

Review

Potato Industry By-Products as a Source of Protein with Beneficial Nutritional, Functional, Health-Promoting and Antimicrobial Properties

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Abstract: Most potato proteins are fractions of albumin and globulin, soluble in water and in water and salt solutions, respectively; these are patatin glycoproteins, with a pIs in the range of 4.8–5.2. This group of proteins is typical of potato and they are referred to as patatin or tuberin. Around 30–50% of soluble potato proteins comprise numerous fractions of protease inhibitors with a molecular weight in the range of 7–21 kDa; they are often heat-resistant, showing a wide spectrum of health-promoting effects. The nutritional value of proteins is related to the content of amino acids, their mutual proportions and digestibility. Natural proteins of the patatin fraction are characterized by favorable functional properties, including foam formation and stabilization, fat emulsification or gelling. Native potato proteins may also exhibit beneficial non-food properties, such as antimicrobial or antitumor, as well as antioxidant and antiradical. Depending on the method of isolation and the applied factors, such as pH, ionic strength and temperature, the directions of using potato protein preparations will be different.



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Keywords: potato protein; fractions of patatin and protease inhibitors; nutritional value; functional properties; biological properties; isolation method; direction of usage

The importance of isolating potato protein from potato industry by-products, mainly starch, was recognized by various authors over 65 years ago. This was a result of issues connected with the management of huge amounts of waste products, potentially posing a threat to the human environment due to the high concentration of nutrients that feed microorganisms and enable unfavorable changes in water and soil. Initially, attempts were made to separate proteins from juice water in the starch industry and then, after the introduction of pulp centrifuges, from potato juice. Research on the nutritional value and structure of potato proteins, including the identification of fractions differing in molecular weight, solubility or isoelectric point, conducted during this period proved the high nutritional value of most potato protein fractions, especially the patatin fraction, and their significant differentiation in terms of functional characteristics. Moreover, depending on the factors used, such as temperature, pH, type of acid used, ionic strength of the protein solution, and the properties of the preparations obtained, the efficiency of their production and the directions of potential use were differentiated. The introduced techniques for the separation of protein fractions, as well as the attempts to modify the obtained preparations, e.g., through their enzymatic hydrolysis, led to the increased possibility of obtaining preparations with specific compositions and properties. The specific, rich chemical composition of potato juice, including the presence of non-starchy carbohydrates, polyphenols and natural toxic substances, such as glycoalkaloids, significantly hindered the production of potato protein preparations; the different features of foodstuffs, with high purity, good digestibility and optimal functional characteristics, thus necessitated the use of several different isolation techniques in parallel, which significantly increased the price of such preparations.

A new, interesting direction in this field is the development of research on methods of obtaining and modifying low-molecular-weight proteins due to their previously unknown or insufficiently documented antimicrobial properties, or the use of certain potato protein preparations as technological factors, such as in filtration processes in winemaking. Moreover, the wider use of potato pulp for obtaining potato protein preparations, especially with the use of multi-enzyme systems, seems interesting. Therefore, the purpose of this article was to trace the results of research relating to the nutritional, functional, pro-health, antimicrobial and technological properties of potato proteins, as well as the directions of their use resulting from the structure, molecular weight and fractionality of proteins, the raw material used and the methods of isolation and modification.

The processing of potatoes in the starch industry requires the management of large quantities of protein by-products. These include the potato juice separated from the potato pulp and the pulp left over after washing the starch from the potato cells. Annually, the European Union processes around 8 million tons of potatoes in this way, which generates around 6 million m³ of potato fruit juice (PFJ) and potato pulp in large quantities [1,2]. Potato fruit juice as a by-product in the starch industry contains only around 1–2% of crude protein. Obtaining proteins from such large-volume by-products is a challenge for the industry but, at the same time, enables the production of protein with high nutritional value and significant added value due to the use of raw material with a potential threat to the human environment [3–5]. The management of waste by-products from the starch industry contributes also to the acquisition of relatively inexpensive and non-allergenic protein preparations [6] with favorable functional properties, allowing for the replacement of soy or milk proteins traditionally used in food production. Depending on the conditions of the isolation process of proteins from waste products in the starch industry, especially potato fruit juice, the obtained preparations have different properties and there are different potential directions for their use.

