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BIOCHEMICAL COMPOSITION OF MILLET GRAIN AND ITS CHANGES DURING STORAGE

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Abstract. The total fat content, the fatty acid composition of oil (11 fatty acids), and the total carotenoid content in grain of 8 millet varieties harvested in 2020–2021 have been investigated. The mean value of the total fat content in millet grain was 4.4±0.12%, and the varieties did not differ significantly in this parameter. The total carotenoid content averaged 6.7±0.94 mg/kg. The oil from modern millet varieties was notable for high contents of linoleic (62.4±1.32%) and oleic acids (25.1±1.67%), indicating their high nutritional value. The fatty acids in the millet grain were ranked in order of descending contents as follows: C18:2 > C18:1 > C16:0 > C18:0 > C18:2 > C24:0 > C20:0 > C16:1 > C22:0 > C20:1 > C14:1. A high content of linoleic acid in grain was recorded in the varieties Omriyane (63.6±0.07%), Sozh (62.7±0.34%), Yulin 1 (62.4±0.03%), Bila Altanka (62.8±0.04%), Kharkivske 57 (63.2±0.45%), and Zhodynske (63.3±0.31%). The varieties Sozh and Yulin 1 had an above-average content of oleic acid (26.1±0.19% and 26.0±0.01% respectively). The highest oleic acid content was observed in the variety Bohatyrske (28.5±0.15%). These data prove that high levels of linoleic and oleic acids can be combined in one genotype. The carotenoid content in the varieties Slobozhanske, Omriyane, Bila Altanka, and Yulin 1 was significantly higher than the mean value (by 0.6, 0.7, 1.1, and 0.5 mg/kg respectively). Correlation analysis has allowed determining genetic relationships between the total oils, carotenoids, and fatty acids of grain, and has shown weak to very weak correlations between their total contents. However, the oleic acid content was moderately and negatively correlated with the carotenoid content (r=-0.550) as well as with linoleic (r=-0.717), stearic (r=-0.574), eicosanoic (r=-0.590), and lignoceric (r=-0.533) acid contents. This means that breeding of high-quality millet cultivars is complicated. During long-term storage (5 years) of Kharkivske 57 grain, the total oil content decreased by 0.25%, but this change was not statistically significant. After the five-year storage, the carotenoid content decreased by 4.74 mg/kg. The acid value of oil in 2020 was 5.10 mg KOH/100 g of substance. After the five-year storage, it increased to 9.53 mg KOH/100 g of substance. No significant changes were observed in the quantity or quality of fatty acids in Kharkivske 57 oil. Nevertheless, the storage of millet grain was accompanied by a slight increase in palmitic, stearic, linoleic, linolenic, eicosanoic, eicosenoic, behenic, and lignoceric acids.

Keywords: millet, fatty acid composition, fat, carotenoids, correlation, acid value, storage.

Introduction. Formulation of the problem

According to the FAO's data, global warming and its consequence, aridity, which affects the cultivation of major crops and leads to shortfalls in crop production, have made the problem of food security especially acute. Worldwide, this results in the increased number of starving people [1]. Along with the global pandemics, it may lead to catastrophic consequences. Under these conditions, intensive transfer of high-yielding, highly drought-tolerant and heat-resistant crops may be a solution to the food problem. More attention should be paid to valuable cereals, including millet (*Panicum miliaceum* L.), which is notable for its exceptional drought resistance, is of high nutritional value, and allows obtaining low-glycaemic food products (this is especially important with the increasing frequencies of diabetes) [1,2]. Recently, an interest in millet has arisen in the food industry, due to this crop's valuable nutritional and health-improving properties [3].

Traditionally, millet is used to make porridge, but more and more studies [4,5] consider the possibility of adding millet flour to wheat flour, in order to improve the bread quality, or even making 100% millet bread (gluten-free bread). Thus, adding 15% wholemeal millet flour to wheat flour, besides improving the organoleptic characteristics of bread, also allows increasing its nutritional and biological value [6]. One of the problems preventing using millet flour in the food industry is the very short storage period, because millet and millet flour rapidly age and turn rancid [7]. This is caused by hydrolysis of oil by lipolytic enzymes. During millet storage, the fatty acid composition of oil changes: its acid number increases, the carotenoid content declines, etc., which has negative effects on the nutritional value [8].

This problem has mostly been studied on pearl millet (*Pennisetum glaucum*), and very rarely on proso millet (*Panicum miliaceum* L.). In Ukraine, studies of this matter have never been conducted, so they are novel and topical.

Analysis of recent research and publications

Millet grain has valuable biochemical and therapeutic properties. The protein content in grain depends on the genotype, weather, and soil fertility. Proso millet grain contains 1.5–13.0% of protein, which is higher than in other millet species, such as *S. Italica*, *E. Colona*, *Pasp. Scrobiculatum*, *P. Miliare* (6.5–11.5%), and the quality of millet protein is better than that of protein from other cereals (maize, wheat, rice, barley, and oats) [2,9]. Millet protein plays an important role in human cholesterol metabolism, because it increases the level of beneficial cholesterol (high density lipoproteins), especially with HDL_2 subfraction, without increasing harmful cholesterol (low and very low density lipoproteins). Rats that were fed on millet had lower levels of blood glucose, cholesterol, and triglyceride, as compared with rats that were fed on rice. Thus, millet can prevent cardiovascular diseases [10]. Immunological tests of its grain showed a low proportion of gliadins (10 mg per 100 g) in millet protein. This means that people suffering from gluten-sensitive enteropathy can eat millet or millet products [2]. Thus, millet protein is not only of high nutritional value, but also has medicinal properties.

