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PRODUCTION AND COSTABILITY ANALYSIS OF BIOACTIVE HYDROLYZATES OF WHEY PROTEIN

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Abstract

Due to the fact that individual whey proteins have their own unique nutritional, functional and biological characteristics, the latest research in this area draws attention to the possibility of synthesizing a wide range of bioactive compounds derived from whey proteins. There are various ways to release bioactive peptides from precursor proteins or whey parent proteins. The paper will present the production of bioactive whey protein hydrolyzates (BHPS), which exhibit high antioxidant activity, and their economic profitability of production. Research is focused on the enzymatic and microbiological process of modifying whey proteins. Whey proteins can be easily modified under mild conditions of temperature and Ph values. For the purpose of this research, two scenarios were modeled: Scenario A, which involves the use of whey, and Scenario B, which involves the use of whey protein concentrate as a protein source. The entire process for both scenarios, which includes pre-treatment, whey protein modification and product separation, was modeled using the SuperPro Desinger program, which is equipped with a wide range of processes, and can be used for mathematical evaluation of the economic performance of the process.

Key words*: *whey, protein, production of bioactive whey protein hydrolyzates

INTRODUCTION

Milk production as a part of livestock production has always had a significant place in the development of Serbian agriculture. The developed Strategies for the Development of Agriculture in Serbia at the macro and micro level indicate that there are available potentials that have not been used, both for the development of livestock production and milk production in the plains, hilly and mountain areas. However, due to the overall situation with Covid 19, there is a decrease in the number of cattle, as shown by the results presented in the Statistical Yearbook for 2021 [16], where the decrease is 3% compared to the previous two years (2019 and 2020), while for the ten-year average of 2011-2020, the total number of cattle is lower by 5.1%. With the decrease in the number of cattle, there is a decrease in the number of dairy cows, so that the production of milk and dairy products is reduced as well as the production of different types of cheese, and we know that investing in cheese production is very profitable more tha production of raw milk. In addition to earnings in cheese production, whey is also obtained as a by-product, which nowadays has a number of commercial applications [13].

Whey, which is produced in the process of cheese production, is a very important waste product of the dairy industry, due to its very rich nutritional composition, which is generated in very large quantities in milk processing processes [2, 3]. Whey contains more than half of the dry matter present in milk, including whey protein (20% of total protein) as the most attractive ingredient. Due to the wide range of bioactivity (antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral and antibacterial) [11] and excellent functional properties (eg high solubility, water absorption, gelatinization and emulsifying capacity) [5] whey can be considered a very valuable by-product with wide possibilities of application in the food and pharmaceutical industry [7]. Therefore, they can be used as potential food ingredients and food supplements and as auxiliary drugs, where their bioactivity would help in the prevention and control of diseases [3].

Apart from the industrial production of the pure fraction of whey proteins, it is also possible to obtain bioactive protein substances by hydrolysis of whey proteins. Enzyme-controlled hydrolysis of whey proteins is one of the fastest, safest and most easily controlled techniques for obtaining bioactive peptides [10].

The main goal of research related to the production of bioactive whey peptides using the hydrolysis process is their commercialization, but the lack of a case study is a major obstacle in the development of this area. Therefore, the commercialization of bioactive whey protein hydrolysates requires comprehensive business case studies that include a clear understanding of the market situation, development of market strategy, analysis of technical work plan, management and staff, legal issues, preparation of financing plan, action plan, risk analysis and exit opportunities.

The basic information for any business plan for the production of industrial capacity includes an analysis of the economic feasibility of the process. Through the analysis of economic justification, it is necessary to accurately analyze the impact of key production parameters, such as production capacity, equipment costs, raw material costs, operating costs and selling price of products on total investment, repayment time and payback period [12, 9, 17]. There is a number of techniques that can be applied to assess the economic viability of a targeted process. Simulation software, such as SuperPro Designer [18], equipped with a wide range of processes, is a powerful tool that can be used to mathematically estimate the economic performance of a process. This process simulator shortens the time required for process development, allows comparison of alternative processes and provides an opportunity for interactive analysis of a large number of processes in a short time [14]. SuperPro Designer is widely used for simulation of industrial production of various bio-products and analysis of economic justification of such processes [4, 8, 15].

