JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2023, 24(12), 235–246
https://doi.org/10.12911/22998993/173065
ISSN 2299–8993, License CC-BY 4.0

Received: 2023.09.19
Accepted: 2023.10.20
Published: 2023.11.04

Protein, Amino Acid and Carbohydrate Content of Fungal Treated Annual and Perennial Wheat Straw

Meruyert Kurmanbayeva[1], Zhaksylyk Makhatov[2], Adil Kusmangazinov[1,3*], Dina Karabalayeva[1], Nurgul Yerezhepova[1,3]

[1] Department of Biodiversitty and Bioresources, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, 050038, Al-Farabi Ave., 71, Almaty, Kazakhstan

[2] Department of Life Safety and Environmental Protection, Faculty of Architecture, Construction and Transport, Mukhtar Auezov South Kazakhstan University, 160012, Tauke khan Ave., 5, Shymkent, Kazakhstan

[3] Scientific Research Institute of Biology and Biotechnology Problems, Al-Farabi Kazakh National University, 050038, Al-Farabi Ave., 71, Almaty, Kazakhstan

* Corresponding author's e-mail: adil_06.1996@mail.ru

ABSTRACT

The use of wheat straw as a cellulose containing raw material for the production of sugars and other biologically valuable products can solve the problem of food products shortages, by supplying oligosaccharides, xylose and other valuable metabolites of microbial synthesis. In our experiment, straw of annual spring wheat of the Tulkibas variety and perennial wheat of the Sova variety was added to the enzyme solutions of *Trichoderma harzianum* 121 and *Aspergillus awamori* F-RKM 0719 strains. As a result, the variant *A. awamori* F-RKM 0719 + Sova straw showed the highest level of nitrogen (1.05%) and protein (6.5%). The highest concentration of amino acids, 7.14 mg/ml, was found in the perennial wheat sample, while the lowest concentration, 1.32 mg/ml, in the annual wheat sample. The total carbohydrate content in the straw of the annual and perennial wheat varieties differed, namely, the perennial wheat straw with the addition of *A. awamori* F-RKM 0719, contained fructose in the amount of 0.0079 mg/g, while in the annual wheat it was absent. The glucose content in the perennial wheat straw was three times higher than in the annual wheat straw, 0.0144 and 0.0035 mg/g, respectively. Based on our results, we recommend wheat straw for the use as a raw material for chemical and microbiological processing.

Keywords: annual wheat, perennial wheat, protein, nitrogen, cellulase.

INTRODUCTION

In the last decades, new technologies have emerged for plant biomass processing. Plant biomass is rich in valuable components that could be used as a source of valuable substances and products for various industries and agriculture, including monosaccharides, feed protein, ethyl alcohol, furfural, and xylitol [Zhapayev et al., 2023]. The widespread use of plant agricultural waste in industry is hampered by its scatter across vast territory, difficulty of transportation, and seasonality of harvesting. This entails the need to store a large amount of raw materials, which over time loses its commercial properties as a result of natural biochemical decomposition. When using such raw materials on an industrial scale, it is necessary to use large-volume carrier devices due to a low bulk density of raw materials, even of crushed ones [Boltovsky, 2014]. The prospect of using these resources and the choice of rational ways of industrial processing depends significantly on the plant biomass composition [Shah et al., 2021].

One of the major problems faced by the Kazakh agriculture is insufficient amount of vegetable protein harvesting. Despite a sharp decline in livestock in recent years, the shortage of high-quality, nutritionally balanced feed still exists [Sushkova et al., 2007; Khazipov et al., 2012]. In Kazakhstan, wheat occupies a leading position

among grain crops in terms of the area under cultivation [Karatayev et al., 2022]. Although technologies for the processing and recycling of wheat straw have been developed, most of them have not been implemented yet. In many cases, straw is used for cattle feeding and as animal litter, while the rest is mixed with soil or burned in the field [Tian et al., 2018]. In Kazakhstan, this bulky, easily detectable, annually generated waste is considered secondary resources for the future use in agricultural production and is highly relevant in practical terms.

The use of straw as a substrate for solid-phase fermentation by microorganisms in order to increase digestibility and protein content provides opportunities for obtaining plant fodder additives rich in carbohydrates. When microbiological pretreatment to increase the reactivity of cellulose (destruction of the crystalline structure of cellulose or removal of lignin) is implemented, an increase in the cellulose surface area and dissolution of hemicellulose occurs, which leads to an increase in the rate of hydrolysis and partial depolymerisation of cellulose, dissolution of hemicelluloses and lignin, and a change in the structure of the latter [Boehlje and Broring, 2011].

The requirements for wheat straw chemical composition depend on its intended use. For the straw intended for agricultural use, the protein and amino acid content is of primary importance. Wheat variety, fertilizers and agricultural machinery used, as well as soil fertility and weather conditions all affect the yield and quality of straw [Wang et al., 2015; Mukherjee et al., 2019; Xue et al., 2019; Zörb et al., 2018]. In Kazakhstan, the key factors affecting the yield of spring wheat are the amount of precipitation and soil nitrogen availability [Schierhorn et al 2014]. There is a relationship between the protein content and the amino acid composition of wheat: with an increase in the mass fraction of protein, the glutamine and proline content increases, and the arginine content often decreases. Glutamic acid and glutamine are the first amino acids to be synthesized in wheat. Plants use glutamine as an amino group source in the synthesis of other amino acids [Kovačević et al., 2013]. The increase in proline content is directly related to the characteristics of wheat proteins. Proline is a component of prolamin proteins, which in turn are the main components of gluten [Hu et al., 2022]. The decrease in arginine is due to its catabolism mobilizing the stored nitrogen and regulating the synthesis of nitric oxide (NO), polyamines, and potentially proline [Winter et al., 2015]. When growing and processing wheat, only 10% of the straw is used as livestock feed and as litter, the rest remains in the field [Ma et al., 2007]. When feeding animals, wheat straw is often used as an additional bulk feed. When using straw as animal feed, the main problem is that the straw nutrients are converted into a strong lignin-cellulose complex, which decomposes poorly in the gastrointestinal tract of animals; as a result the straw nutrients are poorly digested [Ghaffar, 2020]. Wheat straw has a limited use in replenishing organic soil matter, due to a high ratio of carbon to nitrogen, (70–90):1, which slows down the rate of the straw decomposition. Therefore, at the initial stages of the straw decomposition, immobilization of soil nitrogen occurs; in addition, natural harmful for plants substances can accumulate, including saprophytic, pathogenic fungal and bacterial microflora [Rathour et al., 2023; Makhatov et al., 2017].

