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Study of changes in antioxidant activity during fermentation of various types of legumes

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ABSTRACT

Background: Diacylglycerol (DAG) oil is a natural component of various edible oils. DAG has been reported to prevent obesity through a variety of potential mechanisms in comparison with triacylglycerol (TAG) in humans. An increase in postprandial energy expenditure (EE) is proposed to be one of the mechanisms underlying this effect of DAG. Up-regulated mRNA expressions associated with EE by DAG in the small intestine may explain increased postprandial EE. The small intestine seems to contribute to changes in EE by DAG. We previously studied plasma serotonin, which is mostly present in the small intestine and mediates sympathetic thermogenesis. We found that DAG ingestion increases plasma serotonin levels by approximately 50% compared to TAG ingestion.

Objective: To understand the molecular mechanisms for DAG-induced increase in serotonin and EE, we investigated effects of DAG on serotonin release and expressions of genes associated with EE, using the human intestinal cell line.

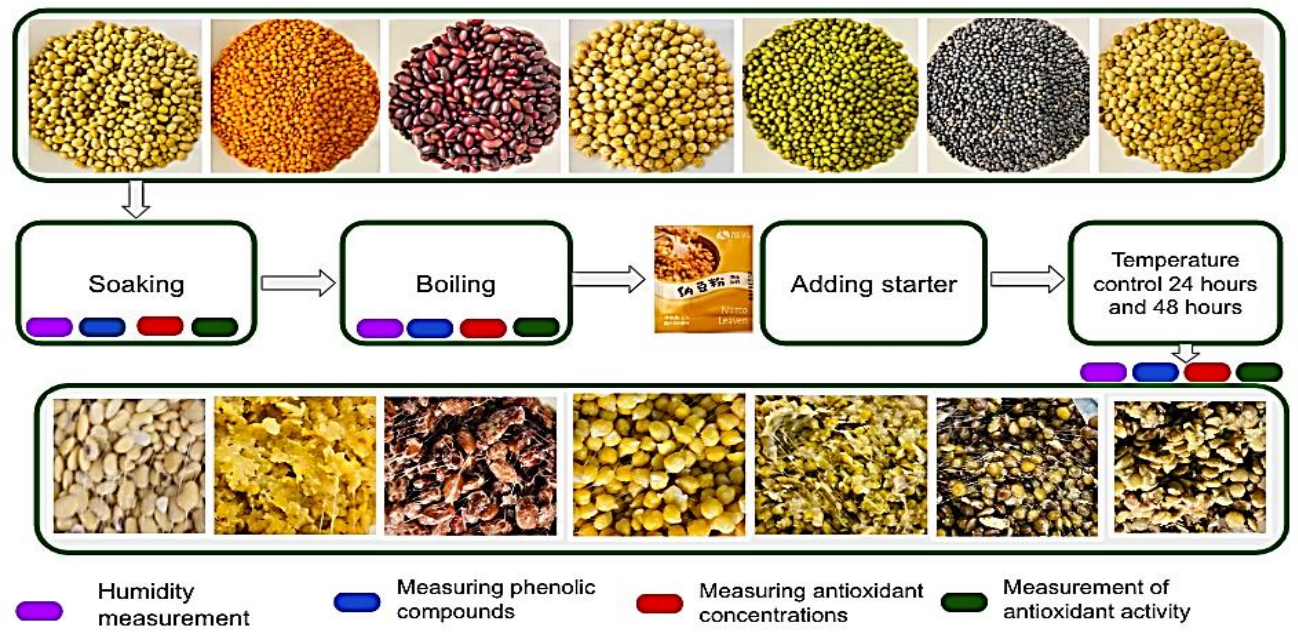
Methods: The intestinal cell line, the Caco-2 cells, was incubated with medium containing 1-monoacylglycerol (1-monooleyglycerol [1-MOG]) and 2-monoacylglycerol (2-monooleylglycerol [2-MOG]), distinctive digestive products of DAG and TAG, respectively. We measured serotonin release from the Caco-2 cells using a newly developed high-performance liquid chromatography. Further, we studied effects of 1-MOG, 2-MOG, and serotonin on expressions of

mRNA associated with EE (acyl-CoA oxidase [ACO], medium-chain acyl-CoA dehydrogenase [MCAD], fatty acid translocase [FAT], and uncoupling protein-2 [UCP-2]), by the Real-Time quantitative RT-PCR system.

