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Waste-free integrated processing of invertebrates into feed additives using proteolytic enzymes

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Abstract. This paper presents a study on integrated and waste-free processing of various invertebrate species such as molluscs and crabs. The main focus is on the use of the proteolytic enzyme preparation ‘Krusenzyme’, isolated from crab liver, for the processing of raw materials. The study showed that the use of Crusenzyme was effective in removing skin from cephalopods, softening the muscle tissue of molluscs and deproteinising the shell of crabs. The enzymatic treatment improved the texture of the products, increased the yield of finished products and reduced energy consumption compared to traditional methods. The possibility of obtaining feed additives from mollusk processing wastes rich in proteins, calcium and microelements was also studied. It was shown that inclusion of flour from soft tissues and mollusk flaps in the diet of farm animals contributes to the increase in weight gain and improvement of productivity indicators. The developed technology allows to obtain a wide range of valuable products, including chitin, chitosan, fat, carotenoids and protein hydrolysates, contributing to environmentally safe production and rational use of hydrobiont resources.

1 Introduction

Traditional seafood processing methods often result in significant losses of valuable nutrients and high energy costs, which emphasises the importance of research aimed at sustainable resource utilisation [1]. At the same time, not all components extracted from aquatic organisms are utilised. To make matters worse, a large proportion of seafood, especially shellfish, is a difficult raw material to process, characterised by rigidity and difficulties in processing. All these factors make aquatic bioresource products not always accessible to consumers.

Along with traditional approaches, a promising direction in solving the problem of effective processing of hydrobionts is the development of biotechnological methods. The use of enzyme preparations for the processing of raw materials can improve its structure and consumer qualities, reduce energy consumption and increase the yield of finished products [2]. However, the introduction of biotechnological methods into practice still faces a number of difficulties. These include the lack of available and effective enzyme preparations, and the lack of technologies that allow controlling the process of protein hydrolysis and obtaining a product with specified properties. In addition, many methods do not involve inactivation of enzymes, which can lead to deterioration of the quality of the finished product and shortening of its shelf life. The problem is also the need to develop technologies for integrated processing of waste generated during the processing of hydrobionts.

In the context of the above-mentioned, the direction under consideration is of particular relevance and importance. Studies on the use of proteolytic enzymes isolated from crab liver for complex processing of invertebrates, in particular, molluscs, open wide opportunities for solving a number of problems. Firstly, the use of the enzyme preparation Krusenzyme makes it possible to effectively remove the skin cover of cephalopod molluscs, soften muscle tissue and obtain high-quality food products with improved consumer properties. Secondly, the use of enzymatic hydrolysis makes it possible to utilise previously unused wastes such as soft tissues and shells of clams and crabs for processing, yielding valuable feed and technical products. Thirdly, the application of biochemical methods contributes to the environmental safety of production by reducing waste. An important point is the development of technology for inactivation of enzymes in the final product, which provides a long shelf life and stable quality. The use of invertebrate shells as a source of easily digestible calcium and other trace elements is

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also an important aspect in achieving waste-free production. Finally, the biomedical and clinical trials conducted on corbicula extracts proved their efficacy in the prevention and treatment of hepatitis, expanding the range of products obtained. Thus, the results of the research are aimed at solving a whole set of problems related to the processing of hydrobionts and correspond to modern trends in the development of the food industry.

The purpose of the presented work is to summarise the results of research in the field of waste-free complex processing of invertebrates, conducted in the laboratory of biotechnology of food and technical products. Within the framework of the work the efficiency of biochemical methods use in technologies of food, feed and technical products manufacturing from squid, octopus, spisula, anadara and crab was analysed. The possibilities of using the food additive Krusenzyme for skin removal, softening of muscle tissue, waste processing were studied. As a result, a number of solutions were proposed aimed at rational, efficient and environmentally safe utilisation of hydrobiont resources, which meets the needs of the modern agricultural industry.

2 Method and materials

Experiments on enzymatic treatment of various species of molluscs and crustaceans using the proteolytic enzyme preparation 'Krusenzyme' isolated from the liver of crab were carried out in the course of research. This complex includes various proteinases with broad substrate specificity, which allows it to effectively hydrolyse proteins of skin, muscle tissue, and connective tissue proteins. Crusenzyme also contains thermostable enzymes. In addition to proteinases, there are other enzymes in the preparation that contribute to protein hydrolysis, but the main focus is on proteolytic enzymes. The main objective of the research was to study the effect of enzymatic treatment on the structure and properties of muscle tissue, skin and shell of invertebrates in order to develop effective technologies for the production of food, feed and technical products.

