Hexanal as a Measure of Rancidity in Low Fat Foods

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ABSTRACT AND SUMMARY

The concentration of n-hexanal in low fat, dehydrated foods can be determined in 10 min by the gas chromatographic analysis of the vapors from a 15 g sample suspended in boiling water. With 4-heptanone as an internal standard good day-to-day reproducibility was obtained and the results could be expressed as ppm hexanal in the sample. The onset of rancid odors was found to occur when the hexanal concentration increased to between 5 and 10 ppm. With an oat cereal good correlation between sensory evaluations and hexanal concentration between 0.3 and 5 ppm was obtained. The rate of hexanal increase at 55 and 45 C for a corn and a wheat cereal could be reliably projected to 37 and 21 C. Antioxidants applied to a wheat cereal reduced the rate of hexanal increase.

INTRODUCTION

Lipid oxidation frequently contributes to flavor changes which occur during the storage of foods (1-3). For low fat foods the usual measurements for lipid oxidation such as peroxide determinations (4), the thiobarbituric acid method (5), or oxygen uptake (6) are either difficult to apply or are not sensitive enough. Buttery and Teranishi (7) showed that the gas chromatographic determination of n-hexanal in the vapors of dehydrated potatoes suspended in boiling water was a measure of linolenate oxidation. The concentration of hexanal in dehydrated potatoes (8) and green peas (9) were shown to follow closely the development of off-flavors. With some method modifications, the hexanal determination was found to be a very simple and rapid yet effective analytical tool for measuring oxidative deterioration in low fat foods, especially ready-to-eat breakfast cereals.

EXPERIMENTAL PROCEDURES

Hexanal Determination

The internal standard was prepared each day by adding 25 µl of 4-heptanone (bp 144 C) to a 1000 ml volumetric flask filled about three-fourths with distilled water. After mixing, the flask was made up to volume with distilled water.

If the sample was not a powder, 50 to 100 g was ground to obtain a representative sample. After mixing, 15 g was weighed into a 250 ml Erlenmeyer flask and 2 ml of the internal standard solution added. Boiling distilled water was then added up to the 150 ml mark and the flask immediately capped with four layers of aluminum foil. The mixture was then swirled for 45 sec. Five cc of headspace gas was then withdrawn with a gas tight syringe and injected into the gas chromatograph at a rate of about 1 cc per second.

Several different makes of gas chromatographic equipment with hydrogen flame detectors were used over the years with equally good results. The data reported in this paper were obtained with a 10 ft x ¼ in. aluminum column with 10% Silicone OV-101 on acid washed 60-80 mesh Chromosorb W, a column temperature of 100 C, an injection port temperature of 200 C, a helium flow rate of 40 cc/min, a detector block temperature of 150 C, a detector air flow of 400 cc/min, a detector hydrogen flow of 55 cc/min, and an electronic digital integrator for measuring peak areas.

The hexanal concentration for samples with 3% or less fat were expressed as ppm hexanal in the sample. This value was obtained by multiplying the peak area ratio of hexanal to the internal standard heptanone by 3.31.

To check whether the instrument was functioning properly, the first sample analyzed each day was a "check sample." For the latter a box of commercial oat cereal aged to contain from 1 to 4 ppm hexanal was ground, mixed, and stored in a refrigerator in a moisture tight container. A new check sample was prepared every 2 to 3 wk.

Storage Evaluations

To check whether lipid oxidation occurs in a given product, samples of 100 g or less were placed into 16 oz glass jars or metal cans which were then tightly sealed. The maximum sample weight of 100 g was selected to assure that oxygen would not be a limiting factor in the test. The containers were then stored at one or more of the following temperatures: 55, 45, 37, and 21 C. Periodically samples were removed from storage. After the containers had reached room temperature, they were opened, the odor noted, and hexanal determination carried out.

Sensory Evaluations

Fresh cereals in their commercial packages were stored at 37 C and at -18 C. The latter were placed into metal containers to prevent moisture changes. After 3 wk some of the packages stored at -18 C were transferred into the 37 C room. Sensory evaluations were carried out after 6 wk, using samples stored for 6 wk at -18 C (control), samples stored for 3 wk both at -18 C and 37 C (transfer sample), and samples stored for 6 wk at 37 C. After 12 wk, sensory evaluations were again carried out with samples now stored for 9 and 12 wk at 37 C.

