Odor Generation in Ground, Stored Pearl Millet[1]

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ABSTRACT

The characteristic mousy, acidic odor generated in ground pearl millet during brief storage was investigated and found not to be associated with oxidative rancidity of kernel lipids. Odor generation required relatively high moisture levels in the grits, suggesting that the process is enzymatic. Fractionation and reconstitution experiments showed the odor precursor to be extractable with methanol (but not petroleum ether) and retained on C-18 reverse-phase preparatory columns. When the methanol extract was further separated into water-soluble and water-insoluble fractions, the water-soluble fraction retained the ability to support odor generation. Ultraviolet scans of this active water-soluble fraction showed absorption maxima similar to apigenin, the aglycone of the major C-glycosylflavone present in pearl millet. The characteristic, odor associated with storage was formed when apigenin was added to methanol-extracted millet grits.

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Pearl millet (*Pennisetum americanum* (L.) Leeke) is grown extensively in the dry areas of western and southern India and along the periphery of the Sahara, where it is used as food by an estimated 400 million people (Hoseney et al 1981). In these areas, traditional methods of decortication (Fig. 1) are still practiced (Varriano-Marston and Hoseney 1983). Pearl millet can be stored for long periods without deterioration in quality if the kernels are intact. However, once the grain is decorticated and ground, the quality of the resulting meal deteriorates rapidly (Varriano-Marston and Hoseney 1983). It often develops a unique mousy, acidic odor within a few hours. Recent investigations of this objectionable odor (Kaced et al 1984) showed that the off-odor does not result from the classical oxidative changes in lipids. The studies reported here were undertaken to identify and characterize the compounds (or processes) responsible for the off-odor in ground, stored pearl millet.

MATERIALS AND METHODS

Millet and Sorghum Samples

Pearl millet samples were composited from varieties grown at the Kansas State Agricultural Experiment Station sites at Hays and Garden City during the crop years from 1980 to 1983. White sorghum was grown at the Kansas State Agricultural Experiment Station site at Manhattan, KS, in 1984. All grain samples were stored at 4° C until used.

Reagents and Solvents

Methanol, *n*-butanol, and hexanes for fractionation, reconstitution, and chromatography were reagent grade and purchased from Sigma Chemical Co. (St. Louis, MO). Chloroform and petroleum ether were reagant grade and purchased from Alltech Associates, Inc. (Houston, TX). Solvents for HPLC (*n*-butanol, isopropanol, methanol, and water) were HPLC grade and purchased from Burdick and Jackson (Muskegon, MI 49442).

Sep-pak C-18 reversed-phase preparatory columns were purchased from Waters Associates (Millipore Products Division, Bedford, MA).

Standards

Apigenin and phenolic acid standards for HPLC (coumaric, caffeic, vanillic, ferulic, cinnamic, protocatachuic, and *p*-hydroxy benzoic acids) were obtained from Sigma Chemical Co. Hexanal was obtained from Aldrich Chemical Co. (Milwaukee, WI).

Milling

Millet or sorghum grits were produced by grinding cleaned grain with a Ross Laboratory mill (Ross Industries, Division of Cargill, Inc., Wichita, KS) equipped with corrugated first break rolls. Rolls were operated at a 0.13-in. gap and 2.5:1 differential. Grits were

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fractionated or tested for odor generation immediately after production.

Odor Generation

Freshly milled or treated grits were placed in flat glass trays and mixed with 30% (w/v) water. The resulting mixture was then air-dried at ambient temperature for 12–15 hr with the aid of a laboratory hood or table fan. Treated and dried samples were sealed in mason jars for at least 1 hr before being evaluated for odor generation.

Odor Evaluation

Organoleptic assessment of odor production was performed by a trained panel. For routine evaluations, a three- to five-member panel was asked to sniff the head space over treated samples and compare it with that of control (untreated) and positive (untreated but wetted and dried) samples. For evaluation of apiginen-supplemented grits, a 10-member panel was trained with fresh grits (no odor) and wetted and dried grits (odor present). Evaluations were carried out by presenting each panel member with the above positive and negative controls plus the various treatments (see below). Panel members were required to rate each coded sample on a numerical scale of 0 (no odor) to 5 (strong odor present).

