Prospects for Producing a Protein Additive with Antifreeze Properties from Germinated Faba vulgaris Moench Seeds Through Low-Temperature Bioactivation

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Abstract. In the present study, using endotechnology, certain aspects of germination of dehulled broad bean seeds under low-temperature conditions were investigated, and the theoretical and practical prospects for obtaining a protein supplement with antifreeze properties from them were substantiated. Local beans, widely used in the diet of the population, are considered for the isolation of a protein supplement in the form of an extract with antifreeze properties from their sprouts, obtained by bioactivation of dehulled seeds grown in the southern regions of the country, known as "pakhla". An extraction regime for obtaining a protein supplement was proposed, and its chemical composition and some other quality indicators were determined. The liquid supplement is suggested for potential use in new food technologies.

Keywords: Faba Bean (Faba vulgaris Moench), Endotecnology (Bioactivation), Protein Extract.

1 Introduction

Before discussing scientific data on the possibilities and applications of antifreeze protein systems, it is interesting to provide an explanation. Their advantage is primarily explained by their ability to induce vitrification, i.e., preventing ice formation during freezing and maintaining structural organization in the system. This property has manifested in products during both freezing and thawing processes [1, 2]. Considering these properties of certain proteins, literature extensively covers methods for maintaining cellular viability under low temperatures in moist environments.

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Antifreeze proteins-as cryoprotectors reduce the retention of intermolecular water, thereby increasing the overall concentration of solutes. This allows cells (e.g., in plant seeds) to resist damage caused by the formation of ice crystals under cold conditions, facilitating the formation of hydrogen bonds [3]. It is noted that bound water, which cannot be osmotically separated from cells, interacts with the hydrophilic surface of macromolecules (proteins, etc.). As a result, these proteins participate in ice crystal formation processes, preventing damage in living tissues and thus playing a protective role.

Typically, dehydration processes in the cytoplasm lead to denaturation and structural modification of proteins. Antifreeze proteins can slow these processes for a certain period. Ultimately, they prolong the lifespan of seeds (or products) during storage.

The cytoprotective principles of antifreeze compounds were studied by many scientists, particularly Mazur and colleagues, as early as 1963 [4]. According to these studies, optimizing the slowing of freezing processes enables controlled osmotic water exit from cells and delays crystallization in certain proteins.

The most important feature of these proteins is their ability to increase solute concentration inside cells under cold conditions and reduce osmotic water, thereby enhancing cold resistance. Of course, these compounds (antifreeze proteins) can have potentially harmful effects on biomaterials, for which scientists have developed protective methods. Carrier solutions, salts, buffers, and inhibitors are used for this purpose.

Since 1959, solutions have been widely used in research to provide cryopreservation of cells in marine organisms during freezing [5]. This method has been applied in medicine for tissue plantation, as documented by A. Karamullayev and A. Tikhomirov (2017), and has proven effective in the cryopreservation of carp sperm, maintaining high viability compared to standards [6].

Due to their natural efficacy, solutions such as ethylene glycol and sugar mixtures have also played a positive role as antifreeze agents in freezing human and animal embryos [7]. In general, the study of antifreeze proteins (AFPs) was first observed in Arctic fish in 1957. The principle of AFP action is associated with their thermal hysteresis (TH) properties, which inhibit ice growth during freezing.

One of the most widespread AFPs is the water-soluble protein “sericin,” obtained from silk produced by the cocoon of the silkworm (mulberry) [8]. Currently, AFPs are used more in modern medicine. Nevertheless, their application in food technology began to gain popularity after the 20th century.

Numerous studies have been conducted in this direction in different countries. Russian scientist B. G. Sushik conducted experiments in his research on protein extraction aimed at regulating ice structure for use in ice cream [9]. For this purpose, he first ground the material to 0.5 mm particle size and then performed a two-step extraction of the resulting mass with a 5% NaCl solution at 10 °C for 0.5 hours using a 1:16 solid-to-liquid ratio.