Results: 100 mM 1-MOG significantly increased serotonin release from the Caco-2 cells compared with the same concentration of 2-MOG by approximately 37% ($P<0.001$). Expressions of mRNA of ACO, FAT, and UCP-2 were significantly higher in 100 mM 1-MOG-treated Caco-2 cells than 100 mM 2-MOG-treaed cells by approximately 13%, 24%, and 35%, respectively. Expressions of mRNA of ACO, MCAD, FAT, and UCP-2 were significantly increased in 400 nM serotonin-treated Caco-2 cells as compared with the Caco-2 cells incubated without serotonin by approximately 29%, 30%, and 39%, respectively.

Conclusion: Our study demonstrated that a hydrolytic product of DAG increases serotonin release from the intestinal cells and enhances expressions of genes associated with b-oxidation (ACO, MCAD), thermogenesis (UCP-2) and fatty acids metabolism (FAT). Furthermore, this study revealed that serotonin also enhances expression of these genes, proposing a new molecular biological mechanism for DAG-mediated anti-obesity effect. Serotonin may play an important role in DAG-mediated prevention of obesity.

Keywords: Fabaceae functional food, sustainable food, fermentation, antioxidant activity, osteoporosis prevention, obesity prevention, B vitamins, natto, vegetable protein.



INTRODUCTION

Food products made from legumes are valuable sources of easily digestible protein, fiber, iron, folic acid, calcium, zinc, and B vitamins [1-3]. In the context of the global tendency to achieve the Sustainable Development Goals and population growth, resource shortages, the population's diet will undergo significant changes over the coming decades to meet sustainability criteria [4-5]. First and foremost, it is essential to reduce our consumption of animal products, particularly beef, as its production has the most significant negative impact on the environment. Additionally, a high intake of beef is linked to various health issues, including cardiovascular diseases and cancer, and it contributes to the development of antibiotic resistance in pathogenic microorganisms [6-8]. Legume-based products as well as other plant-based products may serve as good replacement for beef and as basis for a sustainable diet [8-10]. Alongside the pressing issues of population growth, urbanization, and the reduction of agricultural land, the trend of overconsumption of calories among the population—an issue that inevitably leads to obesity—is becoming increasingly critical [11-12]. At the same time, the problem of protein deficiency, micro and macroelements, which provoke several socially significant diseases, such as diabetes, osteoporosis, cancer and obesity, becomes obvious, which leads to economic losses and reduced working capacity of the population [13-17].

Considering the problems described above, it becomes obvious that there is a need for the development of biotechnologies and the production of food products based on plant raw materials, including legumes, satisfying requirements for affordability for the population, organoleptic indicators, as well as the content of important components, such as protein, fiber, vitamins, phenolic substances, antioxidants, etc. A variety of legumes, which have differing levels of these nutrients, can serve as compensatory or functional foods to address deficiencies in specific nutrients. Fermentation of legumes makes it possible to operate with qualitative or quantitative indicators of certain nutrients, allowing the use of fermentation as a tool for correcting indicators to the required parameters, including the elimination of antinutrient factors [18-20]. For example, the traditional Japanese dish natto, made from soybeans fermented with *Bacillus subtilis*, acquires new functional characteristics compared to non-fermented soybeans, such as probiotics, the nattokinase enzyme, and vitamin K2 content.

This product has proven effectiveness as a preventive type of nutrition for diabetes, obesity, osteoporosis, thrombosis, protein and B vitamins deficiency [].

The content of antioxidants and phenolic compounds is an important indicator for preventive nutrition products, prevention of cancer, in particular prostate, ovarian, cervical and breast cancer, as well as Alzheimer’s and Parkinson’s [31-39].

Table 1 provides an overview of some legumes used in the food industry, where their huge role in the human diet and great potential for the creation of new products is clear. These crops will be studied in this work.

