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Macronutrients, Amino and Fatty Acid Composition, Elements, and Toxins in High-Protein Powders of Crickets, Arthrospira, Single Cell Protein, Potato, and Rice as Potential Ingredients in Fermented Food Products

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Abstract: Due to the increasing global population and climate change, new sustainable food sources are being intensively sought to replace less favorable livestock production. Especially new protein sources and their food applications are being focused on. In this paper, several selected protein sources that may have potential application in future functional foods, such as fermented foods, were examined and compared. These sources include single cell protein (SCP), *Arthrospira platensis* (Algae), *Acheta domesticus* (edible insect), potato, and rice protein. The above sources were compared to whey proteins. The parameters studied were total nutritional value, amino acid profile, fatty acid profile, the content of some elements, and the presence of toxins.

Keywords: unconventional proteins; novel proteins; alternative protein sources; novel food; fermented products; functional food

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1. Introduction

The requirement to explore and research new protein sources is motivated by current trends in food technology and human nutrition. These trends are primarily dictated by the need to solve global problems, such as increasing world population, dynamic climate change, and limited availability of raw materials like water, cropland, and electricity [1,2]. Increased food production in response to a growing population should also seek to reduce food and waste losses, greenhouse gas emissions simultaneously, and water and electricity use. By 2050, it is estimated that there will be 9 billion people worldwide, and the demand for food will increase by up to 60% [3–5]. Currently, the primary source of protein in the form of meat from slaughtered animals is not sustainable, and its production is harmful to the environment. Livestock production is responsible for a large amount of land use because crops are cultivated for feed and animal husbandry. Animal production is estimated to take 70% of the world's arable land [6]. The most amount of land area is taken up by beef production (144–258 m^2/kg protein), followed by pork production (47–64 m^2/kg protein), and lastly, slightly less than pork, by poultry production (42–52 m^2/kg protein) [7]. To produce 1 kg of animal protein, 6 kg of plant protein is used as feed [8,9].

The growing popularity of meat-free diets also supports the need to introduce new protein sources into food. The number of people interested in limiting meat consumption or excluding it from their diet is proliferating. The reasons include a greater interest in climate protection and counteracting the effects of climate change. Flexitarianism, vegetarianism, and the most restrictive veganism have become the most popular dietary models [10]. Plant-based diets' primary protein sources are legumes and cereals, which must be skillfully combined to obtain a complete protein. Unfortunately, these sources present an incomplete protein [11].

Proteins of plant origin usually have a lower digestibility than those of animal origin. The main reason for the reduced digestibility of plant proteins is the presence of anti-nutritional factors (ANFs) such as phytates, tannins, trypsin inhibitors, and lectins. One way to increase the digestibility of plant proteins is fermentation. For this reason, combining plant proteins or other non-conventional proteins with fermented beverages is justified [12].

New sources of protein that are most commonly cited in the scientific literature and may have the potential for use in human nutrition include the following listed below.

Single cell protein (SCP) is derived from single-cell microorganisms such as bacteria, fungi, and molds. SCPs can be used in future foods due to their high production efficiency and protein content, with bacteria containing the most protein (50–80%) [13,14]. Organic waste left over from the production of other food products can be used to produce SCP, which eliminates another problem of excessive waste products in the food industry [13]. Despite the wide variety of compositions depending on the type of microorganism, there are some standard features, such as high protein content with low fat and carbohydrate content and high thiamine, riboflavin, glutathione, and folic acid content. SCPs can also be a source of phosphorus and potassium [15].

Edible insects have recently gained popularity and are also increasingly described in the scientific literature. Entomophagy in the world is not new, but insects are consumed only in some parts of the world, and in Europe, for example, they are still a novelty, and only a small part of the population of this continent has the opportunity to taste edible insects. They are now being studied for their potential use in human nutrition [16,17]. Insects are much more environmentally friendly compared to livestock production. They require much less water, space, and electricity to produce the same amount of protein. Insect production also produces much less CO_2 than animal production [2,18]. Nutritionally, edible insects, according to available sources, have a high protein content (50–76%), although the results vary widely among species. Furthermore, they show some health-promoting features, such as high content of vitamins and minerals [19]. Moreover, insects exhibit some antioxidant activity, an added benefit of human consumption in place of products from slaughtered animals [20].

