

KAUNAS UNIVERSITY OF TECHNOLOGY

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**PROCESSING OF RICE MILLING BY-PRODUCTS INTO
HIGHER VALUE-ADDED COMPONENTS USING
ULTRASOUND TREATMENT AND MICROBIAL
FERMENTATION METHODS**

Summary of Doctoral Dissertation
Technological Sciences, Chemical Engineering (T 005)

2023, Kaunas

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KAUNO TECHNOLOGIJOS UNIVERSITETAS

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**RYŽIŲ MALIMO ŠALUTINIŲ PRODUKTŲ PERDIRBIMAS Į
DIDESNĖS PRIDĖTINĖS VERTĖS KOMPONENTUS TAIKANT
APDOROJIMO ULTRAGARSU IR MIKROBINĖS
FERMENTACIJOS METODUS**

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ABBREVIATIONS

AA	amino acid
Ag	agar
AL	sodium alginate
AMG	amyloglucosidase
CCD	central composite design
CEAA	conditionally essential amino acid
CFU	colony forming units
CYS	cysteine
D/L	D(-)- and L(+)- lactic acid isomer ratio
DNS	3,5-dinitrosalicylic acid
EAA	essential amino acids
FT-IR	Fourier transform infrared spectroscopy
GLY	glycine
HIS	histidine
LA	lactic acid
LAB	lactic acid bacteria
LEU	leucine
LP	cranberry pomace
LYS	lysine
MET	methionine
MRS	de Man, Rogosa and Sharpe
PGS	plant growth substrate
RDS	rapidly digestible starch
RE	relative error
RP	rice milling by-products
RS	resistant starch
RSM	response surface methodology
SD	standard deviation
SSF	semi-solid state fermentation
TS	total starch content
TSM	transplantation soil mixture
TTA	total titratable acidity
UFLC	ultra performance liquid chromatography
US	ultrasound treatment
VAL	valine
Xg	xanthan gum
X-ray	X-ray diffraction

1. INTRODUCTION

Motivation for the research

Due to the development of agro-innovations and improving food biotechnology, the aim is to improve the quality of food products and reduce the amount of industrial waste, using the concept of biorefining, processing by-products that are generated in the agricultural and food industry and using them to provide the added value.

For this reason, in the world of science, more and more attention is paid to the by-products that were formed during the processing of various grains, fruits/berries and vegetables, large amounts of which remain unused or are used irrationally. Valuable grain waste food materials (proteins, fibers) and fruit/berry pomace bioactive compounds, characterized by antioxidant activity, antitumor, antiviral, antibacterial, and antimutagenic effects, could supplement food components, thus protecting food from microbiological spoilage and contributing to reducing the risk of various diseases as well (1, 2).

Rice bran is one of the main by-products of rice milling, with a global amount of 76 million tons per year (3); therefore, it is receiving increasing attention from the scientific community not only because it is widely available and cheap, but because of its valuable components, such as protein, dietary fiber, resistant starch (RS), vitamins, minerals, and phytochemicals (4).

Recently, RS has been characterized as a functional biopolymer with physiological effects that are similar to those of dietary fibers (5). By-products of rice processing can be used as a source of RS, but the natural amount depends on the rice variety and may be relatively low (6). The content of RS can be increased during the technological processes (7, 8) as well as by applying physical or hydrothermal modification methods (9, 10).

Ultrasonic cavitation is one of the innovative methods used for the stabilization and modification of various biomass of plant origin, activation of enzymatic systems of microorganisms and more efficient extraction of valuable ingredients (11). Most of the scientific research in the area of increasing the amount of RS has been focused only on the modification of pure starch (12, 13); therefore, it is relevant to investigate the possibilities of an ultrasonic modification of starch in the entire matrix of rice milling by-products.

Semi-solid state fermentation (SSF) with antimicrobial lactic acid bacteria (LAB) is an effective biotechnological process that improves product sensory properties and nutritional value and ensures product safety (14). The ability of LAB to grow on substrates of grain processing by-products without additives and viability during storage are important criteria in maintaining the technological and physiological properties of the food products.

From this perspective, new food systems from RP biopolymers and antimicrobial components are being developed using processing US technology and microbial fermentation. By implementing these technologies, it is possible to expand the possibilities of secondary use of rice milling by-products, optimizing the conditions for the production of RS, evaluating the changes in both antimicrobial activity and antinutritional factors in RP.

One of the possibilities for the rational use of valuable components of RP would be the development of functional food products from RP and fruit/berry pomace with immobilized antimicrobial lactic acid bacteria. The products, in which bioactive components are contained in the immobilization material or can be immobilized, have nutraceutical properties, are an innovative concept in the production of functional food products.

Another possibility, which allows for the rational use of valuable materials from the by-products of grain processing, is the development of substrate components for the cultivation of vegetable plants. It is a potential alternative to the synthetic additives that can help solve the problems related to ecology and the excess by-products in the industry.

The aim of the thesis is to investigate the influence of ultrasound treatment and microbial fermentation on the changes of bioactive components in rice milling by-products and evaluate the possibilities of using treated rice milling by-products for the production of food products and plant growing substrate.

The following tasks were set in order to achieve the aim of the thesis:

1. To select lactic acid bacteria that are suitable for semi-solid state fermentation of rice milling by-products and determine their influence on the formation of lactic acid isomers, antimicrobial and enzymatic activities, and changes in the phenolic compounds and antinutritional factors.
2. To investigate the influence of ultrasound treatment on increasing the amount of resistant starch in rice milling by-products and evaluate the influence of ultrasound and temperature on the morphological, physicochemical, and technological properties of resistant starch.
3. To evaluate the possibilities of using rice milling by-products for the production of a functional food product with lingonberry pomace by selecting thickeners or thickening agents and evaluating the viability of bacterial cells and the stability of the product during storage.
4. To investigate the possibilities of using rice milling by-products for the production of substrate for vegetable plants, evaluating the influence of their additives on the prevention of diseases of vegetable plants (tomatoes and beans) and the intensity of growth.

Novelty of the study

The suitability of rice milling by-products for the fermentation by various *Lactobacillus* spp. and *Pediococcus* spp. by lactic acid bacteria, evaluating the formation of L(+)-lactic acid, the qualitative and quantitative composition of amino acids, and the inhibitory effect of fermentation metabolites of some *Penicillium* spp., *Fusarium* spp., *Aspergillus* spp. for the growth of microorganisms have been analyzed for the first time.

The effect of high-frequency (850 kHz) ultrasound treatment on the formation of resistant starch in rice milling by-products (bran) depending on the US parameters has been evaluated for the first time. Ultrasonic treatment parameters (temperature, duration, power) were optimized for the maximum formation of starch resistant to digestion, and a mathematical model equation was created for predicting its amount.

The practical value of the work

Rice milling by-products can be used as a fermentation medium for the propagation of various lactic acid bacteria and the production of bioactive metabolites with enzymatic, antifungal, and antibacterial activity without the addition of hydrolytic enzymes or nutrients.

High-frequency (850 kHz) ultrasonic treatment at 40 °C can be applied to increase the amount of functional component, i.e., resistant starch, in rice milling by-products. Thus, the functionalized by-products of rice milling can be used for the immobilization of antimicrobial lactic acid bacteria and the production of functional food products.

Based on the obtained results, a recipe for a new product (bar) was created from rice milling by-products and lingonberry pomace, treated with ultrasound and fermented with antimicrobial lactic acid bacteria, choosing the optimal combination of these components and thickeners that ensure texture stability and the viability of lactic acid bacteria during storage. The product was presented at KTU Technorama 2022, where it received great interest and favorable evaluation.

Fermented by-products of rice milling with antimicrobial lactic acid bacteria can be recommended as bioactive components of the growing substrate of garden plants, such as beans and tomatoes, increasing their resistance to pathogens that are causing root rot as well as improving the seed germination and growth intensity of these plants.

Statements for the defense

1. Rice milling by-products are a suitable medium for the growth of lactic acid bacteria and the production of bioactive metabolites with enzymatic, antifungal, and antibacterial activity.

2. High-frequency (850 kHz) ultrasound can increase the amount of resistant starch (type 3) in rice milling by-products.

Structure and content of the dissertation

The thesis is written in the Lithuanian language. It consists of a list of abbreviations, an introduction, a literature review, materials and methods, results and discussion, conclusions, a list of references, and a list of publications relevant to the subject of the dissertation. The entire dissertation consists of 137 pages, including 31 tables and 30 figures. The list of references includes 339 bibliographic sources.

Publications of the research results

The results of the research have been published in 5 scientific articles in the journals indexed by Thomson Reuters (WoS), and the results have been presented in 9 international conferences.

2. RESEARCH MATERIALS AND METHODS

2.1. Materials

Rice milling by-products

Rice milling by-products were obtained from UAB Ustukių malūnas (Pasvalys, Lithuania) in the period of 2016–2021. The first batch of raw material was used in all RP fermentability experiments. Unfractionated raw material was sterilized for the experiment (121 °C, 15 min). The total mean number of microorganisms after sterilization was found to be $<10^{-1}$ CFU/g with the following characteristics (g/100 g): moisture 11.38±0.12, protein 8.89±0.32, fat 1.27±0.32, starch 68.72±0.36, dietary fiber 7.46±0.96, ash 2.28±0.11.

The second batch of raw material was used for the production of a functional product based on the functionalized RP and LP pomace and in the related experiments. Before the experiment, the raw material was fractionated into 3 fractions (sieve mesh diameter: 200, 315, and 500 µm) by using fractionation equipment (EFL 300, Sonic Sifter, Endecotts Limited, London, UK). The fraction of 315 µm particle size, containing the highest amount of resistant starch (RS 8.80 g/100 g d.w.), was selected for the experiment (g/100 g d.w.: protein 14.62, fat 5.66, starch 51.95, indigestible fiber 26.43, ash 1.35).

