Dynamics of physico-chemical properties and safety indicators of fermented wheat bran

A.P. Karmazin [1,*], *O.A.* Mironova [2], *A.A.*Mironova [3]

[1] Institute of environmental engineering of the Peoples' Friendship University of Russia, 6 Miklukho-Maklaya str., Moscow, 117198
[2] Peoples' Friendship University of Russia (RUDN University), 117198, 6 Miklukho-Maklaya str., Moscow
[3] The North Caucasus Zonal Veterinary Research Institute is a branch of the Federal State Budgetary Scientific Institution "Federal Rostov Agrarian Scientific Center. Rostov region, Novocherkassk, Rostov highway, 0

Annotation. The article is devoted to the study of quality and safety indicators of wheat bran under the influence of microbiological fermentation. Wheat bran is the main by-product of deep processing of wheat, formed at the stage of grain purification and crushing, and accounts for about 15% of its weight. The purpose of our research was to study the effect of microbiological fermentation of wheat bran on the quality and safety of the resulting product during fermentation for 12 and 24 hours. It was found that after 12- and 24-hour fermentation of wheat bran with Lesnov's starter culture, the mass fraction of protein, soluble carbohydrates, starch increased; the mass fraction of moisture, fat, and crude fiber content decreased in comparison with the initial level; pH shifted to the alkaline side. CFU/g of mold fungi and yeast cells, the content of mycotoxins, pesticides, nitrates and nitrites, heavy metals, GMOs in wheat bran were in quantities below the minimum MAC level both before and after fermentation, regardless of the fermentation time.

1 Introduction

According to the All-Russian Scientific Research Institute of Feed, the annual protein deficiency in feed is more than 1.8 million tons, including 1,068 thousand tons in bulk and 750 thousand tons in concentrated ones. Traditional forage crops are not able to fully meet the growing needs for full–fledged feeds [1].

Microbiological fermentation of various raw materials is a promising method of replenishing protein deficiency in farm animal feed. Yeast and fungal strains characterized by high growth rate, relative ease of cultivation, and high protein content are used as producing microorganisms [2].

As a substrate for growing microflora producing feed protein, waste from the milling industry is used, most often wheat bran, which is the main by-product of deep processing of wheat, formed at the stage of grain purification and crushing, and accounts for about 15%

* Corresponding author: Fumrostov@mail.ru

of its weight [3]. Wheat bran contains 11-24% starch, 15-18% protein and a large amount of pentosans (up to 20.6%), which, due to their inability to be digested by digestive enzymes, are often called dietary fibers. In wheat, pentosans account for about 2% of the mass of ground grain [4,5]. Bran is rich in biologically active components beneficial to animal and human health, such as phenolic compounds and other antioxidants, therefore it is often used in the production of feed additives and as a source of nutrients for the cultivation of bifidobacteria and lactobacilli [6, 7].

There are reports that fermentation increases the content of biologically active components in bran [8]. Biofermentation using a specially selected association of microorganisms, the action of which is based on the method of solid-phase biofermentation, has been tested on many substrates, including wheat and rye straw, waste from growing oyster mushrooms, cake, meal, etc. with a positive result. It has been proven that the drug increases the nutritional value of coarse feed by 80-100%, starchy and sugary substances by 15-20%, enriches feed with vitamins B, D, PP, K, E, H, does not adversely affect biological and chemical safety indicators [9, 10].

Growing and storing wheat, which is processed and obtained bran does not do without the use of potentially dangerous substances for animal health: pesticides, heavy metals, nitrates, nitrites, mycotoxins, GMOs [11].

The purpose of the research is to study the effect of microbiological fermentation of wheat bran on the quality and safety of the resulting product during fermentation for 12 and 24 hours. To achieve this goal, we have identified the following tasks: 1– to study the physico-chemical quality indicators; 2 – to study the indicators of biological safety; 3– to study the indicators of chemical safety.

