to techniques that measure the release of glycerol. As hydrazinolysis can be carried out at relatively lower temperatures, and does not require any kind of acid catalyst, this approach will have a particular advantage over transesterification where acid- or heat-labile moieties are involved.

That degradation with hydrazine, followed by reaction with acetone, quantitatively converts phospholipids as well as other lipids to isopropylidene hydrazide derivatives of their constituent fatty acids has been shown (3). Therefore, we can assume that the present method should work for quantitation of *O*-acyl lipids other than triglycerides. Moreover, a combination of spectrophotometry and GLC/HPLC should enable accurate determination of total fatty acids and their individual proportions in a lipid sample (4). To use a hydrazinolysis value, determined by absorbance at 229 nm, in the manner that iodine or saponification values are used for characterization of oil samples, should be possible. The method can also be used for analysis of the triglyceride content of blood samples.

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♣Effect of Moisture Content of Oil Type Sunflower Seed on Fungal Growth and Seed Quality During Storage

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ABSTRACT

Oil-type hybrid sunflower seed exposed to relative humidities of 65%, 84% and 93% in environmental chambers at 10 C attained equilibrium moisture contents (mc) of 7.5±0.2%, 10.1±0.2% and 13.4±0.5% and were stored under these conditions for up to 60 weeks (wk). At 7.5% mc, germinability of seed changed very little during storage, but at 10.1% mc and 13.4% mc, germination significantly decreased during storage. At 7.5% mc, free fatty acid (FFA) levels in extracted oil did not change significantly during 60 wk of storage. However, at 10.1% mc, FFA increased significantly during 40 wk of storage and were significantly correlated with the invasion of seed by the storage fungus *Aspergillus* (r = 0.81). At 13.4% mc, FFA increased significantly during storage and were positively correlated with the invasion of seed by *Aspergillus* and *Penicillium* and negatively correlated with germination percentage. Invasion of surface-disinfected seed by fungi decreased from 83% to ca 66% of total seed during storage at 7.5% mc. The predominant fungus was *Alternaria alternata* (Fr.) Keissler. A previously unreported *Alternaria* sp., morphologically similar to *A. ricini* (Yoshii) Hansford and *A. macrospora*, was isolated from 9% of the seed. At 10.1% mc, fungal invasion also decreased for 24 wk and then began increasing again. At 24 wk of storage, *Aspergillus* began invading the seed. At 13.4% mc, 100% of the seed were invaded with fungi within 8 wk of storage. Total *Alternaria* rapidly decreased during storage; and after only 4 wk of storage, the seed were invaded by both *Aspergillus* and *Penicillium*. After 24 wk of storage, the predominant genus was *Aspergillus*, followed by *Penicillim* and *Alternaria*. Other fungi invading the seed were *Cladosporium, Phoma, Mucor, Rhizopus* and several unidentified fungi.

INTRODUCTION

Sunflower seeds are sometimes harvested at high moisture content and stored without adequate drying. Fungal invasion, seed-mass heating, high levels of free fatty acids (FFA) in extracted oils and a decrease in seed germinability are problems encountered when sunflower seeds are stored under high moisture conditions (1-7). Fungal invasion and decreased germinability are proportional to increased moisture content, elevated seed-mass temperature and length of storage (1). Poisson et al., (4) reported that molds begin growing on whole sunflower seed above 65% relative humidity (RH) and 6.5% moisture content (mc), whereas yeast and bacteria required seed moistures above 10%. Christensen (8) reported that 100% of open-pollinated sunflower seed (cv. Peredovik) stored at moisture contents of 9.5% and 11% were invaded by storage fungi within 82 days and the decrease in germinability was approximately proportional to increased moisture content. Baudet (9) reported that sunflower seed with 12% moisture will maintain seed quality for 4 months when stored at 5 C and only 3 months when stored at 10 C. He also found that sunflower seed at 10% moisture will maintain seed quality for ca. 5 months when stored at 10 C and about 3 months at 20 C. No information about maintaining quality was given for seed moisture contents of less than 10%.

