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RESEARCH ARTICLE

Changes in the biochemical composition of the seed material of sunflower hybrids during long-term storage

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The crop saving is no less important task for farmers than growing it. Crop losses reach 15 percent when sunflower seeds are stored incorrectly. We have conducted an experiment to determine changes in the biochemical composition of sunflower seeds of Lohos and Persei hybrids during long-term storage. We have used laboratory analytical and mathematical statistical methods of research, according to generally accepted methods. The analyzed research on this problem by both national and foreign scientists indicates the relevance of this issue. Our experiments have not revealed a significant difference in the biochemical composition of different hybrids seeds during storage. Throughout the first 6 months of storage in the sunflower seeds post-harvest maturation occurs. It is set that due to antioxidant components in the seed the peroxide processes are inhibited at the end of the first year of storage. At the end of the second year of storage the content of free fatty acids in the sunflower seed of the investigated hybrids increased and was higher than the initial values. According to the results of laboratory researches it has been proven, that the seed material of sunflower does not have substantial changes in biochemical composition during two years under standard storage conditions.

Keywords: Enzymes; Fatty acids; Sunflower seeds; Vitamins; Storage

Introduction

Identifying the potential longevity of seeds in different genotypes is of great interest when creating insurance funds (Walters C. et al., 2007; Desheva G. et al., 2017). The solution of the problem is not possible without finding out internal physiological and biochemical mechanisms underlying the processes of seed aging (Krestikov I.S., 1990).

It is known that reserve nutritives play an important role providing nutrition of plantlets at the first stages of their development (Kalenska S.M. et al., 2017). As early as at the first stages of germination strengthening of intensity of breathing and processes of mobilization of reserve nutritives is specific. In this period many hydrolytic enzymes of protein, carbohydrate and lipid metabolism, Krebs cycle enzymes, glycolytic, pentose-phosphate and glyoxylate cycles are activated (Kuznetsov V.V., et al., 2005).

The main components of the seeds are proteins, carbohydrates, lipids (fats and fatty compounds), nucleic acids, minerals, water, etc. Their longevity is mainly determined by the ratio of these components (Krasilnikova L.A. et al., 2005).

Fats are heterogeneous in seeds, they consist of a mixture of triglycerides and fatty acids (FA). Seeds of different crops are specified by a certain composition and ratio of components, characterized by numerical indices (Sakhno L.O. et al., 2012). To keep seeds of oil-bearing crops is considerably more difficult than cereal grains.

The seeds of oil-bearing cultures differ in the high content of glycerides of highly saturated FAs (linoleic and linolenic acids), which are inclined to accumulate toxic products by the peroxidation (Shpaar D., et al., 2006). In the lipid complex of seeds that are stored, certain enzymatic processes occur phospholipids, glycerides are split; at the same time free FAs are accumulated. Under the influence of air oxygen and the enzyme lipoxygenase, they are oxidized, forming peroxides, hydroperoxides and other oxidation products. This is due to the high content in the seeds of oil-bearing crops of fat, which cannot bind and retain moisture (like protein and starch). It leads to a large moisture saturation of other substances and to its uneven distribution (Marcos Filho, 2005).

Therefore, in modern seed production a great role is given to physiological properties and biochemical features in the predicted realization of the seed quality.

Storage conditions are decisive in ensuring the physiological seed quality, and although its quality cannot be improved, good conditions during this time will help to keep the seeds viable for longer, slowing down the deterioration process (Almeida et al., 2010). This has resulted in seed producers being concerned with the use of techniques that may help to minimise those factors that cause deterioration.

Among the factors affecting the seed quality during storage: the initial quality of the seed lot; the environment for conservation (with its variations on temperature, moisture, oxygen availability; and the packaging) as well as characteristics inherent to the species, should be taken into account (Tonin et al., 2006).

We conducted an experiment to determine the change in the biochemical composition of sunflower seeds of Lohos and Persei hybrids under storage conditions.

Materials and Methods

The seeds grown on hybridization sites (without irrigation) according to the technology recommended for the Steppe zone of

Ukraine were laid in storage. The predecessor was winter wheat. The sunflower was harvested with a John Deere combine harvester at the stage of technical maturity. After harvesting, the seeds were cleaned from the garbage impurities using the PST-1.0 pneumatic sorting table. The resulting seeds were poured into 30 kg sackcloth bags at seed moisture of not more than 7%. Bags were stacked on piles on board decks of 20 cm high above the floor. The height of the seed bag stacks was 1.5 m (6 rows of bags). The aisles between the piles and between the piles and the walls were 1.2 m (Gavrilyuk M.M. et al., 2002).

