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Streszczenie w języku angielskim (Abstract)

Food wastes are generated at every stage of the "food life cycle" including agricultural production, storage, processing, distribution, consumption and withdrawal from the market. Food waste production has a negative impact on the environment. It entails unnecessary greenhouse gas emissions and affects global water resources.

Brewers' spent grain (BSG) is the most abundant by-product of the beer production process, account for about 85% of all waste generated in breweries. BSG is a source of protein, fiber and fat, as well as vitamins, essential amino acids and phenolic compounds. Globally, around 70% of the BSG produced is used as feed for livestock, while another 10% for biogas production. The huge scale of beer production creates problems with the BSG disposal. Due to the short shelf life and difficulties related to storage and transport, approximately 20% of BSG ends up in landfills. Therefore, the search for new, economically profitable methods of BSG utilization is of decisive importance.

Oilcakes are main by-products of the vegetable oil manufacturing. When pressing, around 250-350 g of oil is generated per one kilo of seeds, while 65-75% of their weight is transformed into a by-product. Oilcakes are characterized by a high content of protein, fatty acids, carbohydrates, minerals and vitamins. Traditional methods of oilcakes utilization include livestock feeding and the application as soil compost. In the perspective of biotechnological applications, cakes are used as substrates for the production of antibiotics, biopesticides, vitamins and microbial enzymes. Moreover, cakes are increasingly applied as a substrate for the biosurfactant production.

The aim of the current work was to develop a biotechnological method of the agro-food by-products valorization. Therefore, a microbiological conversion of BSG as well as sunflower and rapeseed cake into biologically active compounds was performed.

In the first work, the production of BSG protein hydrolysates was described. Initially, *in silico* analysis was carried out to determine whether BSG could be a source of biologically active peptides. Subsequently, BSG proteolysis was performed in bacterial cultures with confirmed proteolytic properties. The degree of protein hydrolysis (DH%) was calculated, and the enzymes spectrum, participating in proteolysis was examined. Finally, the antioxidant activity of hydrolysates was investigated.

With the aid of bioinformatics tools, a wide spectrum of bioactive peptides, including natural antioxidants, generated as a result of BSG protein hydrolysis was detected. Under laboratory conditions, *B. cereus* PCM 2849, *B. subtilis* PCM 2850, *B. polymyxa* ATCC 842, *B. lentus* PCM 450, *B. licheniformis* PCM 1847 and *K. rhizophila* PCM 2931 were found to perform efficient BSG proteolysis. The DH% in the most effective bacterial cultures was in the range of 30-45%. Size exclusion chromatography was found to be a convenient tool to monitor the progress of proteolysis. A wide spectrum of proteolytic enzymes involved in the protein degradation has been detected. Finally, significant antioxidant activity of the BSG hydrolysates was determined. The best results were obtained using the ABTS method (2,2'-azino-bis (3-ethylbenzothiazolin-6-sulfonic acid) in *B. cereus* PCM 2849 (1621.31 µM TEAC/g peptides) culture medium.

In the next stage of the research, BSG was used for the production of protein hydrolysates in cultures of the *Yarrowia* clade. Due to the low state of knowledge in the field of *Yarrowia* yeast proteolytic enzymes, a detailed characterization of alkaline proteases was performed. A phylogenetic tree was prepared and the study of enzymes synteny was implemented. Expression of genes encoding putative alkaline proteases was studied *in silico*, in various culture conditions and experimentally, in a medium supplemented with BSG. Moreover, the proteolytic activity of *Yarrowia* clade was investigated. Enzymes involved in BSG proteolysis were selected and proteomic analysis was performed. The process of BSG protein hydrolysis was characterized in detail and the antioxidant activity of the obtained hydrolysates was determined.

Based on the bioinformatic analysis, thirteen groups of putative alkaline proteases of the *Yarrowia* clade were distinguished, including the *XPR2* group, which is a set

of homologes to the extracellular alkaline protease (Aep). Seven of the putative alkaline proteases were expressed in BSG medium and the highest expression was detected for *XPR2* and *YALI0B16500* gene. The lack of proteolytic activity of *Y. phangngaensis*, *Y. deformans* and *C. hispaniensis* and the extraordinary properties of *Y. lipolytica* W29, *Y. alimentaria* and *Y. keelungensis* was determined. Moreover, a broad spectrum of enzymes, involved in the BSG protein hydrolysis was determined and the protease of a key importance were identified. Based on the proteomic analysis, a high homology of *Yarrowia* enzymes to Aep *Y. lipolytica* E150 (CLIB122) was demonstrated, confirming the common ancestry of *Yarrowia* clade. The antioxidant activity of hydrolysates was significantly higher, than that of proteolytic bacteria. It was related to exceptionally high DH% of BSG proteins. In yeast cultures, the DH% was almost twice as high as in the culture of proteolytic bacteria and reached 60-73%. The best antioxidant properties were determined in *Y. divulgata* (2606.13 µM TEAC/g), *Y. galli* (1771.87 µM TEAC/g), *Y. keelungensis* (1730.73 µM TEAC/g) and *Y. lipolytica* (1722.63 µM TEAC/g) post-culture media and the ABTS method was found to be the most appropriate for this type of studies.

Finally, research on the production of surfactin was performed. Initially, chemical composition of sunflower and rapeseed cake, used as a substrate for biosurfactant production was analysed. Surfactin production in both sunflower and rapeseed cake medium was monitored using surface tension (ST), emulsification index (E24) and pH. The kinetics of surfactin production was determined. The profile of proteolytic and lipolytic enzymes, involved in cakes utilization was analyzed. The properties of surfactin, as the oil-recovery agent was examined. The profile of surfactin homologues as well as antioxidant and inhibitory activity of surfactin against angiotensin converting enzyme (ACE) were tested. Finally, *in silico* molecular docking of surfactin to the C and N domains of ACE was performed.

Sunflower and rapeseed cake, due to the high contend of protein and fatty acids are a prospective substrates for surfactin production. In the sunflower and rapeseed cake medium, a significant decrease of ST, respectively to the value of 30.1 mN/m and 29.7 mN/m was observed. At the same time, significant increase of E_{24} to the value of approximately 63% was noted. In addition, a logarithmic increase in surfactin concentration was detected. In the sunflower and rapeseed cake medium, the maximum surfactin concentration reach 1.19 g/L and 1.45 g/L respectively. Preliminary studies confirmed the proteolytic and lipolytic

properties of *B. subtilis* #309. Therefore, a wide spectrum of enzymes, involved in cakes utilization was established. Surfactin was found to be an effective oil recovery agent, while applied technology was described as economically profitable. Determination of the profile of surfactin homologues showed, the highest content of surfactin of the fifteen hydrocarbon chain length (C15) in both tested media. The relationship between homologues proportion and the fatty acid composition of the substrate as well as bioavailability of specific amino acids was reported. Finally, the lack of antioxidant activity of surfactin was determined. On the contrary, significant inhibitory activity against ACE was determined. The half-maximal inhibitory concentration (IC_{50}) was determined to reach 0.62 mg/mL. Molecular docking of surfactin to the C and N domains of ACE confirmed the possible interaction of these compounds.