

QUALITY EVALUATION OF LIPID FRACTION OF MILLET GROATS (*PANICUM MILIACEUM* L.)

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Summary. The aim of the study was quality assessment of lipid fraction extracted from millet groats. The composition of fatty acids was determined by means of gas chromatography, distribution of fatty acids was defined using the Brockerhoff method with partial hydrolysis of triacylglycerols. Additionally, oxidative stability was tested by the use of pressure differential scanning calorimetry. For better investigation of oil quality, acid and peroxide values were also determined. Millet oil turned out to be a rich source of unsaturated fatty acids. Fresh millet oil was characterized by the longest oxidation induction time. During the storage test, the oxidation induction time decreased, while the acid and peroxide values increased. No significant changes were detected in the fatty acid composition. After six weeks of storage, oil from millet groats was characterized by good quality.

Key words: millet groat, fatty acid composition, fatty acid distribution, oxidative stability, acid value, peroxide value

INTRODUCTION

One of the most important groups relevant to preparing a balanced diet are cereal products. Recently, one of the products highly valued by consumers is millet groat. It is called “queen porridge”, what results from many of the health-related properties attributed to it [Abdalla et al. 1998, Chethan et al. 2009, Shobana et al. 2009, Coulibaly et al. 2011, Singh et al. 2012, Saleh et al. 2013]. Current dietary norms indicate that special attention should be paid to lowering the intake of saturated fatty acids. On the other hand,

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the products present in usual meals are low in omega-3 and omega-6 fatty acids. Taking the above into account, it is important to study food products in terms of the quality of the lipid fraction. Millet is not a typical grain in our climate zone and for this reason there is still little research on its chemical composition. Therefore, it is reasonable to conduct research aimed at understanding the properties of the grain.

The aim of the study was to assess the quality of fat fraction of millet groat and to determine the impact of three and six weeks storage on the changes occurring in the sample. The experiments included: extraction of lipid fraction from millet groats and evaluation of the quality of fat fraction by examining such parameters as: peroxide value, acid value, oxidation induction time, composition of fatty acids and their distribution in triacylglycerol structure.

MATERIAL AND METHODS

Material

Millet (*Panicum miliaceum* L.) was purchased from one of the polish leading producers. Millet and the extracted lipid fraction were stored at room temperature (20°C) to ensure standard storage conditions for cereal products.

Methods

After homogenising in a laboratory mill, the millet sample (50 g) was placed in 250 ml flasks, then 100 ml of hexane was added. The samples were placed in a shaker. After an hour of shaking, to separate the resulting precipitate, the solution was filtered off. Then, the hexane was evaporated under reduced pressure. To remove residual hexane, the sample was dried under nitrogen.

Determination of fatty acid composition by means of gas chromatography

Fatty acids were converted to volatile methyl esters in accordance with PN-EN ISO 5509: 2001. The method of gas chromatography (GC) with a capillary column and a flame ionization detector was used to determine the composition of fatty acids in the fat fraction of millet groat.

Distribution of fatty acids in the sn-2 and sn-1,3 positions of triacylglycerols

One of the methods of studying the distribution of fatty acids in specific triacylglycerol positions is the Brockerhoff method – partial hydrolysis of triacylglycerol carried out in the presence of an enzyme – pancreatic lipase [Brockerhoff 1965; Haduka et al. 2003]. The content of fatty acids in the sn-1,3 positions was calculated from the following formula on the basis of information on the initial composition of triacylglycerols and the composition of the sn-2 monoacylglycerols obtained:

$$\text{sn-1,3} = [3 \times (\text{FA in TAGs}) - (\text{FA in sn-2 MAG})] / 2$$

where:

sn-1,3 – content of a given fatty acid in the sn-1 and sn-3 positions [%],

FA in TAGs – content of a given fatty acid in starting triacylglycerols [%],

FA in sn-2 MAG – content of a given fatty acid in sn-2 monoacylglycerols [%].