However, to our knowledge, analysis of economic feasibility of industrial production of bioactive whey protein hydrolysates has not been done yet. This shortcoming should be overcome in order to commercialize bioactive whey protein hydrolysates as very valuable products.

With that goal in mind, SuperPro Designer can be used to conduct an analysis of the economic feasibility of the production process of bioactive whey proteins at the industrial level and to establish fundamental knowledge related to the business plan of the production process of bioactive whey protein hydrolysates [1].

MATERIALS AND METHODS

Whey protein concentrate (WPC, whey protein concentrate) with 80.0% (w/w) protein (DMV International, 5462 GE Veghel, The Netherlands) was used. The WPC solution was prepared as a 5.0% (w/w) suspension of WPC in water. The paper also used whey left over from cheese production (Imlek ad, Belgrade). The chemical composition of whey consists of: dry matter 9.8 ± 0.03% (w/v); proteins 2.6 ± 0.012% (w/v); fat 1.05 ± 0.08% (w/v) and lactose 5.6 ± 0.114% (w /v). Also, the bacterial strain *Lactobacillus rhamnosus* ATCC 7469 (American Culture Collection, ATCC, Rockville, USA) was used. The commercial enzyme Trypsin (porcine pancreas, EC 3.4.21.4) was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Licensed software SuperPro Designer (as in my previous works) was used to test the possibility of using whey in various technological processes. This program provides opportunities to find the optimal procedure for profitability analysis, and by adjusting the basic parameters it is possible to analyze and predict costs for many industrial processes.

RESULTS AND DISCUSSIONS

Description of the process model

In order to convert whey protein into value-added products, microbiological and enzymatic transformation of whey protein was used to produce high quality bioactive whey protein hydrolysates (BHPS) that exhibit high antioxidant activity. The research is focused on enzymatic and microbiological processes of whey protein modification. Whey proteins can be easily modified under mild temperature and pH conditions. The availability of different microorganisms and enzymes from different sources allows the manufacturer to choose the best option based on the desired quality of the final product. For the purpose of this research, two possible scenarios were modeled: Scenario A which involves the use of whey and Scenario B which involves the use of whey protein concentrate as a protein source. Both processes involve three processing steps: pre-treatment, whey protein modification and product isolation. Each step was optimized in a previous study by the author [5, 6]. A simplified flow diagram for the production process of 96% (w/w) bioactive whey protein hydrolyzate is shown in Figure 1.

