RUDN Journal of Ecology and Life Safety
Вестник РУДН. Серия: Экология и безопасность жизнедеятельности
ISSN 2313-2310 (Print); ISSN 2408-8919 (Online)
2025 Vol. 33 No. 1 21–28
http://journals.rudn.ru/ecology

DOI: 10.22363/2313-2310-2025-33-1-21-28
EDN: BALIGH
UDC 631.95

Research article / Научная статья

Recycling of white lupine meal using solid-phase microbiological fermentation

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Abstract. The protein content in white lupine seeds is 35–38%, so it is considered as an alternative to soybeans. After separating the oil from the lupine seeds, the meal remains. Lupine meal is a waste product of lupine oil production. An environmentally friendly method of lupine meal utilization by solid-phase microbiological fermentation is proposed with the prospect of using the fermented product for animal feed. The results of laboratory studies of white lupine meal fermented using Lesnov's starter have proven that, regardless of the fermentation time, the physicochemical properties of the resulting product are significantly improved in comparison with the native substrate; the obtained fermented product meets the requirements of biological and chemical safety for animal feed.

Keywords: white lupine meal, Lesnov's starter, microbiological fermentation, quality, safety, recycling.

Authors' contribution. All authors made an equal contribution to the preparation of the publication.

Article history: received 10.11.2024; revised 18.11.2024; accepted 03.12.2024.

Conflicts of interest. The authors declare no conflicts of interest.

For citation: Mironova OA, Kirichuk AA, Karmazin AP, Mironova LP. Recycling of white lupine meal using solid-phase microbiological fermentation. *RUDN Journal of Ecology and Life Safety.* 2025;33(1):21–28. http://doi.org/10.22363/2313-2310-2025-33-1-21-28

Утилизация шрота люпина белого методом твердофазной микробиологической ферментации

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Аннотация. Содержание белка в семенах люпина белого составляет 35–38 %, поэтому он рассматривается как альтернатива сое. После отделения масла от семян люпина остается шрот. Люпиновый шрот является отходами производства люпинового масла. Предложен экологически чистый способ утилизации шрота люпина методом твердофазной микробиологической ферментации с перспективой использования ферментированного продукта для кормления животных. По результатам лабораторных исследований ферментированного с использованием закваски Леснова шрота люпина белого доказано, что независимо от времени ферментирования достоверно улучшаются физико-химические свойства полученного продукта в сравнении с нативным субстратом; полученный ферментированный продукт отвечает требованиям биологической и химической безопасности, предъявляемым к кормам для животных.

Ключевые слова: шрот люпина белого, закваска Леснова, микробиологическое ферментирование, качество утилизации, требования безопасности к кормам, утилизация

Вклад авторов. Все авторы сделали эквивалентный вклад в подготовку публикации.

История статьи: поступила в редакцию 10.11.2024; доработана после рецензирования 18.11.2024; принята к публикации 03.12.2024.

Заявление о конфликте интересов. Авторы заявляют об отсутствии конфликта интересов.

Для цитирования: *Миронова О.А., Киричук А.А., Кармазин А.П., Миронова Л.П.* Утилизация шрота люпина белого методом твердофазной микробиологической ферментации // Вестник Российского университета дружбы народов. Серия: Экология и безопасность жизнедеятельности. 2025. Т. 33. № 1. С. 21–28. http://doi.org/10.22363/2313-2310-2025-33-1-21-28

Introduction

Protein deficiency in animal feeds is mainly solved by using soya, but the search for alternative sources of plant feed protein continues worldwide. In this regard, extensive scientific and production research is being conducted on the high-protein fodder crop lupin, the protein content in its seeds is 35–38% [3; 11].

Numerous studies have proved that white lupin grain has better nutritional properties than full-fat soya as it contains soluble and easily digestible nitrogen-free extractive substances – polysaccharides, starch and sugars [9].

In white lupin grain, up to 20% is accounted for the low nutritive outer shell, in which fibre predominates, and about 30% are ballast non-nutritive carbohydrates (hemicellulose and pectins) [1; 4].

Numerous search experiments are being conducted to improve the fodder qualities of lupin grain, for example, a method of hydrobarothermal treatment is

proposed, which promotes the destruction of cellulosolignin compounds, starch dextrinisation and inactivation of anti-nutritive substances [8].

Crumbling of lupine seeds to form particles of a given size makes it possible to produce high-protein forage containing up to 42% crude protein and significantly reduce the amount of crude fibre [10].

After separating the oil from the lupine seeds, a meal remains that can be used for animal feed after it has been carefully examined.

A promising way of processing cellulose-containing raw materials is microbiological fermentation, which allows to obtain high-protein feed enriched with biologically active components formed by microorganisms in the process of life activity: vitamins, amino acids, enzymes, bacteriocins, prebiotics [6]. The most effective microbiological fermentation is microbiological fermentation by special microorganisms collected in an ensilage. Lesnov's starter, which includes biologically active substances, mycelia of microscopic fungi, macro- and microelements, has been studied on such substrates as rye, bran, whey, beer pellets [5].