The percentage of selected fatty acids in sn-2 positions in relation to the total content of a given fatty acid was calculated basing of the following formula:

$$\text{sn-2} = (\text{FA in sn-2 MAG}) / [3 \times (\text{FA in TAGs})]$$

Determination of oxidative stability

The experiment was carried out by placing a fat sample (3–4 mg) in an aluminum pan. The sample thus prepared was placed in the chamber of the ThermoAnalysis DCS Q20, together with the empty pan as a reference sample. The test was carried out under isothermal conditions at temperatures of 100°C, 120°C and 140°C. The fat was oxidized at a pressure of 1400 kPa.

Determination of acid value

To determine acid value of the tested fat fraction, 5 g of extracted fat was prepared and placed in a 250 ml flask. Then 50 ml of a neutralized mixture of ethyl alcohol and diethyl ether and three drops of phenolphthalein were added to the flask. The sample was titrated with a solution of 0.1 mol·dm⁻³ potassium hydroxide. The acid number was calculated according to the formula:

$$LK = [(a-b) \times 5.611]/m$$

where:

a – volume of 0,1 molar KOH solution used for titration of the correct sample [ml],

b – volume of 0,1 molar KOH solution used for blank titration [ml],

m – the weight of fat [g],

5.611 – amount of mg KOH in 1 cm³ of a 0.1 mol·dm⁻³ potassium hydroxide solution.

Determination of peroxide value

Peroxide value was determined according to PN-EN ISO 3960:2012. Peroxide value (*PV*) expressed in millimols of active oxygen per kilogram of fat [meqO₂·kg⁻¹ fat] was calculated according to the formula:

$$PV = (V - V_0) \times c/m$$

where:

V – volume of the sodium thiosulphate solution used for titration of the fat sample [cm³],

*V*₀ – volume of the solution of sodium thiosulphate used for blank titration [cm³],

c – concentration of sodium thiosulphate solution [mmol·dm⁻³],

m – mass of fat [g]

Statistical analysis

Test results were analyzed using the STATGRAPHICS Plus software and the Excel spreadsheet. The statistical analysis of the results was carried out using a one-way analysis of variance with the Tukey test at the significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Vegetable fats, as compared to animals, are characterized by a lower content of saturated fatty acids and a higher content of mono- and polyunsaturated fatty acids. Unsaturated fatty acids present beneficial effects on the human body including reducing the level of triacylglycerols and LDL cholesterol in blood [Achremowicz and Szary-Sworst 2005]. Extremely important from the nutritional point of view is to provide the body with the essential unsaturated fatty acids (EFA). In addition, special attention should be paid to the appropriate ratio of n-6 to n-3, which should reach 4-5:1. In the research the composition of fatty acids of millet oil was determined by gas chromatography. The results are presented in Table 1.

Table 1. Fatty acid composition and their percentage content of the millet oil. SFAs: saturated fatty acids, MUFAs: monounsaturated fatty acids, PUFAs: polyunsaturated fatty acids

Tabela 1. Skład kwasów tłuszczowych i ich procentowa zawartość w oleju z kaszy jaglanej. NKT – nasycone kwasy tłuszczowe, JKT – jednonienasycone kwasy tłuszczowe, WKT – wielonienasycone kwasy tłuszczowe

Fatty acids Kwasy tłuszczowe	% total fatty acids % kwasu tłuszczowego						
SFAs	C16:0	C17:0	C18:0	C20:0	Σ SFAs		
NKT	7.58 ±0.17	0.06 ±0.02	1.97 ±0.04	0.71 ±0.02	10.32 ±0.24		
MUFAs	C16:1	C17:1	C18:1 (n-9)	C20:1 (n-9)	Σ MUFAs		
JKT	0.12 ±0.01	0.13 ±0.07	24.99 ±0.02	0.76 ±0.03	26.00 ±0.12		
PUFAs	C18:2 (n-6)	C18:3 (n-3)	C20:2 (n-6)	C20:3 (n-3)	C22:2 (n-6)	C20:5 (n-3)	Σ PUFAs
WKT	59.66 ±0.35	1.00 ±0.02	0.15 ±0.02	0.61 ±0.09	1.86 ±0.19	0.40 ±0.05	63.68 ±0.12

Data represent mean ± standard deviation of 3 independent samples. Podane wartości stanowią średnie ± odchylenia standardowe z 3 analiz.

