Storage Fungi and Fatty Acids in Seeds Held Thirty Days at Moisture Contents of Fourteen and Sixteen Per Cent

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ABSTRACT

In seeds of rice, barley, wheat, corn, soybeans, and sunflowers, neither individual major fatty acids nor total fat acidity increased with increasing invasion by storage fungi until invasion had become obvious. The relative amounts of different major fatty acids varied with kind of seed, but the relative proportion of the total contributed by the different fatty acids remained constant. It seems unlikely that analyses for either individual or total fatty acids would aid in detecting early invasion by storage fungi, or in evaluating storability. Phytopathology 60:1775-1777.

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Fat acidity in grains and seeds (usually referred to by cereal chemists as fat acidity value, or FAV) is expressed as the number of mg of KOH required to neutralize free fatty acids from 100 g of grain on a dry matter basis. Fat acidity value increases with increasing invasion of seeds by fungi (7), but such increase may depend upon the fungus involved and on the moisture content of the grain, since heavy invasion of the embryos of wheat by *Aspergillus restrictus* was not accompanied by any appreciable increase in FAV (4). Fat acidity value has been suggested as a simple measure of market quality of corn (9), but has not been accepted as such, partly because of the great range in FAV in any given numerical grade of grain. Fat acidity value can be measured by various techniques, but all of these involve extraction of the sample with benzene or a similar solvent which might also extract other organic acids. It was thought that precise quantitative measurement of individual fatty acids in seeds undergoing invasion by storage fungi might yield information of value for judging storability, or deterioration risk. The work here described was undertaken to determine whether this was so.

MATERIALS AND METHODS.—Samples of certified seed grade of barley (*Hordeum sativum* Jess.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), soybeans (*Glycine max* [L.] Merr.), and sunflower (*Helianthus annuus* L.) were conditioned to moisture contents of 14 and 16%, wet wt basis. Replicate samples, including those at the original moisture contents (below 12.0%), were stored for 30 days at 25 C, then tested for moisture content, numbers and kinds of fungi, and amounts of fatty acids.

Moisture content was determined by drying 5- to 10-g samples at 103 C for 3 days, and is expressed on a wet wt basis. Numbers and kinds of storage fungi present were determined as follows: 100 seeds were shaken for 1 min in 2% NaOCl, rinsed in sterile water, plated on T-6 agar (Difco tomato juice agar, 25 g; Difco-Bacto agar, 15 g; NaCl, 60 g; distilled water, 900 g), and incubated at 27 C until the fungi that grew out could be identified.

To determine amounts of major fatty acids present, samples of approx 15 g were ground for 2 min in a Stein mill, and 10 g of meal were placed in a Soxhlet extractor and extracted for 4 hr with 30/60 petroleum ether (1). The extract was concd in a flash evaporator, and methyl esters of the fatty acids were prepared quantitatively with diazomethane (8). Appropriate dilutions of the esterified extract were made with a solution of 10% methanol in ether to obtain concn suitable for analysis in a gas chromatograph. All extracts were kept under N_2 at all times to avoid oxidation. The diluted sample was passed through a 45-mm stainless steel column packed with H_3PO_4-treated Chromosorb W solid phase and diethylene-glycoladipate polyester liquid phase (6). The column temp was 210 C, the carrier gas was N_2, and the flow rate was 30 ml/min. The fatty acids were measured quantitatively by means of an Aerograph Hy-Fy Hydrogen Flame Ionization Gas Chromatograph. Calibration curves were made for the major fatty acids to be identified, palmitic, stearic, oleic, linoleic, and linolenic, by injecting known quantities of these into the chromatograph and recording the corresponding peak heights. Three replicate samples of each of the extracts were injected, and the average value was used to calculate the amount of fatty acid present (expressed as μg/g of dry wt of the seed). In no case did the variation among a set of three replicate samples exceed ± 5%. The amounts of individual fatty acids in each sample were added together to obtain total fatty acid content.

RESULTS AND DISCUSSION.—Percentage of surface-disinfected seeds yielding storage fungi, and the total amounts of fatty acids in the samples after 30 days, are given in Table 1. The amounts of major individual fatty acids are given in Table 2.

In the samples with approx 14% moisture, percentage of surface-disinfected seeds yielding the group species *Aspergillus glaucus* appeared to increase in rice, but did not increase in barley, wheat, or corn. We question the apparent increase of *A. glaucus* in rice, since in previous tests (5) no increase in *A. glaucus* was detected in samples of rough rice stored for a month at

TABLE 1. Percentage of surface-disinfected seeds yielding storage fungi and total fatty acids in different kinds of seeds stored for 30 days at different moisture contents and 25 C[a]

Seed	Moisture content	*Aspergillus glaucus*	*Aspergillus candidus*	Total fatty acids
	% *wet wt*	%	%	μg/g *dry seed*
Rice	8.3	13	0	360 ± 30
	14.0	81	0	290 ± 5
	16.1	98	29	530 ± 60
Barley	9.0	1	0	640 ± 200
	13.7	1	0	500 ± 20
	15.9	90	3	700 ± 15
Wheat	8.3	15	0	750 ± 5
	13.9	10	1	910 ± 10
	16.0	100	5	1,260 ± 70
Corn	10.1	0	0	680 ± 40
	13.9	3	1	1,116 ± 40
	15.9	98	3	1,510 ± 50
Soybean	11.6	43	0	1,120 ± 255
	13.7	100	0	1,800 ± 70
	15.7	100	10	2,730 ± 200
Sunflower	4.3	24	5	4,880 ± 200
	13.7	91	10	9,720 ± 1,080
	15.0	25	80	20,460 ± 2,020

[a] Each figure is the average of three tests each consisting of two replicates.