Potato proteins, isolated under industrial conditions by acid-thermal coagulation, are primarily used as a valuable feed. In 2017, 63.900 tonnes of fodder potato protein were produced in Europe [7]. In the starch industry, traditionally obtained dried potato protein for fodder purposes has an important position in the market, being a relatively cheap source of protein for pigs and young calves [8]. Some studies in this area also show a positive effect of potato proteins on the health of farmed pigs [9].

The use of thermally coagulated potato proteins in food production is very limited due to the low solubility, often too-high content of glycoalkaloids and unfavorable organoleptic characteristics of these preparations [10,11]. Many years of research and laboratory-scale methods of obtaining native potato proteins with favorable functional and nutritional properties [12–15] have not been implemented into the industry. This could be due to the high costs of isolation and purification of protein preparations, resulting from the complex chemical composition and high conductivity of potato fruit juice [14], the too-high content of glycoalkaloids and potassium, as well as partial denaturation of proteins and loss of functional properties. There are high hopes associated with the developed methods of separating the two main groups of potato proteins, i.e., patatin and inhibitory proteins, which can be applied on an industrial scale. As the research of many authors has shown [1,2,14,16–20], preparations of each of these fractions are characterized by different properties, more favorable compared to preparations containing all potato proteins. Intensive research in the field of isolation and modification of potato proteins, from both potato fruit juice (PFJ) and pulp, creates more and more opportunities for their use. They result both from the high nutritional value and valuable functional, antioxidant, antiradical and anti-obesity properties of potato protein preparations. The growing interest of both consumers and producers in enrichment with plant protein, including vegetable protein, of highly processed foods is also important [5,9,21].

1. Structure and Fractions of Potato Proteins (*Solanum tuberosum* L.)

Most potato proteins are fractions of albumin and globulin, soluble in water and in water and salt solutions, respectively; these are patatin glycoproteins, with a pI in the range of 4.8–5.2 [4,5,22,23]. According to various authors [5,19,24,25], globular proteins constitute 75–85% of all soluble protein fractions found in potatoes, while approximately 25% of all potato proteins are insoluble proteins that build the potato cell walls. Potato juice also contains smaller amounts of glutelin protein fractions (around 9%) soluble in dilute alkali and prolamines (2–4%) soluble in aqueous alcohol. Research conducted by various authors over the course of around 40 years allowed for a detailed characterization of the protein fractions present in potato juice. This showed that the main proteins dissolved in potato fruit juice (PFJ) can be classified into three groups. These include the patatin group proteins with a molecular weight in the range of 40–45 kDa [4,5,14,18,22,26], including the 41 kDa family of glycoproteins, occurring in the form of dimers with a molecular weight of around 88 kDa. They constitute 35–40% of soluble proteins and coagulate in an acidic environment. The second, no less numerous group of potato proteins is acid-soluble fractions with a molecular weight in the range of 16–25 kDa [4,5,19,25], predominantly the protease inhibitor fractions of 7–21 kDa. The remaining proteins found in potato fruit juice (PFJ) are of high molecular weight (above 87 kDa) [27] and form the third group of protein fractions. The proteins of the protease inhibitor family have been divided into seven groups due to the active site of the enzymes [20,28–30]. Pouvreau et al. [31], analyzed the inhibitor proteins from Elkana cultivar tubers, which released the highest amounts of potato serine protease inhibitor (PSPI/PI-2), which accounted for around 22% of total nitrogen; potato cysteine protease inhibitor (PCPI), whose share in total nitrogen was on average 12%; and a large group of inhibitory proteins present in a total amount of around 12%, such as starch synthetase, polyphenol oxidase or potato multicystatine. In the amount of 4–6% were such protease inhibitors as potato aspartate protease inhibitor (PAPI) (6%), potato inhibitor I (PI-I) (5%) and potato Kunitz-type protease inhibitor (PKPI) (4%). The individual fractions of potato proteins showed significant differences in terms of nutritional value and functional properties [5,19,32–35] and biological activity [18,36]; therefore, it is preferable to separate the protein fractions of the patatin group and protease inhibitors [19,37,38]. In addition, potato proteins intended for food purposes must be subjected to heat treatment, which affects their structure and biological activity, including individual protein fractions [18]. Fluorescence spectroscopy allowed the determination of the temperature at which the conformation of proteins of protease inhibitors clearly changed. As shown by the research of Sun et al. [18], a temperature of 80 °C not only damaged the hydrophobic interactions but also caused the proteins to stretch, as evidenced by the presence of a greater number of tryptophan residues located on the proteins' surfaces. These studies showed that the inhibitory protein family fractions showed mainly a β structure [18].