Ten to twelve tasters previously trained and selected for their ability to detect differences in cereal flavors were used for each evaluation. Each taster was presented with three coded and one uncoded sample. The latter was known to be the control—the sample stored at -18 C. One of the coded samples was also the control. All samples were evaluated in milk. On the questionnaire the taster first indicated which of the coded samples was the control. Then the extent of the deterioration of all three coded samples was rated on a nine point scale where 1 was defined as fresh and 9 as edible but deteriorated to a point where the product should not be sold.

RESULTS AND DISCUSSION

Method

Buttery and Teranishi (7) obtained satisfactory day-to-day reproducibility when they used benzene in water to calibrate their instrument each day. This procedure was judged too time consuming and found unnecessary with an internal standard. At first methyl iso-butyl ketone, which elutes before hexanal, was used. With some product, interference from naturally occurring compounds was obtained. Heptanone, which elutes after hexanal, was found to be a much more reliable internal standard. A typical chromatogram of a deteriorated cereal product to which both internal standards had been added is shown in Figure 1.

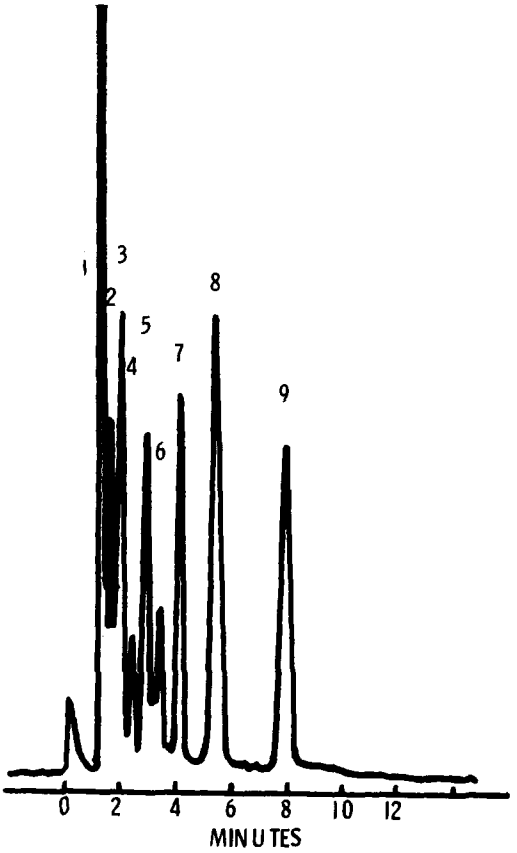

FIG. 1. Chromatogram of a stored cereal sample. Peak 9 is the internal standard 4-heptanone, peak 8 is n-hexanal, and peak 7 is iso-butyl ketone which previously was used as an internal standard.

TABLE I

Hexanal Increase in Breakfast Cereals

Weeks of storage at 37 C	ppm Hexanal			
	Oat	Corn	Wheat	Mixed cereals
0	.31	.38	.03	.05
6	1.25	1.72	.81	.11
9	2.07	4.63	2.47	.08
12	3.85	8.01[a]	15.40[a]	.23

[a]Samples had developed rancid odors.

TABLE II

Sensory and Objective Evaluation of an Oat Breakfast Cereal[a]

Weeks of storage at 37 C	Mean sensory ratings[b]		ppm Hexanal	
	A[c]	B[c]	A[c]	B[c]
0	1.0	1.0	.30	.23
3	3.1	3.3	2.09	2.02
6	4.0	4.0	2.98	2.81
9	5.3	5.6	3.61	3.71
12	6.9	7.6	5.06	5.00

[a]Regression analysis yielded a correlation coefficient of 0.99.

[b]A nine point rating scale was used where 1 was defined as fresh and 9 as edible but deteriorated to a point where the product should not be sold.

[c]Results A and B are replications with different boxes from the same shipping case.