The oil tested was characterized by a high content of polyunsaturated fatty acids, among which linoleic acid (C18:2) dominated (59.66%). In the group of monounsaturated fatty acids, the highest amount of oleic acid (C18:1) was found and accounted for 24.99%. Among saturated fatty acids, palmitic acid (16:0) was detected on the highest level (7.58%). The similar content of polyunsaturated fatty acids was obtained in the Ghodsizad and Safekordi [2012] study. The oil examined was characterized by a higher content of linoleic acid (64.58%). However, oleic and palmitic acid occurred in compa-

rable amounts, amounting to 24.18% and 6.11%, respectively. According to McDonough et al. [2000] the content of linoleic acid and palmitic acid in millet oil was on higher level, 65.40% and 9.8%, respectively, while oleic acid accounted for 21.10%. The stearic acid content determined was the same as in this study (2%). However, the linolenic acid content was significantly higher and amounted to 2.1%.

In Figure 1, the comparison of fatty acids composition of millet oil with commonly used edible oils – rapeseed and sunflower oil – has been presented. Both compared oils are characterized by a lower content of palmitic acid compared to millet oil, in the case of rapeseed oil – 4.6%, and sunflower oil – 6.2% [Krygier et al. 1998]. The highest content of monounsaturated fatty acids was observed in rapeseed oil, in the case of which oleic acid accounted for 50.7%. In millet oil, its content was 25.0%, and in sunflower seeds – 18.6%. The largest group of fatty acids, both in millet and sunflower oil, were n-6 polyunsaturated fatty acids. In millet oil, the share of linoleic acid was 59.7%, while in sunflower fruit – 66.1%. Rapeseed oil proved to be significantly poorer in linoleic acid, as its content was only 19%. The richest source of omega-3 acids from the compared oils is rapeseed oil (9.9%), whereas millet oil contains only 1.0% and sunflower oil – 3.1%. In addition to the fatty acids from the n-6 and n-3 families, their ratio, which should be 4:1, is extremely important. The closest to this result is rapeseed oil with the ratio of n-6/n-3 amounting 2:1. It is worth noting that the oil provides a significant amount of omega-3 acids, which are missing in a typical Polish diet. Millet oil and sunflower oil present an unfavorable from nutritional point of view ratio of n-6 to n-3, which is 30:1 and 20:1, respectively.

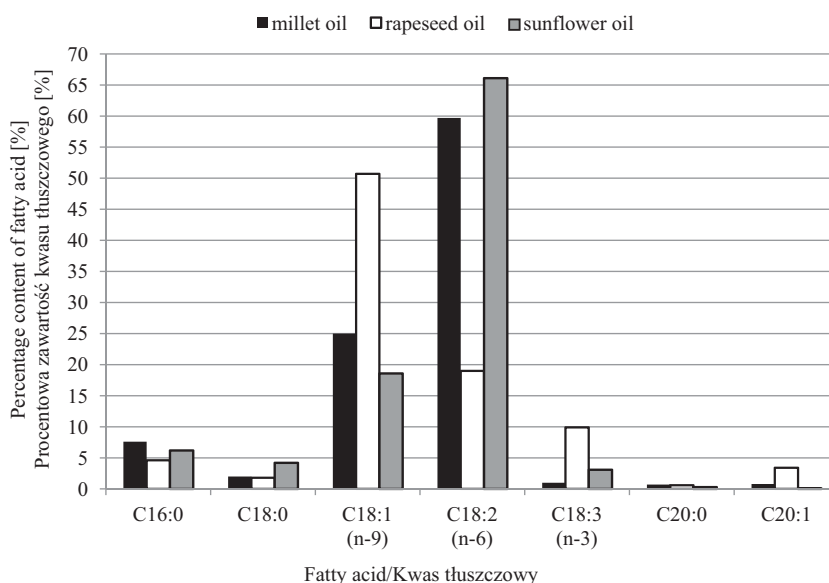


Fig.1. Comparison of percentage content of selected fatty acids present in selected oils: millet oil [our study], rapeseed oil and sunflower oil [Krygier et al. 1998]