Biological methods of pre-treatment of plant raw materials are based on the use of microorganisms capable of decomposing lignocellulosic substrates [Mannekote et al., 2018]. Many microorganisms including basidiomycetes, ascomycetes, microscopic fungi, bacteria, and yeast can decompose polysaccharides of plant raw materials. The best results were obtained when treating wheat straw with fungi such as *A. niger, A. awamori,* and *T. harzianum* [Patel et al., 2007]. In one of the studied cellulase complexes produced by *T. viridemicromycetes*, five endoglucanases and two cellulobiohydrolases were identified. About 30 different forms were found in the *T. harzianum* strain. Many species of microorganisms can act as cellulose degraders [Ahmad, 2010]. The biological role and sources of many types of cellulolytic enzymes are not fully understood. Endoglucanases play an important role in the activity of polyenzymatic systems, since they are the first to attack cellulose. Hydrolysis of glycosidic bonds caused by endoglucanases may be accompanied by preservation and transglycosylation of the disintegrating bond configuration. In addition, some aerobic fungi (not lignin fungi), are able to break down cellulose. For example, *T. harzianum* breaks down cellulose, and can be used to determine the mechanism of action of free cellulase, while pathogenic fungi (e.g., brown rot pathogens) secrete cellulases along with peroxidases, sometimes denoting progressive activity

[Martinez et al., 2005]. When *T. harzianum* and *A.awamori* are co-cultured, the activity of cellulase, β-glucosidase and hemicellulase increases two- or three-fold compared to single culture [Bhardwaj et al., 2021] To obtain a highly active enzyme preparation of cellulase, alternate introduction of cultures and joint cultivation of *T. harzianum* and *A. awamori* fungi are carried out using a cellulose component, which is an inducer of enzyme biosynthesis in a nutrient medium [Jiang et al., 2011]. For the deepest bioconversion of complex plant polysaccharides, such as wheat straw, the most effective approach is to form an association of microorganisms with a complex of enzymes that destroy cellulase and break down pectins, which, when combined with the plant tissue, secrete easily digestible carbohydrates and break them down [Blieva et al., 2015].

A community of microorganisms created for the production of a complex of cellulolytic enzymes is based on a previously active high producer of a complex of pectolytic enzymes that synthesize cellulase and β-gluconase [Manna et al., 2020]. Thus, the complex of cellulolytic enzymes synthesized by *T. harzianum* is induced by cellulose, from which low molecular weight products are formed as weak inducers. The latter can quickly break down into glucose and affect the inhibition (or slowing down) of enzyme synthesis [Prasanna et al., 2015]. The purpose of this study was to determine the amino acid and hydrocarbon composition of annual and perennial wheat straw that have been treated with enzyme solutions of the association of *T. harzianum* 121 and *A. awamori* F-RKM 0719 strains.

MATERIALS AND METHODS

Materials

The research was carried out from 2019 to 2022 at the Department of Biodiversity and Bioresources of Al-Farabi KazNU. As the main study object, wheat straw of the annual wheat variety Tulkibas zoned in the South Kazakhstan region and the perennial wheat variety Sova were used. Straw samples were taken after the harvest. The variety of perennial wheat Sova was grown at the experimental sites of Al-Farabi KazNU and at the Department of Biotechnology of the South Kazakhstan State University named after M. Auezov; Tulkibas annual wheat variety was grown in the experimental fields of the university. The straw composition was determined in the laboratory of Enzymes of Microorganisms of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan.

As sources of hydrolytic enzymes, *Trichoderma harzianum* 121 culture was used, obtained as a result of multistage selection and mutagenesis at the Department of Biotechnology of the South Kazakhstan State University named after M. Auezov. This culture is deposited on the REM of the State Enterprise "Republican Code of Microorganisms" of the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan. *Aspergillus awamori* F-RKM 0719 formed as a result of multistage selection and mutagenesis was produced at the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan in the laboratory of Enzymes of Microorganisms.

Based on the enzyme solutions of *Trichoderma harzianum* 121 and *Aspergillus awamori* F-RKM 0719 strains with the addition of straw waste of the annual wheat variety Tulkibas and perennial wheat variety Sova, four experimental variants and two control variants were prepared. The variants were: No.1 – *Trichoderma harzianum* + straw of the annual wheat Tulkibas*;* No.2 *Trichoderma harzianum* + straw of the perennial wheat Sova; No.3 *Aspergillus awamori* F-RKM 0719 + straw of the annual wheat Tulkibas*;* No.4 *Aspergillus awamori* F-RKM 0719 + straw of the perennial wheat *Sova;* No.5 (Control) straw of the annual wheat Tulkibas*;* No.6 (Control) straw of the perennial wheat Sova.

Preparation of monosporial cultures of microorganisms

To obtain monosporial cultures of micromycetes, the substrate was diluted in a disinfected water medium of grafted Czapek's agar, where on average one spore should fall per drop. Then, 3–4 drops of spore suspension were sown on agar in a petri dish filled with disinfected nutrient medium, placed in a thermostat, and kept at a temperature of 28–30 °C for 4–7 days. Test tubes with colonies of fungus cultures were placed in a refrigerator and stored at a temperature of 4–10 °C.

To obtain vegetative grafting material, nutrient agar medium was used, which was disinfected in an autoclave at 0.05 MPa for 30 minutes and poured into tubes. The nutrient agar medium was

then tested for purity by keeping it in a thermostat at 37 °C for 48 hours. After that, micromycetes were sawn and grown at a temperature of 28–30 °C for 2-9 days. The resulting culture of micromycetes was stored at 20–22 °C for 2.5–3 months and used in studies as needed.

Cultivation of *T. harzianum* 121, followed by the introduction of *A. awamori* F-RKM 0719 lasted for 24–72 h in a 300 ml Erlenmeyer volumetric flask. A suspension of micromycete cells was obtained by washing the spores from the agar medium and introduced at a concentration of 0.4–1.0 g/l in an amount of 10 ml of inoculation material. Cultivation was carried out for 72–120 hours in a thermostat shaker (200–220 rpm) at a temperature of 28–30 °C.

