Technical

Changes in Oil-Type Sunflowerseed Stored at 20 C at Three Moisture Levels

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ABSTRACT

Oil-type hybrid sunflowerseed held at 63%, 83% and 93% relative humidities (RH) at 20 C attained equilibrium moisture contents (mc) of 6.7 ± 0.1%, 9.8 ± 0.1% and 13.4 ± 0.3%, respectively, and were stored under these conditions for up to 60 weeks (wk). Germinability was inversely related to both storage duration and RH. Initially, the seed contained only field fungi (mainly *Alternaria* spp. and *Phoma macdonaldii*) and 0.5% free fatty acids (FFA). At 6.7% mc, the amount of *Alternaria* spp. and *P. macdonaldii* isolated declined slowly with time, storage fungi did not invade the seed, and FFA in extracted oil did not change significantly during 60 wk storage. No significant correlation was found between FFA, germination, mold count (cfu/g) or fungi recovered. At 9.8% mc, field fungi declined more rapidly than at 6.7% mc, storage fungi (mainly *Eurotium rubrum* and *E. repens*) were isolated from the seed after 4 wk, and FFA increased to 2.5% after 40 wk. FFA content was negatively correlated with germination (r = -0.91) and with *Alternaria alternata* (r = -0.90) but was positively correlated with *E. rubrum* (r = 0.78). At 13.4% mc, field fungi vanished and storage fungi invaded the seed more rapidly than at 9.8% mc, and FFA increased to 6.3% after 24 wk storage. FFA content was positively correlated with storage fungi such as *Aspergillus versicolor* (r = 0.74) and negatively correlated with germination (r = -0.88) and *A. alternata* (r = -0.82). After 14 wk storage the fungus recovered most frequently was *A. versicolor*, followed by *Eurotium rubrum*, *E. repens*, *A. alternata*, *E. amstelodami*, *Penicillium* spp. and *Malbranchea sulphurea*.

INTRODUCTION

Sunflowerseeds 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 sunflowerseeds are stored under high moisture conditions (1-9). Fungal invasion and decreased germinability are proportional to increased moisture content, elevated seed-mass temperature and length of storage (2).

Conflicting information exists, however, on the optimum and safe storage conditions for sunflowerseed. A wide range of "safe" moisture contents currently are recommended (6-10%) that supposedly minimize microfloral growth and invasion, and maintain good seed quality during storage (9-11). Scientists at North Dakota State University recommended that seed intended for storage for up to 6 mos should be at 10% moisture or less, whereas seed to be stored up to a year should be 8% or less (9). In a previous study in which we investigated the effect of moisture content (mc) of sunflowerseed on fungal growth and seed quality during storage at 10 C and 7.5%, 10.1% and 13.4% moisture content, we found that at 7.5% mc and FFA levels in extracted oil did not change significantly during storage and germinability changed only slightly (12). However, at 10.1 and 13.4% moisture content, FFA increased and germinability decreased significantly during storage. In addition, only a few genera of fungi were identified. Temperature has a significant effect on deterioration of sunflowerseed at moisture contents above the "critical level" (>6-7% mc). Polchaninova et al. (8) reported that sunflowerseed with moisture content up to 10.8% could be stored for 4 mo at 5-10 C without spoiling; no data was reported for storage longer than 4 mo. At 20 C, they reported that in seed with 8.4% mc the FFA doubled (to an unacceptable level) in 4 mo and in seed with 10.8% mc it rose more than 10-fold. The objectives of this study were to investigate the effect of moisture content on the quality of sunflowerseed during storage at 20 C and to identify the species of fungi invading the seed.

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MATERIALS AND METHODS

Sunflowerseed used in this study were mixed hybrid commercial seed harvested from a 1981 crop in North Dakota which had been stored at -15 C. Seed characteristics were: moisture, 9.1%; oil, 45.8% dry basis; FFA (% as oleic), 0.50%; germination, 97%; dockage, less than 2%. Seed tested contained 10% insect holes and germination of seed with insect holes was 60%.