In another experiment, he processed winter rye leaves ground to 1 mm particle size under the same conditions but used distilled water instead of NaCl solution for extraction, also for 0.5 hours at a 1:16 solid-to-liquid ratio and 10 °C. The experiments showed that in both samples (faba bean and rye leaves), the isolated proteins exhibited cryoprotective properties.

Subsequently, the dosage of these extracted proteins was optimized, and their application in ice cream technology was investigated at 0.07% (from faba beans) and 0.09% (from leaves). Technical specifications for the obtained ice cream (TY9228-059-02068640-09) were developed and approved [10] (Sushik V. G., Moscow, 2009, Abi. R. disser). Based on these studies, two patent applications related to the protein extraction methods were also prepared.

In recent years, interest in obtaining so-called ice-structuring proteins for low-temperature technologies has significantly increased. These proteins are also referred to as antifreeze proteins (AFPs) or antifreeze glycoproteins (AFGPs). The ability of these proteins to exist freely in solution is a fundamental physical property of AFPs [11, 12].

Numerous studies have shown that adsorption of AFPs onto ice surfaces inhibits the difference between freezing and melting points i.e., thermal hysteresis (TH) and prevents ice recrystallization (IRI). While the antifreeze activity of AFPs lowers the freezing point of the solution without significantly affecting the melting point, IRI activity reduces tissue damage by preventing the continuous growth of large ice crystals from smaller crystals during freezing.

The first reports of antifreeze activity in plants were made by M. Urrutia [13]. AFPs prevent freezing caused by external ice, inactivate ice nucleators, and stabilize the supercooled state, motivating many researchers to explore ways to apply them in isolated form. The properties of AFPs allow their use in food preservation, agricultural and technological production, and in medical cryopreservation.

Based on literature sources, this study aimed to investigate the potential of obtaining food compositions containing antifreeze proteins through germination of faba bean (Faba vulgaris Moench) seeds under low-temperature conditions. Germination conditions and temperature (3 ± 5 °C) were adopted from the work of L.V. Golubeva et al. for soft wheat and were verified in our laboratory [14]. A specific modification developed by us for faba bean (produced under the name “pakhla”) germination was also applied [15].

Preliminary experiments in our other published works demonstrated that the bioactivation method we proposed, based on short-term hydrothermal treatment for dehulling the seeds and subsequent germination of dehulled faba bean seeds in a low-temperature environment, may be suitable for obtaining protein extracts with antifreeze properties. It was determined that after three weeks of germination in a cold environment, the dehulled faba bean seeds become a suitable semi-finished product for extracting a liquid supplement containing antifreeze proteins.

In our research, in theoretical terms, we proceeded from the fact that antifreeze proteins, as a rule, in plants are synthesized during low-temperature stress and allow the plant to prevent the formation of large ice crystals during the freezing of extracellular water, i.e., under the influence of low temperature, the expression of genes changes, as a result of which the synthesis of structural proteins is inhibited. At the same time, the synthesis of stress proteins is enhanced [16]. The development of this idea is shown in the work of Fei et al., 2008, conducted with soft wheat samples [17].

2 Materials and Methods

As objects of the study, natural, cleaned, and dehulled germinated seeds of horse beans (paxla) grown under the conditions of the Lankaran zone of Azerbaijan were used (see Fig. 1).

a b c

Fig. 1. a) Broad beans in their initial form; b) Dehulled seeds; c) Germinated dehulled broad bean seeds in a cold chamber at +3–5 °C.

Preparation for dehulling and germination of dehulled seeds was carried out according to our modified method, as described in [15]. The conditions and temperature regime for low-temperature germination of dehulled beans were adopted from the works of Golubeva et al. [14].

The active acidity and overall chemical composition, including proteins, fats, and carbohydrates, were determined using standard methods for biochemical plant analysis [18]. The amino acid composition of the extract from germinated samples was determined by chromatographic method using a Hitachi instrument [18]. Microbiological parameters of the liquid supplement were determined using conventional methods [19].

Next, dehulled germinated horse bean (paxla) seeds were mashed into a puree after being kept at +3–5 °C for three weeks in a well-ventilated refrigeration chamber, and were used for extraction of water-soluble substances, including antifreeze proteins.