Table 1. Legumes studied in this work [40-44]

Name/Systematized name (Latin)	Photo of the studied samples	Cultivation area	Biological, agronomic and physico-chemical features	Use in food technology
Soybeans / Glycine max		Cultivated in more than 60 countries on all continents except Antarctica. The leaders in growing and exporting soybeans are Brazil, the USA, Argentina and China.	High protein content 38-42%. High content of phospholipids. isoflavones. Low carbohydrate content 22-35%, very little starch 1-1.5%. Antinutritional components (protease inhibitors, lectins).	Producing soy sauce, tofu, natto. Bread industry. Production of sausages. Canning industry.
Chickpeas / Cicer arietinum		More than 90% of the harvest comes from South and Western Asia. The leaders in chickpea cultivation are India, Australia, Türkiye, and Pakistan.	A source of zinc and folic acid, the essential amino acid lysine, vitamins B1 and B6. Contains about 20-30% protein, 50-60% carbohydrates, up to 7% fat (mostly polyunsaturated).	The basis for preparing traditional Middle Eastern dishes - hummus and falafel. Canning industry.
Mung beans/ Vigna radiata		Mainly grown in East, Southeast and South Asia. Leaders in cultivation are India, Pakistan, China.	Contains about 55-65% carbohydrates, rich in protein 20-30%, vitamins and minerals. Mung bean is considered the main source of dietary proteins. The proteolytic breakdown of these proteins is even higher during germination.	It is used in Asian and Indian cuisine in whole, shelled and sprouted form, as well as to obtain starch, from which so-called glass noodles are prepared.
Beans/ Phaséolus vulgáris		The top five producers are India, Brazil, Myanmar, China and the USA.	Bean contains proteins (in some varieties up to 31%), 50-60% carbohydrates (mono- and oligosaccharides, starch), up to 3.6% fatty oil, carotene, potassium, phosphorus, a significant amount of copper and zinc, nitrogenous substances (including essential amino acids), flavonoids (quercituron), sterols (β- and γ-sitosterols, stigmasterol) and organic acids (malic, malonic, citric). Contains vitamins: pyridoxine, thiamine, pantothenic and ascorbic acids.	Used in Mexican, Indian, Creole cuisine. Soups, side dishes, and canned food are made from beans. Raw beans, especially red beans, contain significant amounts of lectins, which have toxic effects. To neutralize them, long-term (30 minutes) boiling in water is used.
Green lentils/ Lens culinaris		The largest areas of lentil cultivation are in Canada, India, Australia and Turkey.	Contains dietary fiber, up to 25% protein. Increased content of microelements - potassium, iron and phosphorus.	Lentils are used to make soups, side dishes (often mixed with grains, such as rice), bread, and added to crackers and cookies.
Red lentils/ Lens culinaris		It is especially widely cultivated in India.	Of the fat-soluble vitamins, red lentils contain vitamin A and beta-carotene. Among the water-soluble ones are vitamins C, B1, B2, B3 (PP), B5, B6 and B9. Red lentils are rich in copper, manganese and iron.	Red lentils have a sweeter taste, and a softer texture compared to green lentils. Porridges, side dishes, soups, stews are prepared from red lentils. You can use it to make lean cutlets and pancakes, healthy salads and vegetarian pate.
Black lentils/ Lens culinaris		Black lentils were developed in Canada. The variety quickly spread to the USA, India and Asian countries.	Contains up to 35% protein; up to 53% carbohydrates, including complex ones; about 2% fat, vitamins A, C, group B - especially a lot of folic acid; minerals - potassium, phosphorus, iron, zinc, magnesium, calcium, etc. The black color of lentils is due to their high content of pigment with antioxidant properties.	Catering enterprises and food industry. Soups, vegetable stews, side dishes, salads, sauces, sweet dishes.

Fermentation of legumes makes it possible to operate with qualitative or quantitative indicators of certain nutrients, allowing the use of fermentation as a tool for correcting indicators to the required parameters, including the elimination of antinutrient factors [18-20]. For example, the traditional Japanese dish natt, made from soybeans fermented with *Bacillus subtilis*, acquires new functional characteristics compared to non-fermented soybeans, such as probiotics, the nattokinase enzyme, and vitamin K2 content.