2 Materials and methods of research

The research was carried out in 2023-2024 on the basis of flour mills in the Moscow and Voronezh regions. The objects of research were 30 samples of wheat bran. The third part of the samples was examined in unprocessed form (control). 20 samples were treated with Lesnov's starter culture according to the method proposed by the authors: for 1 hour of raw materials, 0.000005 parts of Lesnov's starter culture were added at a raw material humidity of 45-55%, a temperature of 50-550C, exposures of 12 and 24 hours. Physico-chemical quality indicators, mycotoxin content: aflatoxin B1, deoxynivalenol, zearalenone, ochratoxin A, T-2 toxin; pesticides, nitrates and nitrites, toxic elements and GMOs were studied in the Testing Laboratory of the Federal State Budgetary Institution "Center for Grain Quality Assessment" in Moscow and the Moscow region; microbiological indicators: mold fungi and yeast - in the Voronezh branch of the Federal State Budgetary Institution "Center for Grain Quality Assessment" according to the current regulatory documentation (ND) using methods and techniques of laboratory research test substrates: qualitative and quantitative chemical analysis; high–performance liquid chromatography (HPLC); gas chromatography (GC); atomic absorption spectrometry, etc. Laboratory methods of quality research (GOST R 54951-2012; GOST 27979-88; GOST 13496.4-2019 p.8; GOST 32905-14; GOST 31675-2012 p.7; GOST 26226-95 p.1; GOST 26176-2019 p.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/C); 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).

3 Research results and discussion

Changes in the physico-chemical quality indicators of wheat bran depending on the fermentation time are presented in Table 1.

Table 1. Comparative characteristics of the physico-chemical parameters of fermented wheat bran.

Indicators, units of measurement	Wheat bran (n=10)		
	before fermentation	after fermentation (12 hours)	after fermentation (24 hours)
Mass fraction of moisture, %	11.3±0.38	4.3±0.32***	4.10±0.32***
The mass fraction of crude fat, in terms of dry matter, is not less than %	4.20±0.44	3.30±0.34**	2.70± 0.26***
The mass fraction of crude protein in terms of dry matter, not less than, %	15.40±0.46	18.0±0.34*	19.79±0.58**
Mass fraction of crude ash, in terms of dry matter, no more, %	5.20±0.25	4.98±0.24	5.60±0.27
The mass fraction of crude fiber in terms of dry matter, no more, %	26.2±1.8	10.5±1.4***	10.0±1.7***
Exchange energy, MJ/kg	12.0	12.2	12.2
Mass fraction of soluble carbohydrates, %	4.8±0.6	6.2±0.7 **	7.2±0.7***
Starch content in terms of dry matter, g/kg %	242.0 24.2	266.6 26.7	272.0 27.2
pH, unit pH	6.35±0.13	5.49±0.11	5.48±0.12

* p<0.05;** p <0.01;***p <0.001

After fermentation of wheat bran with Lesnov's starter culture for 12 hours, the mass fraction of moisture decreased by 2.6 times, after fermentation for 24 hours – by 2.8 times (p<0.001).

The mass fraction of fat in dry matter after processing the substrate with Lesnov's starter culture for 12 hours decreased by 29.1% compared to the initial level (p<0.01), after processing the product within 24 hours – by 35.7% (p<0.001).

After fermentation of wheat bran for 12 hours, the mass fraction of crude protein increased by 16.9% (p<0.05), after 24-hour fermentation – by 28.5%.

The content of soluble carbohydrates after 12 hours of fermentation of wheat bran with Lesnov's starter culture increased by 29.2% (p <0.01); after 24 hours of fermentation - by 50.0% compared to the initial level (p<0.001).

The mass fraction of crude fiber after fermentation of wheat bran with Lesnov's starter culture for 12 hours decreased 2.5 times, after 24-hour fermentation – 2.6 times (p<0.001).

There was a pH shift after 12- and 24-hour fermentation of wheat bran to the alkaline side by 13.5%. The mass fraction of crude ash in terms of dry matter, metabolic energy, after 12 and 24 hours of fermentation of wheat bran with Lesnov's starter culture remained in quantities close to those in the initial substrate.