Rys.1. Porównanie procentowej zawartości kwasów tłuszczowych w wybranych olejach: oleju z kaszy jaglanej [badania własne], oleju rzepakowego i słonecznikowego [Krygier i in. 1998]

The composition of fatty acids in triacylglycerols is an extremely important factor affecting their quality. However, apart from the composition, the distribution of fatty acids in the triacylglycerol molecule is also important. Fatty acids can be located in external (sn-1,3) or internal (sn-2) positions of triacylglycerol [Hazuka et al. 2003b]. The location of a particular acid residue in a given triacylglycerol position is extremely important from the nutritional point of view, since pancreatic lipase, which breaks down triacylglycerols, removed fatty acids only from external positions. The situation in which saturated fatty acids occupy the position of sn-2 positively affects the absorption of fats [Ziemlański and Budzyńska-Topolowska 1991, Wirkowska et al. 2012]. In Table 2, the results of fatty acid composition in sn-1,3 and sn-2 positions and the share of individual fatty acids in the sn-2 position were collected. On the basis of the presented data, it can be seen that sn-2 position is occupied mainly by linoleic acid (58.70%) and its share in this position reached 32.80%. The content of oleic acid in the sn-2 position was 21.90% and its share in this item accounted for 29.20%. Among the saturated fatty acids the highest amount of palmitic acid (9.00%) was determined in the sn-2 position and its percentage distribution in the internal position reached 39.70%. It can be seen, that the highest share in the internal position was noted for stearic acid (81.20%), but its amount was only 4.80%.

Table 2. Composition and percentage distribution of selected fatty acids in external (sn-1,3) positions and internal position (sn-2) of triacylglycerols (TAG) from millet oil

Tabela 2. Skład wybranych kwasów tłuszczowych w pozycjach zewnętrznych (sn-1,3) oraz wewnętrznej (sn-2) tłuszczu z kaszy jaglanej oraz udział poszczególnych kwasów tłuszczowych w pozycji sn-2

Fatty acid Kwas tłuszczowy	Fatty acid content in TAG [%] Skład kwasów tłuszczowych w TAG [%]	Fatty acid content in sn-2 [%] Skład kwasów tłuszczowych w pozycji sn-2 [%]	Fatty acid content in sn-1,3 [%] Skład kwasów tłuszczowych w sn-1,3 [%]	Percentage distribution of fatty acid in sn-2 [%] Udział danego kwasu tłuszczowego w sn-2 [%]
C16:0	7.58	9.00	6.80	39.70
C18:0	1.99	4.80	0.60	81.20
C18:1 (n-9)	24.99	21.90	26.50	29.20
C18:2 (n-6)	59.66	58.70	60.10	32.80
C18:3 (n-3)	1.00	1.20	0.90	40.90
C22:2 (n-6)	1.86	0.30	2.60	5.60

To test the resistance of millet fat to oxidation, the PDSC (Pressure Differential Scanning Calorimetry) test was carried out. The results are presented in Table 3.

Fresh millet oil was characterized by the longest oxidation induction time of 97.52 min (at 100°C), 38.44 min (at 120°C) and 8.08 min (at 140°C), respectively. After a three-week storage, the induction time was reduced to 84.42 min (at 100°C), 22.04 min (at 120°C) and 4.25 min (at 140°C). The subsequent storage (six weeks) influenced further shortening of the induction time to: 71.56 min (at 100°C), 16.33 min (at 120°C), and 3.40 min (at 140°C). There are significant differences between OIT at applied temperatures due to the fact that the fats oxidize faster at higher temperatures. At all temperatures test-

Table 3. Changes of oxidation induction time (OIT) of millet oil during storage

Tabela 3. Zmiany w czasie indukcji utleniania (OIT) oleju z kaszy jaglanej podczas przechowywania