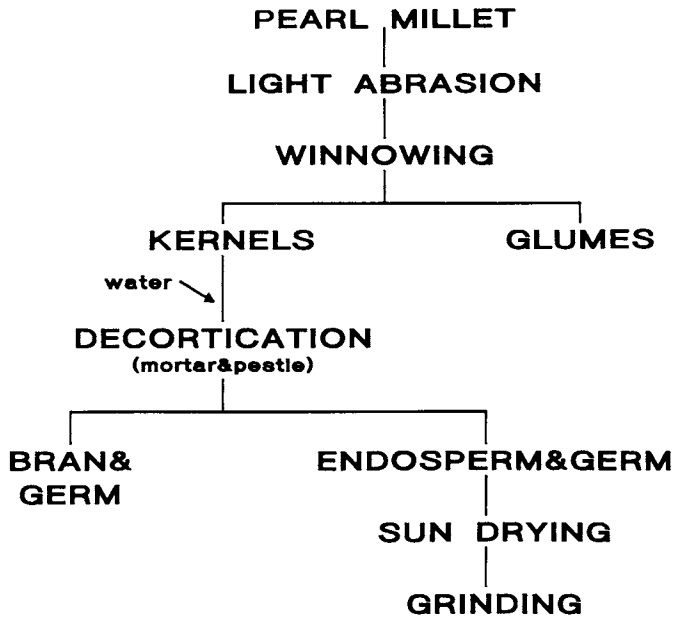

Fig. 1. Traditional processing scheme for pearl millet.

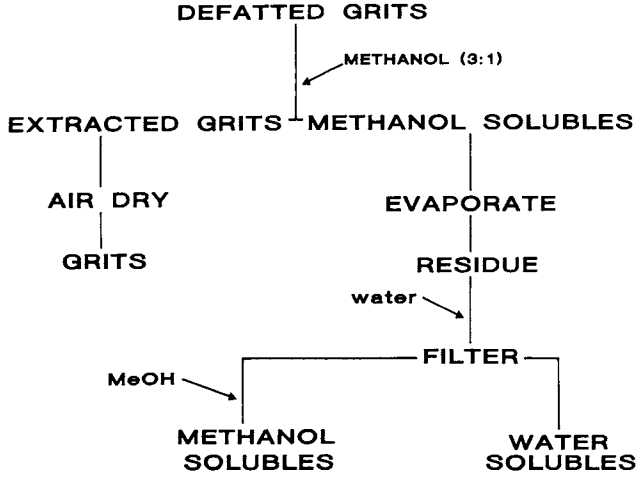

Fig. 2. Extraction and fractionation of methanol extract.

Solvent Extraction

Millet or sorghum grits (500 g) were extracted for 12 hr with 2,000 ml of solvent in a large Soxhlet apparatus. Extracted grits were air-dried to remove residual solvent before being tested for odor production.

Methanol Extracts

Methanol extracts from pearl millet grits were obtained (Fig. 2) and tested as outlined in Figure 3. The methanol extract was fractionated as in Figure 2.

Reconstitution

Millet grits or ground sorghum and their extracted fractions were reconstituted as follows. Extracted grits and an appropriate volume of extract in solvent were thoroughly mixed. Reconstituted grits were allowed to air-dry to remove solvent before being tested for odor production. Ratios of solvent volume to grits weight were adjusted to be equivalent to that used during the original extraction.

Sorghum/Millet Interchange

Extracts from sorghum and millet grits were interchanged and tested as shown in Figure 4.

High-Performance Liquid Chromatography

The phenolic acids present in the methanol extracts of fresh and treated grits were analyzed by the method of Hahn et al (1983) except isopropanol replaced *n*-butanol in eluting solvent B. Ten-microliter samples were analyzed with a Varian 5000 chromatograph (Varian Instruments, Sunnyvale, CA) with an

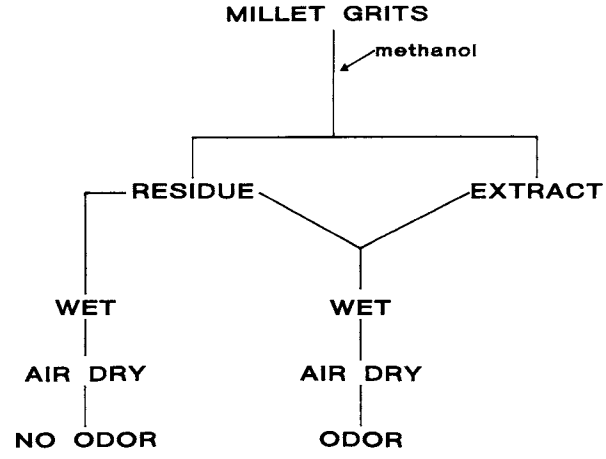

Fig. 3. Extraction and reconstitution procedure for pearl millet grits.