Approximately 130 g aliquots of seed were put in perforated polyethylene bags and placed in environmental chambers maintained at 20 C and RH's of 63%, 83% and 93%. Samples were replicated three times by placing aliquots of seed in each chamber at 3-day intervals. Seed stored under these condtions attained average mc of 6.7 ± 0.12%, 9.8 ± 0.10% and 13.4 ± 0.31%. Seed at 13.4% mc were stored for 24 weeks (wk), 9.8% mc for 52 wk and 6.7% mc for 60 wk. Sample bags of seed were removed every 2 wk for 16 wk, then every 4 wk for the duration of the study 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 (13). 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 5 days. Any seed that produced a radicle was counted as germinated. The numbers and kinds of fungi present within the seed were determined by the procedure of Christensen (2) as modified by Robertson et al. (14). Yeasts that appeared on isolation plates were not considered; the term fungi in this paper refers exclusively to filamentous forms. To determine seed borne fungi, 50 seeds were shaken for 1½ min with about 60 ml 2.6% NaOCl, rinsed twice with terile distilled water, plated onto tomato juice agar containing 6% NaCl (10 seeds per plate) and incubated for 7 days at 27 C. The fungi growing from these seeds were identified directly or were subcultured onto other media (usually half-strength V8 juice agar) when the salt appeared to interfere with sporulation. Total mold counts were determined by grinding 11 g of seed in a sterile Waring blender with 99 ml sterile distilled water for 1½ min. Counts were made in duplicate from serial dilutions on potato dextrose agar acidified to pH 3.5 with 1.8 ml 10% tartaric acid per 100 ml agar. Plates were incubated at 27 C for 48-72 hr (12).

Data were analyzed statistically for mean, standard deviation and correlation coefficient, and a first- or second-degree polynomial equation across wk was fitted using the general linear model procedure in SAS (15).

RESULTS AND DISCUSSION

The effect of storage of sunflowerseed under three different moisture levels at 20 C on FFA content and percent germi-

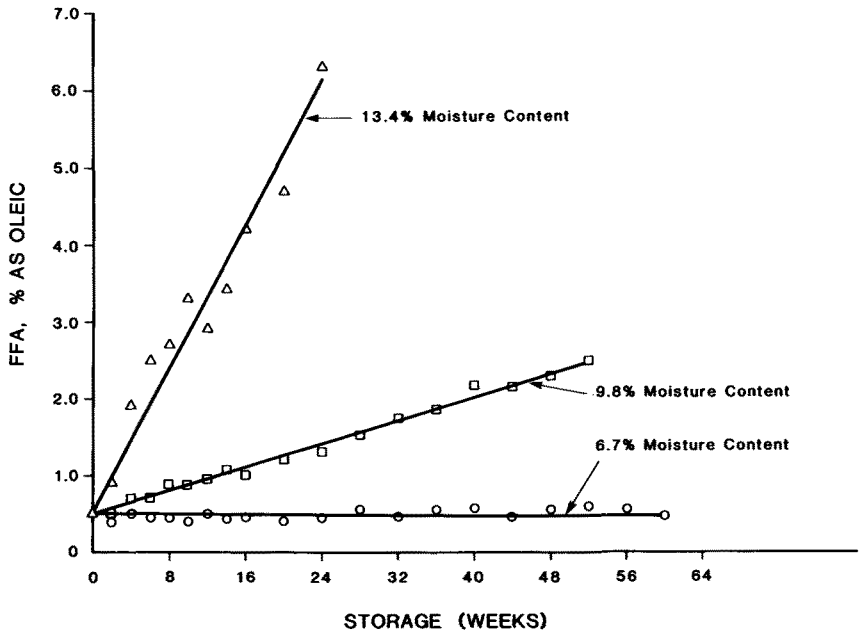

FIG. 1. Changes in free fatty acid content of oil extracted from sunflowerseed stored at 20 C at three moisture levels.