Temperature [°C]	OIT 0 week [min]	OIT 3 weeks [min]	OIT 6 weeks [min]	OIT changes 0–3 weeks [%]	OIT changes 0–6 weeks [%]
Temperatura [°C]	0 tygodni [min]	3 tygodnie [min]	OIT 6 tygodni [min]	Zmiany w OIT 0–3 tygodnie [%]	Zmiany w OIT 0–6 tygodni [%]
100	97.52 ±2.03 ^a	84.42 ±2.88 ^b	71.56 ±2.15 ^c	-13.4	-26.6
120	38.44 ±0.45 ^a	22.04 ±1.62 ^b	16.33 ±0.36 ^c	-42.7	-57.5
140	8.08 ±0.26 ^a	4.25 ±0.64 ^b	3.40 ±0.20 ^c	-50.5	-61.7

ed, a significant reduction in induction time was observed after three and six weeks of fat storage. Thus, it can be concluded that storage of the lipid fraction negatively affects the oxidative stability of millet oil. The percentage change in induction time can be considered as an indicator of loss of oxidative stability of fat. The highest decrease in induction time occurred at 140°C, after three weeks of storage it amounted to as much as 50.5%, while after six weeks the induction time was shorter than the initial one by 61.7%.

Data represent mean ±standard deviation of 3 independent samples. Different letters indicate that the samples are significantly different at $p < 0,05$. Podane wartości stanowią średnie z 3 analiz wykonanych w 3 powtórzeniach. Wartości różniące się indeksami górnymi w kolumnach są statystycznie różne na poziomie istotności $p < 0,05$.

The acid value (AV) of fat informs about the amount of free fatty acids formed as a result of hydrolytic processes taking place in the sample. The results are presented in Figure 2.

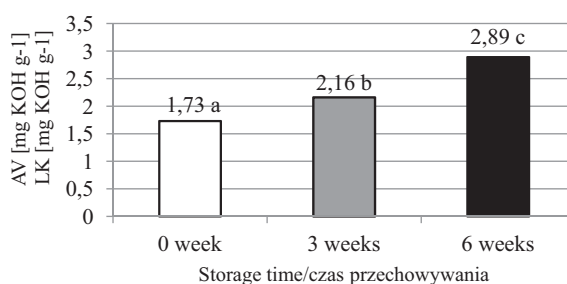


Fig. 2. Changes of acid values (AV) of millet oil during storage. Different letters indicate that the samples are significantly different at $p < 0,05$

Rys. 2. Zmiany w wartości liczby kwasowej (LK) dla oleju z kaszy jaglanej podczas przechowywania. Wartości różniące się literami są statystycznie różne na poziomie istotności $p < 0,05$

Fresh millet oil was characterized by a relatively low acid value (1.73 mg KOH·g⁻¹). During storage, acid value increased and after three and six weeks it reached the values of 2.16 mg KOH·g⁻¹ and 2.89 mg KOH·g⁻¹, respectively. After a six-week storage period, the acid value was still within the recommendations of Codex Alimentarius. The content

of free fatty acids (FFA) in millet oil was calculated based on the acid value. Fresh millet oil was characterized by the lowest content of free fatty acids (0.86%). During fat storage, the amount of FFA increased and after three and six weeks of storage it reached 1.08% and 1.42%, respectively. The increase in the content of free fatty acids results from the hydrolysis process taking place in the fat. The formation of the FFA during storage affects the deterioration of the quality of fat.

The peroxide value (PV) informs about the degree of fat oxidation, it expresses the accumulation of the primary oxidation products, i.e. peroxides. Figure 3 illustrates the changes in the peroxide value during the storage test. Fresh oil was characterized by a low peroxide value ($1.27 \text{ mEq O}_2 \cdot \text{kg}^{-1}$). After three weeks of storage, PV increased to $4.89 \text{ mEq O}_2 \cdot \text{kg}^{-1}$ and after six weeks – to $7.29 \text{ mEq O}_2 \cdot \text{kg}^{-1}$. Codex Alimentarius informs that the peroxide value for cold pressed oils should be lower than $10 \text{ mEq O}_2 \cdot \text{kg}^{-1}$. During the six-week storage test, a significant increase in this ratio was noted, however, the peroxide value did not exceed the permitted norm. Such a significant increase in the peroxide value indicates a progressive process of fat oxidation.

