

EFFECT OF PACKAGE MATERIALS ON ASSOCIATED FUNGI AND CHEMICAL COMPOSITION OF SORGHUM SEEDS DURING STORAGE

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ABSTRACT

Three types of packaging materials i.e., high density polyethylene (143 g/m²-HDP), 0.1-mm thick Kraft paper bags and woven polyethylene (WP) were used in combination with different temperature storage (ambient temperature 25±3 °C, 10-12°C and 5-6°C in refrigerator) during 2 storage periods of 6 and 12 months. The interaction effect of different temperature and storage package types showed significant differences in associated seed borne fungi and chemical composition of the stored seed compared with the seeds in the control treatment stored at ambient temperature determined at zero time of storage. Associated fungi detected with sorghum seed by seed health test methods were *Fusarium verticillioides*, *F. semitectum*, *F. oxysporium*, *Curvularia lunata*, *Aspergillus niger*, *A. flavus*, *Penicillium sp.*, *Alternaria solani*, *A. alternata*, *Bipolaris sorghicola*, *Drechslera oryzae* and *Epicoccum nigrum*. Results indicated that the highest frequency of seed borne fungi associated with sorghum seed was recorded for samples stored in high density polyethylene (HDP) package under room temperature condition after 6 months followed by samples stored in Kraft paper package, the total seed borne count numbers were 129 and 109.33, respectively, while, storage in woven polyethylene (WP) package had the lowest total count (78.67) of seed borne fungi. As regard to sorghum seeds chemical component, data of storage experiments showed that storage at room temperature generally resulted in reduction of crude fiber, ash, oil, crude protein, germination percentage and oil quality while it showed increase of free fatty acid and acidic value especially in seed stored in HDP package. The weight of 100 kernels were also decreased and total infection count were increased under storage at room temperature specially with using HDP package type compared with control treatment (at zero time storage). In brief, the storage of sorghum seeds in woven polyethylene packages type under low temperature (5-6 °C) was very suitable and led to reduction of storage fungi and to keep seed chemical components and oil quality of the seeds. Therefore, the need to maintain the temperature, relative humidity and type of storage package is important criteria of storage.

Keywords: Sorghum seeds, package materials, Storage, seed borne fungi,

INTRODUCTION

Grain mold is the most important and widespread deterioration of grain sorghum (*Sorghum bicolor* L. Moench.) worldwide. It considered as a major constraint during seed production and storage. Damage due to grain mold has been associated with losses in seed mass, grain density, seed germination, storage quality, food and feed processing quality and market value. Moreover, some of the mold fungi are producers of potent mycotoxin that are harmful to human and animal health and productivity. Production losses due to sorghum grain mold range from 30% to 100% depending on cultivar, time of flowering and prevailing weather conditions during flowering

(Singh and Bandyopadhyay, 2000). Bass and Stanwood (1977) and Roberts (1981) suggested that general principle is that low moisture and high quality seeds stored under cool and dry conditions maintain seed quality better than high moisture and low quality seeds under storage conditions. Careful storage may help in alleviating problems of seed viability and large seed crops may be used for several years of re-vegetation activity, avoiding the need to rely on the current crop alone. Seed of most species may be safely stored for several years by careful control of temperature and relative humidity. El-Aidy *et al.* (2001) reported that the germination percentages of cotton seeds affected by interaction of storage periods and packing materials. Significant differences were observed for packing materials. Storage in woven polyethylene package recorded the highest germination values for cotton seeds. Owolade *et al.* (2011) reported that sorghum seeds stored at three different storage temperature (gene bank temperature -5°C), freezing temperature ($-20 \pm 2^{\circ}\text{C}$) and ambient temperature ($25 \pm 3^{\circ}\text{C}$), while the packaging materials were in an aluminum can, plastic container and polyethylene bag. The result showed that sorghum seeds stored inside gene bank and freezer irrespective of the packaging materials and type of accession retain their viability to the tune of 90.67 to 100%. Whereas; seeds stored at ambient temperature had low germination percentage (10.67 to 28.00%) except those stored in aluminum can (41.33%).