This product has proven effectiveness as a preventive type of nutrition for diabetes, obesity, osteoporosis, thrombosis, protein and B vitamins deficiency [21-30].

The content of antioxidants and phenolic compounds is an important indicator for preventive nutrition products, prevention of cancer, in particular prostate, ovarian, cervical and breast cancer, as well as Alzheimer's and Parkinson's [31-39].

Table 1 provides an overview of some legumes used in the food industry, where their huge role in the human diet and great potential for the creation of new products is clear. These crops will be studied in this work.

The purpose of this study is to obtain data on changes in the concentration of phenolic compounds and their relationship with the antioxidant status of legume foods during their fermentation with the probiotic microorganism Bacillus subtilis. Of particular interest is the evidence base for the antioxidant activity of Bacillus subtilis metabolites during the fermentation of legume raw materials.

The data obtained will allow us to assess the potential of these food products as a source of compounds beneficial to human health and will also be useful for building predictive models for the storage of these products.

MATERIALS AND RESEARCH METHODS

The following types of legumes were used as samples for fermentation:

- Soybean "Altaiskaya", harvest 2023. Country of origin: Russia. Proteins - 36.7 g, fats - 17.8 g, carbohydrates - 17.3 g. Energy value 364 kcal. Round grains with a diameter of about 5-6 mm, coloring from light yellow to light brown with dark inclusions.
- Chickpeas - harvest 2023. Country of origin: Uzbekistan. Proteins - 20.1 g, fats - 4.3 g, carbohydrates - 46.2 g. Energy value 309 kcal.
- Round grains with a diameter of about 6-8 mm, coloring from light yellow to light brown with dark inclusions.
- Mung bean - harvest 2023. Country of origin Uzbekistan. Proteins - 23.5 g, fats - 2.0 g, carbohydrates - 46 g. Energy value 300 Kcal.
- Rounded grains, about 3-4 mm in diameter, dark green in color with rare dark brown inclusions.
- Red beans, variety "Rubin", harvest 2023. Country of origin: Russia. Proteins - 21.5 g, fats - 1.6 g, carbohydrates - 52.7 g. Energy value 310 Kcal.
- Large green lentils "Canadskaya" produced by "Mistral", harvest 2023. Country of origin: Russia. Proteins - 19.4 g, fats - 1.1 g, carbohydrates - 59.1 g. Energy value -339 Kcal. Fiber 7.5g. Technical specifications - 01.11.74-036-99621687-2022.
- Red lentils, produced by "Agro-Alliance", harvest 2023. Country of origin: Russia. Proteins - 26.0 g, fats - 2.0 g, carbohydrates-57 g. Energy value 350 Kcal. Technical specifications -01.11.74-006-87345472-

2019.

- Rounded grains, flattened in one plane, about 3-4 mm in diameter and about 2 mm thick, orange in color with rare dark brown inclusions.
- Black lentil variety “Beluga”, producer “Sampo”, harvest 2023. Country of origin: Russia. Proteins - 35.0g, fats - 2.0g, carbohydrates - 53.0g. Energy value - 325 Kcal. STO 21318887-009-2013

Rounded grains flattened in one plane, about 2-4 mm in diameter and about 2 mm thick, color from dark brown to black. *Bacillus subtilis* is a species of gram-positive spore-forming facultative aerobic soil bacteria, which, due to its ability to acidify the environment and produce antibiotics, is an antagonist of pathogenic and opportunistic microorganisms such as salmonella, proteus, staphylococci, streptococci, yeast fungi; produce enzymes that remove putrefactive tissue decay products; synthesize amino acids, vitamins, and immune-active factors. Some strains of Bacillus subtilis are producers of hyaluronic acid. A commercially available powder based on the spores of this culture, Bacillus subtilis natto, and starch, was used as a starter for the fermentation of legumes. The starter is produced in 1 gram portion packets and is designed for 200 g of prepared beans. The country of origin of the starter is China.

METHODS

Determination of humidity, moisture content: The essence of the method is to dry a portion of samples at high temperatures and calculate the mass loss as a result of drying.

