Chemical and Fungal Evaluation of Graded Sunflowerseed

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Thirty-four samples of commercial oil-type sunflowerseed graded No. 1, 10 samples graded No. 2 and 33 samples graded Sample grade (SG) were used to study the relationship between percent heat damage, an important grading character, and percent FFA, percent color and UV absorption of extracted oil, percent germination and number and identities of fungi present. Seventy-two fungal species belonging to 28 genera were isolated. Thirty-seven fungal species in 17 genera were isolated from grade No. 1 seed; 68 species in 13 genera were isolated from grade No. 2 seed, and 68 species in 26 genera were isolated from SG seed. The genera most frequently isolated from grade No. 1 seed were *Alternaria* (85.3%), *Phoma* (4.7%) and *Cladosporium* (4.5%). *Alternaria alternata* was recovered from all No. 1 samples and comprised 75.6% of all isolates. The genera most frequently isolated from grade No. 2 seed were *Alternaria* (74.5%), *Eurotium* (8.4%) and *Phoma* (7.7%). Fewer fungal species were isolated from grade No. 2 than from No. 1 seed, but a greater recovery of storage fungi was found for No. 2 seed (13.3%) than for No. 1 seed (2.5%). *Alternaria* (33.8%), *Eurotium* (33.1%) and *Microascus* (8.3%) were the genera most frequently isolated from SG seed. Species of common storage fungi (*Eurotium, Microascus, Penicillium* and *Aspergillus*) were recovered more frequently from SG than from Grades No. 1 and No. 2. In general, as the quality of the seed decreased from grade No. 1 to grade No. 2 to SG, there was an increase in percent heat damage, percent FFA, Lovibond color and UV absorption of extracted oil, and a decrease in percent germination. Analysis of variance of the quality characteristics data showed no significant differences between grade No. 1 and No. 2 seed except for UV 228 nm absorption. However, the quality characteristics of SG seed all differed significantly from those of grades No. 1 and No. 2 seed with the exception of percent seed yielding fungi. Correlation coefficients of the quality characteristics of SG showed a slight relationship between heat damage and percent FFA (r = 0.63) and UV 228 nm absorbance (r = 0.68). Although many of the SG seed were badly heat damaged, no statistical relationship was found between percent heat damage and percent seed yielding fungi or total isolates of storage fungi. The data in this study show that errors exist in grading decisions when seed are judged visually to be heat damaged.

The quality of marketed sunflowerseed on which market price is established generally has been determined through the use of Minnesota State Standards and separate industry trading rules. Recently, official U.S. standards for sunflowerseed were established to provide federal inspection procedures and to facilitate marketing of the crop (1). Heat damage traditionally has been one of the criteria used to evaluate a variety of grains and seeds and is defined as damage (discoloration) caused by heat as measured by visual evaluation of seed discoloration (2,3).

Discoloration of seed may be caused by both field and storage fungi (4). Invasion of seed by storage fungi causes heating, discoloration and decay (5). Discoloration of the seed may also result from overheating during artificial drying, or from microbial thermogenesis that occurs when storage conditions permit rapid fungal growth. Robertson et al. (13) reported that oil-type hybrid sunflowerseed could be stored for 60 weeks at 7.5% moisture content at 10 C without a significant change in FFA content or percent seed germination. When the moisture content was increased to 13.5%, however, there was a significant decrease in germination and significant increase in FFA after only 24 weeks storage. They also reported that the invasion of the seed by storage fungi began after four weeks storage. In a preliminary study using a limited number of graded sunflowerseed samples, Robertson et al. (6) did not establish an adequate relationship between heat damage and other quality parameters. They reported, however, that for No. 1 and SG seed, the percent free fatty acids (FFA) showed a positive correlation when percent seed yielding fungi, total damaged and total number of *Aspergillus* isolates were combined. Percent FFA is an important factor of oilseed quality. The National Cottonseed Products Association Trading rules state that grade No. 1 sunflowerseed shall contain..."not more than 1.8% free fatty acids in the oil and seed... and seed containing over 3.0% free fatty acids shall be Sample grade and subject to rejection" (7). Therefore, it has been suggested that FFA be included as part of the Federal standards for sunflowerseed (6).

The purpose of the study was to evaluate a larger number of graded sunflowerseed samples in order to determine the relationship between heat damage and other quality factors of grade No. 1, grade No. 2 and SG sunflowerseed.