Forbes *et al.* (1992), Shetty *et al.* (1995), Farahat (2001), and Bandyopadhyay *et al.* (2002) found that sorghum grains should be dried to 10-12% moisture after harvest using drying technology or sun-drying to avoid molding during storage and further processing. Tolba and EL-Sayed, Soad (2002) added that percentage of fungal infection of maize grains were positively correlated with grains content of free fatty acid (F.F.A), acidic value (A.V), acidity and crude protein of grains during storage. In the reverse, the percentage of fungal infection was negatively correlated with maize grain content of endosperm. On the other hand, El-Sayed *et al.* (2004) reported that, the lowest values of free fatty acid (F.F.A), acidic value (A.V.) acidity percentages and the highest germination were determined in seed stored inside high density polyethylene packages. Moreover, EL-Sayed, Soad and Tolba (2005) Shobha *et al.* (2008) reported that, the storage at 10°C condition gave the highest values for germination and best oil characters by decreasing free fatty acid percentage and acidic value.

This work aims to study the effect of storage package types and storage conditions on the associated seed borne fungi and chemical content changes during 6 and 12 months of storage.

MATERIALS AND METHODS

The present investigation was carried out in Seed Technology Laboratory and Plant Pathology Laboratory at Agricultural Research Station at Sakha, Egypt during 2012 and 2013 seasons. One commercial susceptible hybrid (sorghum hybrid 888) was used. A weight of 15 kg from seeds were taken at random and packaged in bags made from different materials i.e., high density polyethylene ($143 \text{ g/m}^2\text{-HDP}$), 0.1-mm thick Kraft paper bags and woven

polyethylene (WP). Each package was filled with 1/2 kg of tested hybrid seeds in three replicates and stored at different periods of 6, 12 months and control at zero time. Packages were stored at different conditions at room temperature $25 \pm 3^\circ\text{C}$, $10-12^\circ\text{C}$ and at $5-6^\circ\text{C}$ of storage inside refrigerator. All samples were tested for germination, physical and chemical composition. Seed health test on agar plates according to ISTA rules (1985) were done for detection of associated fungi and to determine the effect of different storage conditions on seed fungal infection load. The collected data were analyzed according to the factorial completely randomized design with three replicates. Standard germination test was carried out under optimum conditions according to the international rules of testing (ISTA, 1999).

Chemical composition analysis:

As for chemical composition characters, seed samples were taken at random from each sorghum seeds package and grinded to fine powder to pass through 2 mm mesh. The following analysis were determined according to procedures outlined in AOAC (1990) for , oil, crude protein, crude fiber, ash, free fatty acids (FFA) contents, acid value (AV) acidity and seed moisture (ISTA rules, 1999) .

a. Moisture content: Samples of 5 g of sorghum seeds were taken randomly and weighed to three decimal places. The grains were grinded before drying using a grinding mill so that at least 50% of the ground material passed through a wire sieve with meshes of 0.50 mm and not more than 10% was remained on the wire sieve with meshes of 1 mm. High constant temperature oven method was utilized. The working sample was evenly distributed over the surface of the drying container which was weighed with its cover before and after filling. The container was placed on top of its cover in an oven maintained at a temperature of $130 \pm 3^\circ\text{C}$ for two hours. At the end of prescribed period the container was covered and placed in desiccators to cool for 30 minutes.

