ANNOTATION

of the dissertation work by Shaimenova Bakhyt Sailauovna on the topic:

«IMPROVING THE PRODUCTION TECHNOLOGY OF CITRIC ACID BASED ON CARBOHYDRATE-CONTAINING RAW MATERIALS BY OPTIMIZING THE FERMENTATION PROCESS»

submitted for the degree of Doctor of Philosophy (PhD) in the specialty 6D072800 – Technology of processing production

1. Relevance of the dissertation work. Technologies for the deep processing of raw materials are regarded in global scientific practice as relevant and advanced. In many foreign countries, deep processing has become an established industry generating stable and high revenues. For Kazakhstan, it represents a strategic direction for the development of the agro-industrial complex and is one of the key priorities of state policy, which has been repeatedly emphasized in the Addresses of the President of the Republic of Kazakhstan.

A product of deep processing of plant raw materials – citric acid – is one of the most widely used acidity regulators. As a natural antioxidant, citric acid is widely applied in the food industry, the production of synthetic detergents, agriculture, as well as in the cosmetic and pharmaceutical industries and other sectors.

In the republic, three large enterprises for deep grain processing are currently operating, while four additional projects are at the stage of implementation: «Zharkent Starch and Syrup Plant» LLP (150 thousand tons of corn processing per year), «AsiaAgroFood» LLP (60 thousand tons of corn per year – production of starch, molasses, and syrups), and «BioOperations» LLP (300 thousand tons of wheat – production of starch, gluten, bioethanol, and feed). These enterprises form the basis of the domestic starch and molasses industry and ensure the processing of significant volumes of locally produced carbohydrate-containing raw materials. By 2028, the enterprises plan to launch several major new projects, which will contribute to the expansion of deep grain processing [2].

Currently, citric acid is imported into the country, resulting in the domestic market being dependent on external supplies. At the same time, Kazakhstan possesses significant reserves of carbohydrate-containing raw materials, including corn and wheat starch, which can be used as a raw material base for its production.

Commercial production of citric acid is generally based on submerged cultivation of carbohydrate substrates using strains of *Aspergillus niger* (*A.niger*). This method is characterized by high productivity, environmental safety, and process stability at low pH values, which helps to prevent the formation of toxic by-products. However, natural strains of *A. niger* have a limited ability to hydrolyze starch, which reduces the efficiency of biosynthesis. In this regard, a key objective is the development of mutant strains capable of efficiently utilizing starch hydrolysates as the main carbon source.

Thus, the prerequisites are being formed for the development and implementation of domestic technologies for citric acid production based on locally available resources.

2. Research Objects. Carbohydrate-containing substrates were used as the main carbon sources, including native corn starch (GOST 32159–2013 «Corn Starch. General Specifications», produced by «Zharkent Starch and Syrup Plant» LLP, Zharkent) and wheat starch (GOST 31935–2012 «Wheat Starch. Specifications» produced by BioOperations LLP, Tayynsha).

A mutant strain *A. niger* R5/4, isolated from soil samples collected at sugar and starch-processing enterprises of the Republic of Kazakhstan, was used as a citric acid producer.

3. The aim of the dissertation work. Improvement of citric acid production technology from carbohydrate-containing raw materials through optimization of the fermentation process using a new highly efficient strain *A.niger* R5/4.

To achieve this goal, the following objectives were addressed:

1. Screening of active citric acid-producing strains, investigation of their molecular genetic characteristics, and enhancement of their citric acid production capacity;
2. Study of the physicochemical properties of starch-containing raw materials;
3. Determination of the optimal composition of the nutrient medium and cultivation parameters;
4. Application of ozonation for sterilization of fermentation equipment and determination of optimal process parameters;
5. Optimization of the citric acid biosynthesis process under pilot-scale conditions;
6. Determination of the quality characteristics of the final product and evaluation of its functional properties;
7. Assessment of the economic efficiency of citric acid production technology based on corn starch;
8. Development of an improved technology for citric acid production using a new highly efficient strain.

4. Research methods and methodology. A set of physicochemical, microbiological, biotechnological, and molecular genetic methods was used in this study. The composition of raw materials and biosynthesis products was determined using titrimetric, spectrophotometric, and gravimetric methods in accordance with standard procedures.