MATERIALS AND METHODS

Sunflowerseeds used were mixed commercial oilseed hybrids obtained from and graded by the North Dakota Grain Inspection Service, Fargo, North Dakota, using Minnesota Department of Agriculture grade standards (8). Samples (approximately 450 g) were of unknown geographical origin. Thirty-four samples were graded No. 1, which has maximum limits of 10% moisture, 5% total damaged or 0.5% heat damaged. Ten samples were graded No. 2, which has maximum limits of 10-12% moisture, 10% total damaged or 1.0% heat damaged seed. Thirty-three samples were graded Sample grade (SG), which has more than 12% moisture, 10% total damaged or 1.0% heat damaged seed.

FFA content and Lovibond color of extracted oil from the samples were determined by AOCS methods (9). Spectroscopic examinations were made with a Cary

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TABLE 1

Genera of Fungi Isolated from 34 Grade No. 1, 10 Grade No. 2 and 33 Sample Grade Sunflowerseed Samples

Class and genus	Grade No. 1		Grade No. 2		Sample Grade	
	Number of isolates[c]	Number of samples yielding genus	Number of isolates[d]	Number of samples yielding genus	Number of isolates[e]	Number of samples yielding genus
Zygomycetes						
Mucor	1	1	—	—	1	1
Rhizopus	6	6	1	1	32	11
Deuteromycetes						
Alternaria	1657	34	398	10	622	27
Arthrinium	1	1	—	—	—	—
Aspergillus[a]	12	5	7	6	106	20
Botrytis	2	2	1	1	—	—
Cephialophora	—	—	—	—	3	1
Cladosporium	87	24	10	7	11	7
Epicoccum	1	1	—	—	1	1
Fusarium	10	8	2	2	20	9
Paecilomyces	—	—	—	—	14	6
Penicillium	14	10	5	4	106	11
Phoma	92	30	41	9	62	18
Phomopsis	21	11	3	2	8	6
Scopulariopsis[b]	1	1	1	1	22	4
Stemphyllium	—	—	—	—	1	1
Thermomyces	—	—	—	—	1	1
Trichothecium	—	—	—	—	1	1
Verticillium	—	—	—	—	1	1
Ascomycetes						
Chaetomium	—	—	—	—	4	3
Emericella[a]	1	1	—	—	19	8
Eupenicillium	—	—	—	—	2	1
Eurotium[a]	28	13	45	9	609	31
Microascus[b]	7	7	19	7	153	23
Monascus	—	—	—	—	1	1
Thermoascus	—	—	—	—	34	9
Basidiomycetes						
Schizophyllum	—	—	—	—	1	1
Mycelia sterilia						
Rhizoctonia	1	1	1	1	6	4
Total	1942	34	534	10	1841	33

[a]*Aspergillus* isolates producing telemorphs are counted with appropriate isolates of *Eurotium* or *Emericella*.
[b]Isolates of *Scopulariopsis* producing telemorphs are counted with isolates of *Microascus*.
[c]From 3,400 seeds.
[d]From 1,000 seeds.
[e]From 3,300 seeds.

15 low-UV recording spectrophotometer. Absorbances were measured on approximately 0.1% solutions of the oils (1-cm cell) in cyclohexane and normalized to 0.1%. Germination percentages were determined by placing 100 seeds of each sample between wet paper towels that were rolled up loosely and incubated at room temperature at a relative humidity above 95% for five days. Any seed that produced a radicle was counted as germinated. The number and identities of fungi present within the seed were determined as previously described (6). All analyses were conducted in duplicate, and the data were analyzed by using correlation and linear regression methods from SAS (10).

RESULTS AND DISCUSSION

Seventy-two fungal species belonging to 28 genera were isolated (Table 1). Thirty-seven species in 17 genera were isolated from grade No. 1 seed; 28 species in 13 genera were isolated from grade No. 2 seed, and 68 species in 26 genera were isolated from SG seed.

The genera most frequently isolated from grade No. 1 seed were *Alternaria* (85.3%), *Phoma* (4.7%) and *Cladosporium* (4.5%). These genera are considered to be field fungi and are known to invade seed before harvest. Thus, any damage done by field fungi occurs by the time the seed are harvested, and no further

damage is likely to occur in storage because these field fungi require moisture contents in equilibrium with relative humidities (RH) above 95% for growth (5,11). *Alternaria alternata* was the species most frequently recovered of all isolates from No. 1 seed (75.6%) and was found in all 34 samples. *A. helianthinficiens* Simmons, Walcz and Roberts, which was recently described from sunflowerseed (12), was recovered from 26 of the 34 seed samples (6% of all isolates). The mycofloral data indicate that the No. 1 seed in this study were of higher quality than the No. 1 seed in our previous study (6), from which there was a higher recovery of storage fungi. The high percentage of field fungi and the low level of storage fungi indicate that the seed were of high quality when harvested and were properly stored. In our previous study, the low percentage of *Alternaria* spp. isolated (49.6%) and the relatively high percentage of storage fungi of the genus *Aspergillus* (21.4%) indicated that some of the grade No. 1 samples were of questionable quality (5).

