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Influence of bioutiluzation on quality and safety indicators of sunflower husk

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Abstract. Sunflower husk is formed during the production of sunflower oil and due to its poor digestibility by the body, cannot be widely used for animal feed. The authors studied the effect of microbiological fermentation during fermentation for 12 and 24 hours on the quality and safety indicators of sunflower husk. The results of laboratory studies have proven that 1) regardless of the time of microbiological fermentation, the mass fraction of moisture, crude fat, and crude fiber significantly decreased; the mass fraction of crude protein, crude ash, and starch content increased; the level of metabolic energy for all animal species and the content of soluble carbohydrates increased, but the difference was not significant; 2) the resulting fermented product meets the biological and chemical safety requirements for animal feed.

Key words: utilization, microbiological fermentation, physicochemical quality indicators, mycotoxins, chemically hazardous substances, GMO, Genetically Modified Organism

Authors' contributions. *Mironova O.A.* – conceptualisation, development of research methodology, work with software, data curation, writing – preparation of draft manuscript; *Mironova L.P.* – visualisation, conducting the study; *Kirichuk A.A.* – scientific supervision of the study; *Karmazin A.P.* – software, data validation. All authors were familiarised with the final version of the article and approved it.

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Влияние биоутилизации на показатели качества и безопасности лузги подсолнечника

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Аннотация. Подсолнечная лузга образуется при производстве подсолнечного масла и из-за плохой усвояемости организмом не может широко использоваться для кормления животных. Авторы изучили влияние микробиологического ферментирования при ферментации в течение 12 и 24 ч на показатели качества и безопасности лузги подсолнечника. По результатам лабораторных исследований доказано, что, во-первых, независимо от времени микробиологического ферментирования, достоверно уменьшились массовая доля влаги, сырого жира, сырой клетчатки; увеличились массовая доля сырого протеина, сырой золы, содержание крахмала; показатель уровня обменной энергии для всех видов животных и содержание растворимых углеводов выросли, однако разница была недостоверной; во-вторых, полученный ферментированный продукт отвечает требованиям биологической и химической безопасности, предъявляемым к кормам для животных.

Ключевые слова: утилизация, микробиологическое ферментирование, физико-химические показатели качества, микотоксины, химически опасные вещества, ГМО, генетически модифицированные организмы

Вклад авторов. *Миронова О.А.* – концептуализация, разработка методологии исследования, работа с программным обеспечением, курирование данных, написание – подготовка черновика рукописи; *Миронова Л.П.* – визуализация, проведение исследования; *Киричук А.А.* – научное руководство исследованием; *Кармазин А.П.* – программное обеспечение, валидация данных. Все авторы ознакомлены с окончательной версией статьи и одобрили ее.

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Introduction

Solid wastes in sunflower oil production are sunflower seed cleaning wastes, which are annually generated about 100 thousand tonnes, a quarter of which is disposed of in landfills. These wastes create environmental and economic problems for oil and fat enterprises and the territories where they are located [1; 2]. Another solid waste, sunflower husk, is produced during sunflower oil production in the amount of 1–2 million tonnes annually. The husk, separated from sunflower seeds in the process of their preparation for oil extraction, is a woody plant tissue, homogeneous in physical structure, with a constant chemical composition and physical and mechanical properties [3].

The husks are pelletised, briquetted or burned in pure form to obtain process steam and even electricity for its use in production [4; 5], produce thermal insulation and abrasive materials [6; 7, p. 155].

In addition, husks are used in animal feeds to reduce feeding costs, but the percentage of utilisation is low [8; 9].

The husk contains high amounts of crude fibre and other nutrients important for proper digestion of animals and for increasing the content of additional nutrients in feed. However, the nutrients contained in the native husk are not readily available for animal digestion [10].

Scientists are working on finding ways to improve the nutritional properties of sunflower husk and its availability for the digestive function of the body. Thus, there are known scientific developments on creation of animal feed and feed additives based on sunflower husks[1] [11; 12].