2. Nutritional Value of Potato Proteins

Potato proteins are characterized by high nutritional value, comparable to proteins of animal origin. This has been confirmed by the research of many authors over several decades [4,5,10,32,35,39–44]. Despite the beneficial nutritional, health-promoting and functional properties of potato proteins, they are relatively rarely used in food production. This is due to a number of technological and economic limitations; however, the constant progress in methods of isolating proteins from plant materials, including potatoes, and in increasing the purity and homogeneity of the obtained preparations creates the basis for the development of food preparations with high nutritional and health-promoting potential, as well as technological potential.

The nutritional value of proteins is related to the content of amino acids, their mutual proportions and digestibility. In potatoes, apart from protein-building amino acids, there are significant amounts of free forms of amino acids, which increase the nutritional value of potatoes as a food product [39,40]. The high nutritional value of potato protein and its good digestibility has been confirmed by many studies [4,5,10,32,35,41–44]. Basic fractions that

build potato protein, i.e., albumin, globulins, glutelins and the so-called residual proteins, are characterized by high nutritional value, as evidenced by the values of indicators such as chemical score (CS), the index of essential amino acids or PER at the level of 57–69, 48–83 and 0.95–2.3, respectively [5]. The rather wide ranges of values of these indicators showed, first of all, the varietal differences. With respect to nutritive value, potato proteins are similar to proteins of animal origin and exceed most plant-based proteins [35]. On the other hand, the low amounts of the prolamine fraction proteins (around 4% soluble protein) show lower nutritional value. The studies presented by Peřksa et al. [45] showed that the nutritional value of potato proteins was influenced by the potato variety, regardless of the color of the tuber flesh. Storage conditions such as time and temperature, especially in the interaction with the cultivar, also influenced the differentiation of potatoes in terms of the content of specific proteins and selected amino acids [39,40]. Potato protein is characterized by a particularly high content of the essential amino acid lysine, which is distinguished among plant proteins, while the limiting amino acids are usually tryptophan, methionine and cysteine; in colored potatoes, leucine is found [45]. The most abundant in potatoes are the amino acids aspartic and glutamic acid and their amides.

The protein fractions of albumin and globulin, glycoproteins with a degree of glycosylation of around 4% [46], belong to the group of proteins typical of potato and are referred to as patatin or tuberin [22]. These proteins, due to their presence in significant amounts (40–60% soluble potato proteins), are considered as storage proteins. They are distinguished from other fractions by their high nutritional value, good solubility in water or aqueous salt solutions and enzymatic and antioxidant activity [14,17,23,27,46]. Patatin proteins are characterized by a good balance of the amino acid composition and a higher content of the essential amino acid, lysine, than other plant-based proteins. For these reasons, their nutritional value exceeds most plant proteins, including lysine-poor cereal proteins, and is close to the nutritional value of hen egg proteins [34], considered to be one of the reference proteins. Isolated from potato juice in its natural or only partially denatured form, it can be a potentially beneficial food additive [10,19,47,48].

The second-largest group of proteins, accounting for around 30% of all potato proteins, are protease inhibitor proteins. They show great diversity in terms of structure, molecular weight, isoelectric point pH and biological activity [31]. Their nutritional importance is limited to exerting a positive therapeutic effect in the feeling of satiety in weight loss therapy. This may be related to the lower digestibility of these proteins, e.g., with the participation of trypsin. It is believed that the potential anti-obesity properties of proteins of protease inhibitors result from the release of the cholecystokinin peptide under the inhibitory effect of trypsin [49,50]. For these reasons, potato protein inhibitors, such as the protease inhibitor II-proteins (21 kDa), are considered a possible dietary supplement [3,4,18].