Determination of the amount of total protein

The nitrogen content was measured according to Kjeldahl, followed by conversion to protein content. The essence of the method is in the decomposition of the organic matter of the sample with boiling concentrated sulfuric acid to obtain ammonium salts, conversion of ammonium into ammonia, distilling the latter into an acid solution, quantitative accounting of ammonia by the titrometric method and measuring the nitrogen content in the material under study. From an averaged crushed homogeneous sample, an accurate sample was taken and weighed in a test tube, with an error of not more than 0.1%. The weighed amount was transferred to a Kjeldahl flask. Further, the experiments were carried out according to the methodological instructions (Control Methods). The mass fraction of nitrogen (X) in the test sample was calculated as a percentage of its mass during the distillation of ammonia into sulfuric acid was calculated by the formula 1:

$$X = \frac{(V_1 - V_0) \times K \times 0{,}0014 \times 100}{M} \qquad (1)$$

where: V_o – is the volume of 0.1 mol/l of sodium hydroxide solution consumed for titration of 0.05 mol/l of sulfuric acid in the control experiment, ml; V_1 – is the volume of 0.1 mol/l of sodium hydroxide solution consumed for titration of sulfuric acid in the test solution, ml; K – is the correction to the titer of 0.1 mol/l of sodium hydroxide solution; 0.0014 – is the amount of nitrogen equivalent to 1 ml of 0.05 mol/l of sulfuric acid solution; M – is the weight of the sample, d.

The arithmetic mean of the results of five parallel tests was taken as the final test result. The results were calculated to the third decimal place and rounded to the second decimal place. The mass fraction of nitrogen in terms of the dry substance of the product (X_3), percent, was calculated by the formula 2:

$$X_3 = \frac{X_1 \times 100}{100 - W} \qquad (2)$$

where: X_1 – mass fraction of nitrogen in the test sample, %; W – moisture content of the test sample, %.

The mass fraction of protein (Y) in percent calculated by the formula 3:

$$Y = K \cdot X \qquad (3)$$

where: K – the conversion factor of nitrogen to protein with a moderate lipid content – 6.38.

Determination of carbohydrates in wheat straw

The following equipment was used: Agilent 1100 liquid chromatograph equipped with a Degasser G1379A degasser, a QuatPump G1311A pump, an ALS G1313A autosampler, a Colcom G1316A column thermostat, a G1362A rid refractometric detector, and an Agilent ChemStation data processing system B.01.03; Column SupelcosilLC-NH2 5 micron 4.6×250 mm, "Supelco", USA; micropipettes with a volume of 100 and 1000 µl, "VWR", Poland; 5 ml pipette, "Biohit", Finland; analytical balance AnD GR-202 (accuracy 0.00001 g), "AnD", Japan; Millipore Simplicity deionizers, "Millipore", France; ultrasonic bath S 30 H Elmasonic, "Elma", Germany; nylon 0.45 micron 13 mm filter. The analysis conditions were: the elution mode isocratic, and the volume ratio of the mobile phase of acetonitrile/water 82/18, without mixing, from two separate containers. The composition of the mobile phase may vary to achieve complete separation of the glucose and fructose peaks. Volumetric elution rate was 1.0 ml/min; injection volume was 10 µl; the column oven temperature was 35°C; and the retention times of standards were: fructose, 4.9 ± 0.2 min; glucose, 5.7 ± 0.2 min; sucrose, 10.4 ± 0.2 min; and maltose, 12.1 ± 0.2 min.

Isolation of free amino acids

The precipitation of proteins and peptides from the aqueous extract of the samples was

carried out in centrifuge beakers. For this purpose, 1 ml (exact volume) of 20% TCU was added to 1 ml of the test sample. After 10 min, the precipitate was separated by centrifugation at 8000 rpm for 15 min. After separating 0.1 ml of the supernatant, it was lyophilically dried. The hydrolysate was concentrated, the dry residue was dissolved in a mixture of triethylamine-acetonitrile-water (1:7:1) and dried. This operation was repeated twice to neutralize the acid. Reaction with phenylthioisocyanate produced phenylthiocarbamyl derivatives (PTC) of amino acids by the method of Steven et al. (1988). Amino acid derivatives were identified by HPLC using Agilent Technologies 1200 chromatograph with DAD detector, and a 75×4.6 mm Discovery HS C18 column. The solution A was 0.14 M CH_3COONa + 0.05% TEA pH 6.4, and the solution B was CH_3CN. The flow rate was 1.2 ml/min, and the absorption, 269 nm. Gradient %B/ min: 1–6%/0–2.5 min; 6-30%/2.51–40 min; 30–60%/40.1–45 min; 60–60%/45.1–50 min; 60–0%/50.1–55 min [Steven A et.al. 1988].

RESULTS AND DISCUSSION

In the present study, the amino acid, carbohydrate and protein composition of perennial and annual wheat straw residues were determined. Using microbiological research methods, screening of promising strains-producers of cellulase was carried out, and the composition of free amino acids and carbohydrates was studied after treating perennial and annual wheat straw with microorganisms in order to intensify the processes of enzyme biosynthesis. The scientific novelty of the study lies in that the sources of enzymes were identified with cellulose, xylanatic, β-glucanatic, and pectnasal action for the effective depolymerization of wheat straw polysaccharides to obtain glucose and sorbitol.

Total nitrogen and protein in the straw of perennial and annual wheat

According to the results of the study, the nitrogen and protein content was higher in the fungal treated annual and perennial wheat straw samples (Table 1). Moreover, fungal treated perennial wheat samples (2 and 4) had the highest nitrogen and protein content. Of the six variants used in the study, No.4 *(A. awamori* F-RKM 0719 + Sova perennial wheat straw) showed the highest levels of nitrogen (1.05%) and protein (6.5%).

Carbohydrates in the perennial and annual wheat straw

The following carbohydrates were identified in the annual and perennial wheat straw: fructose, glucose, sucrose, and maltose (Table 2). However, these carbohydrates were not found in all samples. Thus, fructose was not found in samples 3 and 6, glucose was absent from sample 6, sucrose, from sample 4, and maltose, from samples 4 and 6 (Tables 2, Figures 1-6). In sample 6 (Control: Sova perennial wheat straw), only sucrose was found.

Free amino acids in wheat straw

When the wheat samples were tested for 20 different amino acids, aspartic acid and lysine were absent from all experimental variants (Table 3). Glutamic acid was absent from sample No. 2. Glycine, asparagine, and glutamine were not detected in experimental variant No.2. Cysteine was absent from all samples but one (No.6), and threonine was found only in two samples (4 and 6). Arginine, alanine, and histidine were found in half of the samples, and leucine and tryptophan were absent from one sample (No.5). The smallest number of amino acids (8 out of 20) were found in sample No.2, and the largest (18 out of 20), in sample No.6. The latter sample also had

Table 1. Nitrogen and protein content of the annual and perennial wheat straw

№	Sample	Nitrogen, %	Protein, %
1	*Trichoderma harzianum* + Tulkibas annual wheat straw	0.42	2.6
2	*Trichoderma harzianum* + Sova perennial wheat straw	0.84	5.2
3	*A. awamori* F-RKM 0719 + Tulkibas annual wheat straw	0.35	2.1
4	*A. awamori* F-RKM 0719 + Sova perennial wheat straw	1.05	6.5
5	Control: Tulkibas annual wheat straw	0.28	1.75
6	Control: Sova perennial wheat straw	0.44	2.75