Biofermentation with the help of microorganisms is an effective method of utilisation of cellulose-containing waste from agricultural

[1] Miroshnikov SA, Sheda EV, Duskayev GC, Proskurin DA., Ovetchkin MV, Rahmatullin SG. *Patent 2 817 620 C1 Russian Federation, MPC A23K50/10 A23K10/30. Feed supplement for cattle on the basis of fermented sunflower seed: RU2023131771A; tender no. 12.04.2023*; available only in French. 17.04.2024; applicant and patent holder: Federal State Budget Scientific Institution "Federal Scientific Center of Biological Systems and Agro-Technologies of the Russian Academy of Sciences". Bulletin 11; Stepanova OV, Stepanov VV. *Patent on the invention 2,667,784 Russian Federation, MPC A23K 50/00, A23K 10/30. Feed for animals on the basis of sunflower husk and method of its production: applied for. 12.09.2017; used. 24.09.2018*; patent holder: Society with limited liability Scientific and production association "ROST". Bulletin 27.

production.[2] A specially selected association of microorganisms, named Lesnov's starter in honour of the author, the effect of which is based on the method of solid-phase biofermentation, has been tested on many substrates, including wheat and rye straw, oyster mushroom cultivation waste, bran, etc. with positive results [2; 14]. It is proved that the preparation increases the nutritive value of roughage by 80-100%, starchy and sugary forages by 15-20%, enriches forages with vitamins B, D, PP, K, E, H, and does not adversely affect the biological and chemical safety indicators [6; 4; 8].

In the literature sources available to us, we found no publications on scientific studies on the effect of microbiological fermentation using Lesnov's starter on sunflower husk.

The aim of the study was to investigate the effect of microbiological fermentation during fermentation for 12 and 24 h on quality and safety parameters of sunflower husk.

Materials and methods

Studies of native and fermented sunflower husk samples were carried out in 2024. 18 sunflower husk samples were studied: six samples before fermentation (native), six subjected to twelve-hour fermentation, and six after twenty-four-hour fermentation using Lesnov's starter. Physico-chemical quality parameters, the content of mycotoxins: aflatoxin B1, deoxynivalenol, zearalenone, ochratoxin A, T-2 toxin; pesticides, nitrates and nitrites, toxic elements and GMOs were investigated in the Testing Laboratory of FGBU 'Centre for Grain Quality Assessment' in Moscow and Moscow region according to the current regulations. According to the current regulatory documentation (RD) with the use of methods and techniques of laboratory research of the tested substrates: qualitative and quantitative chemical analysis; high-performance liquid chromatography (HPLC); gas chromatography (GC); atomic absorption spectrometry and others. Laboratory methods of quality research (GOST R 54951-2012; GOST 27979-88; GOST 13496.4-2019 item 8; GOST 32905-14; GOST 31675-2012 item 7; GOST 26226-95 item 1; GOST 26176-2019 item. 9; GOST R 54078-2010 appendix A; GOST ISO 6493-2015; GOST 26483), chemical elements (GOST 32343-2013) and feed safety: mycotoxins (GOST 30711-2001; GOST EN 15851-2013; GOST 31691-2012; GOSTMUK 4. 1 2204-07; instruction P43/B); pesticides (DIN EN 15662 2018); nitrates (GOST 13496 19-2015), nitrites (GOST 13496 19-2015); toxic elements (GOST R 53100-2008; GOST 31 650-2012), GMOs (GOST R 53214-2008).

[2] Lesnov PA. Patent No. 2 122 330 C1 Russian Federation, MPK A23K 1/12. *Method of using leaven in feed mixture. Lesnov's starter for feeder mixture*. Avv. 02.10.1997; published 27.11.1998 (In Russ.) EDN: ZRHZQL

Results of the research

After microbiological fermentation of sunflower husk for 12 h the mass fraction of moisture decreased by 9.6%, after fermentation for 24 h – by 22.3% (*).

The mass fraction of crude fat in dry matter after substrate fermentation for 12 h decreased by 1.3 times (*) in comparison with the initial level, after treatment for 24 h – by 1.8 times (*).

The mass fraction of crude protein in terms of dry matter increased by 6.7% after 12 h of fermentation and by 23.0% after 24 h of sunflower husk processing compared to the native product (*) (Table 1).

Table 1. **Effect of biofermentation duration on physico-chemical parameters of sunflower husks**

Indicators, units of measurement	Sample (n = 6)		
	before fermentation	after fermentation	
		12 h	24 h
Mass fraction of moisture, %	9.4 ± 1.2	8.5 ± 1.3	7.3 ± 1.1*
Mass fraction of crude fat, in terms of dry matter, %	9.8 ± 0.44	7.60 ± 0.46 *	5.40 ± 0.49 *
Mass fraction of crude protein in terms of dry matter, %	12.11 ± 0.66	12.92 ± 0.54	14.90 ± 0.67 *
Mass fraction of crude ash, in terms of dry matter, %	6.80 ± 0.31	8.81 ± 0.52*	10.20 ± 0.44*
Mass fraction of crude fibre, in terms of dry matter	19.4 ± 1.5	15.8 ± 1.7*	12.1 ± 1.9*
Exchange energy, MJ/kg – cattle – pigs – poultry – sheep	 8.4 5.4 10.4 9.3	 8.8 5.9 11.6 10.6	 8.9 5.8 12.5 10.5
Mass fraction of soluble carbohydrates	8.8 ± 0.4	9.6 ± 0.8	10.1 ± 0.8
Starch content in terms of dry matter, g/kg	24.2 ± 3.4	30.5 ± 3.1	31.3 ± 3.4
pH, units	6.7 ± 0.10	6.7 ± 0.10	6.8 ± 0.12

Note: * p < 0,05 regarding control.
Source: compiled by O.A. Mironova, A.A. Kirichuk, A.P. Karmazin, L.P. Mironova.