Algae are another potential source of alternative protein. The most popular types of algae are Spirulina (*Arthrospira platensis*) and Chlorella (*Chlorella vulgaris*). However, Spirulina appears to be a better source because, unlike Chlorella, it does not have a cellulose cell wall that is difficult to digest and which requires additional processing to destroy [21,22]. According to available literature, Spirulina is a rich protein source (>60%). Moreover, it has a health-promoting effect, where 3 g/day supplementations can improve cardiovascular fitness and accelerate body regeneration [23]. A problem, however, may be sensory characteristics such as taste or smell, which are not favorable in Spirulina [24].

Potato protein is an ingredient that is extracted from potato juice. It, in turn, is a waste product in potato starch production, which is the primary purpose of worldwide potato production [25]. According to the literature, potato protein preparations contain up to 80% of protein [26]. This process allows the potato juice to be put to good use and not be a waste product. It is a very economical and environmentally friendly solution that can be much more sustainable than animal production [27]. Potato proteins could be used as an enrichment ingredient in the production of bread, pasta, yogurt, and their analogs. Studies have proved the existence of good technological properties such as water solubility and heat-induced gelation [26,28].

Rice proteins can also be a potential unconventional protein source. Rice is the second most cultivated cereal in the world and is grown in more than 100 countries. The largest share is in China, where 50% of the world's rice is grown [29,30]. Although rice alone contains only 7–9% protein, it is one of the primary dietary protein sources in South and Southeast Asian countries. The best raw material for the extraction of protein from the rice plant is rice bran, which is often a waste product. It is another potential source of protein that can use up waste in the food industry [31]. In protein preparations derived from rice bran, after appropriate technological processing, about 80% of protein can be obtained [32].

Many scientific papers contain descriptions of the individual proteins listed above. However, there is a lack of publications that directly compare these proteins with each other. Therefore, this study aimed to examine all the above mentioned protein preparations (SCP, edible insects, algae, potato protein, rice protein) under the same conditions, using the same methods, and to compare them with a standard in the form of whey protein concentrate, which is currently most commonly used to increase the protein content in food products, especially fermented foods and foods for athletes. The study was based on determining the nutritional value, the physicochemical properties, the content of significant elements, and the toxins. The results can be used to evaluate the suitability of individual protein sources for food production as a protein-enrichment ingredient.

2. Materials and Methods

For this study, high-protein powder formulations were selected as the samples of the selected protein sources. Due to the wide variety of types of protein sources in the case of insects, single-cell proteins (SCPs), and algae, one type of each was selected as a representative. For insects, the choice was made for the domestic cricket (*Acheta domesticus*) because it is readily cultivated and available in many highly developed countries. In the case of SCP, the choice was made for a product derived from *Corynebacterium glutamicum*. It was the only product of this kind available in Poland. Spirulina (*Arthrospira platensis*) was the representative sample for algae due to its lack of a cellulose cell wall, unlike Chlorella, and its easy market availability. In the case of potato and rice proteins, there was no problem with the variety of types. Whey protein concentrate (WPC80) was also tested in parallel with all formulations and was selected as a control sample with an excellent amino acid composition for humans.

Materials

The following high-protein preparations in powdered form were used for the study: *Acheta domesticus* cricket powder (CF Banks Ltd. t/a Instar Farming, Scopwick, UK); Spirulina *Arthrospira platensis* powder (country of origin of raw material—China, distribution: Targroch, Zakliczyn, Poland); potato protein powder (Pepees S.A., Łomża, Poland); single cell protein (SCP) (*Blattin, Izbicko, Poland*); rice protein powder (BENEO-Remy N.V., Leuven, Belgium); whey protein concentrate (WPC80) (Ostrowia Sp. z o.o., Ostrów Mazowiecka, Poland).

Methods

Protein content

The determination of protein content was based on the determination of nitrogen by the Kjeldahl method (International Organization for Standardization [ISO] 5983-1:2005). The determination principle is based on removing organic nitrogen compounds by converting to ammonium sulfate with concentrated sulfuric acid in the presence of a copper catalyst, alkalization of the solution, distillation, and titration with hydrochloric acid of the ammonia bound in boric acid. The samples were mineralized using a Tecator Digestor Auto 20 (FOSS, Hilleroed, Denmark) mineralizer. Distillation and titration were carried out using a Kjeltec 2300 automatic analyzer (FOSS, Hilleroed, Denmark).