The total starch content in millet grain can range 65.8 to 78.9% [11]. The amylose content in millet starch is 17.21–32.6%, which is slightly higher than the amylose content in maize (17–27%). Millet starch is maximally assimilated by the human body (50%), as compared with other millet-like crops. Even maize starch is only digested by as much as 43% [2].

Millet grain is rich in trace minerals: Fe (41–66 mg/kg^{-1}), Zn (26–46 mg/kg^{-1}), Ca (91–240 mg/kg^{-1}), P (156– 230 mg/%), Mg (78–140 mg%) [2,9,12]. Besides, the total content of carotenoids in grain is much higher than that in gluten-free crops like sorghum and amaranth, which significantly increases the nutritional value of millet products [3].

Millet grain is rich in antioxidants and phenolic substances: phytates (0.48%), phenols (0.3–3.0%), and tannins (0.61%), which can have positive effects on human health, for example, boost anticarcinogenic, antidiabetic, anti-inflammatory, antimicrobial potencies, etc. in the body [9].

Millet grain contains approximately 4.0–4.7% of fat [7]. Compared with oil crops, it is but very small an amount. However the biochemical composition of millet lipids significantly influences the nutritional properties of grain and grain products. The fatty acid patterns play an important role, namely the amounts of major fatty acids (myristoleic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, eicosanoic, eicosenoic, behenic, lignoceric) and their ratios. Linoleic acid is known as essential for people: it beneficially affects their health, having anticarcinogenic, anti-inflammatory, neuro-protective and cardiovascular-protective effects. Linoleic acid is involved in synthesis of vitamin D, cellular membrane and various hormones [13]. Proso millet (*Panicum miliaceum* L.) grain contains more linoleic acid than sorghum, lupine, or pearl millet does [14-16]. Thus, millet is not only delicious, but also a healthy food raw material, and owing to the high content of linoleic acid in grain, it can improve human health.

Myristoleic, palmitic, palmitoleic and, stearic acids play important roles in the human body, but since human cells cannot biosynthesise them, it is very important to consume them in sufficient quantities from plants [17]. Millet grain contains by 2–5 times more palmitic acid than stearic acid, depending on the variety. F. Al. Juhaimi *et al.*, 2019 [14], reported that the palmitic acid content in millet grain was 7-fold higher than the stearic acid content. Eicosenoic acid belongs to the group of omega-9 fatty acids, which intensify glucose assimilation in blood and on the whole positively affect the human immunity. Linolenic acid is omega-6, it reduces cholesterol levels in human blood and normalises blood pressure. Unfortunately, as evidenced by other scientists' research [14], only 1.69±0.09% of it is detected in millet grain. K. Lorenz *et al.*, 1986, originated millet samples with the linolenic acid content up to 2.20% [7].

It is known that during storage of millet grain or millet products (groats of millet, millet flour), their biochemical composition changes, organoleptic properties deteriorate, and unpleasant smell appears. One of the causes is the increase of the acid value of oil during storage, which is associated with increased lipase activity. These biochemical processes make millet rancid and, as a result, such millet cannot be used for food. Thus, a significant increase in the acid value of oil in pearl millet flour is observed as early as on day 30 of storage [18]. Another cause of organoleptic deterioration of millet groats during storage is, in scientists' opinion, alterations in fatty acids amounts [15,19-21]. This problem limits the use of millet and millet products in the food industry.

Studies of the biochemical composition of millet grown in Ukraine will allow identifying varieties with increased nutritional qualities, and determination of biochemical changes during storage will make it possible to substantiate appropriate conditions and terms of storage.

The purpose was selecting the best millet cultivars to be further used in the food industry or in plant breeding, and establishing changes in the biochemical composition of millet grain during storage.

For this purpose, the following **objectives** must be achieved:

– To study the contents of fat and carotenoids and the fatty acid composition in millet grain, and to establish how these parameters vary depending on a cultivar;

– To determine correlations between the fatty acid composition of oil and the total contents of fat and carotenoids in millet grain;

– To determine the changes in the biochemical composition of millet grain during its storage.

Research materials and methods

The total fat content, fatty acid profiles, and total carotenoid content in millet grain were measured in 8 cultivars (Kharkivske 57, Slobozhanske, Omriyane, Bohatyrske, Bila Altanka (Ukraine), Zhodynske, Sozh (Belarus), and Yulin 1 (China)) in 2020–2021. The millet varieties were grown by traditional farming techniques in the experimental field of the Plant Production Institute named after V. Ya. Yuriev of the NAAS. Grain was harvested in 2019 and 2020.

To evaluate biochemical changes in millet grain during storage, the variety Kharkivske 57 grown in 2010, 2015, and 2020 was used.

From 1987 to 2014, Kharkivske 57 was the national standard variety in Ukraine and is still a standard of grain quality in the forest steppe zone of Ukraine. Grain was stored in the Laboratory of Millet Selective Breeding, in special paper bags at 20°C, with the moisture content of grain 14%. Grain was dehulled on a device LUP–1 in the Laboratory of Millet Breeding, and then ground on a laboratory grinding mill LZM.

The total fat content in millet grain was determined by Soxhlet's procedure [23].