20-25 C with moisture contents of 14.2-14.5%. Total fatty acids decreased in rice, barley, and wheat, but increased to almost double the original amount in corn; this increase evidently was not a product of invasion by storage fungi.

In the samples of rice, barley, and wheat with approx 16% moisture, *A. glaucus* increased greatly within 30 days, but neither total fatty acids (Table 1) nor any of the individual fatty acids (Table 2) increased appreciably. An invasion of wheat by *A. restrictus* sufficient to reduce germinability of the seed to zero and produce 100% discolored embryos was not accompanied by an increase in fat acidity (4). In our experience, beginning spoilage in commercially stored grains and seeds almost invariably was preceded or accompanied by invasion by *A. glaucus* or *A. restrictus*, or both.

So far as invasion by storage fungi is concerned, moisture contents of 14 and 16% in soybeans are approx equivalent to moisture contents of 15 and 16.5% respectively, in the starchy cereal seeds (3), sufficient to permit some invasion by *A. candidus* as well as greater invasion by *A. glaucus* than would occur in the starchy cereal seeds at moisture contents of 14 and 16%. *Aspergillus candidus* evidently did increase slightly in the soybeans stored at 15.7% moisture (Table 1), and the total fatty acids as well as each of the major individual fatty acids increased appreciably in this sample.

Similarly, moisture contents of 14 and 16% in sunflower seeds (achenes) are approx equivalent to moisture contents of 18 to 20% in the starchy cereal seeds (2), and permits a more extensive invasion by storage fungi than would occur in the starchy cereal seeds with moisture contents of 14 and 16%. Sunflower seeds stored for 30 days at a moisture content of 16% were, in fact, heavily invaded by storage fungi, and many of the seeds were covered with masses of mycelium and spores of *A. glaucus* and *A. candidus*. The relatively large increase in total (Table 1) and in individual (Table 2) fatty acids in sunflower seeds with 15.0% moisture probably was due to invasion by *A. candidus*.

The proportions of different major fatty acids differed from one kind of seed to another. For example, oleic acid made up 3 to 8% of the total fatty acids in corn, and 40 to 52% of the total fatty acids in rice. Linoleic acid made up from 68 to 84% of the total

TABLE 2. Major fatty acids detected in different kinds of seeds stored for 30 days at different moisture contents and 25 C[a]

Seed	Moisture content	Palmitic μg/g	Palmitic % Of total	Stearic μg/g	Stearic % Of total	Oleic μg/g	Oleic % Of total	Linoleic μg/g	Linoleic % Of total	Linolenic μg/g	Linolenic % Of total
Rice	8.3	32.4	9	0	0	144.0	40	183.6	51	0	0
	14.0	29.0	10	0	0	150.8	52	110.2	38	0	0
	16.1	53.0	10	0	0	249.1	47	227.9	43	0	0
Barley	9.0	147.2	23	0	0	38.4	6	454.4	71	0	0
	13.7	140.0	28	0	0	30.0	6	330.0	66	0	0
	15.9	189.0	27	0	0	28.0	4	483.0	69	0	0
Wheat	8.3	97.5	13	0	0	82.5	11	570.0	76	0	0
	13.9	118.3	13	0	0	154.7	17	637.0	70	0	0
	15.9	138.6	11	0	0	126.0	10	995.0	79	0	0
Corn	10.1	108.0	16	0	0	20.4	3	550.8	81	0	0
	13.9	264.1	19	0	0	83.4	6	1,042.5	75	0	0
	15.9	286.9	19	0	0	120.8	8	1,102.3	73	0	0
Soybean	11.6	145.6	13	0	0	78.4	7	806.4	72	89.6	8
	13.7	216.0	12	18.0	1	144.0	8	1,296.0	72	126.0	7
	15.7	382.2	14	27.3	1	245.7	9	1,856.4	68	218.4	8
Sunflower	4.3	195.2	4	195.2	4	780.8	16	3,708.8	76	0	0
	13.7	388.8	4	194.4	2	972.0	10	8,164.8	84	0	0
	15.0	818.4	4	613.8	3	3,273.6	16	15,754.2	77	0	0

[a] Each figure is an average of three tests of each of two replicate samples.

fatty acids in all seeds except rice, where it constituted from 38 to 51%; linolenic acid was detected only in soybeans (Table 2). Within any given kind of seed, however, the proportion of each individual major fatty acid contributing to the total remained relatively constant, usually remarkably so (Table 2).

Our evidence indicates that the early stages of deterioration caused by storage fungi growing in stored grains and seeds with moisture contents of 14 to 16% were not accompanied by an appreciable increase in any of the major individual fatty acids or in total fatty acids. Tests for these compounds, therefore, probably would be of little value in judging storability or deterioration risk. By the time stored seeds are invaded by *A. candidus,* as in the sunflower seeds stored at 15.0% moisture in these tests, they usually are visibly moldy and often heating, and no tests are needed to indicate that deterioration is in progress. Another limitation of tests of FAV, total fatty acids, or of the individual major fatty acids as indices of storability is that one might not be able to tell whether the figure obtained was derived from a portion of the sample being in a relatively advanced stage of deterioration, or from most or all of the sample being moderately invaded by storage fungi. Tests for numbers and kinds of storage fungi, however, will distinguish between these two conditions.

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