The genera most frequently isolated from grade No. 2 seed were *Alternaria* (74.5%), *Eurotium* (8.4%), *Phoma* (7.7%) and *Microascus* (3.6%). Fewer fungal species were isolated from grade No. 2 than from No. 1 seed, but a greater recovery of storage fungi was found for No. 2 seed (13.3%) than for No. 1 seed (2.5%).

Alternaria (33.8%), *Eurotium* (33.1%) and *Microascus* (8.3%) were the genera most frequently isolated from SG seed. Fungi most often recovered from SG seed were *Alternaria alternata* (29.9%); *Eurotium rubrum* Konig, Speick., and Brem. (14.5%); *E. amstelodami* Mangin (8.9%); *E. repens* de Bary (8.5%), and *A. helianthinficiens* (2.3%). *Eurotium* spp., *Microascus* spp., *Penicillium* spp. and *Aspergillus* spp. are storage fungi and were recovered more frequently from SG than from No. 1 or No. 2 seed. These storage fungi grow when the moisture content of the products is in equilibrium with RH's of 70-90% (11). Thirty-one of the 33 SG seed samples were invaded by *Eurotium* spp. In our previous study, the fungal species invading SG seed were essentially the same as those invading the seed in this study (6). However, in the previous study approximately 92% of the isolates recovered were storage fungi as compared to only 53% in this study. These results indicate that the extent of invasion and growth of fungi in sunflowerseed depend upon production and storage conditions. *Thermoascus crustaceous*, a thermophilic fungus, was isolated exclusively from SG seed that had appreciable heat damage scores, suggesting that heat damage in those samples was caused by self-heating due to fungal growth.

Characteristics of the three grades of sunflowerseed are presented in Table 2. Considerable variability in these characteristics was observed among the samples studied. In general, as the quality of the seed decreased from grade No. 1 to SG, there was an increase in percent heat damaged, percent FFA, Lovibond color and UV absorption of extracted oil and a decrease in percent germination. The percent of seed yielding fungi was approximately the same for all three grades. Analysis of variance of the quality characteristics data showed no significant differences between grades No. 1 and No. 2 seed except for UV 228 nm absorption. However, the quality characteristics of SG seed were all significantly different from those of grade No. 1 and No. 2 with the exception of percent seed yielding fungi. Although SG samples had heat or total damage scores above the official grade limits for No. 1 and No. 2 seed, 11 of the 33 samples had FFA levels which were within the NCPA limits of grade No. 1 seed (less than 1.8%) (7). The low FFA in these samples is evidence that these seed lots, if processed promptly, would produce crude commercial oils of acceptable quality and that heat damage does not always accurately reflect seed quality. These data support the findings of others that the growth of fungi on oilseeds results in lowered oil quality and increased levels FFA (14-16).

The ultraviolet absorption of extracted oil increased

TABLE 2

Characteristics of Graded Sunflowerseed[a]

	% Heat damaged[b]	Germination %	FFA of extracted oil % as oleic	Lovibond color of extracted oil		Seed yielding fungi %	Absorptivity[c]	
				Yellow	Red		UV 270 nm	UV 228 nm
Grade No. 1								
Mean[d]	0.0[x]	78 ± 14[x]	0.60 ± 0.28[x]	12.8 ± 3.7[x]	1.1 ± 0.2[x]	53 ± 20[x]	0.043 ± 0.015[x]	0.305 ± 0.116[x]
Range	—	45 − 94	0.20 − 1.46	6 − 22	0.8 − 1.7	20 − 92	0.027 − 0.086	0.119 − 0.583
Grade No. 2								
Mean[e]	0.6 ± 0.4[x]	72 ± 10[x]	0.98 ± 0.22[x]	12.3 ± 1.4[x]	1.2 ± 0.4[x]	49 ± 9[x]	0.042 ± 0.006[x]	0.494 ± 0.094[y]
Range	0 − 0.9	57 − 87	0.53 − 1.35	10 − 15	0.9 − 2.1	30 − 61	0.028 − 0.050	0.309 − 0.634
Sample Grade								
Mean[f]	16.0 ± 18.1[y]	36 ± 28[y]	3.24 ± 2.34[y]	17.1 ± 8.3[y]	1.7 ± 0.9[y]	44 ± 25[x]	0.068 ± 0.032[y]	0.733 ± 0.370[z]
Range	1.2 − 71.6	0 − 77	0.80 − 10.00	8 − 36	0.9 − 4.1	2 − 100	0.032 − 0.162	0.352 − 1.495

[a]Mean data in rows having different superscripts are significantly different ($P < .05$).
[b]Determined by North Dakota Grain Inspection Service.
[c]Absorbance divided by the product of sample path length (cm) and the concentration of the oil (g/l).
[d]Mean of 34 samples, duplicate analysis.
[e]Mean of 10 samples, duplicate analysis.
[f]Mean of 33 samples, duplicate analysis.