The moisture content as a percentage by weight (fresh weight basis) is calculated to one decimal place, by using of the formulae:

$$\% \text{ seed moisture content (mc)} = \frac{M2-M3}{M2-M1} \times 100$$

Where:

M1 = Weight of the weighing bottle/container with cover in gm

M2 = Weight of the weighing bottle/container with cover and seeds before drying

M3 = Weight of the weighing bottle/container with cover and seeds after drying. The mean of four replicates was considered reliable if the difference between replicates didn't exceed 0.2% other wise, the test was repeated (ISTA, 1999).

b. Protein content: The micro-Kjeldahl method was used to determine the total nitrogen in grain and multiple by 1.63 to obtain the content of crude protein according to AOAC (1990).

c. Oil, free fatty acid (FVA), ash and crude fiber (%): Oil and other components content of grain was determined as the method described by the AOAC (1990) using Soxhlet apparatus and petroleum ether ($40-60^\circ\text{C}$) as a solvent.

d. Acidity: Samples of 15 g of sorghum seeds were taken randomly and grinded before drying using a grinding mill. Acidity percent was determined as described by the AOAC method (1990).

e. Weight of 100 kernels: One hundred seeds from each package under each storage period and condition were taken and weighed

All the aforementioned measurements were estimated before storage (at zero time) and after the tested storage periods (six and twelve months) under all tested storage conditions.

Seed health test: Associated fungi with seed represent different samples in storage experiment were carried out in laboratory using PDA plates according to seed health testing rules of ISTA (1985). Frequency of each isolated fungi were recorded and each isolated fungus was identified in department of Mycology, Institute of Plant Pathology, ARC, Giza, Egypt.

RESULTS AND DISCUSSION

Associated fungi detected with sorghum seed by seed health test methods were *Fusarium verticillioides*, *F. semitectum*, *F. oxysporium*, *Curvularia lunata*, *Aspergillus niger*, *A. flavus*, *Penicillium sp.*, *Alternaria solani*, *A. alternata*, *Bipolaris sorghicola*, *Drechslera oryzae* and *Epicoccum nigrum*. Data presented in Table 1 and Fig. 1 showed that the highest frequency of seed borne fungi associated with sorghum seed was recorded for samples stored in high density polyethylene (HDP) package under room temperature condition after 6 months followed by samples stored in Kraft paper package, the total seed borne count numbers were 129 and 109.33, respectively, while, storage in woven polyethylene (WP) package had the lowest total count (78.67) of seed borne fungi. On the other hand, data presented in Table 1 and Fig. 1 for seed samples stored under 10-12°C temperature, clarified that total infection percent was generally decreased specially in woven polyethylene package on which total infection recorded was 62.00. In the reverse, seed samples stored in HDP package led to high count of total infection (109.0) compared with control treatment of seed borne fungi determined at zero time of storage that recorded 67.67 total fungal infections. Moreover, storage at 5-6°C condition had generally the lowest count of infection especially in seed samples stored in woven polyethylene package kind (51.33), and Kraft paper package (65.67) while, the total count of seed borne fungi was very high (126.33) in seed stored in HDP package compared with control treatment. These results were in the same line with that obtained by El-Aidy *et al.* (2001) and Frahat (2001) who found that seed deterioration increased and life span decreased as storage temperature and moisture content increased. The germination percentage was highly significant decreased after six months of storage period. They added that cotton seeds stored in woven polyethylene packages at low temperature (7-10°C) recorded the highest germination values and lowest infection percent by seed borne fungi.

T 1

F 1

Data presented in table 1 and Fig. 2 after 12 months of storage period showed that the total count of seed borne fungi was high under room temperature in three tested package types of HDP, PP and WP and it recorded 154.67, 121.33 and 94.00, respectively followed by seeds stored under 10-12°C in the three types of tested packages (128.00, 89.67 and 69.00), respectively, compared with seed sample of control treatment (67.67) inspected at zero time of storage. On the other hand, the lowest value of total infection count were obtained under 5-6°C storage condition in woven polyethylene package, it was 57.33. In the reverse, the highest value of total infection count (137.00%) was recorded after 12 months for seed samples stored under condition of 5-6°C in HDP package. This result was in agreement with those of Owolade et al.(2011) who reported the presence of *Alternaria*, *Helminthosporium*, *Fusarium*, *Curvularia*, *Stemphylium*, *Rhizopus*, *Cladosporium*, *Aspergillus* and *Penicillium* species in sorghum seeds. Also, Frahat (2001) indicated that the lowest sorghum seeds infection percent by seed rots causal organisms were obtained in storage at low temperature (7°C) .