Biotechnological investigations included enzymatic hydrolysis of starch, cultivation of microorganisms, and optimization of fermentation conditions. Microbiological and molecular genetic methods were applied for the screening and identification of producer strains.

The quality of citric acid was assessed using standard analytical and instrumental methods. Experimental data were processed using Minitab software with the application of nonlinear regression methods. Data visualization and process optimization were also performed using Minitab.

Statistical analysis of the model developed based on the Box–Behnken design was carried out using analysis of variance (ANOVA) and Duncan's multiple range test.

5. Scientific novelty of the research.

- The technology for citric acid production has been improved through the use of maltodextrin and a highly efficient strain *A. niger* R5/4 obtained by combined mutagenesis, ensuring a high yield of the final product;

- For the first time in citric acid production technology, the use of ozonation for the sterilization of fermenters has been proposed and scientifically substantiated. This approach enhances the productivity and activity of microbial strains, thereby increasing the yield of citric acid.

6. Application Field: The results of the study can be widely implemented in small and medium-sized enterprises of the food and processing industries, as well as in biotechnological production of organic acids, contributing to the expansion of product range, improving the efficiency of carbohydrate-containing raw material utilization, and reducing dependence on imported products.

7. Practical value of the dissertation work. The results of the study have high practical significance for the food and processing industries of the Republic of Kazakhstan. The improved technology for citric acid production based on carbohydrate-containing raw materials can be implemented at food and processing enterprises, including starch-processing and biotechnological facilities.

The practical significance of the work lies in increasing the yield of citric acid, reducing production costs through the use of readily available raw materials, and improving process efficiency by optimizing fermentation parameters and introducing ozonation for equipment sterilization. The feasibility and novelty of the proposed solutions are confirmed by patents of the Republic of Kazakhstan No. 34800 «Mutant strain *Aspergillus niger* R5/4 – citric acid producer» and No. 35429 «Method for producing maltodextrins from wheat starch».

Implementation of this technology will contribute to reducing dependence on imported citric acid and promoting the development of domestic production.

8. **Personal Contribution of the author**. The author's contribution consists in the independent formulation of the research aim and objectives, development of the methodology and experimental program, conduct of theoretical and experimental studies, as well as processing and interpretation of the obtained results.

The author carried out a comprehensive review of the scientific literature, substantiated the relevance and scientific novelty of the research topic, performed the selection and investigation of microorganisms, and optimized the process conditions.

9. Work Approbation. Pilot-scale validation of the developed technology was carried out at «Zharkent Starch and Syrup Plant» LLP, which is confirmed by an act of industrial testing. Based on the results of the dissertation research, scientific papers were published in the proceedings of international and national scientific and practical conferences, including: the International Scientific and Practical Conference «Grain Industry: Current State and Development Prospects» (Almaty,

ATU, 2020), the International Scientific and Practical Conference «Current Issues of Biodiversity and Biotechnology» (Astana, 2019), the International Scientific and Practical Conference «Food Technologies of the Future: Innovations in the Production and Processing of Agricultural Products» (Saratov, 2020), as well as the national scientific and practical conference «Microbiome: Fundamental and Applied Aspects» (Astana, 2020).

The publications are indexed in the Russian Science Citation Index (RSCI) and are accompanied by the corresponding ISBN and ISSN identifiers.

Pilot-scale testing of the developed technology was carried out at «Zharkent Starch and Syrup Plant» LLP, which is confirmed by an official report on production trials.

Based on the results of the dissertation research, scientific papers were published in the proceedings of international and national scientific and practical conferences, including: the International Scientific and Practical Conference «Grain Industry: Current State and Development Prospects» (Almaty, ATU, 2020), the International Scientific and Practical Conference «Current Issues of Biodiversity and Biotechnology» (Astana, 2019), the International Scientific and Practical Conference «Food Technologies of the Future: Innovations in the Production and Processing of Agricultural Products» (Saratov, 2020), as well as the National Scientific and Practical Conference «Microbiome: Fundamental and Applied Aspects» (Astana, 2020).