Since the process of fermentation by microorganisms of Lesnov's starter is carried out under conditions suitable for the development of mould fungi and yeasts, it is necessary to study the fermented product for biological hazard indicators: mycotoxins, mould fungi and yeasts [2; 7].

When growing, storing and processing lupine, chemical preparations may be used: herbicides, growth stimulants, pesticides, fertilisers, which creates a threat of chemical hazard of feed. Based on the above, the use of fermented white lupine meal for animal feed can be recommended only after making sure of their safety.

In the literature sources available to us, we found no information on solid-phase microbiological fermentation of white lupin meal using Lesnov's starter.

The aim of our research was to study the quality and safety parameters of white lupin meal fermented using Lesnov's starter for twelve and twenty-four hours.

Objectives:

1 – to study physico-chemical quality indicators;

2 – to study biological safety indicators;

3 – to study chemical safety indicators.

Materials and methods

Studies on fermented samples of white lupin meal were conducted in 2024. The objects of research were 18 samples of white lupin meal: six samples before fermentation (native), six subjected to twelve-hour fermentation, and six – after twenty-hour fermentation. 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 "Grain Quality Assessment Centre" in Moscow and Moscow region according to the current legislation. According to the current

regulatory documentation (RD) with the use of methods and techniques of laboratory research of tested substrates: qualitative and quantitative chemical analysis; high-performance liquid 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 p.8; GOST 32905-14; GOST 31675-2012 p.7; GOST 26226-95 p.1; GOST 26176-2019 p. 9; GOST R 54078-2010 annex 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 31650-2012), GMOs (GOST R 53214-2008).

Results of the research

We studied the effect of solid-phase microbiological fermentation method of different durations on physicochemical parameters of white lupin meal (Table 1).

Table 1. **Dynamics of physicochemical parameters of white lupine meal at different durations of microbiological fermentation**

Indicators, units of measurement	Sample (*n* = 6)		
	before fermentation	after fermentation	
		12 hours	24 hours
Mass fraction of moisture, %	5.5±1.3	6.5±1.4*	6.3±1.3*
Mass fraction of crude fat, calculated on dry matter, %	6.40±0.44	8.80±0.48*	9.61±0.49*
Mass fraction of crude protein in terms of dry matter, %	32.69±1.25	38.78±1.14*	41.88±1.22*
Mass fraction of crude ash, calculated on dry matter, %	3.9±0.2	4.0±0.3	4.2±0.2
Mass fraction of crude fiber in terms of dry matter, %	19.7±1.8	13.9±1.6*	12.6±1.5*
Metabolic energy, MJ/kg	11.4	12.7*	13.9*
Mass fraction of soluble carbohydrates, %	6.5±1.0	7.3±0.8	10.2±1.2*
Starch content in terms of dry matter, g/kg %	42.0±2.2 4.2	59.0±2.8* 5.9	78.0±3.1* 7.8
pH, pH units	5.91±0.10	5.55±0.10	5.53±0.10

Note: * p < 0,05-0,001 relative to the control.
Source: compiled by O.A. Mironova, A.P. Karmazin.

Depending on the duration of solid-phase microbiological fermentation of white lupin meal using an association of microorganisms collected in the so-called Lesnov's starter, the value of the mass fraction of moisture increased after 12 hours by 18.2% (*), after 24 hours of fermentation – by 14.6% (*) in comparison with the native substrate.

The mass fraction of crude fat increased by 37.5% (*) after 12 hours of fermentation and by 50.2% (*) after 24 hours compared to the initial product.

It was found that the mass fraction of crude protein increased depending on the fermentation time. Thus, after 12 hours the increase was 18.6% (*), after 24 hours – 28.1% (*) in relation to the initial amount in the native sample.

The mass fraction of soluble carbohydrates increased with the duration of fermentation. So, after 12 hours of fermentation the increase was 12,3%, after 24 hours – 56,9% (*) in comparison with the initial product.

The mass fraction of starch in terms of dry matter increased by 40.5% (*) in relation to the initial substrate during 12 hours of fermentation, and by 85.7% (*) for 24 hours.

The metabolic energy index after 12 hours of fermentation increased by 11.4% compared to the native product, after 24 hours of fermentation increased by 21.9% (*).

The level of crude ash changed little during fermentation: after 12 hours it increased by 2.6% and after 24 hours by 7.7% compared to the native substrate.

After 12 h of fermentation, the mass fraction of crude fat, metabolisable energy and starch content increased significantly (*).