As regard to sorghum seeds chemical component, data presented in Tables 2 and Figs 1 & 2 showed that storage at room temperature generally resulted in reduction of crude fiber, ash, oil, crude protein, germination percentage and oil quality while it showed increase of free fatty acid and acidic value especially in seed stored in HDP package.

The weight of 100 kernels were also decreased and total infection count were increased under storage at room temperature specially with using HDP package kind compared with control treatment (at zero time storage). On the other hand, data presented in Table 2 also showed that storage at 10°C led to reduction of certain chemical component of stored seeds which mentioned above especially in seed samples stored in HDP and Kraft paper packages while, seed samples stored in woven polyethylene package led to less reduction in recorded values of seed chemical components that considered near or equal to values of the control treatment (at zero time storage). Moreover, seed storage at 5-6°C condition had the lowest effect in chemical component of seeds especially in seed stored in woven polyethylene package, since the seed chemical component had values equal the control treatment with few non-significant differences. In the reverse, seed stored in HDP package under all tested storage conditions and storage periods led to reduction of crude fiber %, ash %, oil %, crude protein %, germination %, and oil quality while free fatty acid% and acidic value were increased, as well as, the weight of 100 kernels. The reverse was true; when seed samples were stored in woven polyethylene package under all tested storage conditions and at all tested storage periods as presented in Tables 2 and Figs. 1 & 2. The reduction of seeds chemical component and weight of 100 kernel during storage, especially, after 12 months for seed samples stored in HDP package may be led to stimulate seed respiration and resulting in a net loss in dry weight of the seed and a loss in viability as manifested by poor germination, as recorded and reported by Tolba and EL-Sayed, Soad (2002).

T 2

F2

Also, the finding of the study revealed that seeds of sorghum can best be stored under controlled environment for multiplication. Increase in moisture content over a period of time during storage coupled with high infection rates could lead to a substantial loss of sorghum seeds. These results were in the same line with recorded by El-Aidy *et al.* (2001) and EL-Sayed ,Soad and Tolba (2005), They found that, the germination percentages affected by interaction of storage periods and package materials, the seeds which stored in woven polyethylene packages recorded the highest germination values and lowest decrease in seeds compounds (i.e. crude protein, crude fiber, ash and oil quality [it's decrease by increase free fatty acid (FFA) and acidity value (AV)], as well as increasing weight of 100 kernels. They added that, percentage of fungal infection of maize grains were positively correlated with grains content of free fatty acid (FFA), acidic value (AV) and acidity. In the reverse, the percentage of fungal infection was negatively correlated with maize grains contents of crude fiber, endosperm, ash and oil. Therefore, the need to maintain the temperature, relative humidity and type of storage package is important criteria of storage.

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تأثير عبوات التخزين المختلفة على الفطريات المصاحبة لحبوب السورجم وكذلك التركيب الكيماوى لها