The seeds were stored for two years in a stationary, single-storey, dry, well-ventilated, pest-free and garbage impurity free grain storage facility. During storage, the seeds were monitored for temperature, relative air humidity, the appearance of rodents and other depredators. Sampling for analysis was carried out 6, 12 and 24 months after laying the seeds in accordance with the state standard (DSTU Ukrainian national standardization system 10852-86). Seed moisture was checked twice a month, at the same time checking the pest presence, examining seed notches, as well as organoleptic characteristics – the colour and smell (Kalenska S.M., et al., 2011).

Sampling and preparation of samples for the analysis was performed according to the Dospekhov method (Dospekhov B.A., 1985). When using standard techniques the number of measurements was according to the methodology. When using non-standard methods the number of measurements was not less than in five repetitions.

Seed moisture was determined by the gravimetrical method (DSTU Ukrainian national standardization system 10856-96).

The amount of general lipids was determined by a gravimetrical method. It is based on extraction of fats from sunflower seeds with diethyl ether in a Soxhlet apparatus (DSTU Ukrainian national standardization system 10857-86).

The intensity of the lipid oxidative breakdown was evaluated by the content of peroxides (peroxide number) and the content of one of the major byproducts of lipid peroxidation – malondialdehyde (MDA).

The content of malondialdehyde (MDA) was determined by the spectrophotometric method in nmol/g. The principle of the method is that at an increased temperature in an acidic environment, MDA reacts with 2-thiobarbituric acid to form a coloured trimethin complex with a maximum of absorption at λ=535 nm (Musienko M.M., et al., 2001).

Changes in the content of bioantioxidants (carotenoids, vitamin E, phospholipids) and the activity of antioxidant enzymes (peroxidase and superoxide) were investigated to determine the course of hydrolytic and oxidative degradation of lipids during storage in sunflower seed tissues. The vitamin E content was defined by the spectrophotometric method based on the ability of tocopherols to oxidize. One of the modifications of the Emmeri–Engel's method with application of an iron-pyridyl reagent was used (Antonova B.I. et al., 1991).

The carotenoid content was determined in mg/100 g by extraction of pigments with acetone, followed by determination with a spectrophotometer at λ=440 nm (Musienko M.M., et al., 2001).

The content of phospholipids was determined by the gravimetric method, which consists in the precipitation of phospholipids with acetone from a lipid extract by the Folchus method (Yermakova A.N. et al., 1987).

The superoxide dismutase (SOD) activity (EC 1.11.1.7) was determined by photometry of indigo carmine solution, which was oxidized with H_2O_2 in the presence of SOD and was expressed in μmolH2O2/g×min (Zemlyanukhin A.A., 1985).

The activity of superoxide dismutase (SOD) (EC 1.15.1.1) was determined by its ability to inhibit the reaction of adrenaline auto-oxidation in an alkaline medium (Pat. 2144674) with modification in the preparation of raw materials for the research. To measure the SOD activity, 0.5 g of plant material were taken, 5 ml of phosphate buffer with pH = 10.65 were added and ground with glass on ice in a mortar. Then this mixture was transferred into centrifuge tubes, 0.3 ml of chloroform and 0.6 ml of alcohol were added and centrifuged at 8000 rpm for 20 minutes. For spectrophotometry, a centrifuged solution above the precipitate, λ=347 nm, was selected. The SOD activity was expressed in conventional units showing the percentage inhibition of adrenaline auto-oxidation.

The intensity of hydrolytic processes in sunflower seeds was evaluated by changing the content of free fatty acids (the acid number – AN and the peroxide number – PN by the Krischenko method (Krischenko V.P., 1983).

In the Department of Lipid Biochemistry and the Testing Biological Centre of Palladin Institute of Biochemistry of NAS of Ukraine studies to determine the fatty acid composition of sunflower seeds by gas chromatography (Baydalinova L.S. et al., 1977) and amino acid composition by method of ion-exchange liquid-column chromatography (Kozarenko T.D., 1975) were conducted.