When the method was used three or more times per week over a 2 wk period, the results obtained with the "check sample" were used to monitor the day-to-day reproducibility. For 14 of such periods the mean standard error was 6.4% with a low of 4.3 and a high of 8.1. Somewhat better precision was obtained when the same sample was analyzed five or more times on the same day. The reproducibility with samples containing less than 1 ppm hexanal was at times not as good.

To check the peak area response of hexanal to that of the internal standard heptanone, 5 μ of each were added to 1000 ml of distilled water. Ten ml of the latter were then added to 15 g sugar and the hexanal determination carried out in the usual manner. The mean peak area ratio obtained with these known compounds was 0.83. The amount of each known compound added to the 15 g sample was calculated to be 2.75 ppm. To express the results as ppm hexanal in the sample, the hexanal/heptanone peak area ratio was multiplied by 3.31.

Using the solutions with known amounts of hexanal and heptanone and sugar as the sample, the effect of water temperature, salt and fat content of the sample on peak area response was evaluated. As the water temperature was decreased, the absolute peak areas decreased; however, the peak area ratios remained the same. As more of the sugar was replaced with salt, the absolute peak areas increased but again the peak area ratios remained the same. As more of the sugar was replaced with peanut oil, the absolute peak areas decreased. In this case, however, the hexanal/heptanone peak area ratio increased. Hence, the ppm hexanal conversion factor of 3.31 can only be used if the sample contains 3% fat or less.

Another advantage of the internal standard was that the relative retention time of hexanal to heptanone, which was 0.68, could be readily checked for each determination. In some freshly packaged products an unknown compound which elutes just prior to hexanal and which had a relative retention time of 0.63 was observed. This unkown was at first mistaken for hexanal in samples which contained no hexanal. In storage this unknown disappeared.

Applications

Hexanal measurements on fresh breakfast cereals, both ready-to-eat and instant hot, dehydrated potatoes, breading mixes, dried wheat gluten, defatted wheat germ and soy products showed that such products usually contain less than 1 ppm hexanal. The amount of hexanal in fresh products varied somewhat with the nature of the product. Some contained almost none (0 to 0.1 ppm), others always had a somewhat higher amount (0.2 to 1.0). As most of these products age, an increase in hexanal was observed. The results obtained with four different commercial breakfast cereals, which showed different rates of lipid oxidation, are shown in Table I. Not all corn, wheat, or oat cereals will deteriorate at the rates shown. The rate of lipid oxidation in dehydrated products is affected not only by its composition but also by the moisture content (1), processing conditions (3), surface area, and other factors not as yet elucidated.

Whenever rancid odors were first noted in a storage test, the hexanal concentration in the sample was found to be between 5 and 10 ppm. Prior to the onset of rancid odors no significant changes in the pattern of the chromatograms were noted except for the increase in the hexanal peak. If the samples were allowed to deteriorate beyond this point, there was an increase in peak #1 (Fig. 1) and other peaks appeared and increased with an increase in deterioration. The relative retention times of these new peaks with respect to heptanone were 0.23 (the same as obtained with pentane), 0.43 (the same as obtained with n-heptanal), 1.18, and 2.12. Samples with strong rancid odors were usually not analyzed because the peaks eluting after heptanone increased the analytical time from 10 to 30 min per sample.

Both sensory evaluations and hexanal analysis were carried out with several cereals, an example of which is

TABLE III

Hexanal Increase at Different Temperatures in Two Cereal Products

Storage		ppm Hexanal			
		Corn cereal		Wheat cereal	
°C	Weeks	A[a]	B[b]	A[a]	B[b]
55	0	.34	.33	.03	.04
	1	.75	.76	.34	.23
	2	1.66	1.76	1.25	1.53
	3	4.23	4.05	10.05	9.95
	k_{55}[c]		.8358 (.8438)		1.8744 (1.9177)
45	0	.34	.33	.03	.03
	2	.72	.79	.31	.24
	4	2.03	1.91	1.62	1.71
	6	4.60	4.64	11.42	11.96
	k_{45}		.4426 (.4378)		.9740 (.9564)
37	0	.34	.35	.03	.03
	3	.81	.75	.19	.17
	6	1.50	1.59	.85	.84
	9	3.44	3.39	4.07	4.27
	k_{37}		.2520 (.2512)		.5410 (.5307)
21	0	.34	.34	.03	.04
	8	-	-	.19	.13
	20	-	-	.72	.74
	26	2.47	2.41	-	-
	32	3.72	3.79	3.97	4.30
	k_{21}		.0752 (.0755)		.1463 (.1485)

[a]Experimental results.