The third batch of RP material (in 2021) was used to optimize the US conditions for the production of resistant starch (RS) and the development of biofertilizer components. Thus, for the RS increment optimization experiment, the 315 µm particle size fraction was selected (g/100 g d.w.: protein 11.20, fat 5.38, starch 66.41, dietary fiber 15.12, ash 1.89). The remaining 500 µm particle size fraction was used for the development of functionalized RP biofertilizer components (g/100 g d.w.: protein 15.42, fat 11.14, starch 61.25, dietary fiber 10.84, ash 1.34).

Lactic acid bacteria (LAB)

Eleven heteroenzymatic LAB strains obtained from the collection of the Food/Feed (Bio)Technology Science Group of LSMU Department of Food Safety and Quality [*Lactobacillus plantarum* (Lp18 and Lp135), *Lactobacillus brevis* (Lb140 and Lb173), *Pediococcus pentosaceus* (Pp22 and Pp100), *Pediococcus acidilactici* (Pa236 and Pa29), *Lactobacillus paracasei* (Lpc244), *Lactobacillus uvarum* (Lu245), *Lactobacillus farraginis* (Lf206)], previously isolated from a spontaneously fermented wheat flour media and identified by phenotypic and molecular techniques (15), were used for the fermentability assay. Prior to each experiment, all bacteria were propagated in a MRS broth (CM 0359, Oxoid Ltd., Hampshire, UK) at 30 °C for 48 h under facultative anaerobic conditions.

Indicator microorganisms

In order to evaluate the antifungal and/or antibacterial effects of LAB metabolites, microscopic fungi and bacteria, including pathogenic microorganisms, were obtained from the Lithuanian Agricultural and Forestry Research Center and the Department of Plant Biology and Food Sciences of Vytautas Magnus University and Kaunas University of Technology. The collections of the Department of Food Science and Technology, Faculty of Chemical Technology include [*Fusarium graminearum* spp., *Fusarium culmorum*, *Fusarium avenaceum*, *Fusarium nivale*, *Mucor mucedo*, *Rhizopus oryzae*, *Penicillium* spp., *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus* spp., *Sclerotinia sclerotiorum*, *Pythium volutum*, *Verticillium wilt*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Salmonella typhimurium*, *Staphylococcus aureus*].

Enzymes

Enzyme preparation Celstar XL, containing cellulase ($\geq 20,000$ AV/ml), xylanase ($\geq 15,000$ AV/ml), and protease ($\geq 1,500$ AV/ml) activities (optimal operating conditions pH 6.0 and 50 °C), produced by Dyadic International (USA), was used for the hydrolysis of RP in plant experiments.

Natural rice starch

Natural rice starch (S-7260, Sigma Aldrich, Saint Louis, MO, USA) was used for the comparative study of the physicochemical and morphological properties of the isolated RP. During the experiment, all samples were stored in sealed packages in a freezer at -18 °C.

Resistant starch

RS was isolated from rice according to the scheme presented in the methods sub-section (Fig. 2.2). The isolated RS and starch was lyophilized, dried, and used for the evaluation of morphological, physicochemical, and functional properties.

Lingonberry pomace

Freeze-dried lingonberry (*Vaccinium vitis-idaea* L.) pomace powder (LP) that was obtained from the Biochemistry and Technology Laboratory of the Institute of Horticulture and Horticulture (Dotnuva, Lithuania) of the Center for Agricultural and Forest Sciences of Lithuania (Dotnuva, Lithuania) was used for the production of a functional product. Lyophilized lingonberries were fractionated for the experiment, and 230 μ m particle size fraction was selected (g/100 g: moisture 3.9, protein 7.8, fat 10.69, ash 1.05, dietary fiber 75.95), pH 2.82, pectin 2.7 g/100 g.

Horticultural plants

The seeds of beans of the variety Aura (*Phaseolus vulgaris* L.) and tomatoes of the variety Promyk (*Solanum lycopersicum* L.) were purchased from UAB Agrofirma (Kaunas, Lithuania).

Functionalized RP extracts

The RP raw material is functionalized in different ways, which are described in the methods sub-section (2.4.). All samples were freeze-dried and used as a vegetable plant substrate additive in potted plant experiments as well as a soil transplant mixture additive in a greenhouse experiment.

Stabilizers

Plant-derived polysaccharides were used to form the gel texture of rice milling by-products, i.e., lingonberry berries: xanthan gum (R&D Laboratories Ltd., Antrim, UK) and agar (Agar agar, Alvo), purchased at a local supermarket, and sodium alginate from Merck (Merck KGaA, Darmstadt, Germany).

The structure of experiment plant is presented in Fig. 2.1.

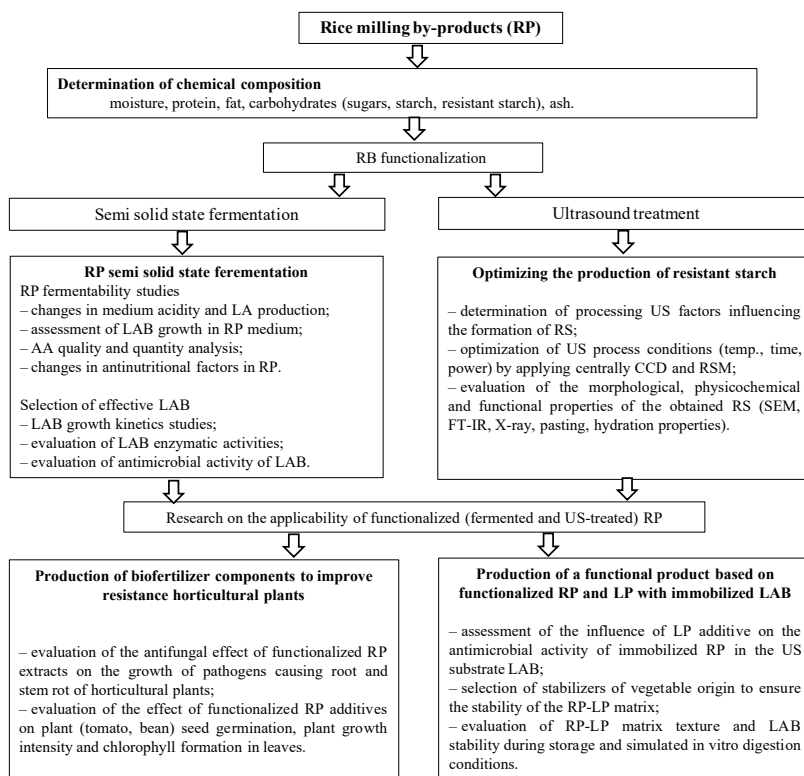


Fig. 2.1. The experiment plant

2.2. Methods

2.2.1. Determination of chemical composition

The moisture was determined by using the drying method according to ICC standard method no. 712:2010.

The total protein content was determined by the Kjeldahl method, according to ICC standard method no. 20483:2007. Crude protein content was estimated by using the conversion factor of 5.95.

The fat content in RP was determined by extraction in the Soxhlet apparatus with hexane of technical grade according to the ICC standard method no. 136:1984.

The starch content was determined by the Megazyme K-RSTAR test (Megazyme Int., Wicklow, Ireland).

Reducing saccharides was determined spectrophotometrically by using 3,5-dinitrosalicylic acid reagent (DNS) (16).

The total fiber content was determined according to AOAC method 985.29.

The ash content was determined according to AOAC method 942.05.

The carbohydrates content was calculated according to the formula: carbohydrates (g/100g) = 100 - moisture - protein - fat - ash.

The determination of pectin was performed by a method based on the pectin extraction with citric acid using the Megazyme Pectin Identification assay kit (Megazyme Int., Wicklow, Ireland).

2.2.2. Fermentability assay

Evaluation of LAB acid tolerance

The ability of LAB strain to survive procedure is described by Jukonyte et al. (17). LAB strain to survive at low pH was evaluated by measuring survival at 37 °C for 3 h in MRS broth, acidified with 1 M HCl to final pH 2.5. Then, aliquots of 0.1 mL were removed at constant intervals (0, 1, 2, 3 h) for the determination of the total viable count. After appropriate dilutions (up to 10^{-4} – 10^{-8}), the cells were plated in duplicate on MRS agar to be incubated at 30 °C for 48 h. The count of viable cells was expressed as a log of colony forming units per milliliter (CFU/mL).

Effect of temperature on the kinetics of bacterial growth in RP medium

In order to assess the kinetics of bacterial biomass growth, the number of LAB was determined at the initial moment, after 6 h, and every 12 h during the entire fermentation period (72 h) (8 times in total). The bacterial growth in RP medium was evaluated at constant temperatures of 30 °C and 37 °C. The number of LAB is determined according to the method for determining the number of lactic acid bacteria (page 15) and is expressed as $\log_{10}$ CFU/g. The bacterial growth kinetics analyzed different phases of the bacterial growth: lag, stationary, and death phases. The specific biomass growth rate was calculated from the change in bacterial content (CFU/g) per unit time (h).

Semi-solid state fermentation

The sample for the assay was prepared by mixing sterilized RP with distilled water at a ratio 1:3 (moisture 68%), wherein 2% (w/v) of the pure culture of each LAB strain was inoculated. The samples were fermented in triplicate for 72 h at 30 °C under facultative anaerobic conditions (in sealed bottles with a closed lid, in a thermostat). The samples were taken every 12 h for pH, TTA, LA content, AA profile, and bacterial count determination.

Determination of medium pH

The pH values were measured directly using pH electrode (Sartorius, Goettingen, Germany). Each sample was analyzed twice.