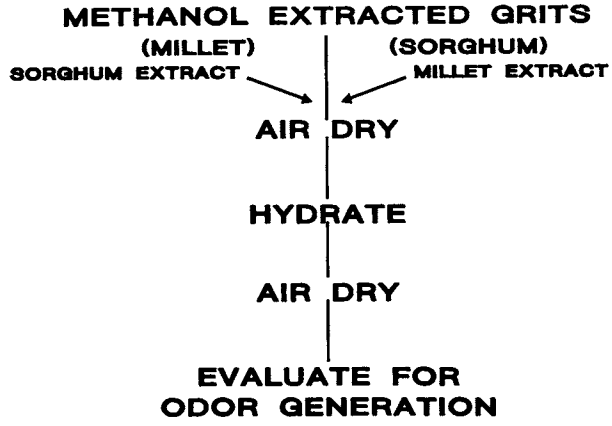

Fig. 4. Procedure for interchanging extracted sorghum or millet grits with their methanol extracts.

Altex 30×4.6 cm R-sil C-18 column with average particle size of 10 μm (Altech Scientific Operations, Berkeley, CA).

RESULTS AND DISCUSSION

Preliminary experiments (data not shown) analyzing the peroxide values of fresh and stored grits as well as the generation of hexanal by stored grits showed that oxidative changes in lipids did not generate the off-odors. This confirmed the conclusions of Kaced et al (1984). Gas chromatographic analysis of the fatty acids present in fresh and stored millet grits were identical, also suggesting that reactions involving lipids were not the source of off-odors.

Extraction of Odor Precursor(s)

To further characterize the source of the odor, we extracted freshly ground millet grits with solvents of increasing polarity in an attempt to remove the odor precursor. By testing extracted grits as well as extracted grits reconstituted with their extracts (Fig. 3), it was possible to evaluate the ability of each solvent to remove the odor-generating compounds or precursors (Table I). Petroleum ether extraction did not affect the ability of the resulting grits to generate the characteristic objectionable odor. This reinforced our conclusion that oxidative rancidity of lipids was not the cause of the odor. Chloroform extraction reduced the intensity of odor generated by treated grits. However, reconstitution did not restore odor production to its control levels. Although it may have removed the odor precursor(s), *n*-butanol could not be evaluated because of the strong residual solvent odor that remained associated with the grits even after prolonged drying.

Methanol, however, was an effective solvent (Table I). When tested, extracted grits failed to generate off-odors. In addition, methanol-extracted grits, when reconstituted with their extract, generated the characteristic odor at approximately control levels.

Freshly ground millet grits were subjected to methanol extraction after they had been wetted and dried to produce the off-odor (Fig. 5). The extracted grits had lost their odor and, when rewetted and dried, were no longer capable of generating the characteristic odor. However, reconstitution of extracted grits with their methanol extracts, followed by wetting and drying, resulted in odor production. These results indicate that methanol extraction was effective at removing not only the precursor(s) of the odor, but also the odor product itself. The odor compound is lost from the extract during rotary evaporation of the extract. However, the ability of the extract to support odor generation when recombined with its parent grits demonstrates that not all of the precursor reacted to produce odor during the first cycle of wetting and drying.

Interchange Studies

Preliminary studies (data not shown) and the results reported by Lai and Varriano-Marston (1980a,b) showed that little or no objectionable odor is generated in samples of low moisture content. This indicated that odor generation might be enzymatic. To test whether the precursor or its putative enzyme were specific to pearl millet, the methanol extracts from millet and sorghum grits were interchanged (Fig. 4). Methanol-extracted sorghum grits, reconstituted with the millet extract, were capable of generating the characteristic odor. The reverse combination, extracted millet grits plus extracts from sorghum, did not produce the odor when wetted and dried. Therefore, the precursor(s) of the odor compound is specific to pearl millet. The enzyme active in generating the odor was not specific and exists in at least one other cereal grain (sorghum).