Total fiber content

The total content of dietary fiber was determined according to the PN-A-79011-15:1998 norm, which consists of the digestion of the test sample with the following enzymes: thermostable α-amylase, pepsin, and pancreatin. Then, the undigested residue of insoluble dietary fiber was determined by weight, and soluble dietary fiber was precipitated from the supernatant solution and determined by weight.

Dry matter/moisture

Dry matter and moisture content were determined by procedure: CLA/PSO/3/2013 v.4 based on PN-ISO 1442:2000 norm. The principle of the method is to thoroughly mix

the sample with sand and dry it to a constant weight at 103 °C. The mass of the residue divided by the mass of the sample × 100 expresses the dry mass in %.

Ash content

The ash content was determined by the weight method based on ISO 2171:2007. The sample was initially dried, charred, and then ashed at 550 °C. After cooling, the weight of the residue was determined. The mass of residue divided by the mass of sample × 100 expresses the ash mass in %.

Fat content

The method for fat determination was liquid–solid solvent extraction (PN-A-79011–4:1998). The analyzed samples were weighed into thimbles and placed in the extraction unit. The extraction vessels were filled with solvent, and the soluble material was extracted in a two-step process followed by a solvent recovery phase. In the final step, the extraction vessels were dried and weighed.

Carbohydrate content

The principle of the method is to calculate the total carbohydrate content after determining the content of the essential food components, i.e., moisture, protein, fat, and total ash. The total carbohydrate complements the sum of the other chemical components to 100%.

Amino acids composition

Acid hydrolysis of proteins for the significance of amino acid composition without oxidation was performed according to Davis and Thomas [33]. The sample was treated with hydrochloric acid at elevated temperatures. Hydrolysis of proteins to obtain separation of sulfur amino acids was performed according to Schramm and Moor [34]. Cysteine was oxidized to cysteic acid and methionine to methionine sulphone using peracid. For tryptophan determination, samples were subjected to alkaline hydrolysis with barium hydroxide according to the method of Sławiński and Tyczkowska [35]. Amino acids were determined using an amino acid analyzer AAA 400 from Ingos (Prague, Czech Republic). Amino acids were separated by ion exchange chromatography. The 0.37 × 45 cm column is filled with a resin ion exchanger. Ostion LG ANB was used for the hydrolysates. It is a strong cation exchanger with an average grain size of about 12 µm in the form of Na cations. The column temperatures are 60 °C and 74 °C. The apparatus detects amino acids using ninhydrin (the detection reagent). A photometric detector identified amino acids at 570 nm for all amino acids; however, for proline, 440 nm was used. Four buffers were used for separation: 1. pH 2.6; 2. pH 3.0; 3. pH 4.25; 4. pH 7.9. After separating amino acids, the column was regenerated with 0.2 N NaOH. The tests were conducted in triplicate for each sample.

Fatty acids composition

Determination of fatty acids was carried out as follows. Sample preparation: 100 mg of fat was transferred into a dry ampule using an automatic pipette. Saponification: a methanolic solution of potassium hydroxide was added to the fat sample, and the ampule was placed in a water bath and brought to the boil and heated until the fat droplets disappeared. After the saponification was completed, the ampule and its contents were cooled. Esterification: methanolic solution of boron trifluoride was added to the obtained potassium fatty acid soaps, brought to the boil, and then cooled immediately. Separation: hexane was added to the cooled contents of the ampule, the mixture was stirred, and a saturated sodium chloride solution was added and mixed thoroughly. The contents of the ampule were made up of saturated sodium chloride solution so that the upper organic layer (hexane) was in the narrow neck of the ampule. Drying: using a pipette, the organic layer (hexane) was taken into an Eppendorf tube, dried by adding anhydrous sodium sulfate, and sealed tightly. Chromatographic separation was then performed using a Varian 450-GC gas chromatograph for which Galaxie Chromatography Data System software is used (Varian

Inc., Walnut Creek, CA, USA). Stationary phase: Select Biodiesel for FAME Fused Silica. Column oven: initial temp 100 °C, final temp 240 °C. FID detector temp. 270 °C. Carrier gas type: helium, carrier gas flow rate: 1.5 mL/min. The tests were conducted in triplicate for each sample.