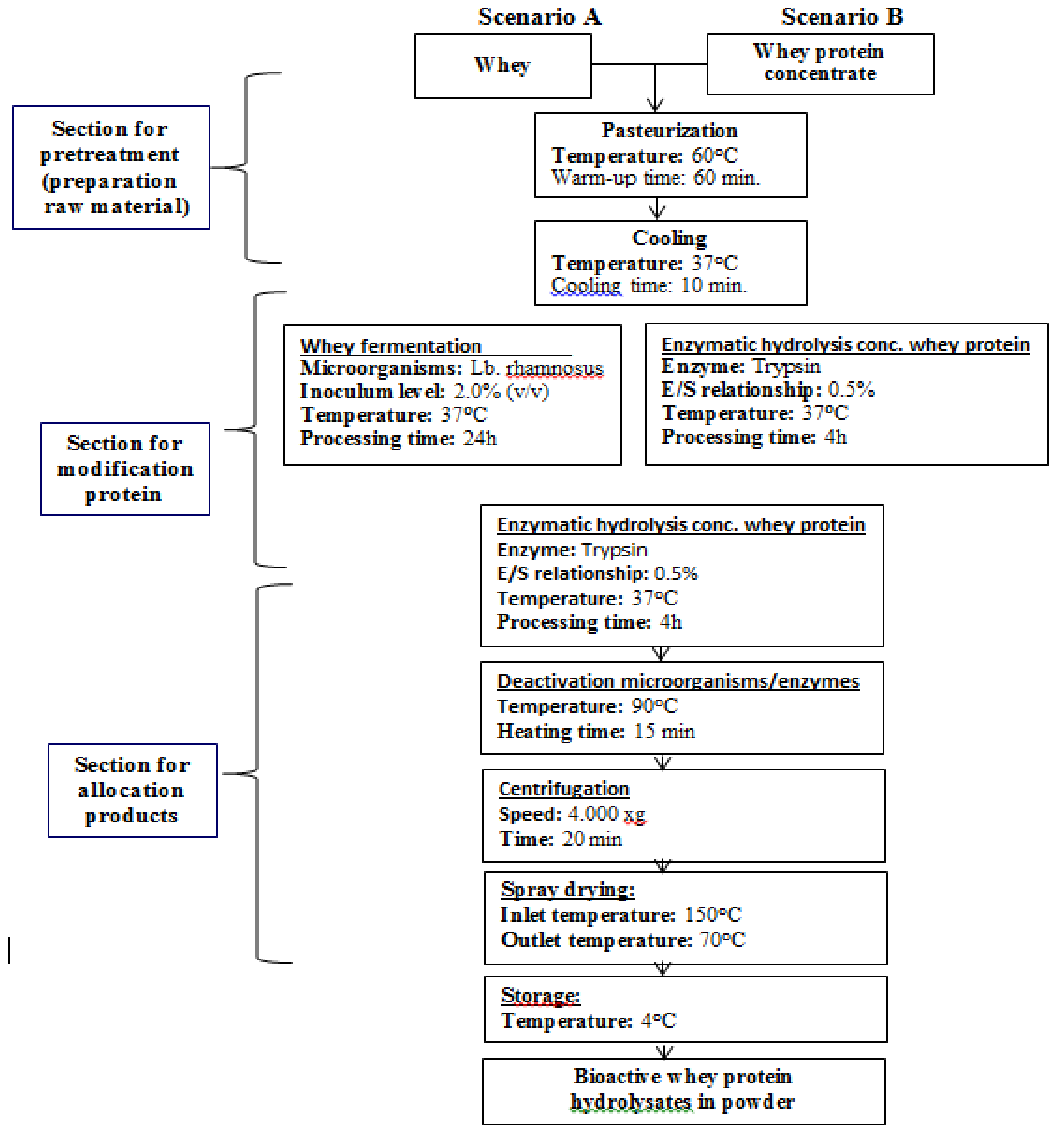

Fig. 1. Block flow diagram of the microbiological/enzymatic process, which is used for production bioactive HPS
Source: Slavica Arsić (2018), Analysis of the techno-economic justification of the exploitation of whey in Serbia. Doctoral dissertation/, University of Belgrade [1].

The entire process for both scenarios, involving pre-treatment, whey protein modification, and product isolation, was modeled using SuperPro Designer, and the flowcharts with the main process devices used in *Scenario* A and Scenario B are shown in Figures 2 and 3 [1]. In general, both processes begin with a step involving pre-treatment of raw materials. The purpose of the pre-treatment procedure is to prepare whey protei n for the next step of modification.To exclude the possibility of external contamination, the raw material was pasteurized at 60°C for 60 min using a HX-101 heat exchanger.