Determination of lactic acid bacteria

The total number of lactic acid bacteria is determined according to LST EN ISO 6887-1:2017.

Determination of lactic acid and L-/D- isomers

The concentration of LA and L- and D-LA isomers was determined using the D-/L-lactic acid Megazyme kit (Megazyme Int. Ireland Ltd., Wicklow, Ireland) (17). Each sample was analyzed with the kit according to the manufacturer's instructions.

Chromatographic analysis of amino acids

Amino acids (AA) content in control and fermented rice polish samples were analyzed by Ultrafast Liquid Chromatography (UFLC) with automated o-phthalaldehyde (OPA)/9-fluorenylmethyl chloroformate (FMOc). The UFLC analysis procedure is described by Jukonyte et al. (17).

2.2.3. Determination of antifungal activity

Determination of antifungal activity

Antifungal and antimicrobial activity of LAB in fermented RP medium against indicator microorganisms was determined by agar diffusion (18) and agar disk diffusion methods (19). The zone of inhibition is expressed in mm (20).

2.3. Determination of starch quality and quantity

Amylose content determination

The amylose content in the RS samples was determined using a Megazyme Amylose/Amylopectin assay kit (Megazyme Ltd., Wicklow, Ireland) according to the manufacturer's instructions.

Starch content measurement

The content of resistant starch (RS) was determined using a Megazyme Assay Kit (K-RSTAR, Megazyme Int., Wicklow, Ireland) for resistant starch determination based on the AOAC method (2002.02).

Starch isolation

The starches were isolated from the RP material by the alkaline extraction before and after the enzymatic digestion step (21). The starch isolation and determination scheme is presented in Fig. 2.2.

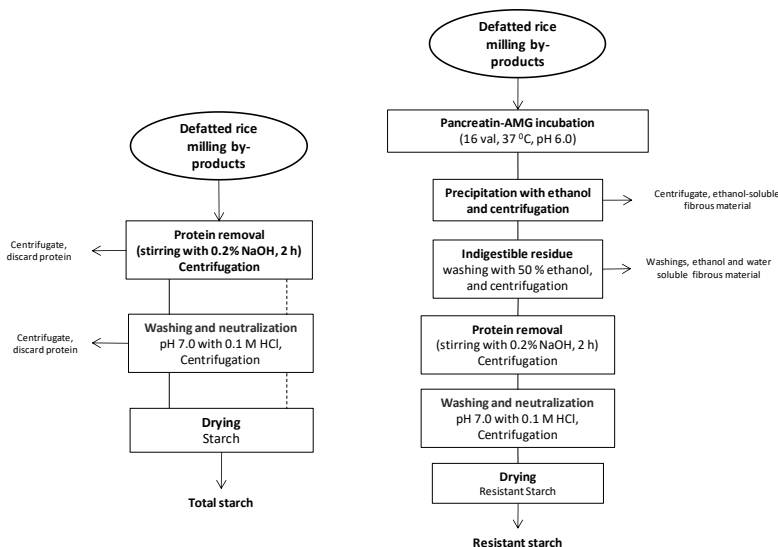


Fig. 2.2. Rice milling by-products starch and resistant starch isolation and measurement scheme

2.3.1. Resistant starch optimization and characterization

Ultrasonication procedure

The RP sample (10 g) and distilled water were mixed at a solid/liquid ratio of 1:3 for the US treatment (22). The mixture was placed in a tightly closed plastic bag (sample thickness 15 mm), and the sample was treated with US in an ultrasonic high-power bath (Type 5/1575) connected to a 120W-HF-Generator (Meinhardt Ultraschalltechnik, Leipzig, Germany). The sonication was carried out at 850 kHz frequency and 50% US intensity for different times (15–35 min) at different temperatures (30–70 °C). After sonication, each sample was subsequently stored at 4 °C for 24 h, then dried in an oven at 50 °C, and

subjected to the isolation and determination of the starch. Each experiment was repeated four times.

2.3.2. Experimental design and statistical analysis

Central Composite Design (CCD) that is consisting of 20 experiments and 6 center points for each power group was employed as the experimental design, performed in a random order at four replications. The factors that are investigated included independent variables: temperature (T , °C), treatment time (t , min), and power (P , W/cm²) at different levels. Response Surface Methodology (RSM) (23) was used to investigate the effect of the US-assisted treatment parameters on the resistant starch (RS) content. Furthermore, the optimization of US processing conditions was performed by developing the simplest possible mathematical model with a coefficient of determination higher than 80%. The response was the RS content that was exclusively obtained under the influence of US action (dY, g/100 g dw). The results were analyzed using Design-Expert trial version 13.0.1.0 software (StatEase Inc., Minneapolis, MN, USA). The analysis of variance (ANOVA) was conducted for the assessment of the suitability of the model using the coefficient of 'lack-of-fit' and the Fisher value (F). All chemical analyses were performed at least in triplicate. The statistical analysis of the data was performed using SPSS software (ver. 27.0, IBM, Chicago, IL, USA). The significant differences between the means were evaluated by one-way ANOVA at a significance level of 0.05.

2.3.3. Characterization of resistant starch

Crystallinity analysis

Starch crystallinity was analyzed using a D8 Advance X-ray Diffractometer (Bruker AXS, Bruker, Karlsruhe, Germany) in Department of Silicate Technology, Kaunas University of Technology. The data were collected using CuK α radiation ($U = 40$ kV and $I = 40$ mA), a 0.02 mm nickel filter, the Bragg-Brentano geometry, and a fast-counting detector Bruker LynxEye. The samples were scanned over the 2θ range of 5–30° at a speed of 6 °/min. The relative crystallinity was determined by calculating the areas below and above the curve that correspond to the amorphous and crystalline regions (24). The ratio of the amorphous area to the total area was taken as the relative crystallinity.

Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra of the samples were recorded using an FT-IR in Department of Silicate Technology, Kaunas University of Technology. A spectrometer (Frontier, PerkinElmer Inc., Waltham, MA, USA) with a single

reflectance horizontal ATR (Attenuated Total Reflectance) cell was equipped with a diamond crystal. The data were recorded in the range from 4000 to 560 cm^{-1} with a resolution of 4 cm^{-1} .

Pasting properties

The thermomechanical behavior of the RS paste (peak viscosity, pasting temperature, final viscosity) was analyzed by a Brabender Micro-Visco-Amylograph (Brabender GmbH, Duisburg, Germany) according to Yang et al. (25) with some modifications (Department of Polymer Chemistry and Technology, Kaunas University of Technology). The suspensions of each sample (10%, w/w) were heated from 25 to 95 °C at a rate of 6.5 °C/min, held at 95 °C for 15 min, cooled to 50 °C at 6.5 °C/min, and held at 50 °C for 2 min with a speed of rotating paddle of 250 rpm. The samples were analyzed twice.

2.4. Functional product matrix composition and texture estimation methods

Modeling of RP-LP pomace matrix structure

In the modeling of the RP-BR matrix structure, different model systems were made from RP and LP pomace powders. RP is prepared according to the description of the SSF (sub-section 2.2.2.). *L. brevis* 173 and *P. acidilactici* 236 bacteria were selected for LAB cell immobilization; their suspensions were prepared according to 2.1. sub-section. LP additive is added by changing the amount of LP additive (10, 20, 30, 40, 50%) of the total mass of dry matter. The structuring of the mixtures was done by US low temperature treatment (40 °C, 20 min, v/m = 1.3 W/cm²) using an ultrasonic bath (model 5/1575) connected to a 120W-HF generator (Meinhardt Ultraschalltechnik, Leipzig, Germany).

Immobilization of microbial cells in the RP-LP matrix

A sample of RP and LP was selected (10 g), and a mass of sterile distilled water (ratio 1:4, w/v) after US treatment was cooled to 30 °C and mixed with LAB cells (48 h, 30 °C, in MRS medium, 10⁹ CFU/ml) according to the methodology presented on page 15, adding so much that the number of cells on average, according to the determination of the LAB number, was 10⁸ CFU/g of the mass. LAB immobilization was performed by mixing the sample for 1 h in a tightly covered container in a thermostat with a shaker at 35 °C, after which the samples were incubated for 5 h in a thermostat with a shaker under the same conditions.

After LAB immobilization, sodium alginate (10%), xanthan gum (5%) in the form of powder are added to the mass (calculated from the mass of dry substances) with vigorous stirring for 30 min at 35 °C. In the case of agar, the

RP-LP sample is mixed with water in a ratio of 1:3, and the remaining amount of water is introduced together with the agar dissolved in it. In order to do this, agar powder is dissolved in appropriate amount of water, heated to 90 °C, and the solution is cooled to 45 °C.

Evaluation of RP-LP matrix stability during storage

In order to evaluate the stability gels and the viability of LAB, the gel samples (diameter 20 mm, thickness 9 mm) were stored in sealed plastic bags at different temperatures (4 °C and 18 °C) for 56 days, determining the number of immobilized LAB cells every 7 days.

2.5. The functionalization of rice milling by-products for the development of plant biofertilizer components

Pre-treatment of rice milling by-products

The RP material before the planting experiment was pre-treated by ultrasound (US), solid state fermentation (SSF), or combined procedure (US treatment following enzyme hydrolysis). The analyzed samples include: control sample – untreated RP (RP), US – RP ultrasonicated (20 min, 40 °C, 1.6 W/cm², m/v ratio 1:4), US+F – RP ultrasonicated (conditions as above) and hydrolyzed with cellulase/xylanase and protease (60 min, 50 °C, pH 6, m/v ratio 1:4, 400 CU/100 g), RP_{SSF} – fermented [36 h at 30 °C, using 2% (w/v) of suspension of pure LAB culture: *P. acidilactici* (Pa), *L. brevis* (Lb), *L. uvarum* (Lu); m/w ratio 1:4]. The pre-treated RP samples were lyophilized and used as a supplement to plant growth substrate (PGS) in potting experiments and the transplantation soil mixture (TSM) in a greenhouse experiment.