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تم استخدام ثلاث أنواع من عبوات التخزين وهى البلاستيك السميك والورق والبلاستيك المنسوج تحت درجات حرارة تخزين مختلفة وهى درجة الحرارة المحيطة أو الغرفة (3 ± 25 م°) ودرجة حرارة تبريد ($10-12$ م°) ودرجة حرارة تبريد ($5-6$ م°) وذلك خلال فترات تخزين 6 شهور و 12 شهر ووجد أن التفاعل بين درجات الحرارة المختلفة ونوع العبوات المختلفة أثناء التخزين أدى إلى اختلافات معنوية فى نسبة الفطريات المصاحبة للحبوب وكذلك التركيب الكيماوى للحبوب المخزنة مقارنة بالحبوب المخزنة تحت ظروف درجة الحرارة المحيطة (الغرفة) والمقدرة فى بداية التخزين (الكنترول) . وكانت الفطريات المصاحبة والمعرفة على حبوب الذرة الرفيعة باستخدام لإختبار صحة البذور هى فيوزاريوم فير تيسليوتس - فيوزاريوم سيميبيكتم ، فيوزاريوم أوكسيسبوريم وكورفيور لاريا لوناتا ، اسبرجلس نيجر ، واسبرجلس فلافس ، بنسيليوم ، الترناريا الترنااتا ، الترناريا سولانى ، بابيولارس سورجيكولا ، دريشليرا أوريزا وابيكوكم نيجرم . وبينت النتائج أن أعلى تكرار للفطريات المصاحبة لحبوب الذرة الرفيعة وجدت فى العينات المخزنة فى عبوات البلاستيك السميك تحت درجة حرارة الغرفة بعد ستة أشهر من التخزين يليها العينات المخزنة فى العبوات المصنوعة من الورق حيث كان عدد الفطريات الكلى فى الحالتين هو 129 ، 109.33 على التوالي بينما كانت 78.67 فى حالة التخزين فى عبوات مصنوعة من البلاستيك المنسوج وبالنظر إلى المكونات الكيماوية لحبوب الذرة الرفيعة فإن النتائج المتحصل عليها بينت أن التخزين على درجة حرارة الغرفة عموماً أدى إلى النقص فى الألياف الخام والرماد والزيت والبروتين الخام ونسبة الإنبات وجود الزيت بينما زادت الأحماض الدهنية الحرة ودرجة الحموضة خصوصاً عند التخزين فى عبوات البلاستيك السميك وفى نفس هذه الظروف من التخزين قلت وزن المائة حبة وزادت نسبة الإصابة بالفطريات مقارنة بالكنترول (عند بداية التخزين) . وخلاصة القول فإن تخزين حبوب الذرة الرفيعة فى عبوات البلاستيك المنسوج تحت درجة حرارة منخفضة ($5-6$ م°) كان مناسبة جداً وأدى إلى انخفاض نسبة الإصابة بفطريات المخزن وحافظ على المكونات الكيماوية للحبة وجودة الزيت بها .
لذلك فإن درجة الحرارة والرطوبة النسبية وعبوات التخزين المناسبة من الاحتياجات الهامة لنجاح التخزين

قام بتحكيم البحث

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Table (1):Effect of package types on seed borne fungi of sorghum under different temperature conditions after 6 and 12 months of storage.