Table 2. Carbohydrates in perennial and annual wheat straw

№	Carbohydrates	№1	№2	№3	№4	№5	№6
		Concentration, mg/l					
1	Fructose	0.0200	0.0029	0.000	0.0079	0.0065	0.0000
2	Glucose	0.0044	0.0042	0.0035	0.0144	0.0512	0.0000
3	Sucrose	0.0130	0.0106	0.0342	0.0000	0.0080	0.0083
4	Maltose	0.0128	0.0151	0.0013	0.0000	0.0030	0.0000
Total		0.0503	0.0329	0.0390	0.0223	0.0686	0.0083

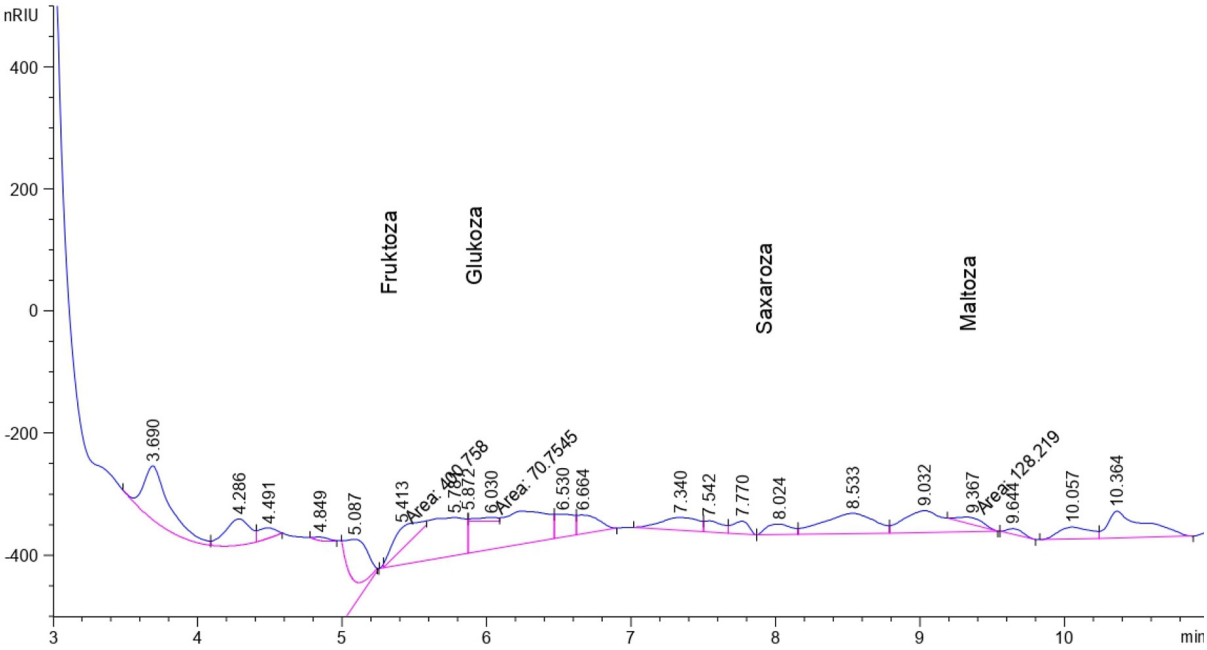

Figure 1. GC chromatogram of sample No.1: *Trichoderma harzianum* + Tulkibas annual wheat straw

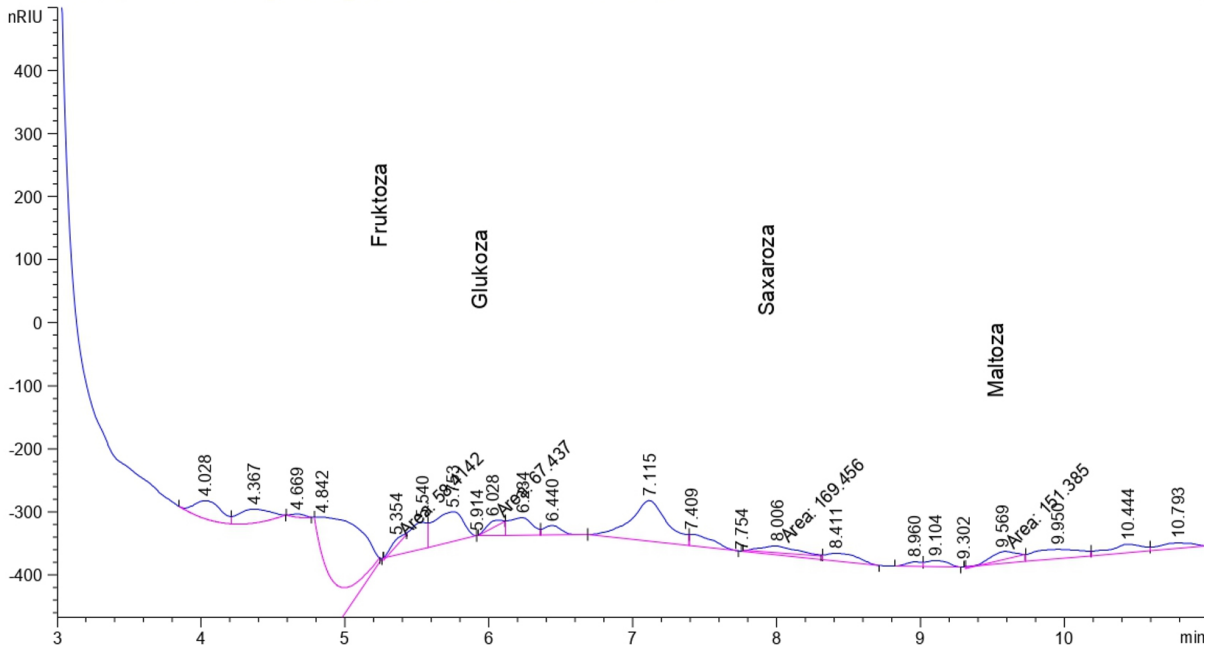

Figure 2. GC chromatogram of sample No.2: *Trichoderma harzianum* + Sova perennial wheat straw