Elements

In this paper, the content of elements was determined: Ca, Mg, K, Na, Fe, Cu, Mn, Se, and P. The determinations were carried out by the Central Research Laboratory of the University of Life Sciences in Lublin (CLB). The elements were determined using the flame atomic absorption spectrometry (FAAS) method, which was based on the procedures described in the CLB internal methodologies CLB/ASA/2/2019 version 4 dated 20 September 2019 and CLB/ESA/5/2019 version 3 dated 10 December 2019 in accordance with PN-EN ISO 6869:2002. However, the phosphorus content was determined using the spectrophotometric method described in the internal procedure CLB/PLC/28/2019 version 3 dated 04 March 2019. The tests were conducted in triplicate for each sample.

Contamination by mycotoxins

The study analyzed samples for the mycotoxins: ochratoxin A, aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2. Tests were performed at the accredited analytical laboratory AGROLAB Poland (Dęblin, Poland) using the QMP_504_KI_52_151:2020-11(LC-MSMS) method in accordance with the standard DIN 10758: 1997-05. The method consists of extracting mycotoxins from the sample matrix with a mixture of acetonitrile, water, and acetic acid, followed by dilution with a buffer containing an internal standard. Analysis was performed by HPLC-MSMS. The tests were conducted in triplicate for each sample.

Statistical analysis

Statistical analysis of the prepared samples was carried out in the STATISTICA 13.0 PL application (StatSoft Polska Sp. z o. o., Kraków, Poland). A one-way ANOVA was performed (amino acid composition and element content), and significant differences between the tested samples were determined by Tukey's post hoc test at $p < 0.05$.

3. Results

3.1. Nutritional Value

Table 1 presents the nutritional value results for all protein sources tested. The last column shows the results of whey protein concentrate (WPC80) as the control sample to which the other protein sources are compared.

Table 1. Nutritional value of alternative and unconventional protein sources compared to the whey protein concentrate (WPC80—control sample).

	Edible Insects	Algae	SCP	Potato	Rice	WPC80 (Control)
	[g/100 g]					
Protein	61.90	59.94	73.20	>81.30	>81.30	73.50
Fat	23.10	4.88	3.40	<0.50	7.00	4.90
Carbohydrates	6.90	14.99	17.30	9.70	5.00	12.70
Fiber	4.80	4.19	8.20	3.40	4.30	3.60
Ash	4.42	12.60	2.36	3.13	0.82	2.48
Dry mass	96.30	92.45	96.30	94.10	94.10	93.60
Energy [kcal/kJ]	473/1981	335/1417	377/1592	357/1516	399/1687	382/1614

It can be seen from the above data that the potato and rice formulations have the highest protein content, as they both exceeded the upper limit of quantification for this macronutrient. Single cell protein has slightly less protein than other proteins and almost as much as the WPC80. Last in terms of protein content are algae (*Arthrospira platensis*) and edible insects (*Acheta domesticus*), with insects showing a slightly higher protein content than

algae. A similar protein content in algae was reported in the work of Liestianty et al. [36]. Despite the considerable variation, it should be mentioned that all preparations have a high protein content, ≥60 g/100 g. All products tested also meet the definition of a high-protein product in the Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods because the amount of energy derived from protein is higher than 20% [37]. In terms of fat content per 100 g of product, the potato protein preparation had the lowest value, and the SCP preparation had slightly more fat, followed by slightly more for algae, which equaled the control sample (WPC80) in fat content. The rice protein had a fat content above the standard, and the edible insect preparation had significantly more, 18.2 g more than WPC80. Most likely, the high fat content of the insects is because ground insects not subjected to defatting were used in the study. In the paper by Kulma et al., the authors examined the composition of *Acheta domesticus* and obtained results ranging from 12.9–21.7 g/100 g dry weight, depending on sex and product batches [38]. This indicates a large discrepancy depending on the production batch and the sex distribution of insects in the whole sample. The protein preparation from rice characterized the lowest content of carbohydrates, slightly more carbohydrates were found in edible insects. The preparation from SCP had the highest amount of this macronutrient; it was 4.6 g/100 g more than the control sample. The amount of dietary fiber in most samples was similar in the range of 3.4–4.8 g/100 g of product, and only the SCP formulation showed a higher value (4.6 g/100 g more than WPC80). The dry matter of all samples was in a similar range between 92.45–96.3 g/100 g. An essential element of nutritional value is the energy content of a given formulation. The highest energy content was in the edible insect protein formulation, followed by rice, WPC80 control protein, and SCP, and the lowest energy content was in the potato and algae formulations. The difference between the highest energy content (edible insects) and the lowest (algae) was 564 kJ (138 kcal). It should be noted that the powder from *Acheta domesticus* was not subjected to a defatting process, so it contains more fat and less protein per 100 g of product. It is noteworthy that more protein can be obtained after defatting the insect powder and the study by Ribeiro et al. proved that the reduced amount of fat has a positive effect on the sensory experience of edible insect bars [39]. All the unconventional protein sources studied can be used to create high-protein products, as they contain a relatively high protein content per 100 g product. Most plant-based protein sources used in the food industry have a much lower protein content per 100 g product [25]. In terms of their composition, all the high-protein formulations studied may be suitable for use in various areas of the food industry. Furthermore, those with a lower protein content than the control protein and from plant sources can be subjected to technological processing that increases protein digestibility, such as fermentation [12]. With the addition of the studied preparations, fermented products could be a good source of protein in the human diet.