Conflicting information exists on the optimum and safe storage conditions for sunflower seed and no research has been reported on storage of the new hybrid seed. A wide range of "safe" moisture levels are currently recommended (6-10%) that supposedly minimize microfloral growth and invasion and maintain good seed quality during storage (7,10,11). Scientists at North Dakota State University recommended that seed to be stored for up to 6 months should be at 10% moisture or less whereas seed to be stored up to a year should be 8% or less (7). Christensen (10)

TABLE I

Changes in Free Fatty Acids, Germination and Mold Counts of Sunflower Seed Stored at 3 Moisture Levels

	7.5% Moisture content			10.1% Moisture content			13.4% Moisture content		
Storage (weeks)[a]	Free fatty acids[b] (% as oleic)	Germination[b] (%)	Mold count (cfu g^{-1})[c]	Free fatty acids[b] (% as oleic)	Germination[b] (%)	Mold count (cfu g^{-1})[c]	Free fatty acids[b] (% as oleic)	Germination[b] (%)	Mold count (cfu g^{-1})[c]
0	0.35	97	4.11	0.35	97	4.11	0.35	97	4.11
4	0.42	98	4.72	0.49	97	3.78	0.59	94	5.45
8	0.39	96	4.89	0.50	95	4.85	0.95	92	6.36
12	0.47	97	4.48	0.54	95	4.40	1.62	88	7.15
16	0.41	98	4.88	0.50	96	4.81	1.97	88	7.60
20	0.43	98	4.81	0.51	97	4.08	2.35	90	6.36
24	0.57	96	4.48	0.69	94	4.11	3.57	87	7.72
28	0.56	98	3.95	0.77	96	3.90	3.20	87	7.41
32	0.47	94	4.36	0.75	92	4.18	–	–	–
36	0.46	97	4.51	0.83	96	3.70	–	–	–
40	0.39	96	4.51	0.96	92	4.20	–	–	–
44	0.39	94	4.38	–	–	–	–	–	–
48	0.49	95	4.23	–	–	–	–	–	–
52	0.41	96	4.08	–	–	–	–	–	–
56	0.41	94	4.23	–	–	–	–	–	–
60	0.56	95	5.14	–	–	–	–	–	–

[a]Temperature of storage, 10 C.
[b]Three repetitions analyzed in duplicate.
[c]Log of numbers of colony-forming units per gram; 2 and/or 3 repetitions analyzed in duplicate.

reported that the lower limit of moisture that permitted invasion of confectionery sunflower kernels by storage fungi was ca. 6%, but at moisture contents below 6.5%, invasion was very slow. Ryazantseva (11) state that sunflower seed should be dried to 7% moisture, and, for long term storage, should be cooled to at least 10 C.

The objectives of this study were to investigate the effect of moisture content during storage on the chemical and microbial quality of new hybrid sunflower seed and to obtain additional information on optimum conditions for storing sunflower seed.

MATERIALS AND METHODS

Sunflower seeds were freshly harvested, mixed hybrid commercial seed from the 1981 crop in North Dakota. Seed characteristics were: mositure, 9.1%; oil, 45.8% dry basis; FFA (% as oleic), 0.35%; germination, 97%; dockage, less than 2%. Seed tested contained 10% insect holes and germination of these seed was 60%.

Ca. 130 g aliquots of seed were put in perforated polyethylene bags and placed in environmental chambers maintained at 10 C and RH of 65%, 84% and 93%. Samples were replicated 3 times by placing aliquots of seed in each chamber at 3-day intervals. Seeds stored under these conditions attained average mc of 7.5±0.21%, 10.1±0.19% and 13.4±0.50%. Seeds at 13.4% mc were stored for 28 weeks (wk), 10.1% mc for 40 wk and 7.5% mc for 60 wk. Sample bags of seed were removed every 4 wk and analyzed for moisture content, germination percentage, FFA, number and kinds of fungi and total mold count.

Moisture content and FFA were determined by AOCS methods (12). Germination percentage was determined by placing 100 seeds of each sample between wet paper towels that were rolled up loosely and incubated at room temperature at RH above 95% for several days. Any seed that produced a sprout or root after a 5-day incubation was counted as germinated. Number and kinds of fungi present were determined by the procedure of Christensen (1). Forty-five seeds were shaken for 1 1/2 min with 60 mL of 2.6% sodium hypochlorite, rinsed twice with sterile distilled water and put on plates on tomato juice agar containing 6% NaCl (15 seeds per plate). Plates were incubated at 27 C until the fungi grew out and could be identified (5-7 days). Total mold counts were determined by grinding 11 g nonsurface-disinfected seed in a sterile Waring blender with 99 mL distilled water for 1 1/2 min. Counts were made in duplicate on potato dextrose agar acidified to pH 3.5 with 1.8 mL 10% tartaric acid. Plates were incubated at 27 C for 72 hr (13).