The share of crude ash in terms of dry matter after 12 h of microbiological fermentation of sunflower husk increased 1.3 times (*), after 24 h of fermentation – 1.5 times (*).

Mass fraction of crude fibre in terms of active dry matter after 12-hour fermentation decreased by 30.6% (*) and after 24-hour fermentation with Lesnov's product – by 60.3% in comparison with the initial level before fermentation (*).

Exchangeable energy after treatment of sunflower husk with Lesnov's starter for 12 h for all kinds of animals increased by 4,8% – for cattle, by 9,3% – for pigs, by 11,5% – for poultry, by 11,4% – for sheep in comparison with native product and after 24 h fermentation of the product respectively by 10,6, 10,7, 12,0, 11,3%.

Mass fraction of soluble carbohydrates after fermentation of sunflower husk with Lesnov's starter for 12 hours increased in comparison with the initial level before fermentation by 10.9%, after 24 hours of fermentation – by 11.5%.

Starch content in terms of dry matter after fermentation of sunflower husk with Lesnov's starter for 12 h increased by 26.2% (*), after fermentation of substrate for 24 h by 29.3% (*) in comparison with the initial substrate.

After 12 h of sunflower husk fermentation the pH did not change, after 24 h of fermentation there was a shift to the alkaline side – by 1.5%.

Thus, after fermentation of sunflower husk with Lesnov's starter, the mass fraction of moisture, crude fat, crude fibre significantly changed: the mass fraction of moisture, crude fat, crude fibre decreased; crude protein content increased; the mass fraction of crude ash, starch content; the index of metabolizable energy level for all types of animals and the content of soluble carbohydrates increased, but the difference was not reliable.

Table 2. **Effect of biofermentation duration on mycotoxin content in sunflower husk**

Indicators, units of measurement	Sample ($n = 6$)		
	before fermentation	after fermentation	
		12 h	24 h
Aflatoxin B1, mg/kg (MPC 0.025-0.1 mg/kg)	< 0.003	< 0.003	< 0.003
Deoxynivalenol, mg/kg (MPC 0.75-1.0 mg/kg)	< 0.058	< 0.058	< 0.058
Zearalenone, mg/kg (MPC max. 1.0 mg/kg)	< 0.1	< 0.1	< 0.1
Ochratoxin A, mg/kg (MPC not more than 0.05 mg/kg	< 0.0005	< 0.0005	< 0.0005
T-2 toxin, mg/kg (MPC max. 0.1 mg/kg)	< 0.05	< 0.05	< 0.05

Source: compiled by O.A. Mironova, A.A. Kirichuk, A.P. Karmazin, L.P. Mironova.

Aflatoxin B1 content in native sunflower husk raw material was 8.3 times lower than the lower MPC level and did not change after microbiological fermentation of the substrate for 12 and 24 h. The content of deoxynivalenol in the original sample was 12.9 times lower than the minimum permissible MPC level and did not change after exposure of the product to microbiological fermentation for 12 and 24 hours. In the native raw material zearalenone was detected 10.0 times lower compared to the recommended MPC; after microbiological fermentation for 12 and 24 h remained at the initial level. Ochratoxin A in the native sunflower husk sample was detected 100 times less than the MPC; after fermentation for 12 and 24 h the content of ochratoxin A did not change. The content of T-2 toxin in the original sunflower husk raw material was 2.0 times lower compared to the recommended MPC; after fermentation of the substrate for 12 and 24 h the level of T-2 toxin did not change (Table 2).

Thus, all tested mycotoxins in native sunflower husk samples were contained in amounts below the minimum MPC level: aflatoxin B1 – 8.3 times, deoxynivalenol – 12.9 times, zearalenone – 10 times, ochratoxin A – 100 times, T-2 toxin – 2 times; after biofermentation of sunflower husk within 12 and 24 h the levels of all tested mycotoxins remained within the MPC.