The fatty acid composition was determined by gas chromatography of methyl esters of fatty acids on a gas chromatograph Selmickrom-1 [22]. Methyl esters of fatty acids were identified by the retentions time of their peaks and compared against reference methyl esters of saturated and unsaturated fatty acids (Sigma). 30–50 μl of lipid extract was transferred into a glass ampoule (Pyrex), 2.5–3 ml of methylating mixture was added, and the ampoules were sealed. Then the ampoules were placed in a thermostat at 105°C for 3 hours. After methylation was over and the ampoules were cooled to room temperature, they were opened, and their contents were transferred to test tubes. Then a pinch of zinc sulphate, 2 ml of distilled water, and 2 ml of hexane were added to extract methyl ethers. After shaking thoroughly and settling, the hexane fraction was filtered and used for chromatography.

The total content of carotenoids was determined by extraction [24]. A weighed portion of flour (4 g) was transferred into a glass flask with a ground stopper. 20 ml of acetone was added to the sample. Carotenoid pigments were extracted at 200°C during 16 hours. The resulting extract was filtered through a paper filter in a fume hood. The absorbance of the acetone solution of carotenoids was measured on a photoelectric colorimeter FEK at 440±5 nm. Concurrently, the absorbance of standard potassium dichromate solution was measured.

$$x = \frac{a \times 0{,}00416 \times 1000}{H} \qquad (1)$$

wherexis the carotene content in the sample under study, mg;
ais the stock solution's equivalent value determined from the chart;
0.00416 isthecarotenecontentin 1 mlofstandard potassium dichromate solution, mg;
1000 is the carotene content calculated as mg/g;
His the weighed portion of a sample, g.

The acid value of fat was found by the quantity of KOH (in m) required to neutralise free fatty acids in 1 g of oil. This parameter was calculated according to the formula [25]:

$$x = \frac{5{,}611 \times V \times K}{m} \qquad (2)$$

where x is the acid value of fat;
5.61 is the coefficient to express the acid value in mg of KOH/g when titrating with 0.1 mol/dm^3 solution of potassium hydroxide;
V is the volume of potassium hydroxide solution used for titration, cm^3;
K is the actual concentration ratio of the potassium hydroxide solution to the normal one;
m is the weight of fat.

The data were statistically processed (analysis of variance, correlation analysis, coefficient of variation) in the statistical software GenStat 12th edition (VSN International LTD, 2009).

Results of the research and their discussion

Though the millet is not an oil crop, its grain contains a small percentage of oil, and this is used to determine the grain quality. The total fat content is determined, and its fatty acid composition is studied. Table 1 summarises the characteristics of eight millet genotypes: the total fat content in grain, fatty acid composition, and carotenoid content. The mean value of the total fat content in millet grain of the studied genotypes was higher by 1.98% than that in R. Shen *et al.*'s findings, 2018 [11] (the reported mean value of fat content in grain was 2.42±0.84%).

Millet grain is also valued due to a significant amount of provitamin A (carotenoids), as compared with other gluten-free crops (amaranth, sorghum, and others) [3]. The carotenoid content in grain of different cultivars ranges within 3.8–13.4 mg/kg [24]. In the millet genotypes under investigation, the total content of carotenoids was medium. However, the maximum value of this parameter was by 5.5 mg/kg lower than ever registered.

The fatty acid composition of millet fat is characterised by the highest contents of linoleic and oleic acids. These results are in agreement with the data of R. Shen *et al.*'s, 2018 [11], who found that the contents of linoleic and oleic fatty acids in millet grains were 61.74±1.71% and 22.16±2.05% respectively. K. Lorenz, Y. S. Hwang (1986) [7], and F. Al. Juhaimi *et al.* (2019) [14] reported that the linoleic acid content in millet grain did not exceed 50.0%, and the oleic acid content amounted to 34.0%. This proves that in millet grain, the level of oleic acid, which is valuable for human health, can be increased. According to their data, millet grain contains small amounts of palmitic, stearic, and linolenic acids. Our results fully fall into line with R. Shen *et al.*'s data, 2018 [11], only the palmitic acid content in the Ukrainian varieties was 3.0% lower. In comparison with F. Al. Juhaimi *et al.*'s findings, 2019 [14], the palmitic acid content was, on average, 3 times as high as the amount of stearic acid (7.2±0.26% vs 2.2±0.91%). Millet grain contains minor amounts of myristoleic, palmitoleic, eicosanoic, eicosenoic, behenic, and lignoceric acids, which agrees with other scientists' data [13,14].

The coefficient of variation has shown that in millet grain, the greatest fluctuations were those in myristoleic, stearic, eicosanoic, behenic, and lignoceric acids. This means that it is possible to create millet varieties with increased contents of myristoleic and stearic acids, which are important for human life too. The most valuable biochemical parameters of grain, such as the total contents of fat and carotenoids and the linoleic acid content, had low coefficients of variation. This indicates that it is difficult to increase the levels of these characteristics via plant breeding.

The total contents of fat and carotenoids in the eight millet varieties are visualised in Fig. 1. The cultivars did not differ significantly in the total fat content. Still, Slobozhanske had an increased fat content, which exceeded the mean value by 0.27%. As to the carotenoid content, the varieties significantly differed at $P<0.05$, and Slobozhanske, Omriyane, Bila Altanka, and Yulin 1 were far higher in carotenoids than the other samples. An increased content of carotenoids in grain, compared with the mean value, was observed in Slobozhanske (by 0.6 mg/kg), Omriyane (by 0.7 mg/kg), Bila Altanka (by 1.1 mg/kg), and Yulin 1 (by 0.5 mg/kg). Thus, a combination of high contents of fat and carotenoids is possible within one genotype. Slobozhanske, Omriyane, Bila Altanka, and Yulin 1 are valuable starting material for plant breeding to improve grain quality.