Plants and potting procedure

In the plant growth experiments, the seeds of bean (*Phaseolus vulgaris* L.) of Aura variety and tomato (*Solanum lycopersicum* L.) of Promyk variety were sowed directly in a potting substrate without or with the addition of pre-treated RP at a concentration of 10% by weight of PGS. The plants were cultivated in plastic pots using 0.5 litter of commercial PGS per pot. The experiment was carried out with 8 bean and 16 tomato plants for each test batch. Bean and tomato seedlings after 21 days of growth were transferred to the TSM. The plants were grown in a greenhouse under controlled conditions (25 °C, 50–60% humidity), irrigating with tap water.

Evaluation of plant growth performance

The growth performance of the tomato and bean plants was determined according to Ranal and de Santana (26).

Statistical data analysis

All tests were repeated at least three times. The average values and standard deviation values of the obtained results were calculated using the Microsoft Excel software package, and the results were presented using graphical representation. The statistical data analysis was performed using SPSS software (ver. 27.0, IBM, USA). Significant differences between the means were assessed by one-way analysis of variance (ANOVA) with a significance level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Evaluation of the rice milling by-products fermentability and lactic acid bacteria metabolite bioactivity

Acid tolerance of tested LAB

All the selected lactobacilli were able to survive at pH 2.5 after 3 h of incubation retaining varying levels (68.7–88.6%) of viability. The tested lactobacilli were found to be more acid tolerant compared to the pediococci. The highest survival (average 88.4%) was shown by the Lpc244 and Lf206, as well as Lb140 and Lb173 (between 81.4 and 82.2%, respectively), followed by Lp18 and Lu245 (75.4%). The least survival after 3 h of incubation at pH 2.5 was observed for Pa29 and Pa236 strains. The low acid tolerance exhibited the Pp22 and Pp100 (32.4% and 48.6%, respectively).

The bacterial growth ability in RP fermentation medium

The results of this study suggested that the rice polish substrate was suitable for growth of the tested LAB strains, differing in the adaptation period. The lowest growth activity during the first 12 h of fermentation was shown by the Lpc244 (from 8.23 to 8.77 CFU/mL) and Lp18 (from 7.80 to 8.52 CFU/mL). The most intensive adaptation in rice polish medium during the first 12 h was observed for all the tested pediococci (average cell count increased from 7.2 to 9.12 CFU/mL) and Lf206 (average cell count increased from 7.50 to 9.39 CFU/mL). All the tested bacterial strains, except for Lp135 and Lpc244, were able to provide considerable growth in rice polish medium, reaching a maximum cell concentration of approximately 9.2–9.5 log CFU/mL in 36 h and up to 9.6 log CFU/mL during 48 h. The highest cell count was recorded for the Pp100 (9.62 log CFU/mL) and was followed by the Lpc244 and Lb173 (9.57 log CFU/mL) at the end of the fermentation process.

The effect of the temperature on the kinetics of bacterial growth in RP medium

In order to evaluate the kinetics of bacterial growth in RP medium during SSF at different temperatures, the strains of different species, showing the best growth in RP medium without additives, were selected (Lb173, Lf206, Pp100, and Pa236). The results revealed that the selected LAB showed typical growth curves (Fig. 3.1).

The bacterial biomass formation was significantly dependent ($p = 0.001$) on the LAB strain and fermentation temperature. After analyzing the kinetic curves, it was noticed that Pp100 and Lb173 strains had the longest lag phase in both cases. Strains Pa236 and Lb173 showed stability in RP medium: the

stationary phase of their development lasted from 12 to 60 h and from 24 to 60 h, respectively, while the stationary phase of strains Pp100 and Lf206 lasted from 12 to 36 h and from 24 h to 48 h, respectively (Fig. 3.1).

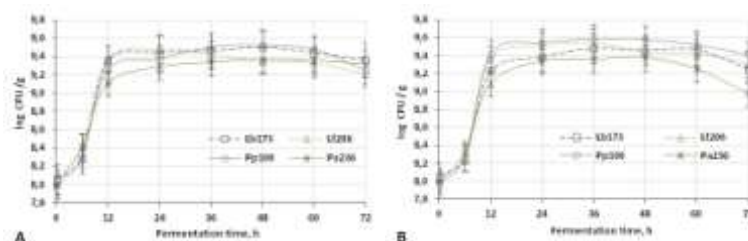


Fig. 3.1. Kinetics of LAB biomass formation during 72 h fermentation period at 30 °C (A) and 37 °C (B)

The mentioned strain reached the maximum biomass growth rate (9.3–9.5 log CFU/g) in 48 h at 30 °C, and at 37 °C, the maximum biomass growth rate of 9.5–9.6 log CFU/g was obtained in 36 h. The death phase of LAB cells (from 60–72 h) depended on the strain and the temperature more suitable for its growth: a sharper death phase was observed at 30 °C for the Pp100 strain, and at 37 °C for the Lb173 and Pa236 strains. The Lb173 strain showed the best growth in the RP medium at 30 °C, while both temperatures (30 and 37 °C) were suitable for Pp100 and Lf206 strains.

Utilization of rice polish to LA at SSF conditions

The Lb173 produced the highest LA content (0.48 g/100 g); slightly lower LA levels were produced by the Lp18 and Lu245 (0.41 and 0.42 g/100 g, respectively) after 60 h fermentation. The LA contents produced by the Lf206, Lb140, Pa236, and Pp100 varied between 0.36 and 0.38 g/100 g (Fig. 3.2).

The tested LAB strains could be indicated as the DL-forming bacterial strains, in which the L(+)-LA ranges from 34.2 to 82.5% from the total LA content (Fig. 3.2), strongly depending on the LAB strain and fermentation period ($F = 5294.263$, $p < 0.0001$, and $F = 4.268$, $p < 0.012$, respectively).

According to the obtained results, the longer fermentation resulted in an increase in total LA concentrations, however, decreasing the L(+)-LA and increasing the D(-)-LA percentage (Fig. 3.2 B). Initial largest L(+)-LA contents were produced by the Lb173 and Lp135 (L/D ratio 4.71 and 2.39, respectively). The highest L/D ratios were recorded for the Lp135 and Pp22 (L/D = 1.78) after 12 h fermentation and for the Lp135 (L/D = 1.53) and Lb173 (L/D = 1.25), at the end of the fermentation (Fig. 3.2 B).

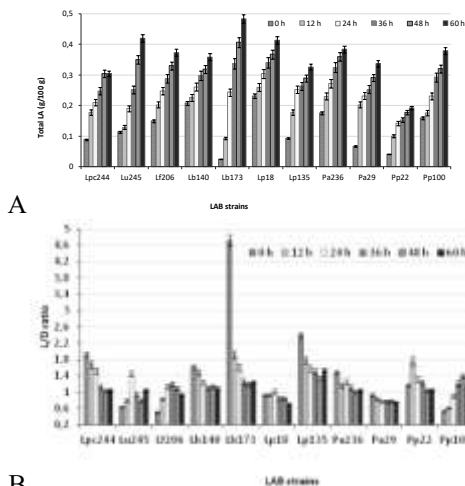


Fig. 3.2. LA production (A) and variation in the L/D ratio (B) in rice polish medium by tested LAB strains

Amino acids profile of rice polish before and after fermentation

The total amino acid (AA) content was 82.9 g/100 g of protein with 32.7% EAA content in rice milling by-products. Among all EAA, the LEU was found at the highest content (5.34 g/100 g), following the LYS (5.06 g/100 g). Significantly lower contents of HIS and VAL (2.91 and 1.92 g/100 g, respectively) were found, while MET was one of the lowest contents (1.76 g/100 g).

Among CEAA, the highest amounts were determined for ASP and GLU (9.52 and 7.58 g/100 g, respectively), and from EAA, CYS was mostly detected (5.86 g/100 g); the amounts of other AA were determined within the limits from 4.46–4.66 g/100 g to 2.22–3.65 g/100 g.

Antifungal activity of lactic acid bacteria in rice polish medium

The tested LAB strains showed different inhibition capability on the growth of *Penicillium* and *Aspergillus* sp. strains. In the tested LAB, Pa236 and Lf206 strains were shown to moderate inhibition and delay spore formation against *P. spinulosum*. The Pp100 and Lb173 produced high (11–12 mm) antifungal activity against *A. versicolor* strain. Among other tested LAB, only Lp18 initiated the delay of fungal sporulation (8 mm) against *P. viridicatum* as well as Pa29 and lactobacilli Lb173; Lpc244 and Lu245 delayed fungal

sporulation (9 mm) against *P. spinulosum*. The Pa29, Pa236, Pp22, Lb173, and Lu245 were slightly active (7–9 mm) against *P. palitans*.

According to the tested strains, Pp100 and Lb173 were able to inhibit *A. fumigatus*, and Lb173 was able to inhibit *A. versicolor*. The other LAB (Lb140, Lp135 and Lf206) did not form the inhibition zones. In previous studies, *P. acidilactici* was found to inhibit the growth of fungi, including species of *Aspergillus* and *Penicillium* spp. (27).