Two portions of samples weighing 10 g each are weighed with an error of no more than 0.001 g into previously prepared weighing bottles. Open bottles with samples and lids are placed in a drying cabinet heated to (103 ± 2) °C. The samples are dried for 6 hours, then the bottles are closed with lids, cooled in a desiccator, and weighed. After weighing, the samples are dried again at the same temperature for 1 hour to constant weight.

The mass fraction of moisture (X) as a percentage is calculated using formula 1:

$$X = \frac{m_1 - m_2}{m} \cdot 100 (1)$$

Where m – sample mass before drying, gr;

m_1 – mass of weighing bottle with the samples before drying, gr;

m_2 – mass of weighing bottle with the samples after drying, gr;

The arithmetic mean of the results is taken as the result of the analysis two parallel definitions, the discrepancy between which does not exceed 0.2%. The result is calculated to the first decimal place.

Determination of the total phenolic compounds content by the spectrophotometric method (with the Folin-Ciocalteu reagent): To determine the total content of phenolic compounds, a spectrophotometric method was used, where the light absorption of complexes of phenolic compounds with the Folin-Ciocalteu reagent was measured as an analytical signal. Optical density measurements were carried out on a SPECTRO star Nano BMG LABTECH spectrophotometer (Germany) at a wavelength of 765 nm (maximum absorption).

Gallic acid was used as a standard sample solution for the determination of phenolic compounds. To construct a calibration curve, a series of 6 solutions of different concentrations was prepared.

To carry out the analysis, 1 ml of the extract was transferred into a 25 ml flask, a fivefold solution of the Folin-Ciocalteu reagent (1:10) was added and after 5 minutes the same volume of a 7% carbonate solution was added and mixed thoroughly. The volume was brought to the mark with the extractant and placed in a dark, cool place for 2 hours for complex formation, after which the optical density of the blue-violet solution was measured at 765 nm.

Determination of the concentration of substances with antioxidant properties SCA total content of antioxidants

Permanganatometric titration method: Antioxidant activity (AOA) was determined by permanganometric titration. The method is based on determining the volume of a titrant solution (in this case, an extract) containing antioxidant substances, which will be spent on decolorization of a 0.05 N solution of potassium permanganate in 0.24 molar sulfuric acid. To quantitatively characterize the value of antioxidant activity, the value B is introduced, which is the content of the amount of biologically active substances of a reducing nature in terms of quercetin in 1 ml or 1 g of the drug (object). The higher the value of B, the higher the AOA the object has.

To calculate the value of B, formula 2 is used:

$$B = \frac{Ck \cdot Vk \cdot Vo}{Vx \cdot m} \quad (2)$$

where B is the concentration of biologically active substances of a reducing nature of the test object, consumed for titration of 1 ml of 0.05 N solution of potassium permanganate, mg/g;

Ck is the concentration of quercetin in the solution used for titration of 1 ml of 0.05 N solution of potassium permanganate, mg/ml;

Vk is the volume of quercetin solution consumed for titration of 1 ml of 0.05 N solution of potassium permanganate, ml;

V is the volume of the test solution, ml;

Vx is the volume of solution of the test object consumed for titration of 1 ml of 0.05 N solution of potassium permanganate, ml;

m is the mass of the sample of the object under study, g.

Spectrophotometric method with DPPH radicals: The so-called DPPH method is based on the ability of antioxidants to interact with the chromogenic radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).

To carry out the procedure, a solution of a standard DPPH sample in methanol (c = 0.01 mmol/l) was prepared; the optical density was measured on a SPECTROstar Nano BMG LABTECH spectrophotometer (Germany) at a wavelength of 517 nm.

To determine the antioxidant activity, 0.1 ml of the test sample was added to 3.9 ml of DPPH, the samples were left for 15 minutes in a dark place, after which the optical density was measured.

The degree of free radical inhibition (X) as a percentage was calculated using formula 3:

$$X = \frac{A_0 - A_x}{A_0} \cdot 100 \quad (3)$$

Where:

Ao - is the optical density of the original DPPH solution;

Ax - is the optical density of the analyzed solution (a mixture of DPPH and the test sample).

The sequence of work when obtaining fermented products is shown in Table 2

Table 2. Sequence of technological operations and measurement methods used.