Table 1 – General characteristics of the fatty acid composition and the total fat and carotenoid contents in millet grain, 2020–2021

Parameter	Mean	Minimum	Maximum	Coefficient of variation, %
Total fat content, %	4.4±0.12	4.24	4.61	2.7
Fatty acids: *myristoleic* C14:1, %	0.06±0.01	0.04	0.08	24.70
palmitic C 16:0, %	7.2±0.26*	6.70	7.67	3.65
palmitoleic C16:1, %	0.5±0.09*	0.30	0.61	19.15
stearic C18:0, %	2.2±0.91*	1.26	3.52	41.65
oleic C18:1, %	25.1±1.67*	22.75	28.57	6.65
linoleic C18:2, %	62.4±1.32*	59.20	63.60	2.11
linolenic C18:3, %	1.3±0.09*	1.20	1.50	7.05
eicosanoic C20:0, %	0.6±0.22*	0.32	0.92	38.20
eicosenoic C20:1, %	0.09±0.01	0.07	0.13	15.51
behenic C22:0, %	0.4±0.14*	0.26	0.75	32.44
lignoceric C24:0, %	0.10±0.04*	0.07	0.20	35.19
Total carotenoid content, mg/kg	6.7±0.94*	5.0	7.9	13.9

*significant difference at $P<0.05$

The total contents of fat and carotenoids in the eight millet varieties are visualised in Fig. 1. The cultivars did not differ significantly in the total fat content. Still, Slobozhanske had an increased fat content, which exceeded the mean value by 0.27%. As to the carotenoid content, the varieties significantly differed at P<0.05, and Slobozhanske, Omriyane, Bila Altanka, and Yulin 1 were far higher in carotenoids than the other samples. An increased content of carotenoids in grain, compared with the mean value, was observed in Slobozhanske (by 0.6 mg/kg), Omriyane (by 0.7 mg/kg), Bila Altanka (by 1.1 mg/kg), and Yulin 1 (by 0.5 mg/kg). Thus, a combination of high contents of fat and carotenoids is possible within one genotype. Slobozhanske, Omriyane, Bila Altanka, and Yulin 1 are valuable starting material for plant breeding to improve grain quality.

The quality of fat in grain depends on its fatty acid composition [11,13]. That is why it was important not only to determine the total content of each fatty acid in millet grain, but also to identify the cultivars with the most beneficial ratio of fatty acids.

The cultivars did not differ significantly in the myristoleic acid content (Table 1). The greatest amounts of myristoleic acid were recorded in Slobozhanske, Omriyane, Bohatyrske, and Zhodynske grain (by 0.01–0.02% higher than the mean value).

Omriyane, Bila Altanka, and Zhodynske had increased contents of palmitic acid (by 0.4%, 0.33%, and 0.24% higher than the mean value, respectively).

As to palmitoleic acid, the millet genotypes could be divided into two groups: with an increased content (0.51±0.02% to 0.60±0.02%) and with a low content of this acid (0.31±0.02% to 0.47±0.02%). Omriyane, Slobozhanske, Bohatyrske, Bila Altanka, and Kharkivske 57 belong to group 1, while Sozh, Yulin 1, and Zhodynske to group 2.

As to stearic acid, the millet varieties were also clearly divided into two groups: high content (3.09±0.03% to 3.50±0.03%) and low content (1.29±0.03% to 1.83±0.03%). Three cultivars (Slobozhanske, Bila Altanka, and Kharkivske 57) belong to the ‘high content’ group, and five (Omriyane, Bohatyrske, Sozh, Yulin 1, and Zhodynske) are in the ‘low content’ one.

Since humans are not able to biosynthesise palmitic and stearic acids, genotypes with the highest contents of these acids and a low ratio between them are very valuable. In our study we have found that the high-palmitic varieties are Slobozhanske, Bila Altanka, and Kharkivske 57. In these cultivars, the palmitic acid content was twice as high as the stearic acid level.

The data show that all millet genotypes differed significantly in the oleic acid content in grain. The lowest value was noted in Bila Altanka. In Slobozhanske, Omriyane, Kharkivske 57, and Zhodynske, this parameter was also below the mean value. In Sozh and Yulin 1, the oleic acid content exceeded the mean value, and Bohatyrske was characterised by the highest value. R. Shen *et al.*, 2018 [11], reported that the oleic acid content in millet grain did not exceed 24.66±0.07%. Thus, Sozh, Yulin 1, and Bohatyrske can be considered high-oleic varieties, which are valuable as sources of this trait in millet breeding for improved nutritional quality of grain.

Linoleic acid is thought to be the most valuable for human health. Six of the eight varieties under study had a high content of linoleic acid in grain: Omriyane, Sozh, Yulin 1, Bila Altanka, Kharkivske 57, and Zhodynske. Only Slobozhanske and Bohatyrske were low in linoleic acid.