In order to optimize the antifungal activity production, five LAB strains showing the highest antifungal activity were tested by an agar disk-diffusion assay for antifungal activity against *Penicillium spinulosum* and *Aspergillus versicolor* (17). The metabolites produced by LAB in rice milling by-products showed various degrees of antifungal activity after 24, 48, and 72 h of fermentation. According to the results, RP filtrates, fermented by tested LAB, showed inhibition zones varying from 7.0 to 11.0 mm. The Lb173 showed the highest antifungal activity against *A. versicolor* (inhibition zones 9 and 11 mm, respectively) and *P. spinulosum* (inhibition zones 7 and 9 mm, respectively) after 48 and 72 h of fermentation. The *L. farraginis* inhibited the growth of *P. spinulosum* (zone 9 and 11 mm) after 48 and 72 h of fermentation.

3.2. The application of high frequency ultrasound to increase the content of resistant starch in rice milling by-products

The research is aimed at the development of a new sustainable food system based on the functional components of RP, evaluating the possible application of ultrasound (US) technology for the production of RS in the RP matrix in order to increase the amount of RS and explain the effect of RP processing in US, applying different temperature regimes on the physico-chemical, structural, and technological properties of RS for changes in properties as well as opportunities for the extraction and wider application of rice milling by-products.

The influence of ultrasonic parameters on the formation of RS in RP raw material

The amount of RS measured at different US processing time points was compared to the initial content in the untreated RP (11.51 g/100 g d.w.). Most of the RS was produced at the early period of ultrasonication (15–20 min); a longer US treatment time (25–35 min) enhanced the degradation of starch, significantly decreasing the content of RS. The sonication at higher temperatures (60–70 °C) caused a slight increase (up to 5.0%) in the RS content during 15–20 min of ultrasonication, but a decrease up to 73.5% at longer sonication times (25–35 min) was fixed. Thus, the rapidly digestible starch (RDS) content increased by 22.7–53.4% with increasing the US time and temperature, increasing the total starch (TS) extraction yield.

The effect of ultrasonication on the RS increment and mathematical model analysis

The effect of the US processing temperature (T), time (t), and power (P) on the RS yield was determined via optimization by CCD and RSM. The yield of RS varied significantly from 1.51 to 12.72 g/100 g d.w. when applying different T (30–50 °C), t (15–25 min), and P (1.3, 1.5, and 1.8 W/cm²) combinations (Table 3.1).

A quartic-order regression model, describing the relationship between the RS content increment ($dY = Y - Y_0$), obtained under the influence of the US processing and independent variables, was constructed according to the following equation:

$$dY = Y - Y_0 = 11.71 - 2.14t + 0.161T + 0.798P - 0.8838tT + 0.0587tP - 0.2063TP - 1.15t^2 - 6.33T^2 + 0.023P^2; \quad (3.1)$$

where dY is the RS content increment, Y is the total RS content, Y_0 is the RS content in raw material, t , T , and P are the values of temperature, time, and US power, respectively.

Based on the statistical analysis, the model was significant for dY , showing that it was reasonably reproducible and well-fitting to the experimental data: Fisher value – 103.24 ($p < 0.0001$), variation coefficient – 7.2%, determination coefficient – $R^2 = 0.9925$. The results showed that the p -value for ‘lack-of-fit’ was not significant ($p = 0.1728$); hence, the model was satisfactory in explaining the obtained data at 95% confidence level. The predicted R^2 value (0.9258) for the experimental design was close to the adjusted R^2 value (0.9829), indicating that 97% of the data could be described by this regression model.

Comparing the experimental data with the theoretical prediction, the relative error (RE) varied between 0 and 0.51 (Table 3.1), indicating that the model could be used to estimate the response for the optimization. To the best of author’s knowledge, there are no reports on the optimization of high-power US-assisted production of RS in rice milling by-products.

Table 3.1. Experimental design and experimental results

Experiment number	Processing Variables			dY* (g/100 g d.w.)		
	<i>t</i> (min)	<i>T</i> (°C)	<i>P</i> (W/cm ²)	Experi-mental	Pre-dicted	RE
Low level (-1)	15	30	1.3			
Medium level (0)	20	40	1.5			
High level (+1)	25	50	1.8			
1	1	1	1	2.02±0.08	2.02	0.00
2	1	-1	-1	1.51±0.04	1.75	-0.14
3	-1	1	-1	6.98±0.13	6.88	0.01
4	1	-1	1	3.82±0.07	3.88	-0.01
5	-1	1	1	8.23±0.18	7.95	0.04
6	1	1	-1	1.09±0.02	0.72	0.51
7	-1	-1	-1	4.42±0.09	4.38	0.01
8	-1	-1	1	5.94±0.13	6.27	-0.05
9	0	1	0	4.87±0.08	5.54	-0.12
10	0	-1	0	5.89±0.11	5.22	0.13
11	0	0	-1	10.75±0.12	10.91	-0.01
12	0	0	1	12.72±0.04	12.51	0.02
13	1	0	0	4.76±0.08	8.42	-0.43
14	-1	0	0	11.34±0.23	12.70	-0.11
15	0	0	0	11.72±0.12	11.71	0.00
16	0	0	0	11.61±0.19	11.71	-0.01
17	0	0	0	11.65±0.14	11.71	-0.01
18	0	0	0	12.07±0.08	11.71	0.03
19	0	0	0	12.11±0.12	11.71	0.03
20	0	0	0	11.12±0.18	11.71	-0.05

dY – resistant starch content, *T* – temperature, *t* – time, *P* – power, RE – relative error.

Optimization and prediction of process parameters

Based on the obtained model, a three-dimensional plot was constructed to predict the relationship between the independent variables. The predictive plotting of the optimum conditions based on the increase in the RS content was

carried out by varying the factors. Fig. 3.3 presents the relationships between the response value (dY) and the independent variables (time, temperature, and power). The elliptical form of the contours and a relatively sharp slope of the surface responses indicate a strong interaction between the analyzed factors and a strong influence of the sonication parameters on the response value.

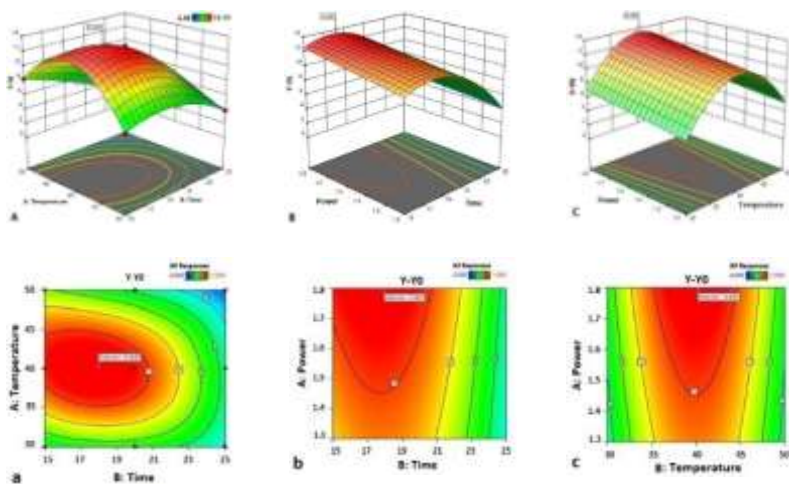


Fig. 3.3. Response surface plots (A–C) and contour plots (a–c) of the interactions between ultrasound temperature and time (A), power and time (B), and power and temperature (C) for the RS increment ($dY = Y - Y_0$)

Based on the RSM, the optimal conditions for the production of RS in RP were set as follows: US power – 1.8 W/cm², temperature – 40 °C, and time – 18 min. However, the optimal conditions proposed for the US power were located at the maximum studied level (+1), indicating that there is a possibility of exploring the production of RS outside the studied level, but this would be outside of the technical characteristics of the device that was used. Under optimal conditions, the predicted RS increment (dY) shows at 13.46 g/100 g d.w. After three repeated tests, the average RS content was 13.44 g/100 g d.w. with a small relative error (RE = 0.42).

The actual value was close to the theoretical value, indicating that the optimization results were reliable and practical. US, as a sustainable processing technique, has the potential to produce RS with desirable properties and mechanical performance. The modification at different US processing temperatures can achieve functional properties that are not found in untreated starches, which may have specific applications in the food industry.

Characterization of ultrasound-modified RP resistant starch

The results of X-ray diffraction (Fig. 3.4) indicate that the untreated RS had the typical A-type crystallinity pattern (corresponding to tight-packed amylopectin double helices) with the characteristic peaks at diffraction angles (2θ) of 15° , 16.9° , 17.9° , a weak peak at $20^\circ 2\theta$, and an additional peak at $23^\circ 2\theta$, as it is similar to the observations of other researchers (28, 29).

The FT-IR spectra of the rice starches are displayed in Fig. 3.4. The intensity of absorption at 995 cm^{-1} (C-O stretching) and $1,149\text{ cm}^{-1}$ (peak E, C-O-C asymmetric stretching) and the intensity of the peak at $1,336\text{ cm}^{-1}$, corresponding to C-H symmetric bending (30), were relatively stronger for the native rice starch and RS_{UN} than for the ultrasonicated RS. The RS samples showed C-H stretching at $2,926\text{ cm}^{-1}$ (peak A2) and at $1,646\text{ cm}^{-1}$ (peak C, C-O bending associated with -OH group) that may be due to the picking of amylose helices during the combined US and thermal treatment.