Data were analyzed statistically for mean, standard deviation and correlation coefficient and a second-degree polynomial equation across wk was fitted using the general linear model procedure in SAS (14).

RESULTS AND DISCUSSION

Storage of sunflower seed under 3 different moisture levels at 10 C had only a small effect on seed germination except under the higher moisture levels (Table I). At 7.5% mc, the germination percentage did not change significantly during storage; but at 10.1% mc, germination decreased significantly ($P<0.01$) from 97% to 92% after ca. 32 wk of storage. At 13.4% mc, germination decreased significantly ($P<0.01$) with increasing storage time up to 12 wk; after 12 wk, germination fluctuated and appeared to have leveled off. Under this high mc, germination was lower than that achieved with lower mc. At 7.5% mc, no significant correlation was found between germination, FFA and the percentage of seed invaded by fungi, but at 10.1% mc, germination was negatively correlated with FFA and invasion by the storage fungi *Aspergillus* ($r = -0.45$ and -0.44, respectively). At 13.4% mc, germinability was negatively correlated with FFA ($r = -0.65$) and invasion of the seed with *Aspergillus* ($r = -0.67$) and *Penicillium* ($r = -0.74$) (Table II).

Under favorable conditions, storage fungi invade the germs or embryo of seed, perferentially causing weakening and death of the embryo (15). Mondal et al., (16) found that as storage of oilseeds (mustard, linseed and sesame) continued, storage fungi increased, accompanied by a reduction in seed germinability, particularly at higher moistures (9-13%). Halder and Gupta (17) reported a rapid loss of sunflower-seed germinability when the moisture level was maintained at ca. 15%. However, Captan-treated seeds stored under the same RH showed a considerably higher percentage of germination. They concluded that the

TABLE II

Correlation Coefficients of Storability Tests of Sunflower Seed Stored at 13.4% Moisture Content for 28 wk

	FFA % as oleic	Seed invaded by fungi, %	Total *Alternaria*	*Aspergillus*	*Penicillium*	Mold count
Germination (%)	-0.65	-0.09	0.68	-0.67	-0.74	-0.56
FFA (% as oleic)		0.15	-0.68	0.72	0.65	0.67
Seed invaded by fungi (%)			-0.78	0.85	0.70	0.41
Total						
Alternaria				-0.90	-0.77	-0.57
Aspergillus					0.72	0.44
Penicillium						0.49

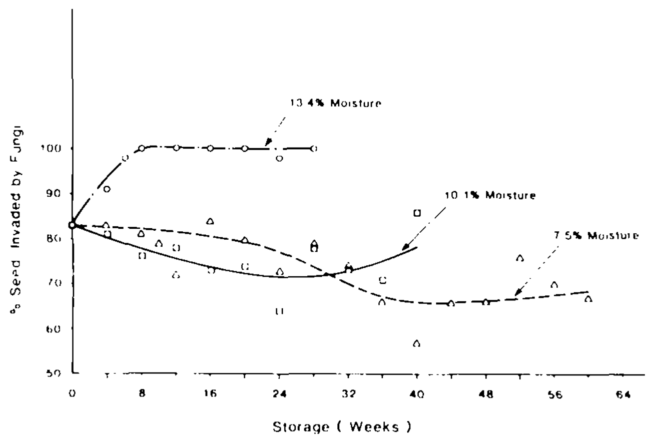

FIG. 1. Percentage of surface-disinfected sunflower seed invaded by fungi during storage at 3 moisture levels.