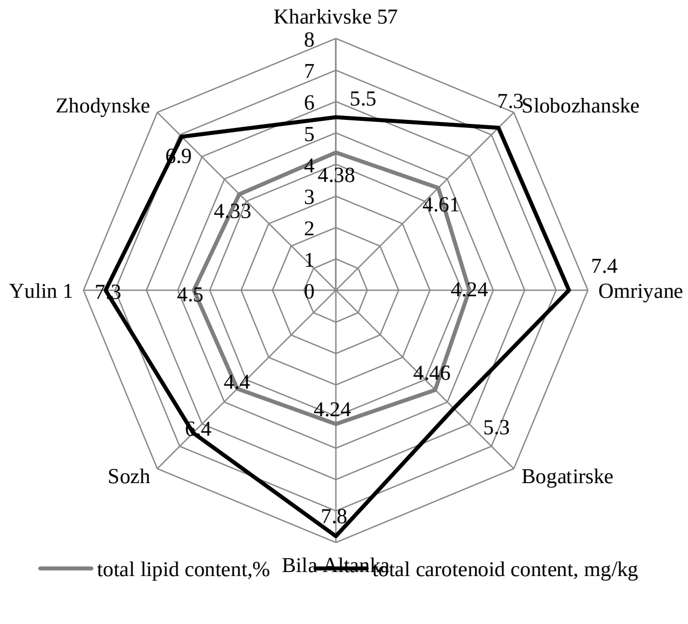

Fig. 1. The total contents of fat and carotenoids in millet grain, 2020–2021

Table 2 – Fatty acid composition of millet grain, % 2020–2021

Fatty acid	Genotypes name							
	Slobozhanske	Omriyane	Bohatyrske	Sozh	Yulin 1	Bila Altanka	Kharkivske 57	Zhodynske
C14:1	0.07±0.01	0.07±0.01	0.08±0.01	0.06±0.01	0.04±0.01	0.06±0.01	0.04±0.01	0.07±0.01
C16:0	7.09±0.07*	7.60±0.07*	7.19±0.07*	7.01±0.07*	7.17±0.07*	7.53±0.07*	6.86±0.07*	7.44±0.07*
C16:1	0.53±0.02*	0.60±0.02*	0.51±0.02*	0.47±0.02*	0.38±0.02*	0.53±0.02*	0.31±0.02*	0.47±0.02*
C18:0	3.50±0.03*	1.44±0.03*	1.83±0.03*	1.51±0.03*	1.29±0.03*	3.30±0.03*	3.09±0.03*	1.51±0.03*
C18:1	24.43±0.12*	24.38±0.12*	28.46±0.12*	26.08±0.12*	25.99±0.12*	22.80±0.12*	23.86±0.12*	24.92±0.12*
C18:2	61.37±0.19*	63.55±0.19*	59.49±0.19*	62.74±0.19*	62.45±0.19*	62.81±0.19*	63.24±0.19*	63.34±0.19*
C18:3	1.26±0.03*	1.38±0.03*	1.34±0.03*	1.25±0.03*	1.52±0.03*	1.28±0.03*	1.33±0.03*	1.26±0.03*
C20:0	0.82±0.04*	0.42±0.04*	0.51±0.04*	0.33±0.04*	0.41±0.04*	0.88±0.04*	0.81±0.04*	0.40±0.04*
C20:1	0.09±0.01	0.09±0.01	0.10±0.01	0.10±0.01	0.09±0.01	0.09±0.01	0.07±0.01	0.11±0.01
C22:0	0.69±0.04*	0.35±0.04*	0.38±0.04*	0.35±0.04*	0.55±0.04	0.51±0.04	0.27±0.04	0.36±0.04
C24:0	0.13±0.01*	0.09±0.01*	0.10±0.01*	0.07±0.01*	0.08±0.01*	0.19±0.01*	0.09±0.01*	0.09±0.01*

*significant difference at P<0.05

The contents of eicosenoic, behenic, and lignoceric acids in millet grain slightly differed with the varieties. Kharkivske 57 was characterised by a low content of eicosenoic acid, and Zhodynske by the highest content among the cultivars considered. Behenic acid increases cholesterol levels in blood, so genotypes with a low content of this acid are the most valuable. In our study, Kharkivske 57 was the lowest in behenic acid, and Slobozhanske showed the highest value. F. Al. Juhaimi *et al.*, 2019 [17], published the data that the average content of lignoceric acid in millet grain was 0.34±0.09%. Of the studied varieties, the highest content of lignoceric acid was detected in Bila Altanka, whereas Sozh and Yulin 1 were found to contain the smallest amounts of this fatty acid. Yulin 1 contained the greatest amount of linolenic acid. The other genotypes did not differ significantly in this parameter.

As to eicosanoic acid, the genotypes studied could be divided into two groups. The ones with its increased content were Slobozhanske, Bila Altanka, and Kharkivske 57, and those low in it were Omriyane, Bohatyrske, Sozh, Yulin 1, and Zhodynske. F. Al. Juhaimi *et al.*, 2019 [17], did not detect eicosanoic acid in millet grain.

Correlation analysis has been conducted to establish genetic relationships between the total contents of fat, carotenoids, and fatty acids in grain (Table 3). There were mostly weak or very weak correlations between these parameters. These data are consistent with B. Sharma *et al.*'s data, 2020 [26], where the correlations between the total fat content and fatty acids in grain were weak and negative. That is, it opens possibilities of breeding each trait individually and creating varieties higher in fat or carotenoids or with an improved fatty acid ratio. At the same time, there were moderate negative correlations between the carotenoid content and that of oleic acid and between the fat content and the linoleic acid level. There was a moderate negative correlation between the contents of fat and palmitic acid and, conversely, a moderate positive correlation between the carotenoid and palmitic acid contents. The behenic acid level was positively correlated both with the fat content and with the carotenoid content. This should be taken into account to select pairs for crossing when designing experiments in breeding.

The correlation coefficients between fatty acids in millet grain are presented in Table 4.