Mathematical processing of research results was performed according to Rozhkov (Rozhkov A.O., et al., 2016). MS Office Excel 2007, Statistica, MATLAB, BorlandDelphi 7 and Agrostat application software were used for statistical processing of research data. (Ushkarenko V.O., 2008; Tsarenko O.M. et al., 2000; Kropotkin A.V., et al., 2010).

Results and Discussion

Our studies have found that at storage in the seed of sunflower, the process of accumulation of fats continues. There is a tendency to increase the fat content by 0.2 - 0.8 relative percent. There has not been a significant difference between the options. Since at rest, the biosynthetic processes in the seeds are very slow, such an increase in the fat content may occur due to hydrolytic processes occurring in the seeds. As a result of these processes, some of lipids related to biopolymers move to the free state (Clarence R., 1961; Jones Q., et al., 1966). One of the reasons for the complex aggregate of biochemical changes in the process of seed aging is lipid peroxidation. The various stresses that arise under the influence of adverse environmental factors (phytopathogenic infections, ionizing radiation, etc.) are, of course, accompanied by increased concentrations in the tissues of superoxide radicals and activation of lipid peroxidation. The content level of one of the products of free radical oxidation of lipids – malondialdehyde – is an integrated indicator. This indicator characterizes the intensity of the passage of the free radical processes and, to some extent, the degree of disruption of the membrane structure (Kolupaev Yu.E., et al., 2011). Thus, we have found that the content of MDA in the sunflower seeds of both studied hybrids during the first 6 months of storage increased 2-fold (Table 1). By the end of the first and second years of seed storage, this index had stabilized and fluctuated within the margin of error.

The indicator of oxidation processes occurring in fats is a peroxide number, and the indicator of hydrolytic processes is an acid number. It is known that at the heart of the processes of fats oxidation is their interaction with oxygen (Belingheri C., et al., 2015). The substrates of this reaction are generally unsaturated fatty acids. During the first 6 months of storage in the seeds of the studied hybrids indicators the peroxide number values increased rapidly (almost 3 times). But over the next six months, these indexes decreased by 2.5 times, which is due to the activation of oxidation processes. During the second year, the storage oscillations of this index were unreliable. The acid number characterizes the content of free fatty acids in the oil. During the storage in the sunflower seeds the intensification of hydrolytic lipid breakdown was observed and at the end of the experiment the acid number increased for the Lohos hybrid by 2.7 and the Persei hybrid by 3.3 times. This fat decay occurred most intensively over the first year of storage. This confirms that the process of hydrolysis in sunflower seeds during storage is irreversible.

Table 1. The variability of the biochemical composition of sunflower seeds depending on the storage life.

Hybrid	MDA content, nmol/g of dry matter				Peroxide number, мгJ$_2$/100 g of dry matter				Acid number, mg KOH/g			
	Storage life, months											
	0	6	12	24	0	6	12	24	0	6	12	24
Lohos	93.7	195.2	154.1	169.4	0.18	0.51	0.21	0.24	0.27	0.39	0.54	0.73
Persei	89.2	181.5	152.7	173.2	0.16	0.48	0.18	0.22	0.21	0.34	0.48	0.69
HIP$_{05}$	2.04	18.4	14.1	17.3	0.03	0.04	0.05	0.01	0.04	0.06	0.07	0.06

Oxidative processes that flow in the seeds result in reduction of the content of tocopherols and carotenoids. Therefore over the first 6 months of storage, there were unimportant changes in the indices of these components (Figures 1 and 2), which is related to the post-harvest maturation of seeds. However, during further storage in the seeds of both investigating hybrids the carotenoids content decreased by an average of 30% and of vitamin E by 25%. At the end of the second year of storage, the carotenoids content in the seeds of the Lohos hybrid was 2.5 times lower and in the seeds of the Persei hybrid 2.7 times it was lower than at the beginning of storage. For vitamin E, these values are equal to 2.5 for the Lohos hybrid and 2.1 for the Persei hybrid.
A reliable difference has not been found between the investigated hybrids. Therefore, when stored in sunflower seeds, the intensive expense of endogenous antioxidants for the binding of free radicals and lipid hydroperoxides occurs that are formed in the reactions of free radical lipids peroxidation.