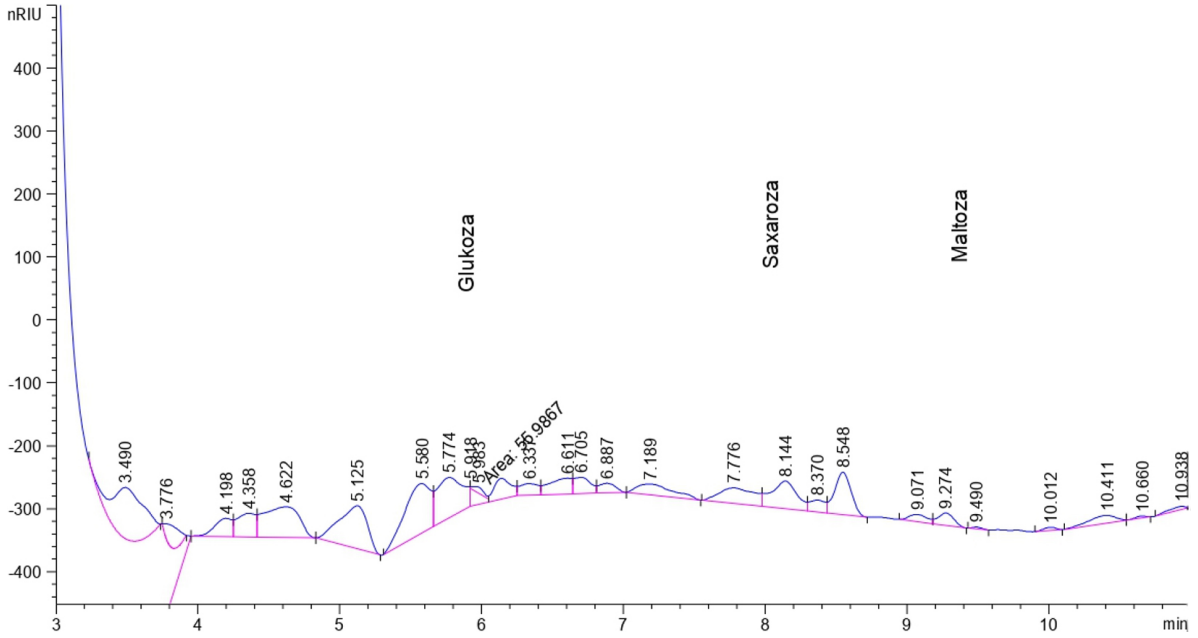

Figure 3. GC chromatogram of sample No.3: *A. awamori* F-RKM 0719 + Tulkibas annual wheat straw

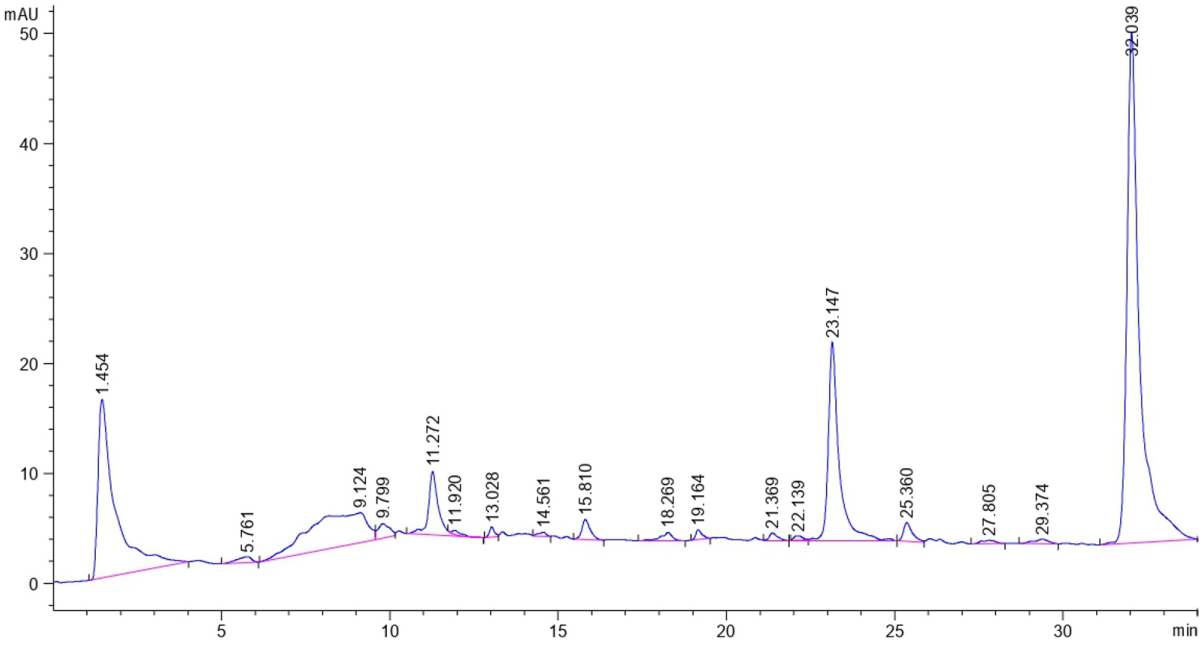

Figure 4. GC chromatogram of sample No.4: *A. awamori* F-RKM 0719 + Sova perennial wheat straw

the highest concentration of amino acids, 7.14 mg/ml, while the lowest concentration, 1.32 mg/ml, was found in sample No. 5.

Over the last decades, industries involved in the recycling of renewable raw materials have been actively developing. Agricultural waste is expected to be utilized more efficiently in the future. In the course of wheat production, two types of waste are generated: straw and bran. Wheat straw is a promising source of raw materials containing more than 30–40% of cellulose. In addition to its valuable chemical composition, wheat straw is produced annually and can be easily detected in the field. The use of wheat straw as a cellulose containing raw material for the production of sugars and other biologically valuable substances can solve the problem of food products shortages, including oligosaccharides, xylose and other valuable metabolites of microbial synthesis. The use of wheat straw as animal feed and as a secondary