Table 3 – Correlation matrix between the total contents of fat and carotenoids, and the fatty acid composition in millet grain, 2020–2021

Fatty acid/carotenoids	Total content	
	fat	carotenoids
Total carotenoid content	-0.180	–
Myristoleic C14:1	-0.112	-0.020
Palmitic C 16:0	-0.612*	0.543*
Palmitoleic C16:1	-0.240	0.421
Stearic C18:0	0.191	0.065
Oleic C18:1, %	0.415	-0.550*
Linoleic C18:2	-0.598*	0.427
Linolenic C18:3	0.102	0.023
Eicosanoic C20:0	0.103	0.110
Eicosenoic C20:1	-0.059	0.105
Behenic C22:0	0.575*	0.522*
Lignoceric C24:0	-0.178	0.382

* significant difference at P<0.05

Table 4 – Correlation matrix between fatty acids in millet grain, 2020–2021

Fatty acid	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0
Palmitic C16:0	0.453									
Palmitoleic C16:1	0.747*	0.681*								
Stearic C18:0	-0.081	-0.209	-0.045							
Oleic C18:1	0.249	-0.239	-0.009	-0.574*						
Linoleic C18:2	-0.366	0.207	-0.177	-0.099	-0.717*					
Linolenic C18:3	-0.370	-0.012	-0.250	-0.414	0.226	-0.004				
Eicosanoic C20:0	-0.177	-0.172	-0.076	0.956*	-0.590*	-0.07	-0.239			
Eicosenoic C20:1	0.241	0.277	0.209	-0.410	0.303	-0.079	-0.351	-0.472		
Behenic C22:0	-0.009	0.027	0.219	0.355	-0.128	-0.286	0.093	0.342	0.001	
Lignoceric C24:0	0.186	0.384	0.339	0.693*	-0.533*	-0.074	-0.323	0.698*	-0.07	0.442

* significant difference at $P<0.05$

There were weak positive or negative correlations between fatty acids. Strong positive correlations were observed between the contents of palmitoleic and myristoleic acids, between the contents of palmitoleic and palmitic acids, between the contents of lignoceric and stearic acids, and between the contents of lignoceric and eicosanoic acids. Thus, it is possible to increase levels of palmitoleic, myristoleic, palmitic, lignoceric, and stearic acids in millet grain via breeding. Unfortunately, there is a strong negative correlation between linoleic and oleic acids. However, the research has demonstrated that, of the studied varieties, Sozh and Yulin 1 have elevated levels of both of these acids. Thus, these varieties can be used in the food industry and in millet breeding as valuable genotypes with increased levels of linoleic and oleic acids in grain. There were weak negative correlations between oleic and stearic acids, between eicosanoic and oleic acids, and between lignoceric and oleic acids. A. Zhang *et al.*, 2015 [27], conducted correlation analysis in foxtail millet (*Setaria italica* L.) and showed that oleic acid, too, was negatively correlated with linoleic and palmitic acids, while linolenic acid was positively correlated with palmitic and oleic acids and negatively with stearic acid.

During storage, millet grain and products undergo significant changes in their biochemical composition. Thus, the largest changes during long-term storage occur in flour, less pronounced changes in groats of millet, and the smallest in millet grain [8,15,20]. Determination of changes in the biochemical composition after five-year storage of millet grain intended for the production of groats or flour was an important part of this study.

The total fat content in Kharkivske 57 grain did not change significantly after 5-year storage and only decreased by 0.11%. P. Goyal *et al.*, 2015 [18], also reported that there was no significant difference in the fat content during storage of pearl millet.

After 5-year storage of Kharkivske 57 grain, the greatest changes occurred in the carotenoid content (Fig. 2).

The acid value is an important parameter determined during storage of flour or grain and indicating their rancidity degree or quality deterioration [28]. Groats and flour from pearl millet turn rancid quite quickly. This phenomenon is associated, first of all, with the fact that the acid value is high (1.1–7.5 mg KOH/g of substance) even in freshly-milled pearl millet flour. During flour storage, 2-fold to 5-fold increase in the acid value was noted, as compared with other crops. [8,18]. In unground grain stored for food purposes, the acid value rises more slowly [20]. Therefore, food millet should be stored unground, especially for long periods.

In our study, the acid value of millet fat from Kharkivske 57 grain harvested in 2020 was 5.10 mg of KOH/g of substance, while it was higher almost by 4.43 mg of KOH/g of substance (i. e. 2 times as high) in grain harvested in 2015. That is, even unhulled millet turns rancid quite quickly.

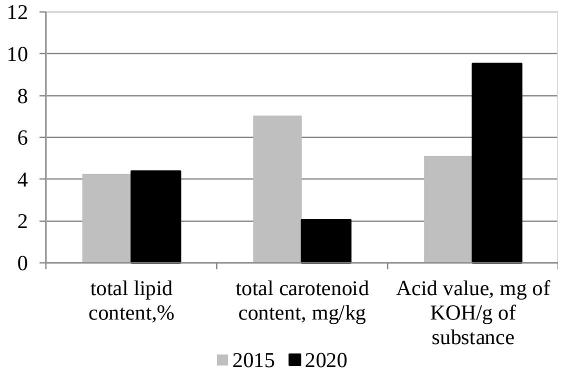

Fig. 2. Dynamics of the total carotenoid and fat contents and acid value in Kharkivske 57 grain during 5-year storage

It is important that during the storage of food-grade millet grain, its fatty acid composition did not change. On investigating pearl millet, C. C. Lai and E. Varriano-Marston, 1980 [15], confirmed that there were no qualitative changes in the fatty acid composition of grain. Nevertheless, they observed a slight increase in linoleic and oleic acids. When grain of different millet species (*Panicum miliaceum*, *Setaria italic*, *Pennisetum americanum*, *Echinochloa frumentacea*,and *Eleusine coracana*) was stored for up to 16 weeks, there was no significant difference in the quantity and quality of fatty acids either [20].