The starch content in wheat bran increased by 10.3% after 12-hour fermentation with Lesnov's starter culture, and by 12.4% after fermentation within 24 hours Thus, after 12 and 24-hour

fermentation of wheat bran with Lesnov's starter culture, the mass fraction of protein, soluble carbohydrates, starch increased; the mass fraction of moisture, fat, and crude fiber content decreased compared to the initial level; the level of metabolic energy, the proportion of crude ash in terms of dry matter remained at the same level; there was a pH shift to the alkaline side.

Table 2. Comparative characteristics of indicators of biological safety of fermented wheat bran.

Indicators, units of measurement, MPC	**Wheat bran (n=10)**		
	before fermentation	**after fermentation (12 hours)**	**after fermentation (24 hours)**
Microbiological indicators			
Mold fungi, CFU/g from less than $1.0*10^2$ before $5.0*10^2$	1.0^*10^1	1.2^*10^1	1.25^*10^2
Yeast, CFU/g, from less $1.0*10^2$ before $5.0*10^2$	2.3^*10^1	3.9^*10^1	2.2^*10^2
Mycotoxins			
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 not more than 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 not more than 0.1 mg/kg	<0.05	<0.05	<0.05

Notes: GOST 10444.12-2013; GOST 34108-2017

It was found that the number of microbial cells of mold fungi (CFU/g) in native samples of wheat bran was 50.0 times lower than the upper limit of MPC. After fermentation of bran with Lesnov's starter culture for 12 hours, the quantitative content of mold fungi increased by 20.0%, while remaining 41.7 times below the upper permissible limit of MPC; within 24 hours, respectively, by 25.0% and 40.0 times. The number of yeast cells (CFU/g) in the initial substrate was 21.7 times lower in comparison with the maximum permissible norm; after fermentation with Lesnov's starter culture for 12 hours, it increased 1.7 times, remained 12.8 times lower in comparison with the upper maximum permissible limit; after fermentation of wheat bran for 24 hours, the number of yeast cells It increased by 1.7 times in comparison with the native sample, while remaining 2.3 times below the upper limit of the permissible norm.

According to Table 2, the content of aflatoxin B1, deoxynivalenol, T-2 toxin, zearalenone, ochratoxin A in the studied samples of wheat bran, both native and at different fermentation periods, does not exceed the established standards.

Thus:

1) CFU / g of mold fungi in wheat bran before fermentation was 50.0 times lower than the upper limit of MPC; after fermentation with Lesnov's starter culture for 12 hours, the number of mold fungi became 41.7 times lower than the upper permissible limit of MPC, after fermentation 24 hours – 40.0 times;

2) CFU/g of yeast cells before fermentation was 21.7 times lower than the permissible upper limit of MPC, after fermentation with Lesnov's starter culture for 12 hours, the number of yeast cells was 12.8 times lower than the upper maximum permissible limit, after fermentation of wheat bran for 24 hours – 2.3 times;

3) all studied mycotoxins in wheat bran were contained in amounts below the minimum MAC level, both before and after fermentation, regardless of the fermentation time.

Table 3. Chemical safety indicators and the presence of GMOs in fermented wheat bran.

Indicators, units of measurement, MPC, ND	**Wheat bran (n=10)**		
	before fermentation	**after fermentation (12 hours)**	**after fermentation (24 hours)**
Pesticides			
Malathion, mg/kg, MPC <0.01 mg/kg, DIN EN 15662:2018 (HPLC)	<0.01	<0.01	<0.01
Pyrimiphos-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 (GC)	<0.01	<0.01	<0.01
Nitrates and nitrites			
Nitrates, mg/kg, MPC <200.0, GOST 13496.19-2015	161.0± 20.25	134.0±34.04	160.0±31.06
Nitrites, mg/kg, MPC <10.0, GOST 13496.19-2015	1.43±0.05	1.36± 0.06	1.64±0.06
Toxic elements			
Lead, mg/kg, MPC<5.0 GOST R 53100-2008	<0.5	<0.5	<0.5
Arsenic, mg/kg, MPC<0.5 GOST R 53100-2008	<0.1	<0.1	<0.1
Cadmium, mg/kg, MPC<0.3 GOST R 53100-2008	<0.05	<0.05	<0.05
Mercury, mg/kg, MPC<0.1 GOST 31650-2012	<0.025	<0.025	<0.025
GMO			
Qualitative determination of regulatory sequences in the genome of GM plants p-35S; nos; p-FMV GOST R 53214-2008	The 35S promoter t-NOS p-FMV not detected	The 35S promoter t-NOS p-FMV not detected	The 35S promoter t-NOS p-FMV not detected