3. Functional Properties of Potato Proteins

The functional properties of proteins are determined by factors such as hydrophobicity, the presence of cross-links, spatial structure (secondary, tertiary and quaternary) and molecular flexibility/rigidity [4,9,51]. According to the definition presented by Kinsella [11], functional properties of proteins are “those physical and chemical properties that influence the behaviour of proteins in food systems during processing, storage, as well as preparation and consumption of food products”. Due to the favorable functional properties, proteins are often used as ingredients to impart rheological and structural properties to products with their participation, to influence the water binding or solubility of products. As it is believed that potato protein allergies are much less common, they may be a replacement for the traditionally used wheat, soybean, egg, fish or milk proteins, with proven high allergenicity [4].

Preparations of potato proteins in non-denatured form, obtained under laboratory conditions by many authors [10,12,13,52], starting from the 1970s, were characterized by favorable functional properties, especially solubility, emulsifying and foaming properties [27,34,38,53]. Stable emulsions with the participation of all potato proteins were ob-

tained by Holm and Eriksen [12], who showed that, in terms of emulsion properties, native potato proteins are comparable to commercial soy protein preparations. Cheng et al. [52] showed that potato protein hydrolysates are active in inhibiting lipid oxidation in soybean-oil-in-water emulsions. Separated fractions of patatin proteins and inhibitory proteins have been shown in studies by various authors [4,9,19,51,53] to possess more favorable functional properties than preparations containing all fractions of potato proteins.

Due to the low denaturation temperature of patatin proteins (in the range of 50–55 °C), the method of their isolation from the raw material influences the development of these properties [46]. Thus, the preservation of the beneficial functional properties of patatin is associated with the use of mild temperature conditions in the method used to obtain them. Van Koningsveld et al. [20], analyzing the functional properties of potato proteins, showed that the activity of protease inhibitor proteins in the formation and stabilization of foams and emulsions is relatively low compared to patatin proteins, which is related to the higher denaturation temperature of inhibitory proteins, i.e., in the range of 55–70 °C.

4. Biological and Health-Promoting Properties of Potato Proteins

Native potato proteins may also exhibit beneficial non-food properties, such as putative antimicrobial or antitumor [54], as well as antioxidant and antiradical [4,22,27,33,54–57]. These authors stated that the proteins of the patatin fraction are active as lipid-acyl hydrolases (LAH) and catalyze non-specific hydrolysis of phospholipids, glycolipids, mono- and diacyl glycerols and esters of long-chain fatty acids. This activity is shown by the hydrolysates of potato proteins. Patatin protein hydrolysates obtained by Waglay and Karboune [2] from potato pulp using multi-enzymatic systems, particularly with the use of Depol 670 L, were characterized by high lipid acyl hydrolase (LAH) activity. In other studies, patatin proteins obtained in the acidic-based precipitation process showed high activity of LAH towards long-chain fatty acids, while those obtained by thermal/acidic and CMC-based protein isolates did not show such activity [33]. The antioxidant activity of patatin protein hydrolysates is also confirmed by the studies of other authors [17,52]. They showed that cysteine and tryptophan, as functional groups of patatin proteins, can influence the activity of these protein fractions against radicals. Studies by Philanto et al. [57] showed that proteins isolated from potatoes, especially from sprouted tubers, as well as those obtained from by-products of the potato industry, had ACE-inhibitory potencies, and this activity increased as a result of hydrolysis of these proteins and reached IC_{50} in the range of 0.018–0.086. The studies of the activity of potato protein fractions showed that patatin fractions were characterized by high antioxidant and antiradical activity. According to Philanto et al. [57], native potato protein isolates could be an important raw material for the preparation of bioactive ingredients exhibiting ACE inhibitors and radical scavenging potencies. In other studies, the proteins of the patatin fraction showed antiproliferative activity against mouse melanoma B16 [18]. It is believed that the glycoproteins from the group of heat-resistant lectins present in potatoes are among the proteins with potential allergenicity [9].

