Original Article

Research of Impact of Direct Bioconversion of Secondary Grain and Fruit Raw Materials by Probiotic Microorganisms on Increasing the Protein Value of Feed Additives

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DOI: 10.47750/pnr.2022.13.S08.292

Abstract

In this article, the prospects of using the germinated grain raw materials and methods of biotransformation (bioconversion) of secondary raw materials with subsequent microbial synthesis for increasing the biological and nutritional compound feed for birds is analyzed. A method for obtaining a protein feed additive for combined feed based on a biotransformed wheat germ product and apple marc powder used for the synthesis of probiotic bacterial microflora is described. The process was carried out on the analogy of the technology of preparation of liquid yeast at bakeries. The prototype of the nutrient medium was a leaven made from wheat flour of the second grade and wholemeal rye flour in a ratio of 7:3. Microbiological screening of products on the experimental and control nutrient substrate showed that the specific growth rate of the lactobacilli population in experimental substrate was almost 2 times higher, and the generation duration was 2 times less than in the control.

Keywords: Feed, Quinoa, Lactobacillus, Biotransformation, Protein Additive, Biological Value.

INTRODUCTION

At present, antibacterial drugs are used in industrial aviculture, which to a some point reduces the food safety degree of products [1]. Probiotic preparations based on biologically active natural strains of lactobacilli and bifidobacteria can be the alternative to antibiotics, which are the natural microflora of the gastrointestinal tract of animals [2; 3], thus research in this field are opportune and relevant.

Impact of direct bioconversion (biotransformation) of secondary grain and fruit raw materials by probiotic microorganisms on increase in the protein value of the feed additive to compound feed for birds has been studied.

As ingredients of the nutrient substrate for the accumulation of microbial biomass, wheat germ product (Uzbekistan State Standard 2928:2015 Wheat germ flakes for food purposes. Technical requirements) and apple marc powder (Technical requirements. 10.61.23-843-37676459-2018 “Powder from fruit and vegetable raw materials), and for the fermentation – biologically active additive “Laktonorm-N” (Uzbekistan State Standard ISO 9001:2015; ISO 9001:2015) produced by “Sog'lomlik nektari” LLC of the Republic of Uzbekistan have been used.

Wheat germ product (hereinafter referred to as WGP) consists of 60…65% of germs and 35…40% of endosperm, shorts and wheat bran fractions [4; 5; 6; 7]. It has been established that in this product, obtained at the mills of Uzbekistan, protein and fat are on average, respectively, 2.4 and 7.8, iron is 4.0 and 2.0, and vitamins are 8.0 and 7.0 times more than in wheat flour of the first grade, and it has increased amount of fiber. Among carbohydrates, sucrose and raffinose are prevailed. The biological value of this product (76.4%) exceeds that of the reference protein according to FAO/WHO (44.0%) by an average of 1.7 times. Bran, which is one of the WGP fractions, contains insoluble prebiotics, i.e. cellulose, hemicellulose and lignin, which improves the nutritional value of feed [8]. In terms of toxicological and microbiological indicators, this object of research complied with the requirements of Sanitary Regulations and Standards No. 0366-19 and Uzbekistan State Standard ISO 6635: 2013.

As an enricher of the nutrient medium, apple marc powder (hereinafter referred to as AP) with the content of glucose and fructose for 11.2–36.8% was used, and that contributes to its efficient and rapid absorption by microorganisms. The presence in apples of pectin, galactooligosaccharides and fructozooligosaccharides, belonging to the group of soluble prebiotics, also

helps to stimulate the growth of microorganisms. The protein content is 3.2…3.8% of DM, essential amino acids is 32.8% of the total volume of amino acids. The increased amount of tryptophan in this raw material, which is necessary to maintain the growth of any organism, is of particular note. Among mineral substances (1.36…2.84% DM), Ca, P, K, Fe, etc. were identified. Among organic acids (1.02…7.5%), succinic, malic, and citric acids dominate [9; 10.11].

Sociological survey of the main producers of juices and canned food from fruit, berries and vegetables practically do not use the marc obtained in their production, but supply it to nearby livestock farms, poultry farms and farms for a nominal fee, which basically covers only transportation costs. Respondents are very interested in more efficient use of this raw material, in particular, in the production of feed for farm animals and poultry, which will significantly increase the profitability of products and expand the possibilities for diversifying production.