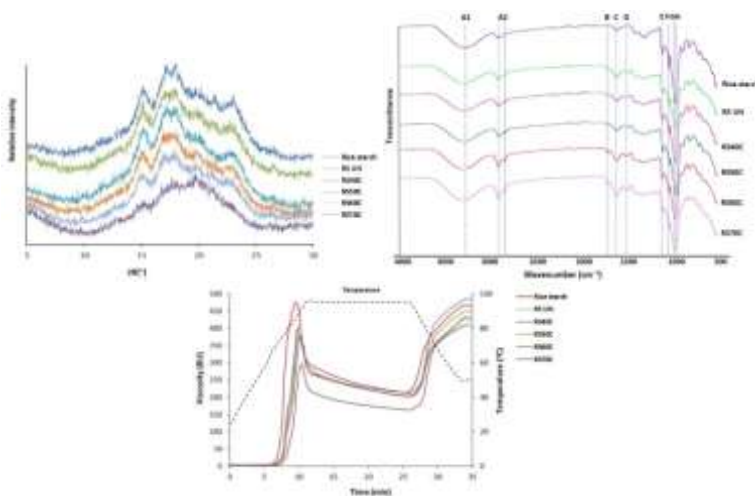


Fig. 3.4. X-ray diffraction patterns, FT-IR spectra, pasting curves of native rice starch and RS isolated from RP that was untreated (UN) and ultrasonicated (1.8 W/cm^2 , 20 min) at different temperatures

In the case of the pasting properties (Fig. 3.4), the native rice starch had the lowest pasting temperature (67.90°C) and the highest peak viscosity (474.02 BU), while the RS_{UN} showed a significantly higher ($p < 0.05$) pasting temperature (72.95°C) and lower peak viscosity (370.63 BU) due to the decrease in the crystalline/amorphous ratio and the double helix content of starch that occurred during the milling of the rice grain (31). The specific viscosity (η_s)

of the RS slurry increased with a 40 °C US temperature and decreased at temperatures between 50 and 70 °C. The RS70C that was isolated after sonication at 70 °C had the highest pasting temperature (75.07 °C), but a strongly reduced peak viscosity (298.51 BU) indicated that the starch granules swelled slowly.

3.3. The use of functionalized rice milling by-products for the production of a functional product with immobilized lactic acid bacteria

The aim of this study is to develop a functional product based on treated US RP in combination with LP pomace rich in phytochemicals, indicating nutraceutical properties.

Creation of an immobilization system based on the treated US rice milling by-products and lingonberry pomace

In order to improve the nutritional value of RP-based product, a lingonberry (*Vaccinium vitis-idaea* L.) pomace was chosen for the experiment. The fraction, containing the highest content of coumaric acid and flavonoids, was selected (32). The matrix of the developed product was composed of raw and US treated RP and LP, changing its amount from 10 to 50%.

The effect of LP additives on the viability of LAB

The analysis of the effect of LP addition on Lb173 viability showed that in all cases, 10–50% LP additives increased the mean cell number by 0.29–0.52 log (Lb173), whereas 10% LP additive increased the cell number by 0.15 log Pa236 in a period of 24–36 h, compared to the US treated RP medium where higher levels of LP (20–50%) reduced the number of LAB cells by 0.38–0.94 log (0.4 log reduction in the control sample) (Fig. 3.5) (32).

The highest number of viable cells after the addition of the LP was observed for Lb173 (9.74 log CFU/g) and was reached after 24 h, using a 50% LP. Higher LP addition (30–50%) slightly reduced the number of viable cells (0.09–0.22 log) during the long fermentation (48–72 h).

In the case of Pa236, 20–50% LP content reduced ($p < 0.05$) the cell survival of this strain. Long-term fermentation reduced the number of viable cells by 0.17–0.32 log compared to the US-treated RP sample without the LP addition, but a greater reduction was observed in the untreated RP sample (0.54 log). The tested LAB strains showed more intensive growth and stability in US-treated RP compared to the untreated RP media. The highest number of Lb173 cells (9.48 log CFU/g) was found in US-treated RP during the 24–36 h fermentation period, compared to the untreated RP (9.38 log CFU/g).

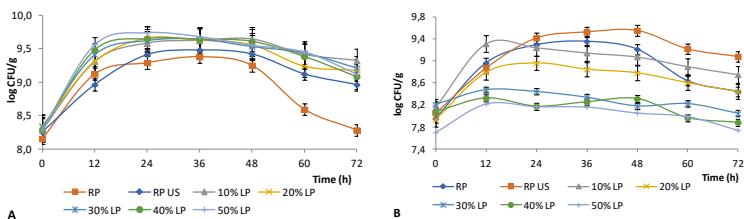


Fig. 3.5. Effect of LP addition (10–50%) on the number of Lb173 (A) and Pa236 (B) bacteria cells (log₁₀ CFU/g) in RP fermentation medium

The US-treated RP medium showed a greater potential to ensure the stability of the tested strains compared to the RP medium: during the fermentation period of 48–72 h, the decrease of LAB viable cells found in the untreated RP medium was 0.40–0.54 log, while in the US-treated RP medium, the LAB viability was the duration of fermentation and had no significant effect ($p \geq 0.05$).

Product stability and viability of immobilized lactic acid bacteria during storage

When evaluating the stability of LAB during storage, it has been found that the duration and temperature of storage had a significant ($p < 0.05$) influence on the viability of LAB cells (Fig. 3.6). After 7 days of storage at 4 °C, the number of Lb173 viable cells in RP-LP mass decreased by the average of 0.102 log CFU/g, and after 56 days of storage, 1.49 log₁₀ CFU/g.

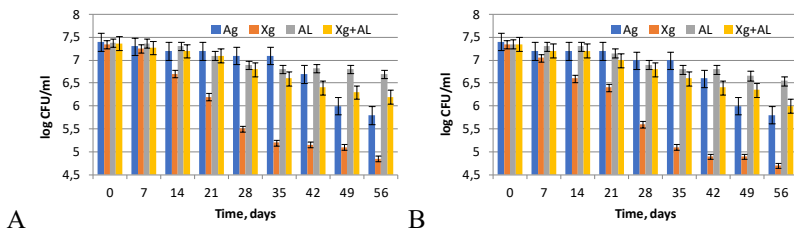


Fig. 3.6. Viable cell numbers of immobilized Lb173 in RP-LP samples with different stabilizers stored at 4 °C (A) and 18 °C (B)

The storage at 18 °C resulted in an average decrease of 0.146 log₁₀ CFU/g after 7 days and 1.58 log₁₀ CFU/g after 56 days. The highest decrease in LAB after 56 days of storage was found in the RP-LP sample with Xg (decrease of 2.464 log₁₀ CFU/g), and the most stable number of LAB cells remained after the immobilization in the gel with AL (average decrease of 0.765 log₁₀ CFU/g) (Fig. 3.6). The RP-LP sample with Ag exhibited a lower protective effect on the

PRB: the number of viable cells after retention was found to be on average 9.7% lower compared to the samples stabilized with AL and Xg.

3.4. The use of functionalized rice milling by-products to improve the resistance and growth intensity of horticultural plants

Antifungal activity of functionalized rice milling by-products filtrates

According to the microbiological analysis, the RP filtrates showed the antifungal activity against 11 pathogens with inhibition zones from 8 mm to 19 mm with the highest effect against *Rhizopus oryzae*.

Table 3.2. The diameters (in mm) of the inhibition zones formed by the untreated and pre-treated rice milling by-products filtrates against plant pathogens

		
FLb against <i>Sclerotinia sclerotiorum</i>	FLu against <i>Pythium volutum</i>	FLu against <i>Verticillium wilt</i>
		
FLb against <i>Alternaria alternata</i>	FLb against <i>Aspergillus versicolor</i>	FLu against <i>Rhizopus oryzae</i>

* significantly different from the control group. RP – untreated, US – ultrasonicated, US+E – ultrasonicated and enzyme hydrolyzed, F_{Pa}, F_{Lb}, F_{Lu}– fermented with appropriate LAB strain. LAB: Pa29 – *P. acidilactici*, Lb173 – *L. brevis*, Lu245 – *L. uvarum*.

US-treated RP filtrates showed inhibitory activity against 9 pathogens (inhibition zones 8–19 mm) with the strongest effect against *Mucor mucedo*. RP_{US+E} filtrates was the most active against *Verticillium wilt* (inhibition zone 14

mm) and less active against *Cladosporium herbarum* (inhibition zone 11 mm) and *Alternaria alternata* (inhibition zone 9 mm) (Table 3.2).

The extract of 48 h *L. brevis* fermented RP demonstrated the strongest growth inhibitory activity against *Pythium volutum* (inhibition zone 22–24 mm), following *F. avenaceum* and *F. graminearum*, *A. versicolor*, *P. syringae* (21–33 mm), and *Sclerotinia sclerotiorum* (19–22 mm). *L. uvarum* fermented RP filtrate was mostly active against *P. volutum*, *Verticillium wilt*, *R. oryzae* (19–29 mm), *P. syringae* (17–26 mm), *A. terreus* (12–20 mm), and *Fusarium* spp. (10–27 mm), but did not show inhibitory activity against *S. sclerotiorum*. The filtrate of *P. acidilactici* fermented RP was moderately active (8–20 mm) against 7 tested pathogenic strains and had no inhibitory activity against *P. volutum*, *S. sclerotiorum* and against 3 of 4 tested *Fusarium* strains.

The effects of functionalized RP additives on bean and tomato seed germination and growth

The results showed that PGS supplementation with RP indicate a significant ($P < 0.05$) positive effect on the seed germination rate and growth performance for both tested plants. In this case, the germination rate varied between 43 and 100% for beans, and between 88 and 100% for tomatoes, while this parameter for PGS (control sample) was significantly lower (25 and 86%, respectively). All RP_F samples, containing higher CYS content among others, improved the germination rate by 42–75% and 2–14% for both bean and tomato seeds.

