J. Agric. Food Chem., Vol. 26, No. 1, 1978 179

Coumarin Off-Odor in Wheat Flour

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A "cinnamon-like" off-odor, first noticed in baked bread and later also detected in the bread wheat flour, was found to be caused by the presence of as much as 3.6 ppm of coumarin occurring in the wheat flour. The coumarin apparently results from the presence of clover (*Melilotus*) seeds occurring with the wheat.

Recently an off-flavor problem was noticed in bread baked with flour from two large independent milling companies in western United States. This appeared as a "cinnamon-like" off-flavor in bread baked from certain batches of wheat flour. The problem was first noticed when there was a considerable number of complaints from the retail customers. Although less noticeable, the "cinnamon" off-odor could also be detected in the wheat flour, especially if the flour was wet. The present study was carried out to determine the cause of the "cinnamon" off-odor.

The volatiles of bread had been previously reviewed by Maga (1974). Some volatiles of flour had been studied previously by Lorenz and Maga (1972) and some of wheat by McWilliams and Mackey (1969). However, coumarin does not seem to have been reported before as a component of bread, flour, or wheat.

EXPERIMENTAL SECTION

Materials. Flour samples, both "normal" and with "cinnamon" off-odor, were obtained from two independent milling companies. One of these companies also supplied samples of clover (*Melilotus*) seeds. Authentic coumarin was Eastman No. 79.

Isolation of Coumarin from the Flour. Flour (750 g) was placed in a 12-L flask together with water (6 L) and treated using vacuum steam distillation continuous extraction with a Likens–Nickerson head, of the type described by Schultz et al. (1977), for 4 h with heptane as the extracting solvent and at 110 mm pressure. The heptane extract was dried over sodium sulfate and concentrated under reduced pressure (110 mm) using a low hold up Vigreux distillation column. The concentrate was made up to 1.0 mL volume and analyzed by gas–liquid chromatography (GLC). To determine the efficiency of the isolation, standard samples were made up by adding weighed amounts of coumarin, dissolved in a small amount of methanol, to the "normal" samples of flour. These were carried through using exactly the same extracting procedure as above.

Isolation of Coumarin from Clover Seeds. Seeds (0.5 g) were weighed out, placed in a mortar with water (10 mL), and ground to a fine paste. After standing at room temperature for 3 h, the slurry was washed into a separatory funnel with additional water (100 mL) and ether (100 mL). The slurry was then extracted with ether (3 × 100 mL) and the ether extract was dried over sodium sulfate, concentrated, and made up to 10.0 mL in a standard flask. This solution was then analyzed by GLC.

Gas Chromatography–Mass Spectrometry Analysis. The GLC column was a 150 m long × 0.75 mm i.d. stainless steel capillary coated with Tween 20 containing 5% Igepal CO-880. For qualitative GLC–mass spectral analysis, the column was coupled to a modified Consolidated 21-620 mass spectrometer using a silicone rubber membrane molecular separator. The column had an inlet pressure of 8 psi He and was programmed from 50–170 °C at 1 °C/min and held at the upper limit.

For quantitative analysis the same column was used at 170 °C constant temperature with an inlet pressure of 20 psi N_2. A standard solution of coumarin was made by dissolving 2.00 mg of coumarin in 10.0 mL of methanol. The amount of standard injected was adjusted so that the peak size of the standard and unknown were of the same order.

Odor Threshold. The odor threshold of GLC purified coumarin was obtained using procedures described previously (Guadagni et al., 1963), with Teflon bottles and tubing as the containers for the odor solutions.

Table I. Quantitative Analysis of Coumarin in Flour Samples

	Sample	Coumarin concn in ppm of flour
Company A	(1) normal sample	<0.1 ppm
	(2) cinnamon off-odor	3.6 ppm
Company B	(1) normal sample	<0.1 ppm
	(2) cinnamon off-odor	2.6 ppm

RESULTS AND DISCUSSION

Samples of flour with both "normal" odor and "cinnamon" off-odor were received from two major wheat milling companies. The volatile components from the flour samples were isolated using vacuum steam distillation continuous extraction. The isolated volatile concentrate was then analyzed by the direct combination of capillary GLC and mass spectrometry. A large number of components were detected in both "normal" and off-odor samples and several characterized by mass spectrometry. However, the GLC patterns for both normal and off-odor samples were generally similar. Only one peak showed a consistent major difference between the normal and off-odor samples, being undetectable in the normal samples. This peak was characterized as coumarin. Its mass spectrum [major ions at m/e 146 (100), 118 (89), 90 (71), 89 (61), 63 (54), 39 (47)] and capillary GLC retention time were consistent with that of an authentic sample of coumarin.

Quantitative analysis of the coumarin was carried out using GLC. The method of isolation was calibrated by mixing known amounts of coumarin with "normal" flour and determining the percent of recovery using the same conditions as used for the unknown samples. Results for "cinnamon" off-odor samples and "normal" samples of flour are shown in Table I. These figures are the averages of at least three GLC analyses. We consider that the figures are accurate within 20% for comparison between samples; however, because of the low recovery of coumarin in the isolation method (see later) they are probably only correct to the right order of magnitude in regard to their absolute value of concentration.