Fractionation of the Methanol Extract

The active methanol extract (Fig. 2) was further fractionated into water and methanol solubles. Each of those subfractions was tested for its ability to support odor production. The methanol-soluble fraction had lost the precursor and was, as a consequence, unable to support odor production. Presence of the precursor in the water-soluble fraction was confirmed by its ability to generate odor when reconstituted with extracted grits.

Some naturally occurring plant phenolics (phenylpropenoids) are soluble in both water and methanol (Harborne et al 1975). Therefore, the phenolic acids present in fresh and stored millet grits were determined by HPLC.

Although at least five phenolic acids could be identified in the methanol extracts of millet grits (Fig. 6), they did not change because of the generation of the off-odor. Thus, chromatograms of the extracts from fresh and stored grits were not distinguishable on

TABLE I
Odor in Millet Grits After Extraction and Reconstitution

Solvent	Extracted Grits	Reconstituted Grits
Petroleum ether	off-odor	off-odor
Chloroform	off-odor (less intense)	no odor
Methanol	no off-odor	off-odor
n-Butanol	butanol odor	butanol odor

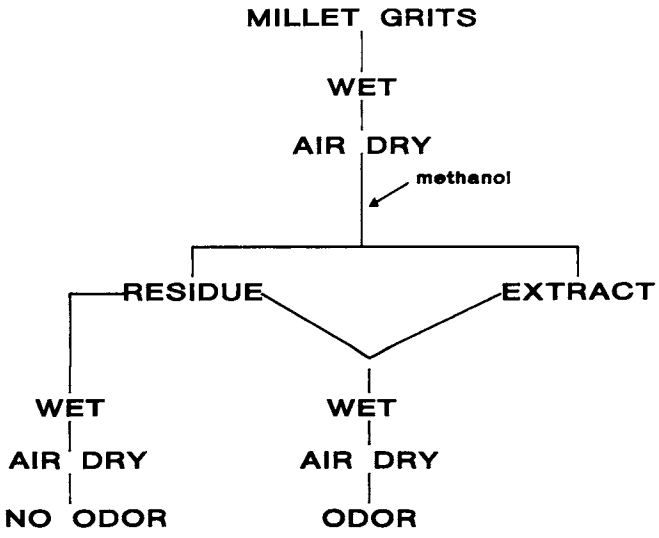

Fig. 5. Procedure for the extraction, reconstitution, and testing of wetted grits.

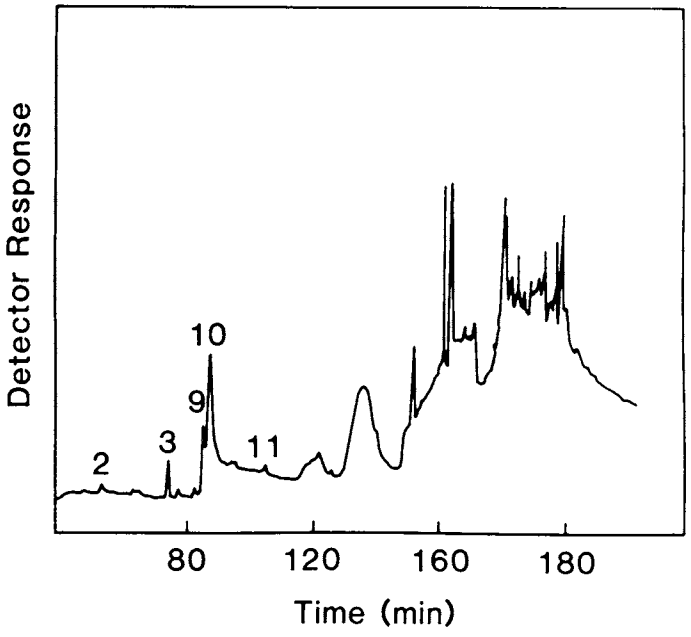

Fig. 6. High-performance liquid chromatographic analysis of phenolic acids from fresh millet grits.