Considering the need for complex processing of food raw materials, the identification of new sources of biologically active substances from the secondary resources of the food industry is of undoubted interest.

"Laktonorm-N" preparation, containing lactobacilli of the strain Laktobacillus fermentum No. 231, was selected because these bacteria are the natural microflora of the gastrointestinal and urogenital tract of humans, animals and birds. Application of ready-made pharmacological preparations fully guarantees the purity of the culture, eliminates the need to purchase special pure cultures of lactic acid bacteria, as well as complex procedures to maintain their viability, which is especially important for regions and farms distant from the center. In addition, fodder yeast is not produced in Uzbekistan, and the strategic development policy of the republic is aimed at the rational use of local raw materials and reducing import dependence. This preparation is produced in Uzbekistan at "Sog'lomlik nektari" LLC and is freely available in the pharmacy network.

METHODS

The following indicators were determined in the culture medium for breeding bacterial microflora: the number of bacteria, the specific growth rate, the duration of population generation; humidity; titrable acidity; mass fraction of reducing substances, total, water-soluble and amine nitrogen.

The number of acid-forming bacteria was determined according to GOST 10444.11-89 "Food products. Methods for determination of lactic acid microorganisms" in Goryaev's counting chamber using a ZSM microscope (Poland).

The specific growth rate of microorganisms was calculated by formula 1:

$$\mu = \ln (A \times P/\tau), \quad (1)$$

Where: A is the content of bacteria at the end of the breeding cycle, g; P is the initial content of bacteria; τ is the duration of the process, h.

The duration of generation of microorganisms was calculated by formula 2:

$$\tau\ d = \ln 2/ \mu = 0.693/ \mu, \quad (2)$$

Moisture content in semi-finished products was calculated by express method by drying a sample on the VNIIKhP-VCh (All-Union Scientific Research Institute of the Bakery Industry) device at a temperature of 160 °C for 5 minutes.

The mass fraction of reducing substances was determined by the iodometric method, based on the ability of reducing sugars (glucose and maltose) to be oxidized by iodine to the corresponding acids, and was calculated using formula 3:

$$P = (V_o - V)\ 9K \times 100 \times 100/\ 200 \times C, \quad (3)$$

where: P is mass fraction of reducing substances in terms of DM, %; V_o is volume of 0.1 n. sodium thiosulfate solution used for titration 25 cm3 0.1 n. iodine solution in a blank experiment, cm^3; V is volume of 0.1 n. sodium thiosulfate solution used for titration during analysis, cm^3; K is the correction coefficient for the normality of the sodium thiosulfate solution; 9 is the mass of glucose corresponding to 1 cm^3 of 0.1 n. sodium thiosulfate solution, mg; 200 is weight of sample, mg; C is the mass fraction of dry matters of the substrate, %.

The mass fraction of total nitrogen was determined by the Kjeldahl method and calculated using formula 4:

$$A = 0.0014 \times 100(a - b) / M, \quad (4)$$

Where: a is quantity of 0.1 n. sulfuric acid solution, ml; b is the amount of 0.1 n. alkali solution used for titration, ml; M is the weight of the sample, g.

The mass fraction of water-soluble nitrogen was determined by the colorimetric method using the Folin-Ciocalteu phenolic reagent and was calculated by formula 5:

$$X = a \cdot b \cdot v_1 \times 100/\ H \cdot v \times 1000. \quad (5)$$

where: X is the amount of water-soluble nitrogen, % DM; a - the amount of nitrogen in the test volume of the extract, mcg; b is the volume of the volumetric flask in which the extract was prepared, ml; v is the volume of the filtrate taken for dilution, ml; v_1 is the total volume of the filtrate after its dilution of 0.01 N. acetic acid solution, ml; H is the sample size, mg; 100 is coefficient of recalculation of results, %; 1000 is the coefficient of recalculation for the amount of nitrogen per mg.

Mass fraction of amine nitrogen was determined by the Pope-Stevens method ("copper method"), described in GPM 1.2.3. 0022.15 [78] and based on the ability of most amino acids and peptides to form soluble complex compounds with copper. The

calculation of the mass of amine nitrogen N, mg per 100 cm^3 of extract, is calculated by formula 6:

$$N = a \cdot 0.28 \cdot 5 \cdot 10. \quad (6)$$

where: a is the volume of 0.01 mol/dm^3 sodium thiosulfate solution used for titration of released iodine, cm^3; 5 and 10 are coefficients of recalculation from 2 to 100 cm^3.