nation is shown in Figures 1 and 2. At 6.7% mc, FFA in the oil extracted from sunflowerseed did not change significantly during 60 wk storage. Though germination decreased significantly ($P<.0001$) from 97% to 80% after 56 wk of storage, germination at the other mc levels was less than 20% when storage was terminated (24 wk at 13.4% mc and 52 wk at 9.8% mc) (Fig. 2). At 6.7% mc, no significant correlation was found between FFA, germination, mold counts (cfu/g) or fungi recovered. Germination was positively correlated with *Alternaria alternata* (Fr.) Keissler ($r = 0.63$). At 9.8% mc and 13.4% mc, FFA increased ($P<.0001$) and germination decreased ($P<.0001$) significantly during storage. At 9.8% mc, FFA content doubled after 16 wk of storage and reached an unacceptable level (14) for grade No. 1 seed, > 1.8%, after 36 wk of storage (Fig. 1). For the 52 wk of storage, FFA content was negatively correlated with germination ($r = -0.91$) and with the field fungus *A. alternata* ($r = -0.90$), but was positively correlated with the storage fungus *Eurotium rubrum* Konig, Spieckerman & Bremmer ($r = 0.78$). At 13.4% mc, FFA reached an unacceptable level after only 4 wk storage. In a previous study in which sunflowerseed were stored at 10 C, we found that the FFA doubled at 24 wk storage at 10.1% mc but were still at an acceptable level after 40 wk storage (12). At 13.4% mc, seed stored at 10 C reached an unacceptable FFA level after 16 wk storage.

At 13.4% mc, FFA increased to 6.3% after 24 wk storage (Fig. 1) and correlated with *Aspergillus versicolor* (Vuill.) Tiraboshi ($r = 0.74$), *Microascus* spp. ($r = 0.62$), *Penicillium* spp. ($r = 0.59$) and *Eurotium repens* de Bary ($r = 0.55$) but were negative correlated with % germination ($r = -0.88$) and *A. alternata* ($r = -0.82$). Individually, these r values explain less than half of the variation in FFA values, but a previous study (14) has shown that when the storage fungi as a group are considered, they adequately explain the variations in levels of FFA observed. Although analysis for FFA probably would not aid in detecting early invasion of sunflowerseed by storage fungi or in evaluating storability (2), this study suggested that FFA content, % germination and fungal species are indicative of sunflowerseed quality and the extent of seed deterioration, confirming and clarifying earlier reports (12,14).

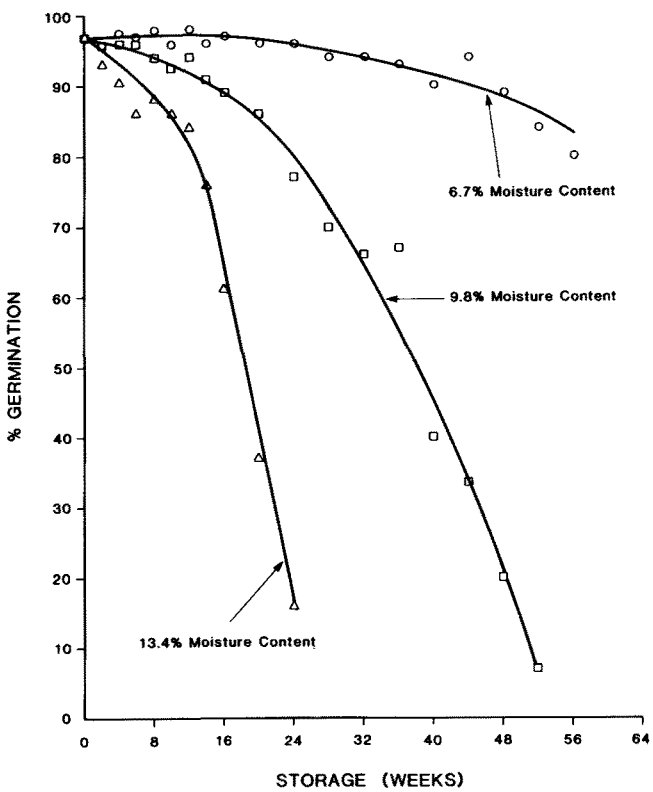

FIG. 2. Change in percentage germination of sunflowerseed stored at 20 C at three moisture levels.

The percentage of surface-disinfected seed yielding fungi during storage at the three moisture levels at 20 C is shown in Figure 3. At 6.7% mc, the fungi isolated gradually decreased from 84% to 30% during 60 wk storage. At 9.8% and 13.4% mc's, the seed yielding fungi increased rapidly, reaching 100% after only 10 wk and 4 wk respectively. In an earlier study (12) in which seed were stored at 10 C, the percent fungi isolated at 7.5% mc and at 13.4% mc was practically the same as the results obtained from this present

study of seed stored at 20 C (12). However, that study (12) also showed that at an intermediate moisture level (10.1% mc), and at 10 C storage, the number of fungi isolated decreased for the first 24 wk before increasing but never reached 100% as reported in this study at 20 C storage.