In the case of root length, the effect of RP additives was fixed on the 21st day of growth for beans (Fig. 3.7 A) and on the 36th day for tomatoes (Fig. 3.7 B). All RP_F samples indicated the greatest effect on the plant root formation (roots were longer by 28–44% for beans and 21–38% for tomatoes), while RP_{US} improved the development of tomato roots slightly (by 8%) (Fig. 3.7 B). The RP_{US+E} additive showed a stronger positive effect on roots formation for tomatoes (by 37%) and did not show a positive effect on the bean roots (Fig. 3.7 A). The longest roots were measured for both plants grown in the PGS supplemented with RP_{Fpa}, which contained the highest content of MET that is beneficial for the root development.

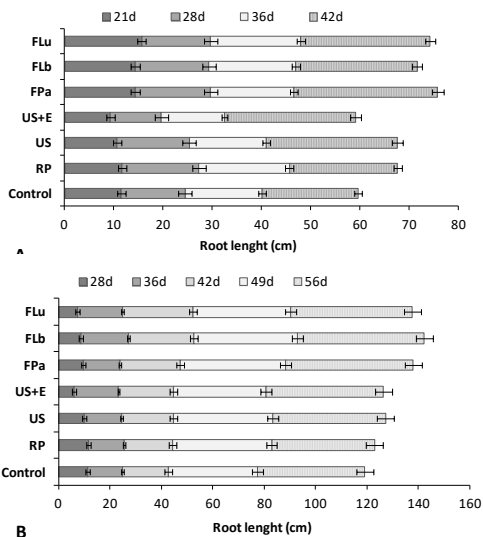


Fig. 3.7. Influence of the RP additives on beans (A) and tomatoes (B) root length

The most positive effect on the bean shoots was observed on the 21st day of growth on the PGS supplemented with RP_F samples (38–47% higher shoots), following the PGS supplemented with RP_{US} (18% higher shoots) compared to the beans grown in PGS and PGS+RP_{UN} (on average 12.16 cm) (Fig. 3.8 A). The notable difference in tomatoes shoots height was fixed on the 28th day of the growth on PGS inoculated with RP_{FLb} and RP_{US+E} (25 and 17% higher shoots compared to the PGS and PGS+RP_{UN} substrates (average height of 13.28 cm) (Fig. 3.8 B). The use of additional organic materials in the substrate can improve plant physiological processes, such as the absorption of water and nutrients (33).

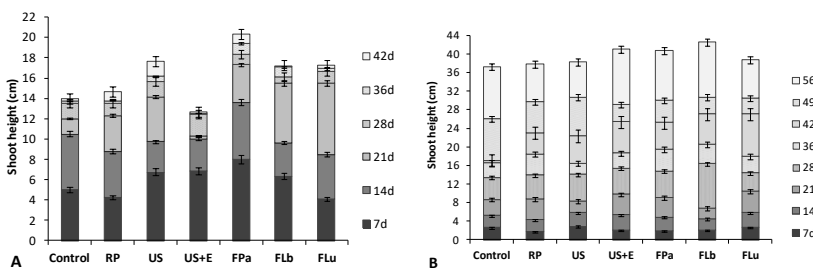


Fig. 3.8. Influence of the RP additives on bean (A) and tomato (B) plant shoot height

CONCLUSIONS

1. Rice milling by-products are a suitable fermentation medium for the cultivation of lactic acid bacteria and production of bioactive metabolites. The efficiency of the process depended on the type of lactic acid bacteria and the duration of the semi-solid state fermentation. In the medium of rice milling by-products under semi-solid state fermentation conditions, after 36–48 h of fermentation, *L. brevis*, *L. uvarum*, *L. farraginis*, and *P. pentosaceus* species produced 0.32–0.48 g/100 g of lactic acid, in which higher L (+)-lactic acid content (L/D ratio 1.05–4.70) was observed, compared to the non-fermented products. *L. brevis* 173, *L. uvarum* 245, *P. acidilactici* 236, and *P. pentosaceus* 100 bacteria produced metabolites with antifungal and antibacterial activity that inhibited growth against *A. versicolor*, *P. viridicatum*, *P. spinulosum*. Applying 24–36 h fermentation in *L. brevis* 173, the amount of condensed tannins decreased by 53.6%, the amount of trypsin inhibitors by 84.3%, and the extraction efficiency of phenolic compounds increased as well.

2. The amount of resistant starch can be increased to 22.13 g/100 g d. m. by treating the rice milling by-products with high-frequency ultrasound (850 kHz) at 40 °C for 20 min. The variance analysis of the obtained results of resistant starch confirmed that the mathematical model could describe 97% of the data. Using the surface response method, the optimal conditions for ultrasonic treatment were determined: duration 18 min, temperature 40 °C, ultrasonic treatment power 1.8 W/cm². Evaluating the influence of ultrasonic treatment on the physico-chemical and morphological properties of resistant starch, it has been found that by controlling the temperature of ultrasonic treatment, it is possible to modify the functional properties of resistant starch by changing its chemical structure, reducing crystallinity and increasing the thermal stability of starch paste.

3. The developed prototype of the product from rice milling by-products and cranberry pomace is suitable for the stabilization of lactic acid bacteria and can be used for the production of food products with specific properties. The selected stabilizers and/or thickener to stabilize the product matrix can successfully maintain a sufficiently high number of viable lactic acid bacteria cells (4.85–6.7 log₁₀ CFU/ml) during storage at 4 °C.

4. Functionalized by-products of rice milling can be used as a substrate additive to increase the disease resistance of vegetable plants and improve growth intensity. Fermented and sonicated rice milling by-products increased tomato and bean seed germination (2–75%), root length (26–52%), and shoot height (28–50%), and significantly increased (up to 32.5%) chlorophyll amount in both plant species. *L. brevis* 173 fermented rice milling by-products filtrates showed the highest inhibitory effect against soil-borne plant

pathogens, such as *Fusarium spp.*, *Pythium spp.*, *Sclerotinia sclerotiorum*, *Aspergillus versicolor*, and *Verticilium wilt*.

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REZIUMĖ

Temos aktualumas. Vykdamt agroinovacijas ir tobulinant maisto biotechnologijas, siekiama ne tik pagerinti maisto produktų kokybę, bet ir sumažinti pramonės atliekų kiekį, pasitelkus biorafinavimo koncepciją – perdirbti žemės ūkio ir maisto pramonėje susidarancius šalutinius produktus ir juos panaudoti, suteikiant pridėtinę vertę.

Dėl šios priežasties mokslo pasaulyje vis daugiau dėmesio skiriama įvairių grūdų, vaisių/uogų ir daržovių perdirbimo metu susidariusiems šalutiniams produktams, kurių dideli kiekiai lieka nepanaudoti arba panaudojami neracionaliai. Vertingos grūdinių atliekų maisto medžiagos (baltymai, skaidulos) ir vaisių/uogų išspaudų bioaktyvieji junginiai, pasižymintys antioksidaciniu aktyvumu, priešnavikiniu, antivirusiniu, antibakteriniu ir antimutageniniu poveikiu, galėtų papildyti maisto komponentus, taip ne tik apsaugant maistą nuo mikrobiologinio gedimo, bet ir prisidedant prie įvairių susirgimų rizikos mažinimo (1, 2).

Ryžių sėlenos (RP) yra vienas pagrindinių ryžių malimo šalutinių produktų, jų kiekis pasaulio mastu sudaro 76 mln. tonų per metus (3), todėl sulaukiama vis didesnio dėmesio iš mokslo visuomenės ne tik dėl to, kad šios sėlenos yra plačiai prieinamos ir pigios, bet ir dėl vertingųjų komponentų, tokių kaip baltymai, maistinės skaidulos, atsparus virškinimui krakmolai (AVK), vitaminai, mineralai ir fitocheminės medžiagos (4).

Pastaruoju metu AVK charakterizuojamas kaip funkcinis biopolimeras, kurio fiziologinis poveikis panašus į maistinių skaidulų (5). Ryžių perdirbimo šalutiniai produktai gali būti naudojami kaip AVK šaltinis, tačiau natūralus jo kiekis priklauso nuo ryžių veislės ir gali būti palyginti mažas (6). AVK kiekis gali būti padidintas technologinių procesų metu (8, 9), taip pat taikant fizinius ar hidroterminės modifikacijos metodus (10, 11).

Ultragarsinė kavitacija yra vienas iš inovatyvių būdų, taikomų įvairios augalinės kilmės biomasei stabilizuoti, modifikuoti, mikroorganizmų fermentinėms sistemoms aktyvinti ir vertingiems ingredientams efektyviau išgauti (14). Didžioji dalis mokslinių tyrimų AVK kiekio padidinimo srityje iki šiol buvo sutelkti tik į grynojo krakmolo modifikavimą (15, 16), todėl aktualu ištirti krakmolo modifikavimo ultragarsu galimybes visoje ryžių malimo šalutinių produktų matricoje.

Pusiau kietafazė fermentacija (PKF) antimikrobinėmis pieno rūgšties bakterijomis (PRB) yra veiksmingas biotechnologinis procesas, pagerinantis produkto juslines savybes bei maistinę vertę ir užtikrinantis produktų saugą (17). PRB gebėjimas augti grūdų perdirbimo šalutinių produktų substratuose be priedų ir gyvybingumas laikant yra svarbūs kriterijai, išsaugant maisto produktų technologines ir fiziologines savybes.

Iš šios perspektyvos, taikant apdorojimo UG technologiją ir mikrobinę fermentaciją, kuriamos naujos maisto sistemos iš RP biopolimerų ir antimikrobinių komponentų. Kai taikomos šios technologijos, galima išplėsti ryžių malimo šalutinių produktų antrinio panaudojimo galimybes, optimizuojant sąlygas AVK gamybai, įvertinant tiek antimikrobinio aktyvumo, tiek antimonybinių faktorių pokyčius RP.