Protease inhibitors play an important role by participating in plant defense mechanisms against the attack of certain microorganisms and insects by neutralizing the proteolytic enzymes secreted by them. The 21 kDa protease inhibitor proteins are believed to have anticarcinogenic activity, and protease inhibitors I and II, potato cysteine protease inhibitor and Kunitz-type inhibitors also show antimicrobial activity [1,4,58,59]. It is all the more important that, among them, there were the two most numerous fractions of inhibitor proteins, i.e., the potato serine protease inhibitors (PSPI) and the potato cysteine protease inhibitors, with a molecular weight of 20.5 and 21.0 kDa, respectively [4,20,31,49]. Additionally, other inhibitory proteins found in potato protein exhibit antimicrobial activity. For example, potato carboxypeptidase and potato cysteine inhibitors have shown to be active in inhibiting the growth of cancer cells and reducing the number of reactive oxygen species [60].

5. Effect of the Potato Protein Isolation Method on the Properties of the Obtained Preparations

The starch industry is investigating the possibilities of using proteins isolated from potato fruit juice (PFJ), mainly due to their promising nutritional and health-promoting properties and also non-nutritional properties [5,51,57].

Extracting proteins from industrial potato fruit juice (PFJ) has been the subject of research for many years. Various factors have been used to coagulate the proteins in the juice. These included increased temperature up to 120 °C, applied to juice with the isoelectric point pH of most potato proteins, most often in the range of 4.5–5.2 [4,5,10,13,20,44,61–63]; low pH in the range of 2–4, causing patatin proteins to coagulate at low temperature [25,64]; ethanol [27,32,65,66] at different pH levels; increased ionic strength in the juice with a pH of 4 and 6–7, combined with a temperature in the range of 50–80 °C [5,19]. Salting out is also an important method of protein isolation. Ammonium sulfate was often used in such a process, the use of which made it possible to obtain preparations with high efficiency, but with a relatively low protein concentration [10,32,33,44,67], while the use of ferric chloride salt accelerated the precipitation of proteins from potato juice and increased the efficiency of its recovery to over 86%. This was due to the strong affinity of this salt to potato proteins. Yet another known method of obtaining native forms of potato protein is complexing them with carboxymethylcellulose. The use of CMC contributed to an increase in the solubility of the preparation and improved the stability of the emulsions formed with its participation [68]. Natural adsorbents, such as clay minerals, were also used in the process of isolating proteins from potato fruit juice (PFJ) [16,69], enabling the reduction of the amount of glycoalkaloids in the obtained protein preparations.

Studies on the extraction of patatin proteins from potato fruit juice (PFJ) have also shown that a significant difficulty in isolating these proteins is the protein–non-starch polysaccharide interactions occurring in the juice, which require pH adjustment [33]. The above-mentioned methods were described in detail in many publications in this field [4,5,9,51].

The coagulated and thermally altered potato proteins did not show the most important nutritional, functional and biological/non-food importance of these proteins. These methods are currently used in industry, mainly due to the high efficiency of the isolation process (over 85%) [10]. At the same time, methods were developed to allow the isolation of potato proteins in their native form. In the late 1970s, such studies were conducted by Eriksson and Sivik [70] using UF ultrafiltration (UF) to treat potato starch effluents for protein recovery. Later, other authors [3,71] applied membrane techniques, including ultrafiltration and reverse osmosis, to obtain native protein preparations of potential food significance. The research of the above authors showed that the use of filtration membranes on an industrial scale led to their fouling by the fiber contained in the potato fruit juice (PFJ). Problems with cleaning membranes, as well as the insufficient filtration efficiency and selectivity of the method, significantly hampered the possible implementation of this technology. In the following years, thanks to intensive research, these problems were minimized by the use of the removal of the pectin fiber in the flocculation process in the presence of calcium ions [4,19].