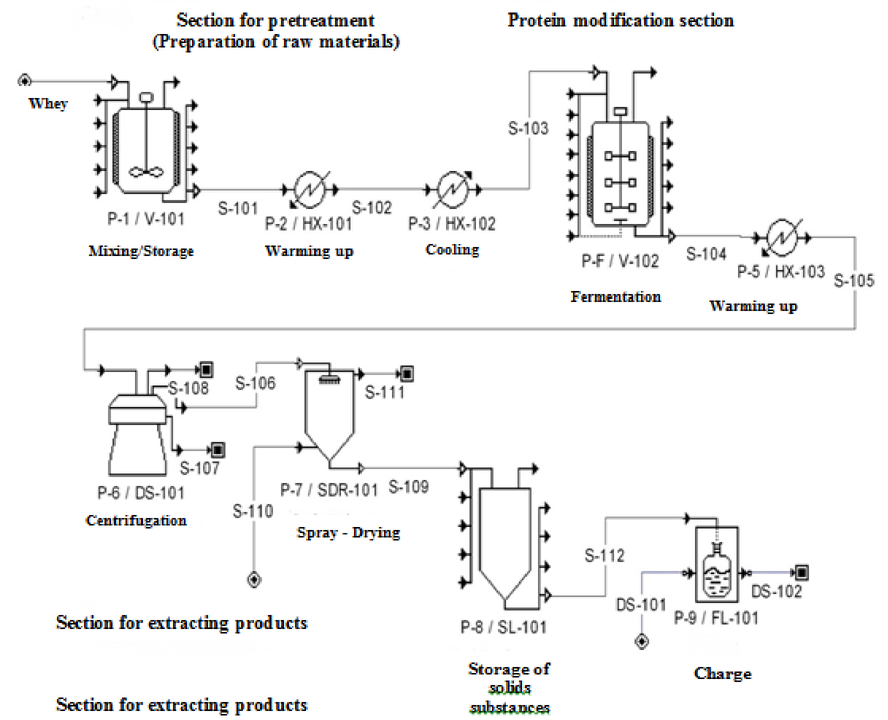

Fig. 2. Scheme of the process based on the use of raw whey (*Scenario* A)
Source: Slavica Arsić (2018), Analysis of the techno-economic justification of the exploitation of whey in Serbia. Doctoral dissertation/ University of Belgrade [1].

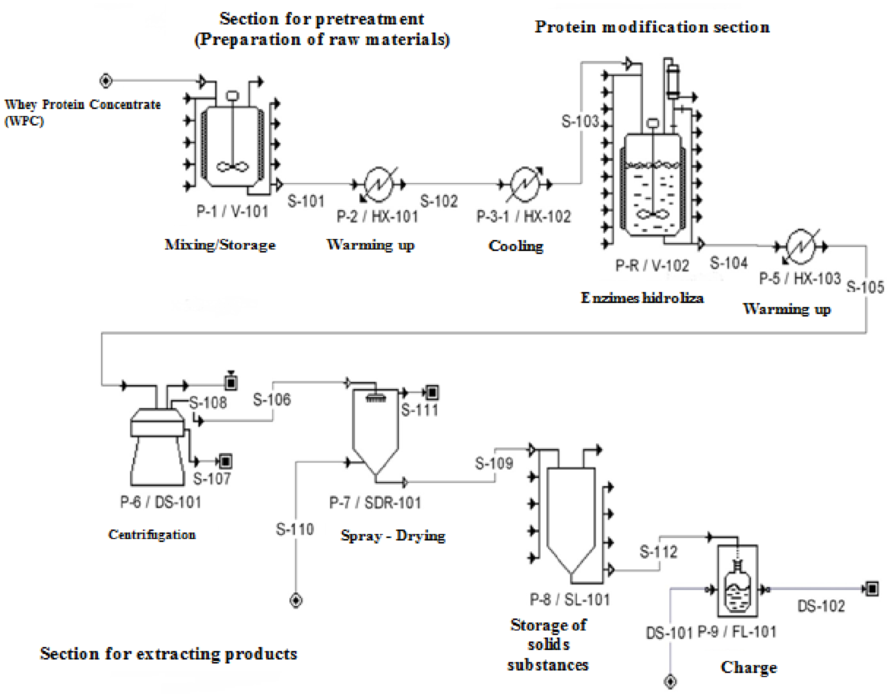

Fig. 3. Process scheme based on the use of whey protein concentrate (*Scenario* B)
Source: Slavica Arsić (2018), Analysis of the techno-economic justification of the exploitation of whey in Serbia. Doctoral dissertation/University of Belgrade [1].

After pasteurization, the mixture was cooled to 37°C using a HX-102 heat exchanger, and then transported to the section, i.e. the unit in which the protein modification will be performed. The protein modification process in *Scenario* A involves a 24-hour fermentation process performed by the addition of 2.0% (v/v) inoculum of strain Lb. *rhamnosus* ATCC 7469 in a unit called P-F/V-102 (Figure 2). The protein modification process in *Scenario*

B involves a 4-hour enzymatic hydrolysis process performed using 0.5% (w/w) of the commercial enzyme Trypsin in a unit called P-R/V-102 (Figure 3). Both processes were performed at a temperature of 37°C as optimal for the activity of microorganisms and enzymes. Both processes are interrupted by inactivation of the microorganism or enzyme at 90°C for 15 min using a HX-103 heat exchanger. After the protein modification process, the mixture is transported to a centrifuge unit (P-6/DS-101) to undergo the following steps involving product separation.