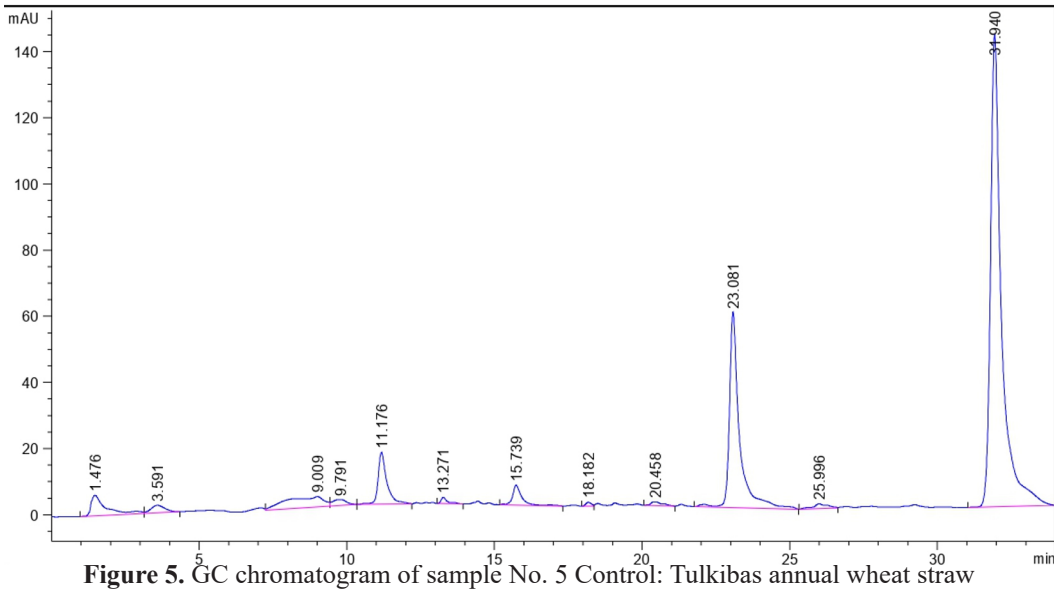

Figure 5. GC chromatogram of sample No. 5 Control: Tulkibas annual wheat straw

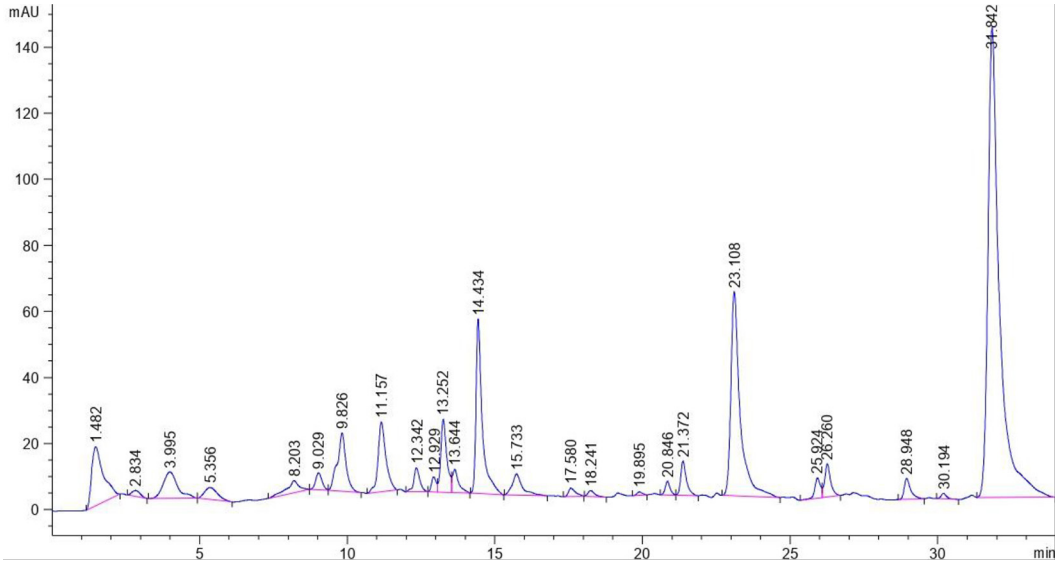

Figure 6. GC chromatogram of sample No. 6 Control: Sova perennial wheat straw

raw material has been growing steadily; the number of scientific publications regarding wheat straw nutritional properties and chemical composition has also increased. A positive customer experience of using straw-based products has led to the increased demand for the development of new wheat straw processing technologies.

Amino acids occupy a central position in cellular metabolism, since almost all biochemical reactions catalysed by enzymes involve amino acid residues. Therefore, better understanding of the amino acid composition of wheat straw and its changes during processing is very important for the future development of straw processing. As demonstrated by our study, wheat straw proteins have deficient amino acid composition, because they contain insufficient amounts of lysine and threonine. Therefore, in order for all essential amino acids to be in the required proportions, wheat straw should be combined with products containing large amounts of lysine and threonine. Nevertheless, the use of wheat straw can be recommended for use in the production of functional foods as a source of essential amino acids. The results obtained in our study can also contribute to the databases of the chemical composition of food products. Amino acid composition of proteins of annual and perennial wheat straw treated with *Trichoderma harzianum* 121 and *Aspergillus awamori* F-RKM 0719 strains demonstrated marked changes when compared to

Table 3. Free amino acids content in perennial and annual wheat straw

№	Amino acids	№1	№2	№3	№4	№5	№6
		Concentration, mg/ml					
1	Aspartic acid	0	0	0	0	0	0
2	Glutamic acid	0.116015	0	0.154792	0.051702	0.12232	0.177018
3	Serine	0.679847	1.189732	0.801339	0.842793	0.31824	0.166932
4	Glycine	0.068766	0	0.048522	0.027153	0.023699	0.03125
5	Asparagine	0.137211	0	0.098008	0.06009	0.047719	0.059608
6	Glutamine	0.281042	0	0.581341	0.398406	0.355412	0.605744
7	Cysteine	0	0	0	0	0	2.144262
8	Threonine	0	0	0	0.024438	0	0.168948
9	Arginine	0.009514	0	0	0	0.044699	0.469194
10	Alanine	0.003732	0	0	0.015239	0	0.079692
11	Proline	0.061121	0	0	0.026037	0.03376	1.716461
12	Tyrosine	0.01866	0.064673	0.045802	0.04877	0.032231	0.100297
13	Valine	0.029796	0.103375	0.0526	0.037093	0.049407	0.211766
14	Methionine	0.060493	0.452735	0.571641	0.021106	0.015755	0.043549
15	Histidine	0.183705	0	0	0.75031	0	0.666976
16	Isoleucine	0.052814	0.067335	0.26497	0.080956	0.06605	0.104857
17	Leucine	0.027018	0.040206	0.028627	0.030235	0	0.224027
18	Tryptophan	0.030257	0.05379	0.029034	0.037897	0	0.148686
19	Phenylalanine	0.130765	0.514968	0.496764	0.260113	0.215615	0.020125
20	Lysine	0	0	0	0	0	0
	Total	1.890757	2.486813	3.173439	2.712339	1.324906	7.139393