Frequency of fungal species associated with sorghum grains														
Storage condition	Package Type	<i>Fusarium verticillioides</i>	<i>F. semitectum</i>	<i>F. oxysporium</i>	<i>Curvularia lunata</i>	<i>Aspergillus niger</i>	<i>A. flavus</i>	<i>Penicillium sp.</i>	<i>Alternaria solani</i>	<i>A. alternata</i>	<i>Bipolaris sorghicola</i>	<i>Drechslera oryzae</i>	<i>Epicoccum nigrum</i>	Total
After 6 months of storage														
Room temperature 25±3 °C	HDP*	18.67b**	14.33 a	8.33 a	11.67 a	15.33 a	12.67 a	8.33 a	11.33 a	10.67 a	3.00 bc	8.33 a	9.67 a	129.00 a
	PP	16.67c	11.33 bc	6.33 b	11.33 a	12.67 b	7.67 c	7.67 a	10.67 ab	8.67 b	2.33 cd	6.33 b	7.67 b	109.33 b
	WP	14.33d	7.67 d	4.67 c	8.67 cd	7.67 cd	5.67 de	4.67 b	7.33 c	6.33 cd	1.67 de	4.67 cd	5.33 c	78.67 c
Refrigerator 10-12°C	HDP	16.33c	12.33 b	6.33 b	9.33 bc	11.33 b	10.67 b	7.33 a	9.33 b	8.67 b	3.67 b	4.33 de	8.33 ab	109.00 b
	PP	15.67dc	10.33 c	4.33 c	8.33 cd	8.67 c	6.33 d	4.67 b	7.67 c	7.33 c	1.67 de	3.67 def	3.67 cd	78.33 c
	WP	13.33d	6.33 e	3.67 c	7.33 de	7.33 cd	5.67 de	2.33 c	5.67 de	6.33 cd	0.67 e	2.33 fg	2.67 d	62.00 d
Refrigerator 5-6°C	HDP	21.33a	5.33 ef	8.67 a	10.33 ab	12.33 b	12.67 a	8.33 a	9.67 b	10.33 a	5.33 a	5.67 bc	9.33 ab	126.33 a
	PP	14.67d	4.33 f	4.67 c	6.67 ef	6.33 d	4.67 ef	3.33 bc	6.67 cd	6.33 cd	1.03 e	2.67 fg	3.33 d	65.67 d
	WP	12.33e	5.67 e	3.67 c	5.33 f	4.67 e	3.67 f	2.67 c	4.67 e	5.33 d	0.67 e	1.67 g	2.33 d	51.33 e
Control (At zero time)		14.33d	5.67 e	4.33 c	7.33 de	7.67 cd	5.67 de	2.33 c	6.33 cd	5.67 d	1.03 e	3.33 ef	4.00 cd	67.67 d
After 12 months of storage														
Room temperature 25±3 °C	HDP	19.33 b	15.33 a	9.33 a	12.33 a	19.33 a	17.33 a	13.67 a	12.67 a	11.67 a	3.33 bc	10.00 a	10.33 a	154.67 a
	PP	17.67 c	11.67 b	7.00 bc	12.33 a	14.33 b	9.33 c	9.67 b	11.33 ab	9.33b	2.67 bcd	7.33 b	8.67 b	121.33 d
	WP	16.67 c	8.33 c	5.67 cd	9.33 bc	10.33 c	6.00 e	6.00 c	8.67 de	8.33b	2.00 c-f	5.33 c	6.00 c	94.00 e
Refrigerator 10-12°C	HDP	21.00 a	11.67 b	7.33 b	10.33 b	15.33 b	14.67 b	9.67 b	10.33 bc	9.33b	4.00 b	5.33 cd	9.33 ab	128.00 c
	PP	14.67 d	8.33 c	5.67 cd	9.33 bc	9.67 cd	8.00 cd	5.67 c	9.00 cd	8.67b	2.33 cde	4.03 de	4.33 d	89.67 e
	WP	12.67 e	6.33 d	4.33 d	8.33 cd	8.33 de	6.00 e	2.67 e	6.33 f	7.33bc	1.03 ef	2.67 ef	3.33 de	69.00 f
Refrigerator 5-6°C	HDP	21.67 a	12.33 b	9.33 a	10.33 b	15.67 b	15.33 b	10.33 b	10.67 b	9.67b	5.67 a	5.67 c	9.33 ab	137.33 b
	PP	10.33 f	5.67 de	5.03 d	7.67 d	8.67 de	6.33 de	4.67 cd	7.33 ef	8.33b	1.33 def	3.33 ef	3.67 de	70.67 f
	WP	9.33 f	4.67 e	4.67 d	6.00 e	7.33 e	4.67 e	3.67 de	6.33 f	6.00c	0.67 f	2.33f	2.33 e	57.33 g
Control (At zero time)		14.33 d	5.67 de	4.33 d	7.33 de	7.67 e	5.67 e	2.33 e	6.33 f	5.67c	1.03 ef	3.33 ef	4.00 d	67.67 f

*HDP = High density polyethylene package, PP = Paper package, WP = Woven polyethylene package

**Mean values followed by the same letter(s) in each column do not differ significantly according to Duncan's test ($P \leq 0.05$);

Table (2): Effect of package types on seed borne fungi and chemical component of sorghum seed hybrid 888 under different temperature conditions after 6 and 12 months of storage .I