Various technological equipment was used to carry out experimental work. Industrial meat grinders were used for grinding raw materials. Fermentation processes were carried out in laboratory thermostats with maintenance of specified temperatures and stirring modes. Laboratory thermometers and digital thermoregulators were used for temperature control. Water baths and laboratory autoclaves were used for hydrothermal treatment. Industrial and laboratory drying cabinets with preset temperature and air circulation regimes were used for drying and wilting of products. Instrumental method was used to determine textural and organoleptic characteristics. Standard analytical instruments and methods were used to analyse the chemical composition of the samples.

The modes of fermentative processing were selected empirically and depended on the type of processed raw material, Crusenzyme concentration, temperature and duration of the process. The temperature regime of fermentation varied in the range of 30-50°C and the duration from several hours to several days. The concentration of Crusenzyme enzyme preparation was varied depending on the task at hand and the type of raw material used. For the treatment of shellfish meat, a short-term fermentation mode was used to soften the tissue, while for skin removal, the enzyme exposure time was increased. For the treatment of crab shell waste, a prolonged fermentation regime was applied to achieve maximum deproteinisation of chitin. To analyse changes in chemical composition, samples were collected and analysed using standard techniques.

3 Results and discussion

In this research work, an attempt was made to comprehensively and non-wastefully process various invertebrate species such as cephalopods (squid and octopus), bivalves (spisula, anadara, corbicula) and crabs to produce a wide range of products. The main objective of this work was to create technologies for the production of food products, feed additives for farm animals, as well as some technical products. The experimental part of the work included application of the enzymatic preparation 'Cruzensim', obtained from the liver of the Kamchatka crab, for the processing of various types of raw materials. This preparation was used to deproteinise, soften tissues and remove unwanted parts such as skin of cephalopod molluscs and rigid structures of bivalves.

At the beginning of the research, the feasibility of using the enzyme preparation Crusenzyme for skin removal in cephalopod molluscs such as squid and octopus was investigated. It was experimentally confirmed that at the concentration of 'Krusenzyme' 0.5% during 30 minutes there is a dissolution of up to 75% of squid skin proteins, while the impact on muscle tissue remains minimal, which guarantees the preservation of native properties of meat. When the processing time was increased to 60 minutes, dissolution of skin proteins reached 90%, resulting in more efficient skin removal, however, there was a negligible (up to 5%) impact on muscle tissue proteins. The optimum processing time was found to be 45 minutes, which achieved 85% dissolution of skin proteins and only 2% impact on muscle tissue, while maintaining high organoleptic characteristics of the product. By adjusting the enzyme concentration and processing time, it was possible to develop production regulations for the products 'Squid skinned frozen' and 'Octopus skinned frozen'. The use of biochemical method with 'Krusenzim' allowed to reduce energy costs by 20% and labour costs by 15% in comparison with the traditional hydrothermal method, as well as to increase the yield of finished products by 8-10% due to the reduction of mass losses during processing. Further studies have shown the effectiveness of this method also in the production of salt-dried cephalopod products. For example, when using 'Krusenzyme' at a concentration of 0.3% and treatment time of 20 minutes before drying, there was observed an improvement in the structure of the finished product by 10-15%, which was manifested in a decrease in brittleness and increase in elasticity, as well as an increase in shelf life by 15-20% due to more uniform drying and reducing the risk of microbial contamination. Thus, enzymatic processing has been shown to be an effective method to improve product properties [3].

Further, the possibility of processing bivalve molluscs such as anadara, spisula, corbicula, mactra, callista and merceria was investigated. Their meat is characterised by considerable toughness due to the high content of connective tissue proteins, particularly collagen. To achieve quality consistency and improve the nutritional value of the products, a complex application of hydrothermal and enzymatic treatment is necessary. During the experiments it was found that when using the enzyme preparation 'Krusenzyme' processing for 60 minutes at 45°C provides degradation of up to 85% of collagen structures, while without enzymatic treatment the collagen dissolution for the same period is only about 15%. The use of fermentation significantly improved the consistency of the muscle tissue, increasing the degree of protein dissolution from 20% to 60-65% at an average 90-minute treatment, whereas hydrothermal treatment provided only 35-40% dissolution during the same time. As a result, the yield of salted products increased from 30% to 70-76% after application of complex treatment. In [4] it was shown that enzymes, changing the structure and composition of tissues, also affect the digestibility of nutrients.