Based on the data of Table 3, the content of pesticides: malathion, pyrimitophos-methyl, cypermethrin, diflubenzuron, most commonly used in the cultivation and storage of sunflower, both in the raw material of sunflower husk before fermentation, and after twelve- and twenty-four-hour fermentation process remained below MPC (below the lower limit of detection by HPLC in accordance with the current RD).

Table 3. **Effect of sunflower husk biofermentation duration on the content of chemically hazardous substances**

Indicators, units of measurement, MPC, RD	Sample (n = 6)		
	before fermentation	After fermentation	
		12 h	24 h
Pesticides			
Malathion, mg/kg (MPC < 0.01 mg/kg) DIN EN 15662:2018 (HPLC)	< 0.01	< 0.01	< 0.01
Pyrimitophos-methyl, mg/kg (MPC < 0.01 mg/kg) DIN EN 15662:2018 (GC)	< 0.01	< 0.01	< 0.01
Cypermethrin, mg/kg (MPC < 0.01 mg/kg) DIN EN 15662:2018 (GC)	< 0.01	< 0.01	< 0.01
Diflubenzuron, mg/kg (MPC < 0.01 mg/kg) DIN EN 15662:2018 (HPLC)	< 0.01	< 0.01	< 0.01
Nitrates and nitrites			
Nitrates, mg/kg (MPC 200.0 mg/kg) GOST 13496.19-2015	289.0 ± 72.0	272.0 ± 52.0	374.0 ± 94.0*
Nitrites, mg/kg (MPC 10.0 mg/kg) GOST 13496.19-2015	1.56 ± 0.12	2. 46 ± 0.11	1.96 ± 0.12
Toxic elements			
Lead, mg/kg (MPC < 5.0 mg/kg) GOST R 53100-2008	< 0.5	< 0.5	< 0.5
Arsenic, mg/kg (MPC < 0.5 mg/kg) GOST R 53100-2008	< 0.1	< 0.1	< 0.1
Cadmium, mg/kg (MPC < 0.3 mg/kg) GOST R 53100-2008	< 0.05	< 0.05	< 0.05
Mercury, mg/kg (MPC < 0.1 mg/kg) / GOST 31650-2012	< 0.025	< 0.025	< 0.025

Note: * p < 0,05 regarding control.
Source: compiled by O.A. Mironova, A.A. Kirichuk, A.P. Karmazin, L.P. Mironova.

Nitrates and nitrites are normative indicators of feed safety. In the study of native sunflower husk samples, the amount of nitrates was above the MPC by 14.5% and fermented 12 and 24 hours, respectively, by 13.6% and 18.7% (*). Nitrite content, both in native sunflower husk samples and fermented 12 and 24 hours did not exceed MPC.

In the study of toxic elements in native and fermented for 12 and 24 h samples of sunflower husk, no differences in the content of lead, arsenic, cadmium and

mercury were found. Thus, the content of lead was below the MPC level 10 times; arsenic – 5 times; cadmium – 6 times; mercury – 4 times.

Thus, the content of pesticides, toxic elements, nitrites in sunflower husk, both before and after fermentation, regardless of the time of fermentation does not exceed MPC norms established by the legislation; the content of nitrates exceeded MPC in the native raw material at 14.5%, at 12-hour fermentation at 13.6% and fermented 24 hours at 18.7%.

Table 4. **Qualitative determination of the presence of GMOs in sunflower husk samples native and fermented 12 and 24 hours (6 samples)**

Samples	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Promotor, terminator	35S t-NOS p-FMV	35S t-NOS p-FMV	35S t-NOS p-FMV	35S t-NOS p-FMV	35S t-NOS p-FMV	35S t-NOS p-FMV
Result of qualitative determination of regulatory sequences in the genome of GM plants (GOST R 53214-2008)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

Source: compiled by O.A. Mironova, A.A. Kirichuk, A.P. Karmazin, L.P. Mironova.

In samples of sunflower husk waste, both before and after fermentation for 12 and 24 h, laboratory tests by the screening method 'Qualitative determination of regulatory sequences in the genome of GM plants (p-35S; t-NOS; p-FMV)' were performed GMO: 35S promoter, NOS terminator; FMV promoter were not detected.

Conclusions

According to the results of laboratory studies of sunflower husk, the following was proved:

1) regardless of fermentation time with the use of microbiological Lesnov's starter significantly decreased the mass fraction of moisture, crude fat, crude fibre; increased the mass fraction of crude protein, crude ash, starch content; the index of metabolic energy level for all types of animals and the content of soluble carbohydrates increased, but the difference was not reliable;

2) the obtained fermented product meets the biological and chemical safety requirements for animal feed.

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