Other studies aimed at extracting native potato proteins from potato fruit juice (PFJ) used the expanded bed adsorption methods, allowing the concentration of proteins at a similar level as membrane techniques [14,18,69,72]. As presented by Løkra et al. [14], multi-modal resins under different pH conditions made it possible to obtain concentrates with high solubility of 70–80%, containing pure protease inhibitor fraction (20–21 kDa) from MIMI I-45 resin, and concentrates containing both patatin (41 kDa) and protease inhibitors differed in terms of color and chlorogenic acid content. Strætkavern et al. [72] showed, for example, that ultrafiltration allowed for obtaining potato protein concentrates with a concentration of more than 75%, similar to the expanded bed adsorption method. The membrane adsorber technique, developed on a laboratory scale, using ion exchange membranes [16] for the separation and concentration of protein fractions, was also pro-

posed. The developed techniques of ion exchange chromatography made it possible to separate the potato protein fractions and obtain preparations with a reduced amount of glycoalkaloids [19,73]. Already in the 1980s, Racusen and Foote [22] used ConA sepharose followed by a DEAE sepharose step to isolate potato proteins. In studies conducted by Sun et al. [18], the ion exchange column Q-Sepharose Fast Flow allowed the separation of proteins with a molecular weight ranging from 16 to 25 kDa from the patatin protein fraction (40 kDa). These authors believed that the combination of UF membrane and Q-Sepharose Fast Flow ion exchange chromatography could be used for the isolation of protease inhibitor proteins from potato fruit juice (PFJ) under industrial conditions.

In the process of isolating proteins from waste by-products of the starch industry, it turned out to be important not only to reduce the degree of protein denaturation but also to reduce the interaction between the isolated protein and phenolic compounds, as well as solanine, which reduced the digestibility and absorption of proteins contained in the obtained preparations. The preparations obtained by thermo-acid methods contained significant amounts of glycoalkaloids, reaching 1612 mg/kg dry matter, which, according to Bártová and Bárta [32], resulted from the formation of complexes between proteins and glycoalkaloids during this process. The conducted research on the preparation of native protein preparations showing specific functional properties—which consisted of protein concentration using cold methods, such as acid coagulation, cryoconcentration, membrane techniques, gel and ion exchange chromatography, using macromolecule separation techniques based on such parameters as size and shape, hydrophobicity, surface charge and affinity—did not contribute to a significant reduction in the glycoalkaloid content in the obtained preparations [16].

Ralla et al. [16] concluded that the use of inexpensive adsorbents meeting criteria such as a reproducible high performance, low cost and availability on a large scale may be a promising possibility for isolating proteins from potato juice on an industrial scale. These authors used hydrated phyllosilicates (clay minerals) as cation exchangers for this purpose and showed that it was possible to reduce the amount of glycoalkaloids in the preparations in one step, together with the separation of the proteins. The phyllosilicates used by them are a natural and inexpensive adsorbent that could form hydrophobic bonds with a variety of organic molecules, including proteins. According to Ralla et al. [16], the mechanism of separation of patatin fractions and protease inhibitors with the use of clay minerals was related to the difference in their charge status. Protease inhibitors as molecules with a positive charge or in the form of molecules of neutral charge status react with negatively charged phyllosilicates, while proteins of the patatin group, being neutrally charged, should not be adsorbed by negatively charged surfaces above their isoelectric point (4.6–5.2). A limitation of this method may be the unequal distribution of charged patches over the surfaces of the protein molecules and the possibility of electrostatic interactions. According to the studies conducted by these authors, most protease inhibitors are adsorbed by the clay minerals at pH 7 to 9, while patatins can be absorbed at pH 4–6. This was an approach for the separation of patatin and protease inhibitors proteins using clay minerals as cation exchangers. An interesting solution of the technology of obtaining native potato proteins is the expanded bed adsorption chromatography modifications, in which membrane adsorber capsules (MA-IEX) are replaced with modules for direct purification of native proteins, skipping the first stages of purification (pre-filtration/microfiltration). The developed process consisted of only three-unit operations and enabled the direct and continuous isolation and purification of native potato proteins from raw potato fruit juice (PFJ) in the form of white powdered products suitable for human nutrition due to the lower content of antinutrients [74].