RESULTS AND ITS DISCUSSION

One of the methods to increase the biological and nutritional value of compound feed is grain sprouting, which helps to simplify the biopolymer structure of carbohydrates, increase the content of water-soluble fractions of non-protein nitrogen and essential amino acids and vitamins.

The next, no less effective way to increase the biological value of feed is to use the method of direct biotransformation (bioconversion) of secondary grain and fruit raw materials by probiotic microorganisms.

Breeding of the bacterial population was carried out following the example of the preparation of liquid yeast at bakery enterprises, that is, with the preparation of a nutrient medium (brewing) and breeding of bacteria. The prototype (comparison sample) was the brews prepared according to traditional technology, from wheat flour of the second grade and wholemeal rye flour in a ratio of 7:3 [12].

In order to prepare a prebiotic nutrient medium in the experimental variants, the flour was replaced with a mixture of WGP, AP, and sprouted quinoa (amaranth) grain in a ratio of 8:1:1 [13]. Previously, this raw material was additionally dried and ground to the size of dietary flour (the passage of sieve No. 38 was at least 60.0%). Further, the prepared mixture was mixed with water (temperature 45…50 °C) in the ratio 1:4. which was then heated to a temperature of 67...70 °C (starch gelatinization temperature). Sprouted quinoa grain was added to the brew saccharified and cooled to a temperature of 45–50 °C and left for 3 hours for the hydrolysis of high-polymer compounds of the raw material [14]. A bacterial preparation was added to the prepared nutrient medium for one dose (not less than 10×10^6 bacteria) per 1.0 kg of the brew. A similar amount of this preparation was added to the control leaven (prototype).

Results of research are shown in Fig. 1.

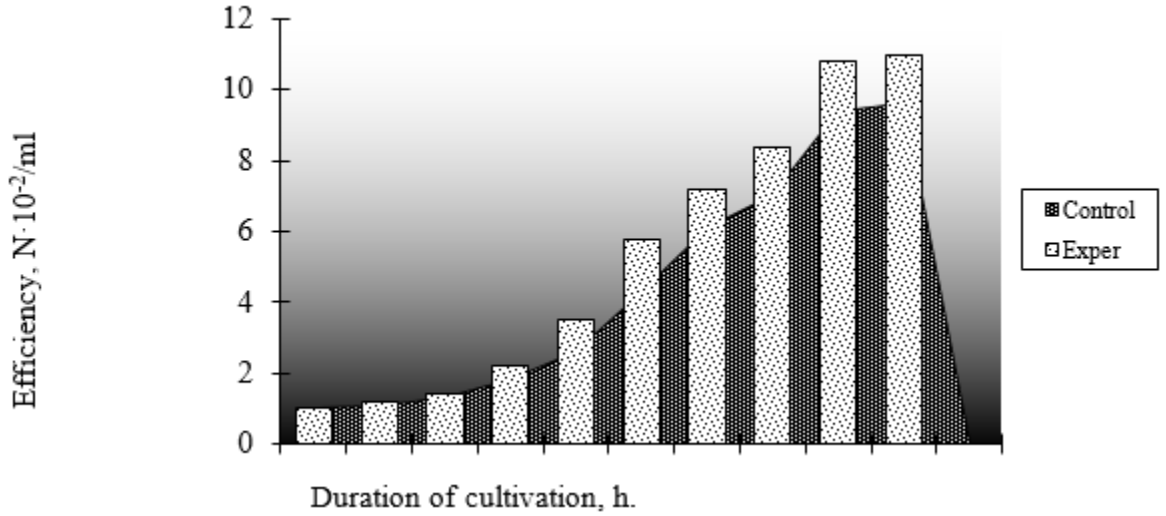

Fig. 1. Influence of the composition of the nutrient medium and the duration of cultivation on the accumulation of bacterial biomass

Cultivation of bacterial microflora was carried out in a constant volume of the nutrient medium (without renewal) for 9 hours with constant stirring (every hour) to aerate the medium at a temperature of 35-40 °C, which is optimal for the reproduction and growth of lactobacilli Laktobacillus ferment No. 231. The growth of the microbial population was determined every an hour of cultivation. Analysis of the results (Fig. 1) showed that both in the control and in the experimental samples of the culture mixture, the duration of adaptation of lactobacilli to the studied nutrient substrate (lag-phase) naturally continued during the first 2 hours. During this time, the biomass of bacteria increased by an average of 1.3 (control) and 1.4 (experiment) times. Then, as expected, a sharp growth (exponential growth phase) of microorganisms was established, which lasted almost up to 8 hours from the start of cultivation. The increase in biomass over this period increased in the control variant in 7.2, and in experimental in 7.7 times. Productivity of lactobacilli in the final control variant was 4.8 billion cells/ml, and in the experimental was 5.4. Further, cell growth practically stopped, the so-called stationary phase set in, so further cultivation is not

Duration of cultivation, h.

advisable, as evidenced by the data.