[b]Points from regression line of ln hexanal on weeks.

[c]Slope of regression line used as reaction rate constants k. Numbers in parentheses are points from regression line of ln k on 1/K (Arrhenius equation.)

shown in Table II. The results show that hexanal is also a measure of deterioration prior to the onset of rancid odors.

In Table III the hexanal results obtained with two breakfast cereals stored at 55, 45, 37, and 21 C are shown. When the ln of these results were plotted against time, relatively straight lines were obtained. The slopes of these lines were then used as reaction rate constants. When the ln of the latter were plotted against 1/K (Arrhenius equation), straight lines were obtained. These results show that good prediction of the time required for the onset of rancidity at any temperature can be made from tests carried out at accelerated temperatures. Prediction of rancidity development from high temperature tests was found not to be possible for products in which both lipid oxidation and nonenzymatic browning may occur at ambient temperatures such as in dehydrated potatoes. Results such as shown were obtained only when the samples were stored in containers which did not allow changes in moisture, when precise temperature control was used, and when hexanal values above 5 ppm when the the chromatograms also showed significant amounts of other decomposition products were not used.

The hexanal method has been used to study the effects of different ingredients, processing conditions, and packaging materials on rancidity development. As an example, the results obtained with a wheat cereal treated with different antioxidants are shown in Figure 2.

Foods are extremely complex mixtures of many organic and inorganic compounds. Any attempt to define the quality of a food from the measurement of a single compound such as hexanal would be presumptuous. The measurement of a single compound can, however, provide valuable information when properly applied and interpreted. In low fat, dehydrated foods whose fat contains substantial quantities of linoleic acid and which has less than 1 ppm hexanal when fresh, an increase to 5 ppm or more hexanal was found to indicate a significant deterioration in quality due to lipid oxidation.

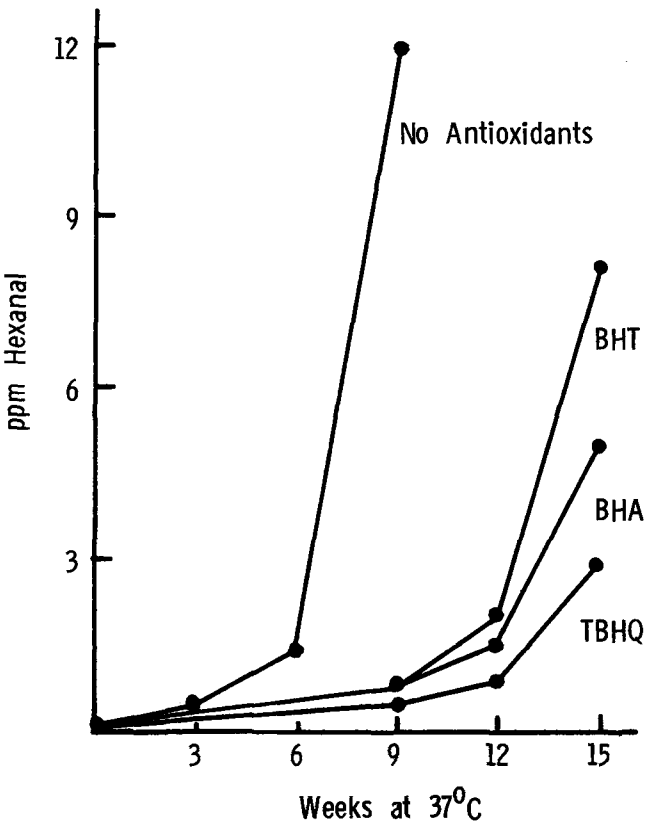

FIG. 2. Hexanal increase in a wheat cereal treated with no antioxidants, with butylated hydroxytoluene (BHT), with butylated hydroxyanisole (BHA) and with tertiary butylhydroquinone (TBHQ). The amount of antioxidants added was 40 ppm.

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