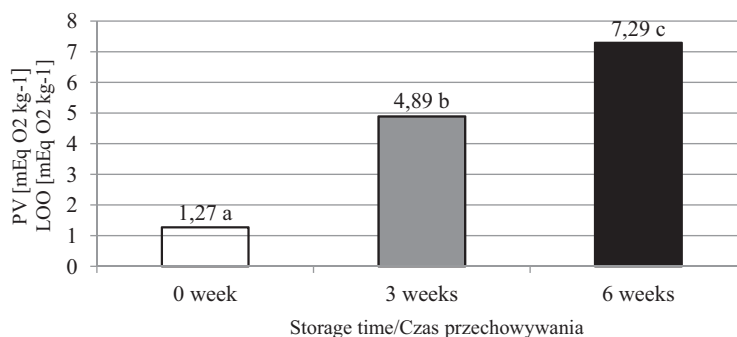


Fig. 3. Changes of peroxide value (PV) of millet oil during storage. Different letters indicate that the samples are significantly different during storage at $p < 0,05$

Rys. 3. Zmiany w wartości liczby nadtlenkowej (LOO) dla oleju z kaszy jaglanej podczas przechowywania. Wartości oznaczone tą samą literą nie różnią się istotnie statystycznie przy poziomie istotności $p < 0,05$

CONCLUSIONS

The study showed that millet oil is a rich source of unsaturated fatty acids, the majority of which are polyunsaturated acids. Three and six-week storage of the lipid fraction did not affect significant changes in fatty acid composition. The proportion of saturated fatty acids in the internal position was high, while the share of unsaturated acids in this position was low. Such structure of triacylglycerols positively influences the digestibility of fat. After three- and six-week storage, the oxidation induction time was shortened. The change in the induction time during the test indicates fat oxidation processes. The acid value increased after three weeks of storage by 25% and was followed by a 65% increase after six weeks. All values were within the norm. Peroxide value increased fourfold after

three-weeks of storage, and was six times higher after six weeks. Despite the significant increase, the peroxide value throughout the test period was within the recommendations.

Acknowledgements

To Paweł Latuszkiewicz for help in carrying out the research.

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OCENA JAKOŚCI FRAKCJI LIPIDOWEJ KASZY JAGLANEJ (*PANICUM MILIACEUM* L.)

Streszczenie. W niniejszej pracy przedmiotem badań była frakcja lipidowa z kaszy jaglanej. Olej wyekstrahowano z kaszy jaglanej za pomocą heksanu. Badany olej scharakteryzowano poprzez wyznaczenie liczby kwasowej, zawartości wolnych kwasów tłuszczowych i liczby nadtlenkowej. Oznaczono również skład kwasów tłuszczowych oraz rozmieszczenie kwasów tłuszczowych w pozycjach sn-2 i sn-1,3 triacylogliceroli. Oceniono stabilność oksydacyjną tłuszczu przy użyciu metody PDSC. Ponadto przeprowadzono badanie przechowalnicze, w którego trakcie zbadano wpływ czasu przechowywania oleju z kaszy jaglanej na zmianę badanych parametrów. Olej z kaszy jaglanej okazał się bogatym źródłem nienasyconych kwasów tłuszczowych. Stabilność oksydacyjna oleju była porównywalna z innymi tłuszczami roślinnymi. Liczba nadtlenkowa oraz liczba kwasowa świeżego oleju z kaszy jaglanej mieściła się w normie. W trakcie próby przechowalniczej czas indukcji utleniania uległ skróceniu, liczba kwasowa oraz nadtlenkowa wzrosły, natomiast w składzie kwasów tłuszczowych nie wykryto istotnych zmian. Po 6-tygodniowym okresie przechowywania olej z kaszy jaglanej cechował się dobrą jakością.

Słowa kluczowe: olej z kaszy jaglanej, skład kwasów tłuszczowych, rozkład kwasów tłuszczowych, stabilność oksydacyjna, liczba kwasowa, liczba nadtlenkowa