A more intensive growth of the biomass of lactobacilli in the experimental variant is conditioned by increased content of reducing substances in the substrate and, most importantly, amine nitrogen (Table 1). In the experimental variant, an increase in the final acidity by an average of 0.3 degree was noted; mass fraction of reducing substances by 5.8, total nitrogen by 12.7, water-soluble nitrogen by 26.4 and, which is especially important, amine nitrogen by 21.2% relative to the control values, which naturally affected the activity of bacteria. Thus, the specific population growth rate in the experimental variant was almost two times higher, and the generation time (the time required to double the biomass) of microorganisms was two times less than in the control.

The data obtained convincingly prove the technological effectiveness of the use of the studied raw materials subjected to bioconversion (biotransformation) and microbial synthesis to obtain feed additives enriched with protein of plant and microbial origin.

Table 1. The influence of the prescription composition on the biotechnological indicators of the quality of the nutrient medium for the synthesis of the bacterial population

Indicators	Indicators value	
	Control (prototype)	Experiment
Humidity,%	78.80	79.20
Acidity, degree	13.70	14.00
Mass fraction of reducing substances, % DM	8.15	8.62
Mass fraction of nitrogen, % DM:		
General	2.68	3.02
water soluble	1.25	1.58
Amine	31.45	38.12
Specific growth rate of bacteria, h^{-1}	0.16	0.30
Duration of the generation, h	4.33	2.31

It is advisable to store this biomass in a dried form. However, this requires appropriate drying equipment, preferably with vacuum drying, which complicates the process and increases the cost of the finished product. Therefore, it is most expedient to mix raw biomass (moisture content 65.0…70.0%) with the rest of the prescription ingredients immediately after 7…8 hours of cultivation of the bacterial population.

The following recommendations have been developed based on research carried out to study the process of direct biotransformation (bioconversion) of secondary grain (WGP), fruit (AP) and germinated quinoa grains,:

- It is advisable to use secondary raw materials of the grain processing, oil and fat and canning industries of the food industry as a nutrient substrate for the synthesis of bacterial microflora;
- As a producer of microbiological protein - lactobacilli or bifidus bacteria, yeasts are the most effective from strains that give a consistently high increase in biomass;
- Dispersion of particles of the main raw materials for the nutrient substrate should not exceed the fineness of particles of dietary wheat flour;
- In order to obtain the required amount of protein supplement without additional costs for the purchase of bacterial preparations or yeast, it is advisable to renew the mixture at certain intervals (depending on the need), adding a nutrient substrate in a ratio of 1:1. This allow bringing the culture mixture to the required volume;
- No additional or special equipment is required to prepare the culture mixture. However, periodic aeration of the mass or its mixing is necessary.

CONCLUSIONS

Reasonability of preparing a nutrient substrate from secondary grain (wheat germ product) and fruit (apple marc powder) raw materials, fermented by the germinated quinoa grains with its own enzymes, followed by fermentation of the mixture with lactobacilli of the strain Laktobacillus fermentum No. 231 to obtain a protein additive with prebiotic and probiotic properties for compound feed has been substantiated. The use of this additive contributes to the formation of a specific normoflora that prevents the development of endogenous bacterial infections. A clear advantage of the use of pharmaceutical biologically active additives is the availability for the consumer even in areas remote from the center, where it is practically impossible and not profitable to produce protein supplements due to biotransformation (bioconversion) of secondary raw materials and microbial synthesis.

Corresponding mode parameters of the process have been developed: the initial humidity of the nutrient medium is 60.0…65.0%; the duration of cultivation of the bacterial (yeast) population is no more than 8 hours; temperature of the culture medium is 35…40 °C; the thickness of the substrate layer is 25…30 mm.

Other species and strains of bacteria and yeast can also be used as a microbial population.

Obtained feed additive can be used to reduce the amount of hormonal drugs and antibiotics.

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