Liquid whey protein hydrolyzate is a highly perishable product due to its high water and protein content as a favorable substrate for bacterial growth. The powder form of whey protein hydrolyzate is a much more favorable form of this product, which is primarily lighter than the liquid form, which greatly facilitates its transport and enables longer storage time. In the product separation section, the bioactive whey protein hydrolysates were centrifuged and dried to produce BHPS in powder form.

Centrifugation performed at 4,000 xg for 20 minutes in a unit called P-6/DS-101 to separate the hydrolyzed suspension into two layers: a protein hydrolyzate solution at the top and a solid layer of non-hydrolyzed protein remaining at the bottom. After centrifugation, the whey protein hydrolyzate suspension was dried in a spray dryer, i. unit P-7/SDR-101 and stored at 4°C (unit P-8/SL-101) until the moment of packing. The end product of bioactive whey protein hydrolysates is a creamy white powder that is characterized by good water solubility and desired functionalities [1].

The basic capacity of 1,000 kg h^{-1} processing of whey or whey protein concentrate results in the production of 8.1 t $year^{-1}$ hydrolyzate obtained by whey fermentation and 165.9 t $year^{-1}$ hydrolyzate obtained by hydrolysis of whey protein concentrate, with a protein content of 96% (w/w). The obtained products can be considered bioactive due to their high antioxidant activity, which confirms the efficiency of the production processes studied in this article.

Enzymatic modification of whey protein according to *Scenario B* reduces the total energy consumption of the production process. The total energy consumption in generation operations in *Scenario A* is 6.46 GW, while in *Scenario B* the total energy consumption is reduced to 5.86 GW. A decrease of 9.1% indicates a higher efficiency of Scenario *B*. The energy is mainly reduced due to the lower electricity demand in the reactor unit (P-R) used in the protein enzyme modification section. On the other hand, the use of enzymes increases the amount of bioactive whey protein hydrolyzate produced from 1.02 kg h^{-1} as obtained by the whey fermentation process to 20.9 kg h^{-1} as obtained by the enzymatic hydrolysis process. An increase of 95.1% increases the economic viability of the bioactive whey protein hydrolyzate production process according to *Scenario B*.

Table 1 shows the results of the economic analysis. For a plant with a base capacity of 1,000 kg h^{-1}, the total capital investment is $ 22,940,000 for *Scenario A* and $ 17,402,000 for *Scenario B*. Assuming that the selling price of the bioactive whey protein hydrolyzate is $ 20 kg^{-1}, the project has an internal return rate (IRR) of 17.73% for Scenario A and 230.55% for *Scenario B*.

Table 1. Summary of economic parameters for the analyzed scenarios A and B.

Parameter	**Scenario A**	**Senario B**
Total investments ($)	22,940,000	17,402,000
Repayment period (years)	3.06	0.09
IRR after tax (%)	17.73	230.55
NPV na 7% ($ · 10^6)	25.38	1,635.5

Source: Results obtained by computer simulation in the program „*SuperPro Desinger*“ [1].

The net present value (NPV) was approximately $ 25.38 million for *Scenario A* and $ 1,635.6 million for *Scenario B* (at a discount rate of 7%).

NPV is an indicator of return on investment, ie. an indicator of whether the investment can bring a profit. In this case, the NPV value is positive, which means that the investment provides an inflow of funds and therefore the project is economically viable and can be accepted and implemented.

Based on these results, the project that assumes Scenario B represents a much more attractive investment compared to *Scenario A*.

CONCLUSIONS

Based on the results presented in this part of the research, enzymatic hydrolysis is the most suitable process for the production of bioactive whey protein hydrolysates. Industrial plant with a capacity of 1,000 kg h^{-1}, which is characterized by the following economic parameters: total capital investment of 17.40 million dollars; direct fixed capital expenditures of $ 15.98 million; annual operating costs of $ 9.14 million and a payback time of 0.09 years, allow the production of a bioactive whey protein hydrolyzate that could have a selling price of $ 20 kg^{-1}, which is significantly lower than the market price, so investing in this way of processing whey can be considered very cost effective.

The presented process offers an environmentally friendly and economically viable solution for the utilization of whey through its transformation into products of the second generation of whey protein-based products.

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