the control group. When treated with microorganism cultures, proteins contained in straw are hydrolysed by proteolytic enzymes to amino acids and peptides, which are used to build new tissues of the germinating seeds and are necessary for metabolism. In our study, among the non-essential amino acids, a high content of serine was noted. Essential amino acids are of particular value in the nutrition of animals. Of these, threonine and isoleucine are the most important. According to our results, threonine was present only in perennial wheat samples, while isoleucine was found in all six variants. Thus, perennial wheat straw is a valuable fodder product and a valuable raw material. In North America, perennial crops, including perennial wheat, attract the most attention as a possible livestock feed due to their agronomic properties, nutritious and tasty grains, synchronous seed ripening, moderate crushing, and acceptable threshing capacity [Cassman and Connor, 2022]. The technical and economic analysis of large-tonnage production aimed at the production of carbohydrate-containing plant raw materials and products based on world biotechnological experience shows that the increase in the profitability of these industries occurs when switching to a comprehensive processing of raw materials, including secondary waste. Such industries, primarily food, pharmaceutical and animal feed industries, require efficient waste processing [Chanda, 2021]. A complex processing of wheat straw to carbohydrates in order to obtain glucose and sorbitol will not only improve the environmental situation, but can also provide raw materials and valuable by-products for agriculture and other industries. The carbohydrate part of wheat straw contains large amounts of easily hydrolysed as well as difficult to hydrolyse polysaccharides. Readily hydrolysable polysaccharides include hemicelluloses and the amorphous portion of cellulose, and complex hydrolysable polysaccharides include cellulose (crystallized part) and a small part of crystallized hemicellulose [Liu et al., 2021].

In our study, *Trichoderma harzianum* was chosen as the most promising strain for further study. This strain is a promising producer of enzymes of the cellulolytic complex for the processing of cellulosic biomass in order to obtain glucose-like sugars, carbohydrates, and xylitol. However, the heat resistance of enzymes produced by fungi is low, their activity varies, and, due to their limited

number, mixing with other sources of enzymes is required. This approach is often achieved by mixing enzyme preparations or co-growing two or more microorganisms that exhibit synergies and complement missing properties of each other. When co-cultured, *Trichoderma harzianum* and *Aspergillus awamori F-RKM 0719* demonstrated the activity of fructose, glucose, sucrose, maltose 2-3 times higher compared to the control group. To obtain an effective enzyme preparation of cellulase, alternate introduction of cultures and joint cultivation of *Trichoderma harzianum* and *Aspergillus awamori* fungi are carried out using a cellulose component, which acts as an inducer of enzyme biosynthesis in a nutrient medium [Jiang et al., 2011]. For the deepest bioconversion of complex plant polysaccharides, such as wheat straw, the most effective approach is to form an association of microorganisms with a complex of enzymes that destroy cellulose and break down pectins.

It should be noted that in the developed countries, technologies for the production of organic agricultural products are becoming increasingly popular. Among them are those aimed at waste reduction, limited use of mineral fertilizers and pesticides, and toxic chemicals. As a result, organic farming systems can be most successful due to large revenues and high prices for organic farming products. The treatment of the residual fraction is a long process that is solved in stages and sequentially. Thus, the development and implementation of environmentally friendly oriented agricultural systems, and obtaining environmentally friendly types of food products is one of the most promising areas for the development of modern agriculture. Using modern agrobiotechnologies, restoration of soil fertility disturbed by long-term application of chemical fertilizers can be achieved in a relatively short time. This in turn will increase the yield of major crops, ensure the quality and safety of products and their waste, extend the shelf life of agricultural products, and enhance other properties of agricultural crops. With this technology, all types of products can be fully used, including phytomass, roots, as well as their waste.

CONCLUSIONS

During the experiment, straw of annual spring wheat of the Tulkibas variety and perennial wheat of the Sova variety was added to the enzyme solutions of *Trichoderma harzianum* 121 and *Aspergillus awamori* F-RKM 0719 strains. As a result, the variant *A. awamori F-RKM 0719* + Sova straw showed the highest level of nitrogen (1.05%) and protein (6.5%). The amino acid amount and composition varied significantly depending on the wheat straw variety and line. In our study, the perennial wheat variety Sova had a higher content of essential amino acids and their total amount compared to the lines of annual wheat; the essential amino acids content was higher than that of the non-essential amino-acids. The total carbohydrate content in the straw of the annual and perennial wheat varieties differed; the perennial wheat straw with the addition of *A. awamori* F-RKM 0719, contained fructose in an amount of 0.0079 mg/g, and in the annual wheat it was absent. The glucose content in the perennial wheat straw was three times higher than in the annual wheat straw, 0.0144 and 0.0035 mg/g, respectively.

Acknowledgements

This research was funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Grant No. AP09259457). The cost of publication will be covered as part of this project.

REFERENCES

1. Ahmad M., Taylor C.R., Pink D., Burton K., Eastwood D., Bending G.D., Bugg T.D. 2010. Development of novel assays for lignin degradation: comparative analysis of bacterial and fungal lignin degraders. Molecular Biosystems, 6(5), 815–821. https://doi.org/10.1039/B908966G
2. Bhardwaj N., Kumar B., Agrawal K., Verma P. 2021. Current perspective on production and applications of microbial cellulases: A review. Bioresources and Bioprocessing, 8, 1–34. https://doi.org/10.1186/s40643-021-00447-6
3. Cassman K.G. and Connor D.J. 2022. Progress towards perennial grains for prairies and plains. Outlook on Agriculture, 51(1), 32–38. https://doi.org/10.1177/00307270211073153
4. Chanda M. 2021. Chemical aspects of polymer recycling. Advanced Industrial and Engineering Polymer Research, 4(3), 133–150. https://doi.org/10.1016/j.aiepr.2021.06.002
5. Sushkova V.I., Vorobyeva V.I. 2007. Wasteless conversion of plant raw materials into biologically active substances. *Kirov*-2007.-204 p. [Rus]