TABLE 3

Correlation Coefficients[a] of Quality Parameters of Sample Grade Sunflowerseed

	Germination (%)	FFA (% as oleic)	Lovibond Yellow	Lovibond Red	Absorbance 270 nm	Absorbance 228 nm	Seed yielding fungi %	Total *Eurotium*	Total *Aspergillus*	Total *Penicillium*
Heat damage (%)[b]	−0.56	0.65	0.32	0.36	0.49	0.64	−0.12	−0.05	0.39	0.29
Germination (%)[b]		−0.61	−0.29	−0.21	−0.40	−0.44	0.25	−0.12	−0.32	−0.45
FFA (% as oleic)[b]			0.56	0.47	0.54	0.52	−0.03	0.03	0.58	0.79
Lovibond yellow[b]				0.82	0.64	0.27	−0.28	−0.29	0.21	0.54
Lovibond red[b]					0.62	0.23	−0.38	−0.37	−0.03	−0.01
Abs 270 nm[b]						0.70	−0.42	−0.30	0.09	−0.01
Abs 228 nm[b]							−0.24	0.02	0.05	−0.15
Seed yielding fungi (%)[b]								0.73	0.53	0.59
Total *Eurotium*[c]									0.37	0.27
Total *Aspergillus*[d]										0.88

[a]$P < 0.5$ for $r > 0.43$ and $P < 0.01$ for $r > 0.51$ ($N = 33$).
[b]33 sunflowerseed samples.
[c]31 sunflowerseed samples.
[d]20 sunflowerseed samples.

as the seed grade decreased (Table 2) and is, as reported by Gray (17), due to oxidative deterioration of the polyunsaturated fatty acids in the oils forming conjugated dienes and trienes, which absorb in the region of 228 nm and 270 nm, respectively. The measurement of diene absorption is a sensitive method of detecting the beginning of oxidation (18). This deterioration may be due to microbial thermogenesis occurring during fungal growth in the stored seed.

Statistical analysis of grade No. 1 seed data revealed no relationship between any of the quality parameters. For No. 2 seed, there was no relationship between heat damage and the other parameters. There was, however, a positive correlation between percent germination and percent of seed yielding fungi ($r = 0.65$) and a negative correlation between FFA content and Lovibond red color of extracted oil ($r = -0.64$).

Correlation coefficients of quality characteristics of SG seed are shown in Table 3. Although heat damage was not significantly correlated ($r > 0.7$) with any other single component of seed quality, a slight relationship was found with FFA content ($r = 0.63$) and UV 228 nm absorbance ($r = 0.64$). Using multiple regression analysis, heat damage could be adequately explained ($R^2 = 0.55$) by combining the variables FFA content and UV 228 nm absorbance into the regression model. In our previous study using a smaller number of SG samples (6), heat damage showed no correlation with percent FFA ($r = 0.19$). Although many of the SG seed were badly heat damaged (Table 2), no statistical relationship was found between heat damage and percent seed yielding fungi or total isolates of *Eurotium* spp., *Aspergillus* spp. or *Penicillium* spp. This would not be expected, because it is well known that stored seed become discolored by heat after seed have been invaded by fungi (4,19). When only the 19 SG samples from which the storage fungi *Eurotium* and *Aspergillus* were isolated were analyzed by multiple regresssion, an R^2 of 0.68 was obtained when the variables FFA, UV 228 nm and total *Eurotium* and *Aspergillus* isolates were introduced in the regression model for heat damage.

Percent germination of SG seed was slightly correlated only with percent FFA ($r = -0.61$) and heat damage ($r = -0.56$), but percent FFA showed greater correlation ($P < 0.01$) than percent germination with all of the quality parameters used to evaluate the seed (Table 3) except Lovibond red color, percent seed yielding fungi and total isolates of *Eurotium*.

The evaluation of a larger number of graded sunflowerseed samples than in our previous study (6) suggests relationships between heat damage and other important measures of seed quality such as percent FFA and UV absorptivity at 228 nm. The data also establishes that errors can be incorporated into grading decisions when seed are judged visually to be heat damaged. Because FFA content is a more quantitative estimation of seed quality than is heat damage, we again (6) recommend that it be used in conjunction with more traditional criteria for grading sunflowerseed. Results in our laboratory indicate that a fairly rapid FFA analysis can be determined by titration of oil removed from seed with a Carver press.

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