Storage condition	Kind of package	Total count fungi	Germ-ination %	Moisture %	Crude protein %	Oil %	Free fatty acids %	Acidity %	Acidic value (AV)	Ash%	Crude fiber %	Weight of 100 kernels (g)
After 6 months of storage												
Room temperature 25±3 °C	HDP	129.00 a	69.67 g	13.03 bc	10.18 d	2.62 f	0.495 a	3.950 a	0.239 a	1.210 j	2.580 g	3.090 h
	PP	109.33 b	73.33 e	12.75 cd	10.43 c	2.76 e	0.396 b	3.650 b	0.196 bc	1.280 i	2.880 d	3.177 g
	WP	78.67 c	80.00 d	12.25 e	10.62 bc	2.85 cd	0.232 c	3.490 d	0.187 bc	1.387 h	3.850 b	3.279 d
Refrigerator 10-12°C	HDP	109.00 b	77.00 e	13.27 b	10.56 c	2.58 f	0.371 b	3.550 c	0.211 b	1.430 g	2.780f	3.191 fg
	PP	78.33 c	83.33 c	12.86 bc	10.68 bc	2.83 de	0.193 c	3.370 e	0.188 bc	1.520e	3.010 c	3.252 e
	WP	62.00 d	90.00 a	12.38 de	10.85 b	3.06 b	0.107 d	3.180 g	0.184 c	1.680 c	3.370 b	3.427 c
Refrigerator 5-6°C	HDP	126.33 a	67.00 h	13.88 a	10.43 c	2.58 f	0.395 b	3.380 e	0.202 bc	1.487 f	2.800 e	3.205 f
	PP	65.67 d	80.00 d	12.95 bc	10.83 b	2.92 c	0.242 c	3.230 f	0.188 bc	1.620 d	3.100 c	3.288 d
	WP	51.33e	87.00 b	12.42 de	10.90 b	3.15 a	0.081 d	3.040 h	0.183 c	1.750 b	3.420 a	3.451 b
Control (At zero time)		67.67 d	87.00 b	12.15 e	11.50 a	3.22 a	0.076 d	3.030 h	0.181 c	1.883 a	3.430 a	3.515 a
After 12 months of storage												
Room temperature 25±3 °C	HDP	154.67 a	60.00 h	13.88 b	9.80 e	2.10 h	0.499 a	4.300 a	0.279 bc	1.000 j	2.170 h	2.970 e
	PP	121.33 d	63.33 g	13.05 d	10.13 d	2.34 f	0.483 a	4.050 ab	0.275 bcd	1.090 i	2.430 f	3.120 d
	WP	94.00 e	66.67 f	12.69 f	10.21 d	2.51 e	0.263 c	3.650 bc	0.257 cde	1.333 e	3.000 b	3.230 c
Refrigerator 10-12°C	HDP	128.00 c	70.00 e	13.95 b	9.98 de	2.33 f	0.396 b	3.720 bc	0.292 b	1.180 g	2.410 f	3.134 d
	PP	89.67 e	80.00 c	12.99 de	10.22 cd	2.49 e	0.198 d	3.530 cde	0.262 cde	1.257 f	2.650 d	3.196 cd
	WP	69.00 f	80.33 c	12.76 f	10.36 c	2.82 d	0.143 e	3.120 ef	0.251 de	1.520 c	2.867 c	3.383 b
Refrigerator 5-6°C	HDP	137.33 b	60.00 h	14.20 a	10.05 d	2.30 g	0.393 b	3.550 cd	0.319 a	1.130 h	2.320 g	3.140 d
	PP	70.67 f	76.67 d	13.50 c	10.47 c	2.88 c	0.271 c	3.400 c-f	0.278 bc	1.360 d	2.560 e	3.222 c
	WP	57.33 g	83.33 b	12.92 e	10.94 b	3.07 b	0.093 f	3.200 def	0.242 e	1.730 b	2.957 b	3.350 b
Control (At zero time)		67.67 f	87.00 a	12.15 g	11.50 a	3.22 a	0.076 f	3.030 f	0.181 f	1.883 a	3.430 a	3.515 a

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