The fungi recovered most frequently from sunflowerseed stored for various lengths of time at the three moisture levels are shown in Figures 4-6. A total of 42 fungal species in 21 genera were isolated from the stored seed. At 6.7% mc, (Fig. 4), the fungi recovered most frequently were *A. alternata*, followed by *Phoma macdonaldii* Boerma and *Alternaria* sp. #1, an undescribed *Alternaria* sp. morphologically similar to *A. ricini* (Yoshii) Hansford and *A. macrospora* Zimm. These field fungi decreased slowly during the 60 wk storage (Fig. 4). According to Christensen and Kaufmann (17), damage in grain resulting from invasion by field fungi (such as *A. alternata*) occurs before harvest and does not continue to increase in storage. They concluded that if the percentage of seed yielding *Alternaria* decreases during storage that undesirable changes may be expected (17). Although there was no significant change in FFA content at 6.7% mc, the significant decrease in germination (from 97% to 80%, Fig. 2) may indicate seed deterioration. A few species of storage fungi were detected on seed stored at 6.7% mc, but they increased little if any during storage.

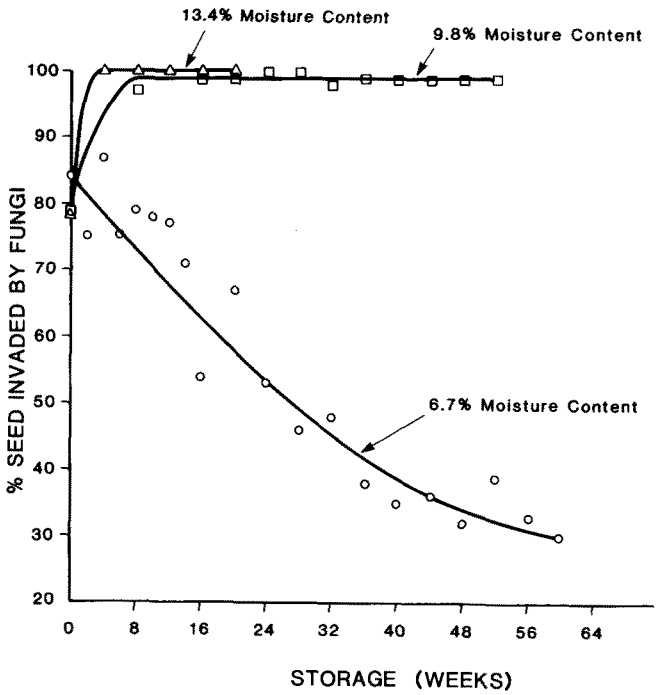

FIG. 3. Percentage of surface-disinfected sunflowerseed yielding fungi during storage at 20 C at three moisture levels.

At 9.8% mc, 29 fungal species were isolated including both field and storage fungi. The levels of the field fungi *A. alternata* and *P. macdonaldii* declined more rapidly at 9.8% mc than at 6.7% mc (Figs. 4 and 5) and were not isolated beyond 32 and 36 wk storage, respectively. Storage fungi (mainly *E. rubrum* and *E. repens*) were recovered from the seed beginning with 4 wk of storage (Fig. 5). Although sunflowerseed yielding *E. rubrum* and *E. repens* increased to 75% and 42%, respectively, by 52 wk storage, the total mold count did not increase significantly during storage. The FFA content (2.49%) and germination (7%) after 52 wk storage show that substantial deterioration of the seed had occurred (Fig. 1). At 9.8% mc, the total mold count of the seed was not significantly correlated with FFA but, while not significant, was higher than seed stored at 6.7% mc. It is well known that FFA increase in oilseeds with increasing invasion of seeds by fungi (6,12,18,19), and the increases probably are caused by microbial lipases, because extracellular lipases are produced by some fungi found in oilseeds (20). However, most oilseeds are also known to have lipolytic enzyme systems which could contribute to the increase in FFA, but it is believed that seed lipases remain inactive in intact seeds unless activated by either germination or mechanical damage (18). Because the seed in this study was not germinated or damaged, it is believed that fungi are related to the increase in FFA. According to Christensen and Kaufmann (17), if seed lots yield *A. glaucus* from 50 to 100% of the surface-disinfected kernels, the lot is partially deteriorated. Fungi of the *Aspergillus glaucus* group, *E. rubrum* and *E. repens*, were isolated from 75% of the 52 wk samples and 50% of the 48 wk samples. Therefore, while the number of storage fungi at 9.8% mc was small (based on total mold count), there were enough present to produce 2.49% FFA if indeed they are involved in the process.