Sequence of operation	Operation name	Technological characteristics and operation parameters
1	Reception, sorting and weighing of raw materials: - soy - chickpeas - mung bean - beans - green lentils - red lentils - black lentils	Selection of substandard raw materials based on visual signs. Weighing the initial mass of samples on a BK-1500 scale (accuracy ±0.02 grams) in an amount of 500 grams ± 1 gram
2	Definition of initial indicators: humidity phenolic compounds content	The method is described above p. 2.1 The method is described above p.2.2 The method is described above p.2.3 The method is described above p.2.4
3	Soaking raw materials	Duration 24 hours the temperature throughout the entire process was maintained at 14 ± 2 °C to prevent spontaneous fermentation in the SM 5/100-80 TSO thermostat (temperature maintenance accuracy ± 2C)
4	Determination of indicators of soaked raw materials: humidity concentration of phenolic compounds antioxidant concentration reduction factor	The method is described above p. 2.1 The method is described above p.2.2 The method is described above p.2.3 The method is described above p.2.4
5	Boiling of raw materials	Heating the boiled biomass until boiling. Boil for 60 minutes with regular stirring (once every 5 minutes). Cooker RDE-1620
6	Cooling and removal of released free moisture	The temperature of the raw material after cooling is 25 ± 1C. Fixed with an LTA-M thermometer (accuracy ± 0.2 C). Cooling duration is 90-120 minutes. Excess moisture is removed by overflow.
7	Determination of indicators of heat-treated raw materials: humidity concentration of phenolic compounds antioxidant concentration DPPH scavenging activity of legume products	The method is described above p. 2.1 The method is described above p.2.2 The method is described above p.2.3 The method is described above p.2.4
8	Inoculation with microorganisms Bacillus subtilis	Addition of the microorganism Bacillus subtilis in the amount of 0.5 grams of pure culture for every 100 grams of prepared raw materials. Weighing was carried out on a VK-300 scale (accuracy ±0.001 grams).
9	Fermentation 24 hours	Fermentation of raw materials for 24 hours at a temperature of 30C in a thermostat SM 5/100-80 TSO (temperature maintenance accuracy ± 2C)
10	Determination of indicators of heat-treated raw materials: humidity concentration of phenolic compounds antioxidant concentration DPPH scavenging activity of legume products	The method is described above p. 2.1 The method is described above p.2.2 The method is described above p.2.3 The method is described above p.2.4
11	Fermentation 24 hours (total 48 hours)	Fermentation of raw materials for 24 (total 48) hours at a temperature of 30C in a SM 5/100-80 TC thermostat (temperature maintenance accuracy ± 2C)
12	Determination of indicators of heat-treated raw materials: humidity concentration of phenolic compounds antioxidant concentration DPPH scavenging activity of legume products	The method is described above p. 2.1 The method is described above p.2.2 The method is described above p.2.3 The method is described above p.2.4

A B C

Picture 1. Changes in the structure of a fermented product during fermentation using soybean as an example. A - soybean before water-thermal treatment, B - soybean after water-thermal treatment, C - soybean after fermentation.

In Picture 1 it is possible to observe a visual change in the structure of the product during the preparation of a fermented product using soybean as an example.

RESULTS AND DISCUSSION

As a result of water-thermal treatment of the product throughout the entire technological process (soaking, cooking, cooling, fermentation for 24 and 48 hours), it was found that changes in moisture content are non-linear and, apparently, are significantly related to the individual structure of legumes (Table 3). At the same time, some patterns seem important. For example, the slight decrease in humidity during the second stage of fermentation (from 24 to 48 hours) in percentage terms was very similar and amounted to 5%.

The humidity indicator is important in the industrial processing of raw materials, since it fundamentally determines the mass of the finished product, and, consequently, its commercial cost. In addition, knowledge of the patterns of changes in product moisture will allow you to accurately predict the characteristics of equipment for industrial production. More precise rheological characteristics of the raw material remain to be established in future studies.

Table 3. Changes in the humidity of raw materials, semi-finished products and ready products.