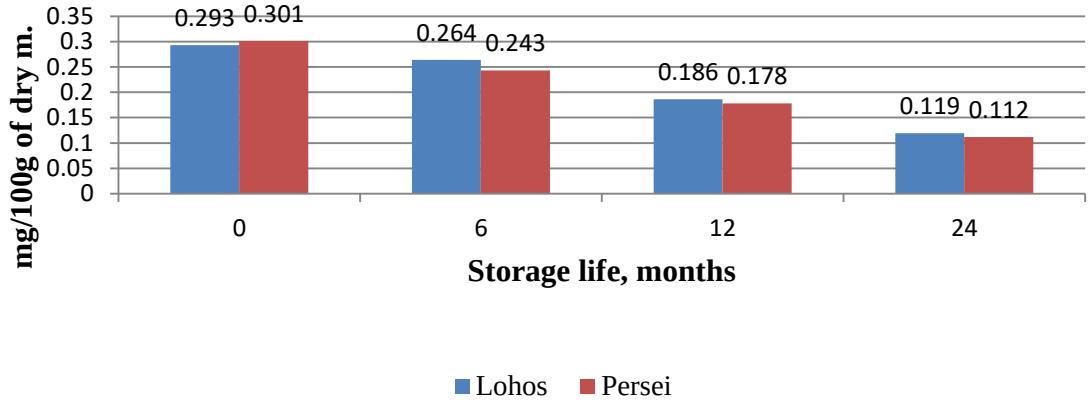

Figure 1. The carotenoid content in sunflower seeds during storage.

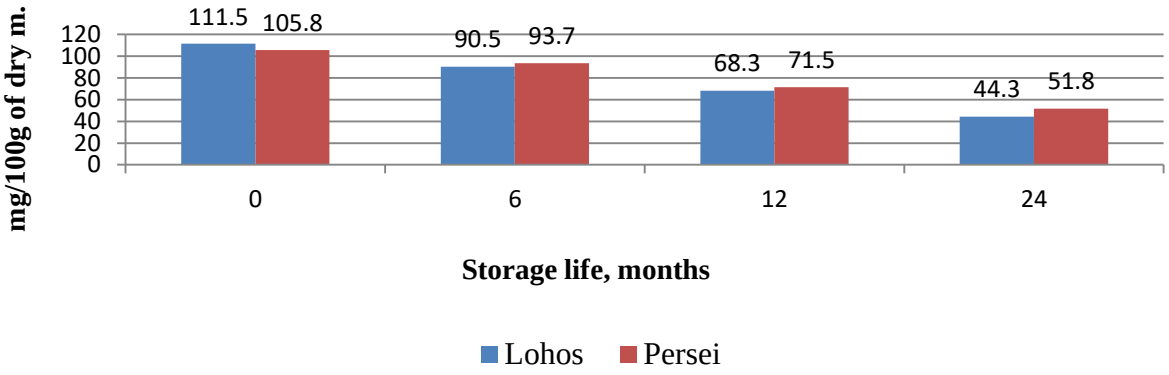

Figure 2. The vitamin E content in sunflower seeds during storage.

During storage of sunflower seeds there is a tendency for gradual accumulation of phospholipids, with no significant difference between the variants (Table 2). We established a significant correlation coefficient between phospholipids content and total lipids content (r=0.879). After two years of storage in the sunflower seeds of both studied hybrids, the phospholipids content increases 3-fold.

Table 2. The content of phospholipids and peroxidase and superoxide dismutase activity in the seeds of sunflower hybrids during storage.

Hybrid	Content of phospholipids, %				Peroxidase, mcmol H_2O_2/g×min				Superoxide dismutase, c.u.			
	Storage life, months											
	0	6	12	24	0	6	12	24	0	6	12	24
Lohos	0.59	0.65	0.97	1.84	0.26	0.21	0.24	0.26	1.84	1.26	0.64	0.31
Persei	0.63	0.68	1.06	1.91	0.28	0.20	0.25	0.26	1.87	1.32	0.67	0.35
HIP$_{05}$	0.02	0.03	0.04	0.03	0.01	0.01	0.01	0.02	0.11	0.14	0.04	0.10

Three enzymes, superoxide dismutase, catalase, peroxidase, are mainly responsible for the enzymatic system of protecting the body from oxidative damage (Sharma P., et al., 2012).