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Table II. Concentration of Coumarin in Clover Seeds

	Coumarin concn, %
Sour clover seeds (*Melilotus indica*)	0.96
White sweet clover seeds (*Melilotus alba*)	1.2

The results in Table I clearly show a much greater concentration of coumarin in the off-odor samples than in the "normal" samples. The amount in the "normal" samples was probably considerably less than that shown, but it was difficult to determine this accurately because of the presence of other interfering peaks at the low levels.

The odor threshold of coumarin in water solution was found to be 34 (95% confidence 25–47) parts/10^9 parts of water. The amount shown in Table I would then seem to be sufficient to cause the off-odor in the flour and bread. With informal qualitative odor evaluation, flour to which ca. 1 ppm of coumarin had been added, was judged by three experienced judges to have a similar odor to that of the samples of "cinnamon" off-odor flour.

Possible Source of Coumarin. As coumarin is a well known constituent of clover (*Melilotus*) foliage, it was thought that an alternate crop or cover crop of this type, used on the wheat fields, might be contributing the coumarin in some way. Investigations by the milling company technologists showed that some batches of wheat contained trace amounts of seeds which were very similar to those of sour clover (*Melilotus indica*) and white sweet clover (*Melilotus alba*). In the particular lots of wheat the clover seeds were apparently harvested inadvertently with the wheat. The clover grows to a similar height as the wheat. Besides its use in agriculture, clover also grows wild in many states and it is possible that the particular lots of wheat involved were grown on recently cleared land where the deep rooted clover still survived. Although considerably smaller than grains of wheat, the clover seeds are about the same density and are not easily separated by the usual cleaning processes.

The sour clover seeds and white clover seeds were analyzed for coumarin by crushing the seeds with a little water, extracting the slurry with ether, and analyzing the ether solution by GLC. The concentration of coumarin found is listed in Table II. The identity of the coumarin in the seeds was confirmed by isolating it by GLC and comparison of its infrared absorption spectrum and GLC retention time with those of an authentic sample of coumarin. Only about 0.02% of these seeds would be needed in the wheat to give the concentration of coumarin listed in Table I. Such low concentrations may be costly to remove from the wheat. Probably the most suitable method would be to eliminate the clover from the wheat field.

Bound Coumarin. It is well known (cf. Hughes et al., 1951) that coumarin in clover foliage occurs largely in a bound form as the glucoside of 2-hydroxycinnamic acid. Moisture and enzymes, released by crushing, hydrolyze the glucoside, giving the free coumarin. This seems to be also true with the clover seeds. The intact seeds have little odor and only give a strong coumarin odor when they are crushed with water. It is possible that the glucoside could be carried through the milling process into the flour without releasing much of the coumarin. The dough-making process for bread may be particularly effective in releasing the bound coumarin because of the presence of the yeast enzymes which are effective in breaking glucoside type bonds.

Recovery with the Isolation Method. Williams (1969) had shown that coumarin was difficult to recover by normal steam distillation and that a special continuous type of apparatus such as the Likens–Nickerson head was necessary. Even with this apparatus, under vacuum in the present work, recovery of only 3% of coumarin added to flour could be obtained using an extraction period of 4 h. With water alone a 5% recovery could be obtained under vacuum. A 10% recovery could be obtained in 4 h from water if the extraction was carried out at atmospheric pressure. Schultz et al. (1977) had shown that, using the Likens–Nickerson head, the recovery was better at atmospheric pressure for poorly volatile, hydrophilic compounds. Longer times of extraction would have given better yields of coumarin. The 4-h period used was, however, a practical limit. Despite the low efficiency, the percentage recovery was quite reproducible and permitted the use of a calibration factor in the calculation of the data in Table I.

The efficiency of isolation of the coumarin using steam distillation continuous extraction is probably dependent on a number of factors, such as the concentration of the coumarin in the water used, the amount of coumarin, the boil up rate of both the solvent and the aqueous medium and the nature of the solvent. Despite its low efficiency, the authors could not find a practical method that was significantly more effective than steam distillation continuous extraction. Direct solvent extraction of the flour gave too much (ca. 1%) lipid material which would interfere with the GLC analysis.

Other Components Characterized. In addition to coumarin, in the present work a number of compounds were characterized by mass spectrometry in all of the samples of flour examined. They are apparently "normal" volatile constituents of flour. These included: hexanal, hept-2-enal, oct-2-enal, nonanal, non-2-enal, deca-2,4-dienal, hexanol, octanol, oct-1-en-3-ol, nonanol, naphthalene, 2-methylnaphthalene, and dimethylnaphthalene. These compounds had mass spectra consistent with those of authentic samples. As far as the authors can determine, of these components, only hexanal had been previously characterized in wheat flour (McWilliams and Mackey, 1972).

ACKNOWLEDGMENT

The authors thank Dan G. Guadagni for determination of the odor threshold of coumarin and Max M. Hanamoto for assistance with flour and bread sample.

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Received for review June 20, 1977. Accepted August 22, 1977. Reference to a company and/or product named by the U.S. Department of Agriculture is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.