The publications are indexed in the Russian Science Citation Index (RSCI) database and are assigned corresponding ISBN and ISSN numbers.

10. Publications A total of 11 scientific publications have been produced on the topic of the dissertation, including 2 articles in journals indexed in the Scopus database; 2 articles in scientific journals recommended by the Committee for Control in the Sphere of Education and Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan; and 7 papers published in the proceedings of international and national scientific and practical conferences. Based on the research results, 2 patents for inventions of the Republic of Kazakhstan have been obtained.

The work was carried out within the framework of program-targeted funding under project IRN BR06249419 (2018–2020) «Scientific and technological support of agro-industrial processing enterprises to improve their efficiency and competitiveness» at NJSC «S. Seifullin Kazakh Agro Technical Research University».

11. Structure and scope of the dissertation. The dissertation includes regulatory references, definitions, symbols and abbreviations, an introduction, a literature review, materials and methods, research results, discussion, conclusions, and a list of references.

The total length of the dissertation is 170 pages. The work contains 25 tables and 33 figures. The reference list includes 169 sources from both domestic and international literature.

12. Conclusions of the dissertation:

As a result of the comprehensive study aimed at improving citric acid production technology based on carbohydrate-containing raw materials, all the set objectives were consistently achieved.

1. Screening and selection of an active citric acid-producing strain were carried out. As a result of multistage mutagenesis of the parent strain *Aspergillus CA20*, a new highly productive strain *A. niger R5/4* with an acid-forming activity of 37.5 g/L was obtained. The strain has been patented, confirming the scientific novelty and practical significance of the research.

2. The physicochemical properties of starch-containing raw materials were studied, and their suitability as substrates for citric acid biosynthesis was substantiated. It was established that the most technologically efficient carbon source is corn starch maltodextrin with a dextrose equivalent of DE = 19 ± 1%, ensuring a stable supply of fermentable sugars during cultivation.

3. The optimal composition of the nutrient medium and cultivation parameters were determined under laboratory conditions. The maximum process efficiency was achieved under the following conditions: fermentation time – 168 h, pH – 5.0, cultivation temperature – 30°C, and concentration of fermentable sugars – 140 g/L based on corn starch maltodextrin (DE = 19%). The optimal concentrations of mineral components were determined as follows: NH_4NO_3 – 3.8 g/L; KH_2PO_4 – 5.0 g/L; $MgSO_4 \cdot 7H_2O$ – 0.17 g/L; $ZnSO_4 \cdot 7H_2O$ – 0.0018 g/L; $FeSO_4 \cdot 7H_2O$ – 0.0170 g/L; $CuSO_4 \cdot 5H_2O$ – 0.063 g/L. It was found that the addition of 3% ethanol and 3% inoculum positively affects the metabolism of *A.niger* and increases citric acid yield.

4. The optimal ozonation parameters were determined, under which the citric acid yield increased from 70.4% to 74.8%.

5. As a result of the study, the optimal process parameters were established: cultivation time – 167.2 h, pH – 5.0, and temperature – 30°C. Under these conditions, the maximum citric acid yield reached 117.71 g/L, confirming the high efficiency of the improved technology under scaled-up conditions.

6. A comprehensive assessment of the quality, authenticity, and purity of the obtained citric acid was performed. The final product complies with the requirements of GOST 908-2004 for food-grade citric acid. It is characterized by high purity, as well as pronounced reducing and chelating activity. Product authenticity and purity were confirmed by capillary electrophoresis. The content of the main substance was 99.36%, while the total content of accompanying organic acids (gluconic and oxalic) was only 0.33%, indicating high purification efficiency. SEM analysis showed the formation of structurally stable and homogeneous citric acid crystals.

7. The conducted techno-economic analysis demonstrated that the citric acid production technology is economically efficient. The total cost of the pilot product is 2097 KZT/kg, which is 803 KZT/kg (27.7%) lower than the average market price of commercial citric acid (2900 KZT/kg). The economic effect for the production of 750 kg reaches 602.25 thousand KZT, confirming the economic feasibility and prospects for industrial implementation.

8. Pilot-scale testing of the technology was carried out at «Zharkent Starch and Syrup Plant» LLP, which is confirmed by an official report on production trials.

.