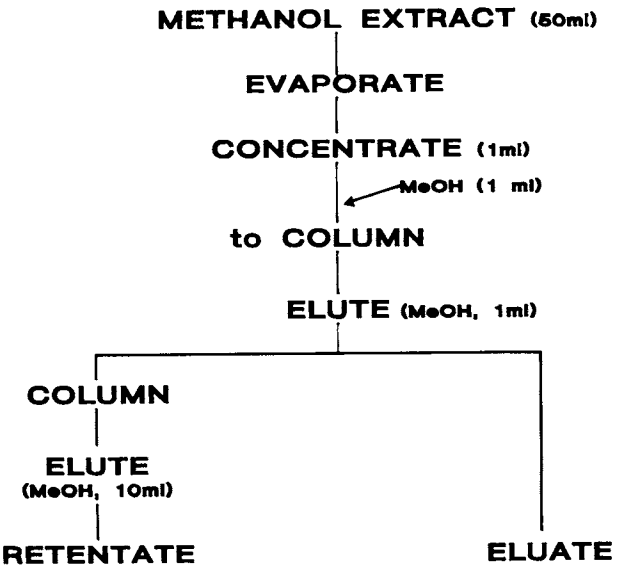

Fig. 7. Reverse-phase preparatory column fractionation of pearl millet methanol extracts.

the basis of their phenolic acids. However, as also seen in Figure 6, the chromatograms contained a large number of late-eluting, unidentified peaks. Those late-eluting peaks are often associated with higher molecular weight, flavonoid compounds (Hahn et al 1983).

Chromatographic Fractionation of the Extract

Methanol extracts of millet grits were further fractionated by passage through small, reversed-phase preparatory columns (Sep-paks, Waters Associates). Higher molecular weight flavanoids are more tightly bound by the column than are phenolic acids and, therefore, take either a greater volume of methanol or a stronger eluting solvent to elute from the column. Using the procedure outlined in Figure 7, methanol extracts were fractionated into column eluant and column retentate. Recombination with extracted grits showed that only the column retentate produced the odor. To this point, the data show that higher molecular weight potentially flavonoid compounds that are specific to pearl millet are the precursor of the mousy, acidic odor.

Reichert (1979) reported the presence of at least four characteristic flavonoid glycosides in pearl millet. These compounds were unique in being c-glycosylflavones. Although usable quantities of the pure glycoside were not readily available, apigenin, the flavone aglycone of these compounds, was available. The ultraviolet spectra of apigenin and the active, water-soluble fraction of methanol extracts both showed similar absorption maxima.

Apigenin Supplementation

We reasoned that, if enzymatic action on one or more of the native c-glycosylflavones were generating the off-odor, the enzymatic modification was more likely to be in the aglycone rather than in the carbohydrate component of the molecule. Therefore, an organoleptic study was designed to test the ability of apigenin to support odor generation in methanol-extracted grits.

TABLE II
Least Significant Difference (*t* Test) of Odor-Intensity Ranking of Millet Grits

Treatment	Mean[a]
Sequential wetting and drying	4.34 a
Methanol extract reconstituted	3.78 b
Apigenin supplemented	3.0 b
Freshly ground	1.0 c

[a] Mean score based on 10 replications rated on a scale of 0 to 5. Means with the same letter are not significantly different.

Panel members were asked to use a continuous scale of 0–5 to rank the intensity of odor resulting from the wetting and drying of the four treatments listed in Table II. Statistical comparison of the mean values for each treatment by least significant difference demonstrated that the intensity of odors produced by apigenin-supplemented grits and grits reconstituted with their methanol extracts was not significantly different (Table II). Further, both of these treatments produced significantly more odor than freshly milled controls.

The data (Table II) also show that the odor generated by wetted and dried unextracted grits was significantly more intense than that generated by either reconstituted or apigenin-supplemented grits. It may be that extraction of the grits by petroleum ether and methanol partially denatures the enzyme responsible for acting on the flavone portion of the substrate molecule. It is also possible that removal of the substrate from its native environment and recombination with solvent (either as methanol extract or methanol solution of the pure compound) changes the ability of the enzyme and substrate to interact. In either case, the result would be production of odor but at intensity levels below unextracted controls.

The results reported here support the contention that off-odor production in ground pearl millet is the result of enzymatic action on the flavone portion of one of the characteristic c-glycosylflavones known to be present in the grain. Further work will be necessary to determine the nature of the enzyme responsible and the structure of the odor-producing product.

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