Raw materials	Initial humidity, %	Humidity after soaking, %	Humidity after boiling and cooling, %	Humidity after fermentation 24 hours, %	Humidity after fermentation 48 hours, %
Soybeans	12,1±0,2	35,2±0,7	47,5±1,0	46,2±0,9	43,9±0,9
Chickpeas	10,5±0,2	46,0±0,9	52,5±1,1	51,9±1,0	49,3±1,0
Mung beans	11,3±0,2	35,7±0,7	50,4±1,0	50,1±1,0	47,1±0,9
Beans	11,9±0,2	35,5±0,7	48,3±1,0	47,5±1,0	45,1±0,9
Green lentils	10,5±0,2	38,1±0,8	49,8±1,0	49,2±1,0	46,3±0,9
Red lentils	11,5±0,2	36,2±0,7	48,6±1,0	48,2±1,0	45,3±0,9
Black lentils	10,2±0,2	36,5±0,7	45,2±0,9	44,4±0,9	42,2±0,8

The concentration of phenolic compounds undergoes significant changes during the process of biological transformation (Table 4). The authors attribute this to the partial loss of this component during hydrothermal treatment. These losses can be estimated at 38-42%.

No significant changes in this indicator were observed during fermentation for all samples. On average, the decrease in the concentration of phenolic compounds under the action of *Bacillus subtilis* was 0.2-0.5%.

These data allow us to draw a conclusion about the dynamic antioxidant potential of the system in the technological process of producing fermented products from legumes and select raw materials depending on the desired antioxidant status.

Table 4. Content of phenolic compounds in legumes at various stages

Raw materials	Content of phenolic compounds, mg/100 g				
	Feedstock	Raw materials after soaking	Raw materials after boiling	Product after fermentation 24 hours	Product after fermentation 48 hours, %
Soybeans	2239,1±22,4	1956,5±19,6	1410,8±14,1	1401,4±14,0	1395±14,0
Chickpeas	2246,3±22,5	1773,9±17,7	1350,9±13,5	1329,9±13,3	1320,4±13,2
Mung beans	3332,3±33,3	2715,9±27,2	1992,7±19,9	1990,5±19,9	1990,1±19,9
Beans	3644,9±36,4	2813,0±28,1	2186,1±21,9	2162,1±21,6	2155,2±21,6
Green lentils	2384,0±23,8	1985,5±19,9	1455,1±14,6	1420,1±14,2	1415,7±14,2
Red lentils	2601,4±26,0	2272,4±22,7	1568,0±15,7	1551,0±15,5	1442,0±14,4
Black lentils	2724,6±27,2	2285,5±22,9	1635,5±16,4	1611,5±16,1	1602,5±16,0

It is reliably known that phenolic compounds make a significant contribution to the total antioxidant status of products [45]. It is also known that metabolites of microorganisms produce substances of antioxidant nature [46]. A study of the antioxidant properties of fermented legumes showed results confirming these properties of living systems (Table 5).

It is important to note the significant accumulation of antioxidant substances because of bacterial activity. This increase by 20 - 30% offsets the loss of the antioxidant properties of the product at the soaking and cooking stage, which is significant.

It is also noted that the behavior of antioxidant status at individual stages of fermentation has its own patterns.

We attribute this to the fact that in fermented legumes, accumulation is associated with the chemical composition of the feedstock, which is predisposed to fermentation of legumes to a greater or lesser extent. In the legumes observed in this study, it can be said that

phenolic compounds rather contribute to the fermentation process, i.e. are a development stimulator for the bacteria *Bacillus subtillis.*

For example, fermented mung beans, beans and black lentils have the highest concentration of antioxidants, while their content of phenolic compounds is also the highest.

We also note that in the period from 24 to 48 hours of fermentation, there is a stabilization of the concentration of substances of antioxidant nature, which may be associated with the accumulation of such metabolites in the first day of fermentation.