Other authors [1] paid attention to the possibility of obtaining a group of thermally stable proteins of the protease inhibitors with potential antimicrobial activity, dissolved in deproteinized fruit water, remaining after thermal protein coagulation in potato fruit juice (PFJ). These authors found a significant impact of the height of the isolation temperature of proteins from industrial potato fruit juice (PFJ) on the composition, structural stability,

as well as on trypsin inhibitors and the antifungal activities of proteins remaining in the waste effluents. In the 80 °C coagulation effluent, they identified the predominant presence of low-molecular inhibitory protein fractions (5–17 kDa), which, compared to the waste effluents after treatment at other temperatures (40 and 60 °C), were characterized by higher antifungal activity against five strains of *Fusarium* species. They included them in the group of protease inhibitors I with a molecular weight of around 12 kDa and II 16 kDa proteins. On the other hand, in the effluent after coagulation at lower temperatures (40 and 60 °C), they identified the presence of various fractions of inhibitor proteins, which they classified as aspartic, cysteine and serine protease inhibitors, including Kunitz-type protease inhibitor proteins with a molecular weight ranging from 20 to 24.5 kDa. The studies conducted by these authors showed that Kunitz-type serine protease inhibitors are thermally stable and fully functional up to the temperature of 80 °C and showed high activity against different strains of *Fusarium*. Additional studies by other authors [31,50] confirmed the activity of these inhibitor protein fractions. The groups of Kunitz-type serine protease inhibitors, AFP-J, PT-1 and Potide-G proteins, isolated by some authors [58,75,76], showed marked antimicrobial activity.

Scientists are becoming increasingly interested in methods for releasing proteins from material rich in non-starch polysaccharides. They use sets of enzymes that break down these compounds, both in the potato juice and in the pulp. This additionally enables the degradation of starch with amylases, such as the α -amylase from *Bacillus licheniformis*. According to Waglay et al. [77], it is possible to effectively obtain proteins from potato pulp produced in the starch industry, using appropriately selected sets of enzymes with a multi-enzyme action on non-protein components. After using the Termamyl amylase, these authors further degraded plant cell wall components with polysaccharide-hydrolyzing enzymes, and then extracted proteins. In another study [2], they showed that, for industrial purposes, it is beneficial to use multi-enzymatic preparations with a broader spectrum of glycosyl-hydrolase interaction, due to the greater efficiency of isolation of highly functional proteins from potato pulp. To prevent hydrolysis of the potato proteins, it was important to select an enzyme system that led to no or a negligible degree of protein hydrolysis (low or no proteolytic activity on potato proteins). The authors obtained favorable efficiency of protein isolation, at the level of 72.9 and 70.7%, using Depol 670 L and Newlase II, respectively. Additionally, other, commercially available multi-enzymatic systems, such as the GAMase preparation and the CER-led preparation, are relatively high in protein recovery yield, at the level of 60–61% [2].

6. Directions of Use of Potato Protein Preparations

Proteins with high nutritional value and with favorable functional properties can be an attractive food additive. Similarly to functional properties, the optimal amino acid composition and digestibility making up the nutritional value of the proteins obtained are closely related to their conformation, which in turn results from the conditions of protein isolation from the raw material. Therefore, depending on the method of isolation and the applied factors, such as pH, ionic strength and temperature [4,5,9,51], the directions of using potato protein preparations will be different. The need to monitor and remove phytochemicals occurring in them in varying amounts is also associated with obtaining proteins from plant raw materials for food purposes. These are naturally occurring polyphenols in raw materials and toxic substances, such as glycoalkaloids α -solanine and α -chaconine [78].

Produced in the starch industry, relatively cheap fodder potato protein, containing approximately 80% of raw protein in dry matter and several percent of carbohydrates and 3–5% of ash ingredients, does not show functional properties and has an intense flavor and aroma as well as a gray color [79]. Thus, it does not exhibit biological activity and its use is limited only to feed purposes. Preparations containing potato proteins with an unchanged structure are proposed for use in food production as nutritional, health-promoting and functional ingredients but also as components with a technological effect, e.g., in filtration processes, or as substances showing a beneficial non-food effect. An important argument

for their use in food production in place of the used protein preparations of other origin is the much less common allergenicity [9] of potato proteins.