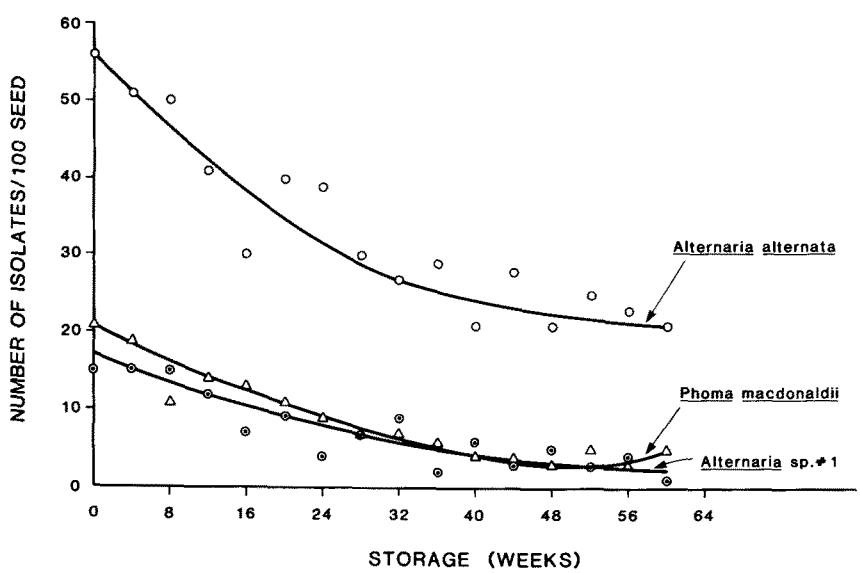

FIG. 4. Number of fungal isolates per 100 surface-disinfected sunflowerseed stored at 20 C at 6.7% moisture content.

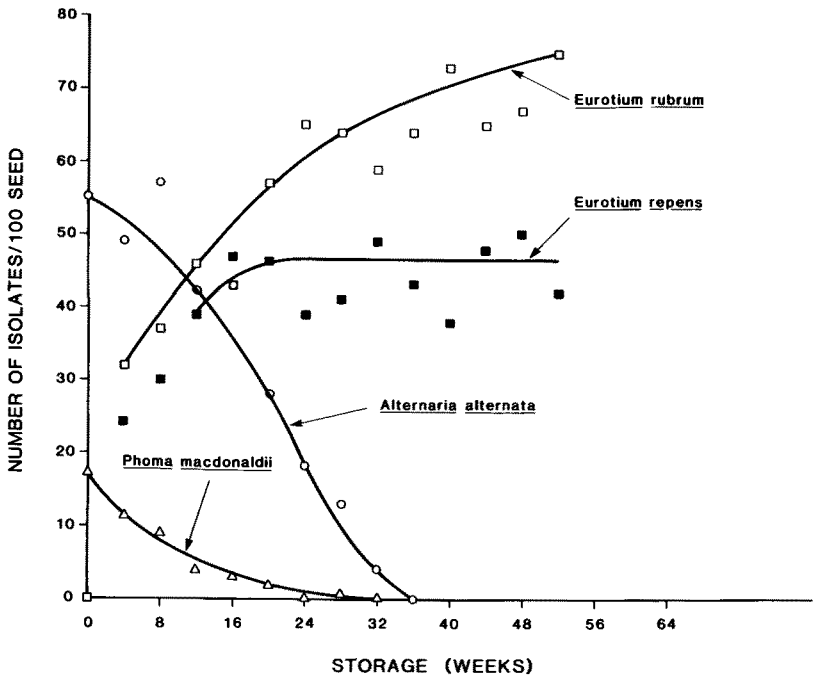

FIG. 5. Number of fungal isolates per 100 surface-disinfected sunflowerseed stored at 20 C at 9.8% moisture content.