After 12 hours of fermentation of white lupin meal the level of crude fibre decreased by 29.5% (*), after 24 hours of fermentation by 36.0% (*). There was a shift of pH after 24 hours of fermentation to acidic side by 6.4%.

Thus, regardless of the time of biofermentation with the use of Lesnov's starter of white lupin meal significantly increased the mass fraction of crude fat, crude protein, soluble carbohydrates, starch, metabolic energy level; the level of crude fibre decreased, pH shifted to acidic side in comparison with the same level in the native product.

Table 2. **Effect of biofermentation duration on mycotoxin content in white lupine meal**

Indicators, units of measurement	Sample (*n* = 6)		
	before fermentation	after fermentation	
		12 hours	24 hours
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 no more than 0.05 mg/kg)	<0.0005	<0.0005	<0.0005
T-2 toxin, mg/kg (MPC no more than 0.1 mg/kg)	<0.05	<0.05	<0.05

Source: compiled by the authors.

The content of aflatoxin B1 in the initial raw material of white lupin meal was 8.3 times less than the lower MPC level and did not change at different duration of substrate fermentation using Lesnov's starter.

The content of deoxynivalenol in the initial sample of white lupin meal was 12.9 times lower than the minimum permissible level of MPC and did not change at different time of exposure to the substrate of microorganisms of Lesnov's starter.

The content of zearalenone in the native sample of white lupine meal was 10.0 times less in comparison with the recommended MPC; after fermentation by microorganisms of Lesnov's sourdough within 12 and 24 hours it did not exceed the MPC.

In the native sample of white lupin meal, ochratoxin A was detected 100 times less than the MPC; regardless of the fermentation time, the quantitative content of ochratoxin A did not change.

The content of T-2 toxin in the native raw material of white lupin meal was 2.0 times lower compared to the recommended MPC; after fermentation of the substrate for 12 and 24 hours, the level of T-2 toxin did not change.

Thus, all tested mycotoxins in native (initial) samples of white lupin meal were contained in amounts below the minimum MPC level: aflatoxin B1 – 8.3 times, deoxynivalenol – 12.9 times, zearalenone – 10.0 times, ochratoxin A – 100.0 times, T-2 toxin – 2.0 times; after fermentation of the substrate with Lesnov's starter for 12 and 24 hours, the levels of all tested mycotoxins did not change.

Table 3. **Effect of fermentation duration on the content of chemical substances in white lupine meal**

Indicators, units of measurement, MPC, ND	Sample (n = 6)		
	before fermentation	after fermentation	
		12 hours	24 hours
Pesticides			
Malathion, mg/kg (MPC < 0.01 mg/kg) DIN EN 15662:2018 (HPLC)	<0.01	<0.01	<0.01
Pyrimifos-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	223.0±56.3	193.5±63.4	196.0±33.7
Nitrites, mg/kg (MPC 10.0 mg/kg) GOST 13496.19-2015	3.56±0.12	2.05 ±0.14	1.85 ±0.16
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

Notes: HPLC – high-performance liquid chromatography; GC – gas chromatography.
Source: compiled by O.A. Mironova, A.P. Karmazin.

Based on the data of Table 3, the content of pesticides: malathion, pyrimiphos-methyl, cypermethrin, diflubenzuron, used in the cultivation and storage of plant products, both in the initial before fermentation and after twelve- and twenty-four-hour fermentation process of white lupin meal remained below MPC (below the lower limit of detection by HPLC in accordance with the current ND).

In the study of native samples of white lupin meal, the amount of nitrates and nitrites was found to be below the MPC; in the study of fermented 12 and 24 hours substrates using microorganisms of Lesnov's starter, the amount of nitrates and

nitrites remained at levels not exceeding the MPC and close to the initial one before fermentation.

In the study of toxic elements in native and fermented for different time samples of white lupin meal no differences in the content of lead, arsenic, cadmium and mercury were found (Table 4). Thus, the content of lead was below the MPC level 10 times; arsenic – 5 times; cadmium – 6 times; mercury – 4 times.

Table 4. **Qualitative determination of the presence of GMOs in native and 12 and 24 hour fermented samples of white lupine meal (6 samples)**

Sample	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Promoter, 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
The result of qualitative determination of regulatory sequences in the genome of GM plants (GOST R 53214-2008)	Not found	Not found	Not found	Not found	Not found	Not found

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

The laboratory GMO screening method "Qualitative determination of regulatory sequences in the genome of GM plants (p-35S; t-NOS; p-FMV)" did not detect 35S promoter, NOS terminator, FMV promoter in samples of white lupin meal both before and after fermentation within 12 and 24 hours.

Conclusions

The results of laboratory studies of white lupine meal proved that 1) regardless of fermentation time with the use of microbiological inoculum Lesnov's reliably improve the physicochemical properties of the obtained product in comparison with the native substrate; 2) the obtained fermented product meets the requirements of biological and chemical safety for animal feed.

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