on Anakrom ABS, column temperature, 100 C; LKB Model 9000.

^c Packed column, 1.83-m by 0.64-cm od, 7.5% EGA with 2% H₃PO₄, column temperature, 85 C; Barber-Colman Model 5000.

The regenerated bisulfite addition products were subjected to GLC and MS. Results of these studies revealed relatively large quantities of the C₆ through C₁₀ straight chain saturated aldehydes in addition to a small peak with an odor highly characteristic of the off flavor. Mass spectral studies on this peak revealed the presence of a molecular ion at M/e 140 and significant fragment ions at M-18, M-28, and M-44 (Table 2) suggesting the presence of one or more nine-carbon monounsaturated aliphatic aldehydes (10). Of the nonenals available, it was possible to eliminate 2-, 3-, and 8-nonenal by a combination of GLC retention studies (Table 1), mass spectra, and especially the odor characteristics of the compounds. The GLC retention data and odor characteristics of the remaining available reference nonenals (4-*trans*-, 5-*trans*-, 6-*trans*-, and 6-*cis*-nonenals) were sufficiently like the unknown peak that it was not possible to differentiate these compounds on the basis of these criteria. Slight mass spectral differences between the remaining reference nonenals exist (relative intensity of low M/e fragment ions) (Table 2) to the extent that this criterion could distinguish between the 4-, 5-, and 6-nonenals. However, the mass spectrum of the unknown peak strongly suggested a mixture was present and, indeed, future results proved this to be the case. On the basis that a mixture existed, separation and identification techniques

were initiated on the 2,4-DNPhydrazine derivatives of the regenerated bisulfite addition products.

Initial separation of the significant 2,4-DNP-hydrazones from the relatively large quantities of the saturated aldehyde 2,4-DNPhydrazones present was accomplished by column partition chromatography employing the method of Corbin et al. (2). By regenerating a portion of each band eluted from the column, the significant off-flavor, 2,4-DNPhydrazone was found to elute with the heptanal 2,4-DNPhydrazone. Keppler et al. (7) have reported that the 4-, 5-, and 6-nonenal 2,4-DNPhydrazones all behave like the heptanal 2,4-DNPhydrazone in a similar partition chromatographic system. Separation of the significant derivatives from the greater quantity of the heptanal 2,4-DNPhydrazone was accomplished by TLC on a series of AgNO₃-silica gel G plates. The areas corresponding to the 4-, 5-, and 6-nonenal DNPhydrazones were scraped from the plates, eluted with benzene, and rechromatographed on a AgNO₃-silica Gel G plate. Two spots were observed, one corresponding to the 5-*trans*-nonenal and the other to the 6-*trans*-nonenal. The spots were scraped from the plates, eluted with benzene, and a portion of each regenerated. The typical odor of the reference compounds was observed. A mass spectrum, following on-column regeneration of the remaining portion of the isolated 6-*trans*-nonenal 2,4-

TABLE 2. Comparison of the most intense and significant mass:charge (M:e) fragment ions of some isomeric nonenals and the significant off-flavor gas-liquid chromatographic peak from the bisulfite addition products.

	6- <i>trans</i> -		6- <i>cis</i> -		5- <i>trans</i> -		4- <i>trans</i> -		Off-flavor peak	
	M/e	%	M/e	%	M/e	%	M/e	%	M/e	%
A	41	100.0	41	100.0	54	100.0	41	100.0	41	100.0
	55	61.2	55	64.6	41	71.7	55	78.2	55	94.0
	67	46.5	67	46.6	67	64.0	84	76.2	54	72.6
	81	43.8	54	45.3	81	61.7	54	51.5	84	57.2
B	54	43.4	81	34.7	55	58.8	67	41.4	67	52.9
	84	12.3	84	10.9	84	7.5	81	29.0	81	51.3
	96	19.9	96	17.3	96	52.6	96	31.0	96	43.3
	112	4.3	112	4.1	112	1.2	112	2.0	112	0.3
C	122	17.7	122	22.4	122	8.9	122	7.6	122	12.2
	140	4.2	140	4.6	140	0.7	140	0.7	140	3.4

A—Most intense fragment ions.

B—Included for comparative purposes.

C—M/e 140—molecular ion

M/e 122—M-18

M/e 112—M-28

M/e 96—M-44

DNPhydrazone, further established the identity of this compound, despite low peak intensity as a result of sample size. Sufficient sample of the isolated 2,4-DNPhydrazone corresponding to 5-*trans*-nonenal was not available for further mass spectral data, but it is tentatively identified on the basis of the TLC behavior of the 2,4-DNP hydrazone and the characteristic odor of the regenerated compound.

The flavor potency of 6-*trans*-nonenal (0.3 ppb in paraffin oil) is 100-200 times greater than 5-*trans*-nonenal, despite identical flavor characteristics (7, 8). Hence, only 6-*trans*-nonenal appears to be of significance in this off flavor, although the concentrations of these two compounds in the off-flavored milk are about equal. The possibility of an additive effect (3) cannot, however, be entirely overlooked. Assuming 100% regeneration and recovery of the parent compound from its 2,4-DNPhydrazone, as described in the experimental procedure, the flavor threshold of 6-*trans*-nonenal in fresh whole milk was found to be 0.07 ppb. The true threshold, however, is less than this value, since the regeneration and recovery procedure is at best semiquantitative. A taste panel of 12 members agreed unanimously that fresh whole milk with 0.7 ppb added 6-*trans*-nonenal was highly characteristic of the off flavor which frequently appears in freshly prepared foam-spray-dried whole milk during the warm summer months.

Reaction mechanisms and precursors involved in the formation of 6-*trans*-nonenal remain to be determined. It is of interest to note that Keppler et al. (7, 8) have demonstrated the formation of 6-nonenal in the early stages of autoxidation from 9,15- and 8-15-octadecadienoic acids, the *cis-cis* forms of which have been reported to occur in milk fat (4). Theoretically, 6-nonenal is also a product of the ozonolysis of 9,15-octadecadienoic acid, and previous studies (9, 10) suggest that ozone is required in the formation of this compound in foam-spray-dried whole milk. In either case, the formation of 6-*trans*-nonenal would require the presence of 8-*trans*, 15-*trans*; 9-*trans*, 15-*trans*; 8 *cis*, 15-*trans*; or 9-*cis*, 15-*trans*-octadecadienoic acid, acids which to the best of our knowledge have not been identified in milk fat.

Acknowledgment

The authors are grateful to J. G. Keppler of the Unilever Research Laboratory, Vlaardingen, The Netherlands, for providing samples of the 4-*trans*-, 5-*trans*-, 6-*trans*-, and 6-*cis*-nonenal 2,4-dinitrophenylhydrazones.

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