Earlier works of the authors show that flour from soft tissues of bivalves is a valuable source of protein containing all essential amino acids, with lysine and methionine accounting for about 30% of the total amount of essential amino acids. This amino acid profile, as well as the presence of a wide range of macro- and microelements, make this meal a promising additive in feeds for farm animals [5]. Experiments with piglets have shown that the inclusion of mollusc soft tissue meal in the diet of suckling and early weaned animals increases their average daily gain by 11.7%, while reducing feed costs by 10.5%. Also, mollusk shell meal, which consists of 98% ash and contains 36-42% calcium, proved to be an effective feed additive. Its use in the diet of pigs resulted in a 16.8% increase in daily weight gain, a 14% reduction in feed costs and a 32% increase in production profitability. When studying the effect of treatment time on protein solubility, it was found that treatment of soft tissues for 30 minutes leads to the dissolution of 55% of protein, and increasing the time to 60 minutes increases this indicator to 80%, after which the increase in protein solubility slows down. The process of protein dissolution when treated for 120 minutes achieves 90% solubility. Flour from flaps also showed a positive effect on laying hens, increasing their egg production by 7%, shell thickness by 4% and stock retention by 2% compared to the control group. As a result of the conducted researches normative documents for production of protein-mineral and mineral feed additives from wastes of processing of bivalve mollusks were developed and approved, that allows to introduce these additives in practice of agriculture. The use of mollusk meal in animal feeding contributes to the improvement of productivity indicators, and also reduces the need for traditional, less sustainable and expensive feed additives [6].

Another part of this work was to scrutinise the disposal of crab processing wastes. Liquid waste (hepatopancreas) and solid shell waste (SSW) constitute a significant portion, namely 40 to 65% of the raw crab weight. Traditional methods of processing these wastes are energy intensive, requiring large amounts of resources, and environmentally hazardous due to the use of harsh chemicals. In this study, a method was used where hepatopancreas is used for two purposes: to obtain fat, which yielded an average of 15-20% of the initial mass of hepatopancreas, and the proteolytic enzyme preparation 'Cruzenzyme'. This enzyme preparation, in turn, was applied for deproteinisation of PCOS, which is a key step in the process. The experiments carried out showed that replacing the traditional alkaline treatment with an enzymatic treatment using Crusenzyme produced impressive results. In particular, the degree of deproteinisation of chitin reached 88-92% after 12 hours of treatment at 50°C, whereas the alkaline method would have required more than 24 hours to achieve a similar result. In addition, the enzymatic treatment promotes efficient degradation of the protein-pigment complex, which resulted in a significant increase in the yield of carotenoid pigments, namely by 30-35% compared to conventional methods, with carotenoid concentrations in the extract reaching 1.2-1.5 mg/ml. It was found that the use of 'Krusenzyme' also makes it possible to control the process of protein hydrolysis, thus the process of obtaining hydrolysates with a given composition and properties was developed, and the degree of protein hydrolysis could reach 70-80% within 8 hours at a temperature of 45°C. As a result of the conducted research, a waste-free technology of complex processing of crab waste was developed, which allowed to efficiently and environmentally safe obtain a wide range of valuable products, including chitin, chitosan (the yield of which from deproteinised chitin was 75-80%), complex of proteolytic enzymes 'Krusenzyme', fat, carotenoid extract, as well as protein-containing compositions, the yield of which reached 60% of the initial protein material after hydrolysis. Such methods aimed at recycling and efficient utilisation of wastes are an important aspect of sustainable development [7]. Methods based on fermentation not only reduce waste but also contribute to the recovery of valuable components [8, 9].

In general, the results of this work demonstrate the prospects of waste-free and integrated processing of various invertebrates to obtain a wide range of products. The application of biochemical methods, and in particular the enzyme preparation 'Krusenzyme', allowed to increase the yield of products, improve their quality and reduce the negative impact on the environment.

4 Conclusion

The presented work deals with the integrated waste-free processing of various invertebrate species, such as cephalopods, bivalves and crabs, using the enzyme preparation 'Krusenzyme' derived from crab liver. The research is aimed at creating technologies for the production of food, feed additives and technical products. The effectiveness of 'Krusenzyme' for skin removal in cephalopod molluscs, softening of muscle tissue and degradation of collagen structures of bivalves was experimentally proved, which allowed to improve consistency and yield of finished products. The application of fermentation significantly reduced energy consumption and labour intensity of the processes in comparison with traditional methods. The technology of obtaining feed additives from soft tissues and shells of molluscs enriched with protein and minerals was developed, which showed a positive effect on the growth and productivity of farm animals [10]. Special attention was paid to the processing of crab waste, where Crusenzyme was effectively used for deproteinisation of chitin, extraction of carotenoids, production of fat and hydrolysates, which made it possible to create a waste-free technology with high economic and environmental efficiency [11]. The study demonstrates the potential of biochemical methods in the processing of hydrobionts, leading to improved product quality, increased yield and reduced negative impact on the environment. The developed technologies make it possible to obtain a wide range of valuable products from previously unused waste, which contributes to the rational use of resources and enhances the sustainability of production processes [12-14].

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