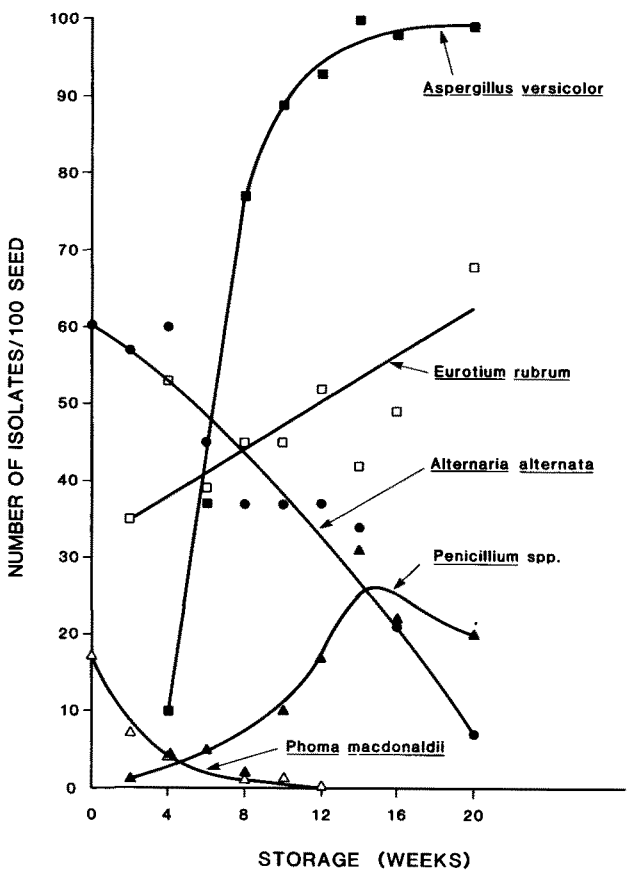

FIG. 6. Number of fungal isolates per 100 surface-disinfected sunflowerseed stored at 20 C at 13.4% moisture content.

At 13.4% mc, 27 genera of field and storage fungi were isolated from seed stored for 20 wk. Field fungi declined and storage fungi invaded the seed more rapidly than at 9.8% mc (Fig. 6). Although the level of field fungi declined, the total number of fungi increased from 5 million cfu/g after 4 wk to 85 million cfu/g after 20 wk of storage. *P. macdonaldii* was not detected after 12 wk of storage, and only 7% of the seed yielded *A. alternata* after 20 wk of storage (Fig. 6). After only 2 wk of storage, sunflowerseed were invaded by storage fungi. The fungus most frequently isolated was *A. versicolor*, which increased from 10% after 4 wk to 100% by the end of the storage period (20 wk) (Fig. 6). Percent seed yielding *A. versicolor* correlated significantly with FFA content ($r = 0.73$), but not with germination ($r = -0.52$). The percent recovery of *E. rubrum* (Fig. 6), *E. repens* and *E. amstelodami* Mangin increased during storage but did not correlate significantly with FFA or germination. The recovery of *Penicillium* spp. and *Malbranchea sulphurea* (Miehe) Sigler & Carmichael increased until the 14th wk and then decreased. In this and in our previous study (12) *Penicillium* spp. were isolated infrequently from sunflowerseed stored at 9.8% mc (83% RH) or 10.1% mc (84% RH) at 20 C and 10 C, respectively. Christensen (16) reported that species of *Penicillium* grow on products in storage at low temperature and at moisture contents in equilibrium with RH's above 85%. In our studies at 13.4% mc, the percent yielding *Penicillium* were greater when seed were stored at 10 C (12) than at 20 C. After 14-16 wk of storage, *A. versicolor* became the most frequently isolated fungus, while the isolation frequency of *Penicillium* spp. and *Malbranchea* spp. decreased. The isolation of *Microascus* spp. gradually increased to 31% after 20 wk of storage, but it was negatively correlated with germination ($r = -0.70$) and positively correlated with FFA ($r = 0.62$), *A. versicolor* ($r = 0.69$) and *Penicillium* spp. ($r = 0.58$).

This study indicates that storage fungi, particularly of the *Aspergillus glaucus* group, invade sunflowerseed of 9.8%

mc or higher at 20 C and suggest that this group can be associated with significant deterioration of seed within 6 mo of storage. This finding extends and confirms our previous study at 10 C (12). Studies are now in progress to determine and clarify the role of seed borne fungi isolated from sunflowerseed in the observed increases in FFA during storage.

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