Viena iš galimybių, leidžiančių racionaliai panaudoti RP vertinguosius komponentus, būtų funkcinių maisto produktų iš RP ir vaisių/uogų išspaudų su imobilizuotomis antimikrobinėmis pieno rūgšties bakterijomis kūrimas. Produktai, kuriuose bioaktyvieji komponentai yra imobilizavimo medžiagoje arba gali būti imobilizuojami, pasižymi nutraceutinėmis savybėmis, yra inovatyvi koncepcija funkcinių maisto produktų gamybai.

Kita galimybė, leidžianti racionaliai panaudoti grūdų perdirbimo šalutinių produktų vertingąsias medžiagas, yra substrato komponentų daržo augalų auginimui kūrimas. Tai potenciali sintetinių priedų alternatyva, kuri padėtų spręsti su ekologija ir pertekliniu antrinių produktų susidarymu susijusias problemas pramonėje.

Darbo tikslas. Ištirti apdorojimo ultragarsu bei mikrobinės fermentacijos įtaką bioaktyviųjų komponentų pokyčiams ryžių malimo šalutiniuose produktuose ir įvertinti apdorotų ryžių malimo šalutinių produktų panaudojimo galimybes maisto produktų bei augalų auginimo substrato gamybai.

Darbo uždaviniai:

1. Atrinkti ryžių malimo šalutinių produktų pusiau kietafazei fermentacijai tinkamas pieno rūgšties bakterijas ir nustatyti jų įtaką pieno rūgšties izomerų susidarymui, antimikrobinių bei fermentinių aktyvumų, taip pat fenolinių junginių bei antimonybinių faktorių pokyčiams.
2. Ištirti apdorojimo ultragarsu įtaką atsparaus virškinimui krakmolo kiekio padidimui ryžių malimo šalutiniuose produktuose ir įvertinti ultragarso bei temperatūros įtaką atsparaus virškinimui krakmolo morfologinėms, fizikinėms-cheminėms bei technologinėms savybėms.
3. Įvertinti ryžių malimo šalutinių produktų panaudojimo galimybes funkcinio maisto produkto su bruknių uogų išspaudomis gamybai, parenkant stingdiklius ar tirštiklius ir įvertinant bakterinių ląstelių gyvybingumą bei produkto stabilumą laikymo metu.
4. Ištirti ryžių malimo šalutinių produktų panaudojimo galimybes daržo augalams skirtu substrato gamybai, įvertinant jų priedų įtaką daržo augalų (pomidorų ir pupelių) ligų prevencijai ir augimo intensyvumui.

Mokslinis darbo naujumas. Pirmą kartą išanalizuotas ryžių malimo šalutinių produktų tinkamumas fermentacijai įvairiomis *Lactobacillus* spp. ir

Pediococcus spp. pieno rūgšties bakterijomis, įvertinant L(+)-pieno rūgšties susidarymą, aminorūgščių kokybinę bei kiekybinę sudėtį ir fermentacijos metabolitų slopinantį poveikį kai kurių *Penicillium* spp., *Fusarium* spp., *Aspergillus* spp. mikroorganizmų augimui.

Pirmą kartą įvertinta apdorojimo aukštojo dažnio (850 kHz) ultragarsu įtaka atsparaus virškinimui krakmolo susidarymui ryžių malimo šalutiniuose produktuose (sėlenose) pagal UG parametrus. Optimizuoti apdorojimo ultragarsu parametrai (temperatūra, trukmė, galia) maksimaliam atsparaus virškinimui krakmolo susidarymui ir sudaryta matematinio modelio lygtis jo kiekiui prognozuoti.

Praktinė darbo vertė. Ryžių malimo šalutiniai produktai gali būti panaudoti kaip fermentacijos terpė įvairių pieno rūgšties bakterijų dauginimui ir bioaktyviųjų metabolitų, pasižyminčių fermentiniu, antigrybiniu ir antibakteriniu aktyvumu, gamybai be hidrolizinių fermentų ar maistinių medžiagų priedų.

Apdorojimas aukštojo dažnio (850 kHz) ultragarsu 40 °C temperatūros aplinkoje gali būti pritaikytas funkcinio komponento – atsparaus virškinimui krakmolo – kiekio padidinimui ryžių malimo šalutiniuose produktuose. Taip funkcionalizuoti ryžių malimo šalutiniai produktai gali būti panaudoti antimikrobinį pieno rūgšties bakterijų imobilizavimui ir funkcinį maisto produktų gamybai.

Gautų rezultatų pagrindu sukurta naujo produkto (batonėlio) receptūra iš apdorotų ultragarsu ir fermentuotų antimikrobinėmis pieno rūgšties bakterijomis ryžių malimo šalutinių produktų ir bruknių uogų išspaudų, parenkant optimalią šių komponentų kombinaciją ir stingdiklius, užtikrinančius tekstūros stabilumą ir pieno rūgšties bakterijų gyvybingumą laikant. Produktas pristatytas KTU parodoje „Technorama 2022“, ten sulaukė didelio susidomėjimo ir palankaus įvertinimo.

Fermentuoti antimikrobinėmis pieno rūgšties bakterijomis ryžių malimo šalutiniai produktai gali būti rekomenduojami kaip bioaktyvieji daržo augalų – pupelių ir pomidorų – auginimo substrato komponentai, padidinantys jų atsparumą šaknų puvinį sukeliantiems patogenams, taip pat pagerinantys šių augalų sėklų daigumą ir augimo intensyvumą.

Ginamieji disertacijos teiginiai

1. Ryžių malimo šalutiniai produktai yra tinkama terpė pieno rūgšties bakterijų dauginimui ir bioaktyviųjų metabolitų, pasižyminčių fermentiniu, antigrybiniu ir antibakteriniu aktyvumu, gamybai.
2. Taikant aukštojo dažnio (850 kHz) ultragarsą galima padidinti atsparaus virškinimui krakmolo (3 tipo) kiekį ryžių malimo šalutiniuose produktuose.

IŠVADOS

1. Ryžių malimo šalutiniai produktai yra tinkama fermentacijos terpė pieno rūgšties bakterijų kultivavimui ir bioaktyviųjų metabolitų gamybai, tai priklauso nuo pieno rūgšties bakterijos rūšies ir pusiau kietafazės fermentacijos trukmės. Pusiau kietafazės fermentacijos *L. brevis*, *L. uvarum*, *L. farraginis* ir *P. pentosaceus* bakterijomis 36–48 h laikotarpiu susidaro iki 0,32–0,48 g/100 g pieno rūgšties, esant L(+)/D(–) santykiui 1,05–4,70. *L. brevis* 173, *L. uvarum* 245, *P. acidilactici* 236 ir *P. pentosaceus* 100 bakterijos gamino antigrybiniu ir antibakteriniu aktyvumu pasižyminčius metabolitus, slopinančius *A. versicolor*, *P. viridicatum*, *P. spinulosum* augimą. Taikant 24–36 h fermentaciją *L. brevis* 173, galima sumažinti kondensuotųjų taninų ir tripsino inhibitorių kiekius ryžių sėlenose, atitinkamai 53,6 % ir 84,3 %.
2. Apdorojant ryžių malimo šalutinius produktus aukštojo dažnio ultragarsu (850 kHz) 40 °C temperatūros aplinkoje 20 min, galima padidinti atsparaus virškinimui krakmolo kiekį iki 22,13 g/100 g s. m. Gautų atsparaus virškinimui krakmolo rezultatų dispersinė analizė patvirtino, kad sudarytu matematiniu modeliu galima aprašyti 97 % duomenų. Taikant paviršiaus atsako metodą, nustatytos optimalios apdorojimo ultragarsu sąlygos: trukmė 18 min, temperatūra 40 °C, UG galia 1,8 W/cm². Vertinant apdorojimo ultragarsu įtaką atsparaus virškinimui krakmolo fizikinėms-cheminėms ir morfologinėms savybėms, nustatyta, kad, kai kontroliuojama apdorojimo ultragarsu temperatūra, galima modifikuoti atsparaus virškinimui krakmolo funkcines savybes, pakeičiant jo cheminę struktūrą, mažinant kristališkumą ir padidinant krakmolo kleisterio terminį stabilumą.
3. Sukurtas produkto iš ryžių malimo šalutinių produktų ir bruknių išspaudų prototipas yra tinkamas pieno rūgšties bakterijoms stabilizuoti ir gali būti panaudotas maisto produktų, pasižyminčių specifinėmis savybėmis, gamybai. Parinkti stingdikliai ir/ar tirštiklis produkto matricai stabilizuoti gali sėkmingai išsaugoti pakankamai didelį gyvybingų pieno rūgšties bakterijų ląstelių skaičių (4,85–6,7log₁₀ KSV/ml) laikant produktą 4 °C temperatūros aplinkoje.
4. Funkcionalizuoti ryžių malimo šalutiniai produktai gali būti panaudoti kaip substrato priedas daržo augalų augimo intensyvumui pagerinti. Fermentuoti ir apdoroti ultragarsu ryžių malimo šalutiniai produktai padidino pomidorų ir pupelių sėklų daigumą (2–75 %), šaknų ilgį (26–52 %) ir ūglių aukštį (28–50 %), taip pat žymiai padidino (iki 32,5 %) chlorofilo kiekį abiejų rūšių augaluose. Fermentuotų ryžių malimo šalutiniai produktai parodė slopinantį poveikį augalų stiebų ir šaknų puvinį sukeliančių mikroorganizmų augimui. *L. brevis* 173 fermentuotų

ryžių malimo šalutinių produktų filtratas pasižymėjo didžiausiu slopinančiu poveikiu prieš dirvožemio augalų patogenus, tokius kaip *Fusarium* spp., *Pythium* spp., *Sclerotinia sclerotiorum*, *Aspergillus versicolor* ir *Verticilium wilt*.

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