Peroxidase (EC 1.11.1.7) controls the level of hydrogen peroxide in cells by oxidizing various compounds (foremost phenols) with hydrogen peroxide (Takahama U., 2004).
Superoxide dismutase (EC 1.15.1.1) is a basic enzyme in the system of antioxidant protection that neutralizes superoxide radicals, converting them into the less toxic hydrogen peroxide and oxygen (Fridowich J., 1995; Baranenko V.V., 2006).
Through catching superoxide dismutase active oxygen radicals on the stage of freely radical overoxidation origin by molecules, the oxidation damage of lipids is slowed down. The activity of antioxidant enzymes in the storage of sunflower seeds during the first months is reduced slightly, and then gradually increases. But by of the intensity of SOD and PR changes is very different. If the SOD activity at the end of seed storage does not reach the initial values, then the PR activity is almost equal to these values. Analysis of changes of the enzyme activity antioxidant protection shows that the activity of SOD throughout the period of seed storage is gradually reduced in both studied hybrids.
We have established the reverse high force cross-correlation dependence between the indices of acid numbers and the SOD activity. For the Lohos hybrid, this indicator was r = -0.976, and for the Persei hybrid it was r=-0.971.
At the beginning of storage, the high levels of oleic and linoleic acids in the seeds of the studied hybrids were observed (Table 3). By the end of the first year of storage, the content of these acids gradually increased (by an average of 12%).
During the second year of storage, the content of oleic acid in the seeds of the hybrid Persei remained unchanged, while in the seeds of the Lohos hybrid this indicator decreased by 15%. The content of linoleic acid during the second year of storage was not significantly reduced in the seeds of both investigated hybrids.
During storage the seeds of sunflower hybrids during the first year, there is an increase in the content of fatty acids (except arachidonic acid). For the seeds of the Persei hybrid this decrease is 2.2 times, and for the seeds of the hybrid Lohos it is 3.2 times. Arachidonic acid belongs to the group of polyunsaturated fatty acids and is one of the components of vitamin F. It "activates" the seed protective reactions, which increases its resistance to the factors of stress during storage. The total amount of fatty acid methyl esters was established in the seeds of the Logos hybrid at the end of the first year of storage, it was 23% more than that one of the Perseus hybrid.

Table 3. Fatty acid composition of sunflower seeds of hybrids for storage, mW* min.

Name of the fatty acid methyl esters	Storage life					
	6 months		12 months		24 months	
	Hybrid name					
	Persei	Lohos	Persei	Lohos	Persei	Lohos
Myristic	0.0181	0.0191	0.0975	0.0943	0.0326	0.0265
Palmitic	2.2136	2.6517	4.3308	5.9457	3.7764	3.4531
Palmitoleic	0.0489	0.0390	0.0782	0.0835	0.0761	0.0588
Margaric	0.0090	0.0079	0.0231	0.0379	0.0166	0.0212
Heptadecenoic	0.0065	0.0074	0.0140	0.0218	0.0128	0.0137
Stearic	1.2434	1.5415	2.5526	3.5741	2.3273	2.1804
Oleic	12.4087	15.1881	14.4702	18.1747	14.3062	15.6207
Linoleic	12.5111	12.8319	13.8649	14.5063	13.1341	13.4911
Linolenic	0.0419	0.0279	0.0527	0.0374	0.0672	0.0395
Arachic	0.1039	0.1091	0.1924	0.2617	0.1799	0.1772
Gondoic	0.0442	0.0645	0.0814	0.1688	0.0803	0.1019
Arachidonic	0.1057	0.1384	0.0484	0.0435	0.3211	0.2043
Behenic	0.3558	0.4311	0.4665	0.8090	0.5673	0.6241
Docosatric	0.0148	0.0078	0.0283	0.0207	0.0164	0.0136
Lignoceric	0.0113	0.1235	0.2291	0.2561	0.2161	0.1764
Total	29.5843	33.2016	36.2642	43.1103	35.6254	36.2571

During the second year of storage, the content of fatty acids varied in the seeds of the studied hybrids in different ways. At the end of storage, the acids content of the seeds of all the tested hybrids increased and was higher than the initial values.

Conclusion

The changes in the biochemical composition of sunflower seeds of the Lohos and Persei hybrids during two years of storage have been investigated. We did not find any significant difference in the biochemical composition of the seeds of the various hybrids. For the first 6 months of storage in the sunflower seeds the post-harvest maturation occurs. It is established that due to antioxidant components by the end of the first year of storage peroxide processes are inhibited in the seeds. It was proved that at the end of the second year of storage, the content of fatty acids in the sunflower seeds of all the studied hybrids increased and was higher than the initial values. According to the results of laboratory studies, it has been established that sunflower seed has no significant changes in the biochemical composition when stored for two years.

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