In food production, preparations of both natural plant-based and modified proteins are used, with the most commonly used proteins being hydrolysates. Wang and Xiong [80], leading the limited enzymatic hydrolysis of heat-coagulated potato proteins, showed an increase in its solubility by 14–19 fold. Hydrolyzed potato proteins used by these authors to produce frankfurters at a dose of 2.5% contributed to the extension of the quality of these products under refrigeration conditions. The products that they obtained showed significantly lower cooking losses, fracture force and lipid oxidation than the products without the addition of potato proteins [9].

Patatin, due to its chemical properties similar to animal proteins, including an apparent molecular weight of around 40 kDa and an isoelectric point at pH 4.6, is widely used as a clarifying agent in oenology [81]. For example, patatin proteins (supplied by Solanic) have been used as a grape must clarifier in place of traditionally used clarifiers such as potassium caseinate and the synthetic PVPP polyvinylpyrrolidone polymer. By using clarifying agents, browning and cloudy musts are prevented. The activity of patatin proteins in reactions with phenolic compounds, plant dyes and pectin compounds causing inappropriate must color and turbidity was comparable to or better than that of potassium caseinate. Patatin proteins also turned out to be an important clarifying agent in the treatment of red wines, reducing their astringency [82], and, in other studies, a clarifying agent replacing egg albumin and casein [83]. The promising flocking properties of patatin require further research to determine the effects of pH and temperature on these properties, as well as the ability to retain must components that affect wine aroma [82].

Partially thermally denatured potato protein preparations were used as nutritional and texturizing additives for mashed potatoes [84], potato snacks [85,86] and corn snacks [87]. As shown by these studies, the addition of potato protein concentrates had a positive effect on the texture of crisps, both fried and extruded. Even a small percentage addition of 80% potato protein preparation significantly improved the consistency of these snacks, increasing their tenderness. Snacks obtained with a 3% share of potato proteins were characterized by a higher protein content by around 30–40% and were better spread and less hard than products without the addition of protein. On the other hand, the addition of partially denatured potato protein to boiled potato puree not only increased the nutritional value but also improved the texture of this dish, especially made of raw material with the wrong texture after cooking.

Waglay et al. [21] applied the potato protein concentrate and isolate to a reduced-gluten cookie formulation. They found that the addition of an isolate was more advantageous due to its purity and thus a lower polysaccharide content. Cookies obtained with the addition of isolate up to 7.5% were better assessed in terms of color, fracturability, crispness, adhesiveness, aftertaste and overall liking than products obtained only with rice flour. Another direction of using potato proteins is the possibility of using them in the process of encapsulating grape seed oil. Potato protein-based microcapsules were successfully formed to encapsulate grapeseed oil, by complex coacervation, particularly when combined with chitosan [88].

Potato proteins are also a valuable addition to livestock feed. Research has been carried out to develop an alternative feed for salmonids, which are usually fed with feed that includes fishmeal. The research showed that replacing up to 40% of fish meal with potato protein concentrate did not deteriorate the digestibility of the ingredients by Atlantic salmon. However, the inhibitors present in the potato protein concentrate should be deactivated [9].

The possibility of using high-purity potato protein preparations (RPP) as an antibacterial substance, instead of antibiotics, was also investigated [87] in the diets of weanling pigs. The authors found that the refined potato protein (RPP) was antimicrobial in vitro and inhibited the growth of the tested microorganisms at a concentration of 150 ppm (*Staphylococcus aureus*, *Salmonella choleraesuis*, *Salmonella gallinarum* and *Escherichia*

coli). The protein preparation used in these studies was isolated from the juice of potatoes of the “Gogu” cultivar, known for its very high resistance to tuber diseases during storage. The antimicrobial activity of the 5.6 kDa protein found in “Gogu” potatoes was confirmed by the study by Kim et al. [58]. Therefore, some authors [58,75,76] suggested that proteins of the inhibitor proteases may serve as useful candidates for the development of novel anti-infective agents or agrochemicals. Jin et al. [89] used a preparation of potato proteins obtained by ultrafiltration through membranes with a 10.000 molecular weight cut-off (MWCO) as a substance that inhibits the growth of selected microorganisms at concentrations between 100 and 150 ppm.

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