As Table 5 shows, there were no significant changes in the quantity and quality of fatty acids in Kharkivske 57 grain. The contents of palmitic, stearic, linoleic, linolenic, eicosanoic, eicosenoic, behenic, and lignoceric acids slightly increased during storage. Thus, the palmitic acid content increased by 24%, the stearic acid content by 1.04%, the linoleic acid content by 1.73%, the linolenic acid content by 0.17%, the eicosanoic acid content by 0.88%, the eicosenoic acid content by 0.04%, the behenic acid content by 0.19%, and the lignoceric acid content by 0.08%. The highest coefficients of variation were noted for eicosanoic, eicosenoic, behenic, and lignoceric acids. A slight decrease in the contents of myristoleic (by 0.01%), palmitoleic (by 0.01%), and oleic (by 0.70%) acids was observed.

Thus, during the storage of millet grain, eicosanoic, eicosenoic, behenic, and lignoceric acids were accumulated.

Table 5 – Changes in the fatty acid composition of Kharkivske 57 grain during long-term storage

Fatty acid	Mean		Coefficient of variation, %
	2020	2015	
Myristoleic C14:1, %	0.05±0.00	0.04±0.01	1.1
Palmitic C16:0, %	6.63±0.20*	6.87±0.27*	4.0
Palmitoleic C16:1, %	0.31±0.02	0.30±0.07	2.8
Stearic C18:0, %	1.79±0.08	2.83±0.06	2.1
Oleic C18:1, %	23.86±0.29	23.16±0.40	1.7
Linoleic C18:2, %	63.24±0.45*	64.97±0.96*	1.5
Linolenic C18:3, %	1.33±0.04*	1.50±0.08*	5.8
Eicosanoic C20:0, %	0.83±0.14	1.71±0.16	29.9
Eicosenoic C20:1	0.07±0.01	0.11±0.02	20.7
Behenic C22:0, %	0.27±0.10	0.46±0.12	29.9
Lignoceric C24:0, %	0.09±0.01	0.17±0.04	31.8

* significant difference at $P<0.05$

Conclusion

The mean value of the total fat content in millet grain was 4.4±0.12%, but the cultivars did not differ significantly in this parameter. In the millet genotypes under investigation, the mean value of the total carotenoid content was 6.7±0.94 mg/kg. The fatty acid composition of millet fat was notable for the highest contents of linoleic (62.4±1.32%) and oleic (25.1±1.67%) acids, indicating high nutritional value of this cereal. Millet grain contained the minimal amounts of myristoleic (0.06±0.01%), palmitoleic (0.5±0.09%), eicosanoic (0.6±0.22%), eicosenoic (0.09±0.01%), behenic (0.4±0.14%), and lignoceric (0.10± 0.04%) acids. Of the biochemical parameters of grain, the total contents of fat (2.7%) and carotenoids (13.9%) as well as the content of linoleic acid (2.11%) had low coefficients of variation, suggesting that increasing these parameters via breeding is quite a challenge. The coefficients of variation have shown that the greatest fluctuations in millet grain were those in myristoleic (24.70%), stearic (41.65%), eicosanoic (38.20%), behenic (32.44%), and lignoceric (35.19%) acids. Thus, it is possible to create millet varieties with increased contents of myristoleic and stearic acids, which are also important for human vital functions.

Slobozhanske had an increased fat content (4.61%), which was by 0.27% higher than the mean value. As to the carotenoid content, the cultivars significantly differed: in Slobozhanske, Omriyane, Bila Altanka, and Yulin 1, this parameter exceeded the mean value by 0.6 mg/kg, 0.7 mg/kg, 1.1 mg/kg, and 0.5 mg/kg respectively. Thus, a combination of high contents of fat and carotenoids is possible within one genotype. The varieties did not differ significantly in the myristoleic acid content in grain. Omriyane, Bila Altanka, and Zhodynske were distinguished because of an increased content of palmitic acid: their values were higher than the mean by 0.4%, 0.33%, and 0.24% respectively. As to palmitoleic acid, the millet genotypes could be divided into two groups: with an increased content of palmitoleic acid (0.51±0.02% to 0.60±0.02%) and with a low content (0.31±0.02% to 0.47±0.02%). Omriyane, Slobozhanske, Bohatyrske, Bila Altanka, and Kharkivske 57 belong to group 1, and Sozh, Yulin 1, and Zhodynske to group 2. As to stearic acid, the millet varieties clearly fall into two groups too: with a high content (3.09±0.03% to 3.50±0.03%) and with a low content (1.29±0.03% to 1.83±0.03%). Three cltivars (Slobozhanske, Bila Altanka, and Kharkivske 57) belong to the group with an increased content of stearic acid. Five of the varieties studied (Omriyane, Bohatyrske, Sozh, Yulin 1, and Zhodynske) belong to the low content group. We have identified high-palmitic varieties (Slobozhanske, Bila Altanka and Kharkivske 57): in their grain, the palmitic acid content is 2 times as high as the stearic acid level. All millet genotypes differed significantly in the oleic acid content in grain. The lowest level of this parameter was detected in Bila Altanka. The oleic acid content in Sozh and Yulin 1 was higher than the mean value, and the highest level of this parameter was detected in Bohatyrske. Thus, Sozh, Yulin 1, and Bohatyrske can be considered high-oleic varieties, which makes them valuable sources of this trait in millet breeding to improve the nutritional quality of grain. Six varieties showed a high content of linoleic acid in their grain: Omriyane, Sozh, Yulin 1, Bila Altanka, Kharkivske 57, and Zhodynske. The contents of eicosenoic, behenic, and lignoceric acids in millet grain slightly differed with the variety.