The technological scheme for the extraction of water-soluble compounds, including proteins, from the puree of germinated broad bean seeds is shown in Fig. 2.

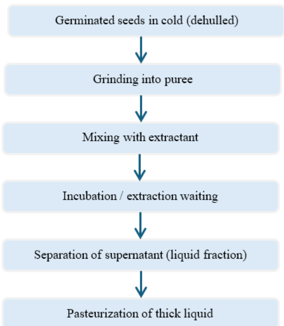

Fig. 2. Process of extracting water-soluble compounds from dehulled broad bean seeds germinated at low temperatures.

Extraction of the liquid fraction of the crushed germinated mass was carried out at a temperature of 40 °C for 2 hours using distilled water in a ratio of 3:2. Separation of the liquid fraction after extraction was performed by centrifugation. The supernatant (liquid) consisted of dissolved substances, including proteins. The separated liquid was subjected to short-term pasteurization in a water bath at a temperature of 65–70 °C. The resulting thick liquid was subjected to analysis.

The extract was subjected to short-term pasteurization and characterized organoleptically and for overall chemical composition, including proteins. Simultaneously, the resulting thick liquid was analyzed for its amino acid composition. The results of their determination are presented in Table 1.

3 Results and Discussion

As studies on the determination of the amino acid composition of the extract have shown, both in our previous studies and in the present case, the germination of beans in a low-temperature environment causes an increase in their quantity and a redistribution towards essential amino acids [20].

The amino acid composition of the extract from dehulled seeds of horse beans (pakhla) grown in a low-temperature environment is presented in Table 1 for comparison with whole seeds.

Table 1. Amino acid composition of the proteins of the extract from dehulled seeds of low-temperature-germinated “pakhla” compared with the amino acid composition of the proteins of whole seeds.

Amino Acids	Whole seeds [20] g/100 g protein	Extract from Sprouted Seeds at Low Temperature, g/100g protein
Valine	4.5	5.3
Isoleucine	4.0	4.8
Leucine	7.7	9.2
Lysine	7.0	9.1
Methionine	0.6	0.57
Threonine	3.5	3.3
Tryptophan	-	-
Phenylalanine	4.3	5.1
Alanine	4.4	5.3
Arginine	10.6	13.5
Aspartic Acid	13.0	14.8
Histidine	2.8	3.5
Glycine	4.5	3.9
Glutamic Acid	18.3	23.5
Proline	4.3	6.0
Serine	5.0	4.6
Tyrosine	3.4	3.2
Cystine	3.4	3.0

From dehulled germinated faba beans ground in cold conditions, water-soluble dry matter, including proteins presumably with antifreeze activity, was extracted. The ratio of extractant to raw material was 3:2. The extraction temperature was considered optimal at 40 °C, with a duration of 2 hours, during which the maximum amount of dry matter was obtained.

It was established that the liquid obtained after extraction and short-term pasteurization at a reduced temperature (65–70 °C) could be used in food systems as a structuring additive. This opaque, viscous liquid contains approximately 5.0% protein, up to 0.3% fat, and 28–30% carbohydrates and other beneficial components. Its taste and aroma are slightly characteristic of faba beans, and the active acidity varies between 6.34 and 6.65 over 24 hours depending on storage conditions. Moreover, it was found that the microbiological indicators of the opaque liquid remained at $1{,}35 \times 10^4$ – $3{,}0 \times 10^4$ CFU/mL during 3 days of storage.

4 Conclusion

The study demonstrates that a food additive derived from dehulled faba beans germinated under cold conditions can serve as a promising functional ingredient for food technologies, including frozen products, due to its favorable physicochemical and organoleptic properties. The bioactivation process enhances protein content and improves the amino acid profile, enabling the production of protein-rich extracts with desirable functional properties such as foaming, emulsifying, and freeze - thaw stability. These extracts show potential for application in a wide range of food products, including ice cream, meat products, and sauces. Overall, the findings highlight the potential of cold-induced bioactivation as an effective approach for developing innovative, protein-enriched ingredients, with further studies ongoing to assess safety and expand applications.

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