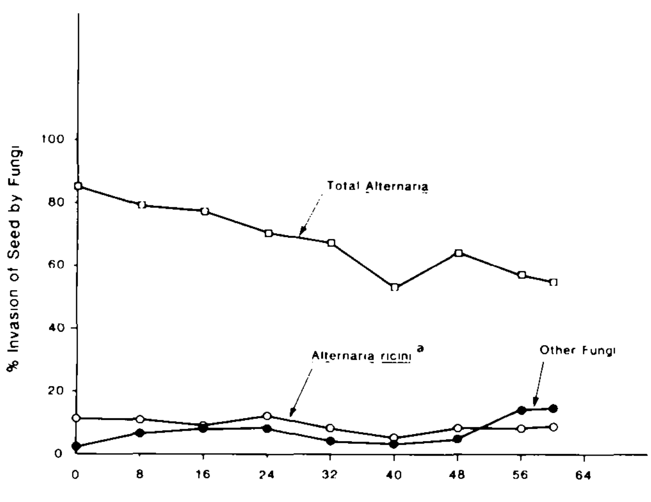

FIG. 2. Percentage of invasion of surface-disinfected sunflower seed by fungi during storage at 7.5% mc and 10 C. [a]Tentative indentification.

decline in germinability in untreated seed appeared to be caused by fungal pathogenicity. Germination data in the present study indicate that germinability in untreated seed depends on both moisture content and the extent of fungal invasion.

At 7.5% mc, FFA in the oil extracted from sunflower seed did not change significantly during 60 wk of storage (Table I). However, at 10.1% mc, FFA increased significantly during storage ($P<0.01$), reaching 1.0% FFA by the end of 40 wk of storage, and showed a positive correlation with the invasion of the seed by *Aspergillus* ($r = 0.81$). At 13.5% mc, FFA increases was highly significant during storage and reached 3.6% after only 24 wk of storage. Increased levels of FFA in the seed were positively correlated with the invasion by *Aspergillus* ($r = 0.72$) and *Penicillium* ($r = 0.65$) and negatively correlated with germination ($r = -0.65$) (Table II). In previous studies, we found that confectionery sunflower kernels were sour when FFA reached 2% (13).

Mold counts were variable with no significant change during 40 wk of storage at 7.5% and 10.1% mc (Table I). However, at 13.4% mc, mold counts increased to 2.3 million/g (6.36 $\log_{10}$ cfu/g) after 8 wk of storage, and between 4-6 wk *Aspergillus* and *Penicillium* began to invade the seed and rapidly increased until 20 and 24 wk, respectively (Fig. 4). The highest FFA levels were found in seed at 13.4% mc, which also had high mold counts. At 7.5% mc and 10.1% mc, mold counts were not significantly correlated with germination, FFA or fungi, but at 13.4% mc, counts were correlated the highest with FFA ($r = 0.67$) (Table II).

The percentage of surface-disinfected seed invaded by fungi during storage at the 3 moisture levels is shown in Figure 1. At 7.5% mc, fungal invasion gradually decreased from 83% to ca. 66% after 36 wk and remained unchanged. These seed were primarily invaded by field fungi of the genus *Alternaria*, which decreased from 85% to 55% invasion by 60 wk of storage (Fig. 2). The predominant fungus was *A. alternata* (Fr.) Keissler. A previously unreported *Alternaria* sp., morphologically similar to *A. ricini* (Yoshii) Hansford and *A. macrospora*, was isolated from ca. 9% of the seed during storage at all moisture levels. Other fungi invading these seed were *Cladosporium*, *Phoma*, *Mucor*, *Rhizopus* and several unidentified fungi. These fungi were grouped as "other fungi" in Figures 2-4. The percentage of seed invaded by fungi did not correlate with FFA or germinability at 7.5% mc.

Fungal invasion of seed with 10.1% mc decreased from 83% to 64% during 24 wk of storage and then increased to 86% by 40 wk of storage (Fig. 1). *Aspergillus* began invading these seed by 24 wk of storage and by 40 wk of storage had invaded 34% of the seed, whereas total *Alternaria* gradually decreased during storage from 85% to 42% at 36 wk (Fig. 3). These seeds were also characterized as not having *Penicillium* as a significant genera because the RH was just below that required for most species to grow (15).