There were moderate negative correlations between the carotenoid and oleic acid contents (r=-0.550) and between the fat and linoleic acid contents (r=-0.598). There were strong positive correlations between palmitoleic and myristoleic acids (r=0.747), between palmitoleic and palmitic acids (r=0.681), between lignoceric and stearic acids (r=0.693), and between lignoceric and eicosanoic acids (r=0.698). The oleic acid content was strongly and negatively correlated with other acids: linoleic (r=-0.717), stearic (r=-0.574), eicosanoic (r=-0.590) and lignoceric (r=-0.533). These negative relationships will complicate breeding to create new cultivars with high grain quality.

The results have demonstrated that one of the causes why the quality of Kharkivske 57 grain deteriorated during 5-year storage was a decrease in the total contents of fat and carotenoids and an increase in the acid value of fat. The total fat content in Kharkivske 57 grain decreased by 0.11%. The total carotenoid content in grain harvested in 2020 was 7.03±0.05 mg/kg, while after 5-year storage, the total carotenoids decreased to 2.29±0.29 mg/kg (by 4.74 mg/kg). The acid value of fat from Kharkivske 57 grain harvested in 2020 was 5.10 mg of KOH/g of substance, and it increased almost by 2 times in grain harvested in 2015 (to 9.53 mg of KOH/g of substance). No significant changes were observed in the quantity and quality of fatty acids in Kharkivske 57 grain.

Thus, the selected sources with increased nutritional qualities of millet grain should be used in plant breeding to obtain grain and products with high nutritional value. It has been established that long-term storage of millet grain results in a sharp deterioration of its quality, indicating the need to use food-grade grain within five years.

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БІОХІМІЧНИЙ СКЛАД ЗЕРНА ПРОСА ТА ЙОГО ЗМІНИ ПРИ ЗБЕРІГАННІ

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Анотація. У роботі досліджено загальний вміст жиру, жирнокислотний склад, загальний вміст каротиноїдів в зерні проса 8 сортів 2020-2021 рр ВРОЖАЮ. Середнє значення загального вмісту жиру в зерні проса становило 4.4±0.12%, зразки за цією ознакою суттєво не різнилися. Середнє значення загального вмісту каротиноїдів - 6.7±0.94 мг/кг. Жирнокислотний склад сортів проса характеризувався найбільшим вмістом линолевої (62.4±1.32%) та олеїнової кислот (25.1±1.67%), що вказує на високу харчову цінність пшона. Вміст жирних кислот в зерні проса за їх кількістю можна показати за рівнем зменшення: С18:2 > С18:1 > С16:0 > С18:0 > С18:2 > С24:0 > С20:0 > С16:1 > С22:0 > С20:1 > С14:1. Високий вміст лінолевої кислоти в зерні був у сортів Омріяне (63.6±0.07%), Сож (62.7±0.34%), Юйлінь 1(62.4±0.03%), Біла Альтанка (62.8±0.04%), Харківське 57 (63.2±0.45%) та Жодінське (63.3±0.31%). Сорти Богатирське, Сож і Юйлінь 1 характеризувалися вмістом олеїнової кислоти вище за середнє (28.5±0.15%, 26.1±0.19% та 26.0±0.01% відповідно). Це свідчить про можливість поєднання високого вмісту линолевої та олеїнової кислот в одному генотипі. Сорти Слобожанське, Омріяне, Біла Альтанка, Юйлінь 1 суттєво перевищили за вмістом каротиноїдів в зерні в порівнянні з середнім значенням на 0.6 мг/кг, 0.7 мг/кг, 1.1 мг/кг, 0.5 мг/кг відповідно. Між загальним вмістом жиру, каротиноїдами та жирнокислотним складом зерна існують, в основному, слабкі або дуже слабкі кореляції. Але олеїнова кислота має середні негативні зв'язки з вмістом каротиноїдів (r=-0,550), з линолевою (r=-0.717), стеариновою (r=-0.574), ейкозановою (r=-0.590) і лігноцериновою (r=-0.533) кислотами. Це свідчить про ускладнення процесу виведення високоякісних сортів проса селекційним шляхом. При довготривалому зберіганні зерна проса сорту Харківське 57 загальний вміст жиру суттєво не змінився. Через п'ять років зменшення вмісту каротиноїдів спостерігали на 4.74 мг/кг. Кислотне число у 2020 р. становило 5.10 мг КОН/г речовини. У процесі зберігання зерна через п'ять років кислотне число було вже 9.53 мг КОН/г речовини. За кількістю і якістю жирних кислот не спостерігали істотних змін.Але на протязі зберігання зерна проса відбулося незначне збільшення пальмітинової, стеаринової, линолевої, ліноленової, ейкозанової, ейкозенової, бегенової та лігноцеринової кислот.

Ключові слова: просо, жирнокислотний склад, жир, каротиноїди, кореляція, кислотне число, зберігання.