6. Ghaffar S.H., Fan M. 2015. Differential behaviour of nodes and internodes of wheat straw with various pretreatments. Biomass and Bioenergy, 83, 373–382. https://doi.org/10.1016/j.biombioe.2015.10.020
7. Ghaffar S.H., Fan M., Zhou Y., Abo Madyan O. 2017. Detailed analysis of wheat straw node and internode for their prospective efficient utilization. Journal of agricultural and food chemistry, 65(41), 9069–9077. https://doi.org/10.1021/acs.jafc.7b03304
8. Hu X., Ma J., Qian W., Cao Y., Zhang Y., Liu B., Liu L. 2022. Effects of low temperature on the amino acid composition of wheat grains. Agronomy, 12(5), 1171. https://doi.org/10.3390/agronomy12051171
9. Jiang X., Geng A., He N., Li Q. 2011. Jiang, X., Geng, A., He, N., & Li, Q. 2011. New isolate of *Trichoderma viride* strain for enhanced cellulolytic enzyme complex production. Journal of bioscience and bioengineering, 111(2), 121–127. https://doi.org/10.1016/j.jbiosc.2010.09.004
10. Karatayev M., Clarke M., Salnikov V., Bekseitova R., Nizamova M. 2022. Monitoring climate change, drought conditions and wheat production in Eurasia: the case study of Kazakhstan. Heliyon, 8(1). https://doi.org/10.1016/j.heliyon.2021.e08660
11. Liu D., Tang W., Yin J.Y., Nie S.P., Xie M.Y. 2021. Monosaccharide composition analysis of polysaccharides from natural sources: Hydrolysis condition and detection method development. Food Hydrocolloids, 116, #106641. https://doi.org/10.1016/j.foodhyd.2021.106641
12. Luković K., Prodanović S., Perisić V., Milovanović M., Rajicić V., Zečević V. 2020. Multivariate analysis of morphological traits and the most important productive traits of wheat in extreme wet conditions. Applied Ecology and Environmental Research, 18(4), 5857–5871. http://dx.doi.org/10.15666/aeer/1804_58575871
13. Ma J., Li X.L., Xu H., Han Y., Cai Z.C., Yagi K. 2007. Effects of nitrogen fertiliser and wheat straw application on CH4 and N2O emissions from a paddy rice field. Soil Research, 45(5), 359–367. https://doi.org/10.1071/SR07039
14. Makhatov Zh.B., Kedelbaev B.Sh., Lakhanova K.M., Aimenova Zh.E., Adilkhanov S.I. 2017. Prospects of use of enzymatic treatment of wheat straw for glucose production. Industrial Technologies and Engineering (ICITE-2017).
15. Martínez Á.T., Speranza M., Ruiz-Dueñas F.J., Ferreira P., Camarero S., Guillén F., Río Andrade J.C.D. 2005. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. Int. Microbiol, 8, 195–204.
16. Mukherjee A., Wang S.Y.S., Promchote P. 2019. Examination of the climate factors that reduced wheat yield in Northwest India during the 2000s. Water, 11(2), 343–355. https://doi.org/10.3390/w11020343
17. Patel S.J., Onkarappa R., Shobha K.S. 2007. Comparative Study of Ethanol Production from Microbial Pretreated Agricultural Residues. J.Appl.Sci. Environ.Manage, 11(4), 137–141.
18. Prasanna B.D., Sathyanarayana N., Gummadi and Vadlani Praveen V. 2015. Biotechnology and Biochemical Engineering. Select Proceeding of ICACE: 978-9811019197. http://dx.doi.org/10.1007/978-981-10-1920-3
19. Rathour R.K., Devi M., Dahiya P., Sharma N., Kaushik N., Kumari D., Bhatia R.K. 2023. Recent trends, opportunities and challenges in sustainable management of rice straw waste biomass for green biorefinery. Energies, 16(3), 1429. https://doi.org/10.3390/en16031429
20. Ghaffar S.H. 2019. Wheat straw biorefinery for agricultural waste valorisation. Green Materials, 8, 60–67. https://doi.org/10.1680/jgrma.19.00048
21. Shah S., Venkatramanan V., Prasad R. 2021. Biorefinery: Potential and Prospects for Utilisation of Biogenic Waste. Bio-valorization of Waste: Trends and Perspectives, 315–325. http://dx.doi.org/10.1007/978-981-15-9696-4_14
22. Steven A.C., Trus B.L., Maizel J.V., Unser M., Parry D.A.D., Wall J.S., Studier F.W. 1988. Molecular substructure of a viral receptor-recognition protein: The gp17 tail-fiber of bacteriophage T7. Journal of molecular biology 200(2), 351–365. https://doi.org/10.1016/0022-2836(88)90246-X
23. Wang X., Cai D., Grant C., Hoogmoed W.B., Oenema O. 2015. Factors controlling regional grain yield in China over the last 20 years. Agronomy for sustainable development, 35, 1127–1138. https://doi:10.1007/s13593-015-0288-z
24. Winter G., Todd C.D., Trovato M., Forlani G., Funck D. 2015. Physiological implications of arginine metabolism in plants. Frontiers in plant science, 6, 534. https://doi.org/10.3389/fpls.2015.00534
25. Azipov N.N., Kamalov B.V., Zakirov I.R. 2012. Preparation of feed for feeding. Agrarian theme,1, 66–69. [Rus]
26. Xue C., Matros A., Mock H.P., Mühling K.H. 2019. Protein composition and baking quality of wheat flour as affected by split nitrogen application. Frontiers in plant science, 10, 642. https://doi.org/10.3389/fpls.2019.00642
27. Zając T., Synowiec A., Oleksy A., Macuda J., Klimek-Kopyra A., Borowiec F. 2017. Accumulation of biomass and bioenergy in culms of cereals as a factor of straw cutting height. International Agrophysics, 31(2), 273–285. https://doi.org/10.1515/intag-2016-0041
28. Zörb C., Ludewig U., Hawkesford M.J. 2018. Perspective on wheat yield and quality with reduced nitrogen supply. Trends in plant science,

23(11), 1029–1037. https://doi.org/10.1016/j.tplants.2018.08.012

29. Schierhorn F., Müller D., Prishchepov A., Faramarzi M., Balmann A. 2014. Schierhorn, F., Müller, D., Prishchepov, A. V., Faramarzi, M., & Balmann, A. 2014. The potential of Russia to increase its wheat production through cropland expansion and intensification. Global food security, 3(3–4), 133–141. https://doi.org/10.1016/j.gfs.2014.10.007
30. Blieva P.K., Sadanov A.K., Shormanova Sh.Sh. 2015. The leading role of macerating enzymes in the utilization of agricultural waste. Reports of the National Academy of Sciences of the Republic of Kazakhstan, 5, 106–113. [Rus]
31. Boltovsky V.S. 2014. Hydrolytic processing of polysaccharide components of plant biomass: problems and prospects. Proceedings of the National Academy of Sciences of Belarus. Chemical Sciences Series, 1, 118–123. [Rus]
32. Korobko V.V., Khakalova D.A. 2007. Features of the organization of the shoot node of soft spring wheat. Bulletin of the Botanical Garden of Saratov State University, 6, 138–141. [Rus]
33. Marchenko D.M., Kostylev P.I., Grichanikova T.A. 2013. Correlation analysis in winter wheat breeding (review). Grain Farming of Russia, 3, 28–32. [Rus]
34. Wang X., Wang Q., Zhang Y., Zhang J., Xia S., Qin H., Bie S. 2023. Influence of decomposition agent application and schedule in wheat straw return practice on soil quality and crop yield. Chemical and Biological Technologies in Agriculture, 10(1), 1-12.
35. Zhapayev R., Toderich K., Kunypiyaeva G., Kurmanbayeva M., Mustafayev M., Ospanbayev Z., Kusmangazinov A. 2023. Screening of sweet and grain sorghum genotypes for green biomass *production* in different regions of Kazakhstan. Journal of Water and Land Development. https://doi.org/10.24425/jwld.2023.143752