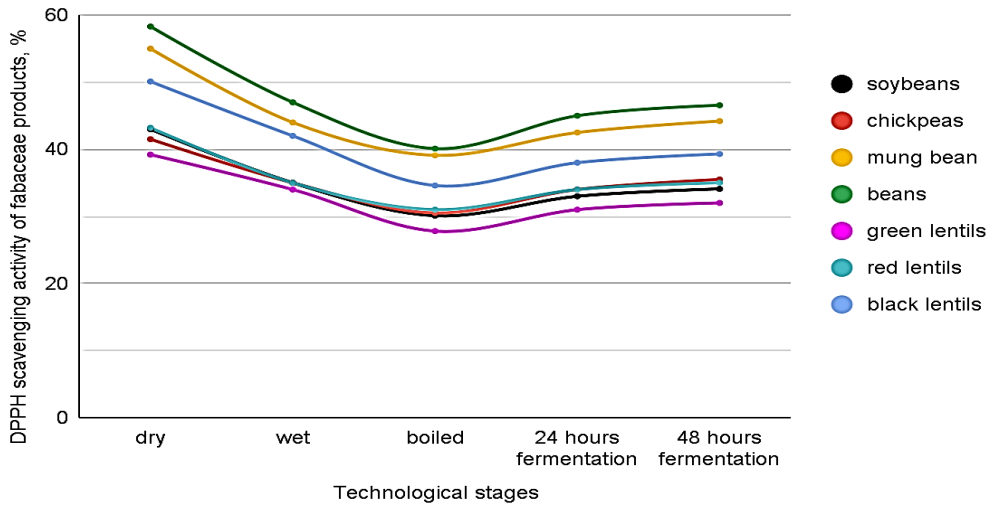
In general, the antioxidant status in products from fermented legumes and their fermentation products can be assessed as quite high. For example, in fruit juices known for their antioxidant properties [47], this indicator is: blueberry juice 291 mg/100 g, apple juice 57 mg/100 g, kiwi juice 45 mg/100 g. In vegetable juices: tomato juice 64 mg/100 g, carrot juice 19 mg/100 g. [48]

Table 5. Content of antioxidants at various stages of production

Raw materials	**Content of antioxidant compounds, mg/100 g**				
	Feedstock	**Raw materials after soaking**	**Raw materials after boiling**	**Product after fermentation 24 hours**	**Product after fermentation 48 hours, %**
Soybeans	65,48±3,25	55,21±2,61	45,09±2,26	48,21±2,46	49,91±2,45
Chickpeas	67,63±3,38	56,15±2,81	47,04±2,35	50,75±2,59	52,98±2,65
Mung beans	82,58±4,13	71,56±3,58	57,58±2,88	62,58±3,18	64,37±3,22
Beans	88,76±4,44	73,84±3,69	62,78±3,14	65,51±3,32	67,29±3,37
Green lentils	62,73±3,14	54,62±2,73	45,63±2,28	47,70±2,44	49,26±2,47
Red lentils	65,63±3,28	57,94±2,90	47,31±2,37	49,21±2,51	51,09±2,56
Black lentils	77,26±3,87	67,32±3,37	55,84±2,79	60,37±3,10	61,64±3,12

The activity of the antioxidant complex of compounds in the Fabaceae - *Bacillus subtillis* system was also determined using the DPPH method, which evaluates the total contribution of all components to the overall status of the system. The results presented in Picture 2 correlate well with the data in Table 5. An important observation can be considered:

- decrease in antioxidant status after soaking and washing legumes by 28-33% of the original,
- an increase of 20-30% from the minimum value after hydrothermal treatment of the system. Probably due to the accumulation of active metabolites of Bacillus subtillis [49-50].
- the total decrease in antioxidant activity in the finished product, compared to the original raw material, can be characterized as 20-25%, which can be considered acceptable, especially since the original raw material is not traditionally used in its native form.
- Increase in antioxidant activity during fermentation.



Picture 2. Kinetics of changes in antioxidant activity when obtaining a fermented product from legumes.

CONCLUSION

During the production of legumes fermented by *Bacillus subtilis*, profound physical and biochemical transformations occur, which have a significant impact on the antioxidant potential of the product.

The humidity of the product determines the change in the amount of antioxidant substances due to losses with water during soaking and boiling.

During fermentation, on the contrary, the antioxidant status of the product increases.

It's important to note that, in addition to their health benefits, antioxidants play a crucial role in the storage of finished products by extending shelf life and preventing undesirable oxidative processes. The relationship between antioxidant potential and storage quality of fermented soy products remains to be established.

List of Abbreviations: STO: standard of organization; DPPH: chromogenic radical 2,2-diphenyl-1-picrylhydrazyl.

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