According to Table 3, the content of pesticides (malathion, pyrimitophos-methyl, cypermethrin, diflubenzuron) used in the cultivation and storage of wheat, the by-product of processing of which is bran, both in the feedstock before fermentation and regardless of the fermentation time remained below the MPC.

In the study of native and fermented wheat bran samples for 12 and 24 hours, the content of nitrates, nitrites, lead, arsenic, cadmium and mercury were within the established standards.

The study of native and fermented wheat bran samples for 12 and 24 hours by the screening method of qualitative determination of regulatory sequences in the genome of GM plants p-35S; t-NOS; p-FMV was not detected.

Thus, the content of pesticides, toxic elements, nitrates and nitrites in wheat bran, both before and after fermentation, regardless of the fermentation time, does not exceed the legal norms of MPC; GMOs p-35S; t-NOS; p-FMV were not detected.

4 Conclusion

In the course of research, it was found that after 12- and 24-hour fermentation of wheat bran with Lesnov's starter culture: 1) the mass fraction of protein, soluble carbohydrates, starch increased; the mass fraction of moisture, fat, and crude fiber decreased compared to the baseline level; there was a shift in pH to the alkaline side; 2) CFU/g of mold fungi and yeast cells, the content of mycotoxins, pesticides, nitrates and nitrites, heavy metals, and GMOs were in quantities below the minimum MAC level, both before and after fermentation, regardless of the fermentation time. Evaluating the results obtained, we believe that it is advisable to further study the fermented product from the point of view of the possibility of its use as feed for farm animals and a substrate for growing beneficial microflora.

References

1. V.M. Kosolapov, I.A. Trofimov, L.S. Trofimova, *Fodder production in agriculture, ecology and rational nature management (theory and practice)* (Wildebeest Research Institute of Feed, Moscow, 2014)
2. P.A. Lesnov, The method of using sourdough in a feed mixture. Lesnov's starter culture for the preparation of feed Patent RU 2 122 330 C1 Russian Agency for Patents and Trademarks. Published on 11/27/1998. - Byul.
3. N.Yu. Khromova, V.D. Smirnova, I.V. Shakir, V.I. *Panfilov, International scientific and practical conference "Biotechnology and quality of life",* 323-324 (2014)
4. Valeeva, R.T. Bulletin of the Kazan Technological University, **17(3)**, 33-38 (2014)
5. Y.W. Carter, R. Madl, F. Padula, Nutricions research, **26(1)**, 33-38 (2006)
6. A.P. Lesnov, E.P. Kudziev, A.G. Puzankov, Tsenovik, **12**, 15 (2006)
7. R.P. John, K.M. Nampoothiri, A. Pandey, Biotechnology letters, **28(22)**, 1823-1826 (2006)
8. K. Katina et al., Food Microbiology, **24(2)**, 175-186 (2007)
9. O.A. Mironova, A.P. Karmazin, A.P. Lesnov, Feed production, **6**, 32-35 (2023)
10. O.A. Mironova, V.N. Vasilenko, L.P. Mironova, A.P. Karmazin, A.A. Mironova, A.P. Lesnov, Compound feed, **9**, 55-57 (2023)
11. M.N. Monodchenko, G.S. Pokhodnya *The use of non-traditional feeds in pig farming* ("Veselitsa", Belgorod, 2011)