At 13.4% mc, 100% of surface-disinfected seed were invaded by fungi at 8 wk of storage (Fig. 1). Total *Alternaria* invasion decreased significantly from 85% to 39% by 8 wk and remained fairly constant for the rest of the storage period (Fig. 4). After only 4 wk of storage, 23% of these seeds were invaded by *Aspergillus* and invasion increased to 75% by 28 wk of storage. *Penicillium* had invaded only 4%

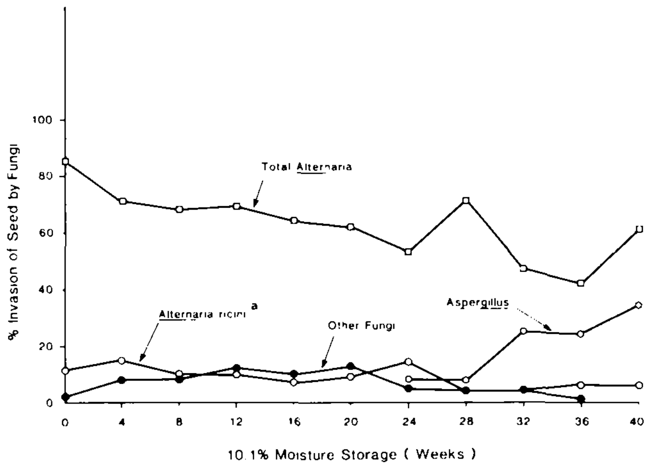

FIG. 3. Percentage of invasion of surface-disinfected sunflower seed by fungi during storage at 10.1% mc and 20 C. [a]Tentative identification.

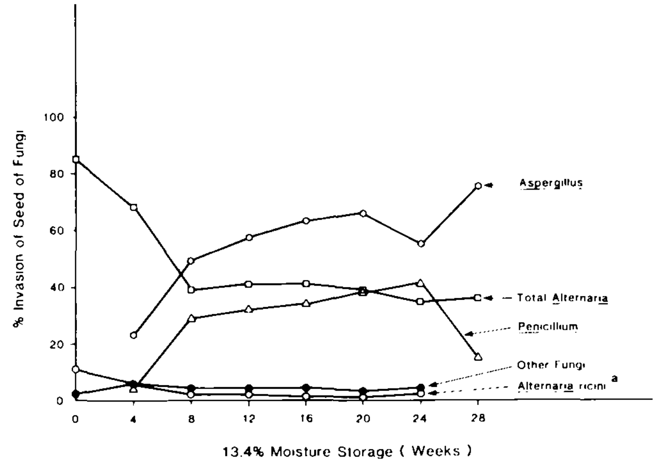

FIG. 4. Percentage of invasion of surface-disinfected sunflower seed by fungi during storage at 13.5% mc and 10 C. [a]Tentative identification.

of the seed by 4 wk and reached 41% invasion by 24 wk. At 13.4% mc, total *Alternaria* was positively correlated with germination (r = 0.68) and negatively correlated with FFA, seed invaded by fungi, *Aspergillus, Penicillium* and mold counts (Table II).

Based on our findings, viable seed with good oil characteristics can be maintained for well over a year at 7.5% mc and storage temerature of 10 C. Seed at 10.1% mc would, for all practical purposes, meet the 10% moisture maximun for Grade No. 1 sunflower seed established by the Minnesota Department of Agriculture, Grain Inspection Division (18). However, as our data show, FFA levels in seed with 10.1% mc reached ca. 1% and germination decreased to 92% after 40 wk of storage, whereas longer storage may result in significantly decreased oil quality. Polchaninova et al., (19) reported that at a temperature of 5-10 C and seed with a moisture of 10.8%, the FFA in the extracted oil showed essentially no change after 4 months of storage. However, when the same seeds were stored at 20 C, the FFA increased more than 10-fold. Our results show that conditions also occur in sunflower seed stored at 13.4% mc that support prolific fungal growth, even though the storage temperature was 10 C. Ten percent of the seed had insect holes, which probably contributed to increased fungal invasion and FFA content.

FFA increase in oilseeds with increasing invasion of seeds by fungi, but such an increase may depend on the fungus involved and on the moisture content of seed (3,20). McGee and Christensen (3) reported that analyses for individual fatty acids or total fatty acids would not aid in detecting early stages of deterioration caused by fungi during storage or in evaluation storage quality. Our data indicated that at both 10.1% and 13.4% mc (2 wk data not reported), FFA levels began to increase before the invasion by *Aspergillus* was detected, which suggests that the initial increase of FFA might not be caused by invading fungi. In fact, by the time the seed were invaded by *Aspergillus,* FFA had already doubled at 10.1% mc. Therefore, based on our findings, we conclude that measurement of FFA is a valid indicator for predicting storage quality of sunflower seed as well as determining the number and kinds of fungi present. More studies are needed to establish the cause for the early increase in the pool of FFA and any relationship of this pool to subsequent fungal invasion.

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