3.2. Amino Acids Composition

Table 2 shows the results of the determination of amino acids, including essential amino acids, of all protein preparations. According to FAO/WHO/UNU [40], the amino acid composition pattern for essential amino acids is also presented.

In most cases, the amino acid composition of the tested protein preparations meets the requirements set by FAO/WHO/UNU [40]. However, there are cases where the amount of amino acids in the tested protein preparations is lower than in the standard. In the case of threonine, the highest amounts were recorded in potato protein (64.6 mg), almost twice the standard's value. Next, a lower value, but still relatively high compared to the standard, was determined in the WPC control sample (56.2 mg). The other preparations had values close to each other (27.7–33.5 mg) but still amounts above those in the standard (23 mg). Cysteine levels were higher than the standard in all cases, with rice protein (23.1 mg) and WPC80 (27.9 mg) having the highest content of this amino acid. However, comparing the other protein sources to the control WPC80, only rice protein has a similar amount of cysteine. Valine levels are also close to the pattern, slightly exceeding the standard value

(39 mg). Only in *Arthrospira* is the valine level lower, but just by 3.6 mg. Methionine content in all samples is above the reference protein; in most cases, the amount of methionine is close to that in WPC80. Only edible insect powder does not exceed the recommended isoleucine content, but the difference is just 0.8 mg. Other samples contain more isoleucine than the recommended standard. Leucine is at a similar, slightly lower level than the standard in the insect protein, *Arthrospira*, and SCP samples, while potato protein, rice protein, and WPC80 contain significantly more leucine than the others and exceed the values in the standard. The reason is most likely that the overall protein content of edible insects, algae, and SCP is lower than that of rice protein, potato protein, and WPC80. The total lower protein content translated into a lower content of individual amino acids. To maximize the amino acid content per 100 g, it would be necessary to obtain protein isolates from these preparations. The recommended content for phenylalanine was exceeded only in potato protein and rice protein. The remaining sources contain less than the recommended value (30 mg) but still at a reasonable level above 25 mg. The histidine level in the algae preparation is below the recommended content by 4.8 mg. Moreover, in the case of lysine, algae protein contains the lowest amount of this amino acid, much below the general pattern (27.8 vs. 45 mg). In this case, the rice protein also falls short of the benchmark (30.1 mg) and the insect protein slightly below the recommendation (41.2 mg). Tryptophan content significantly exceeds the recommended amounts for all samples tested. The sum of all essential amino acids in all samples tested is above the reference value. The highest amounts of essential amino acids were determined in the potato protein (478.31 mg), while the least in the algae preparation (272.63 mg). Although the amino acid content varies from one formulation to another, and not all essential amino acids meet the reference standard, it can be crucial to use the right amount of formulation per serving of the whole product. In the study by Joy et al. (2013), supplementation with 48 g of WPC80 or rice protein was shown to be just as effective in athletes [41]. In addition, individual proteins can be fermented, which can increase their digestibility and bioavailability [12].

Table 2. Amino acid composition of protein samples and amino acid composition pattern according to FAO/WHO/UNU [40]. Different letters (a–f) in the same row indicate a significant difference at $p < 0.05$.

Amino Acid	Edible Insects	Algae	SCP	Potato	Rice	WPC80 (Control)	WHO/FAO/UNU Pattern
	[mg/g]						
Aspartic acid	64.40 ^{b} ± 0.17	56.70 ^{a} ± 0.17	68.40 ^{c} ± 0.17	104.00 ^{f} ± 0.87	80.60 ^{e} ± 0.40	83.90 ^{e} ± 0.40	
Threonine *	27.70 ^{a} ± 0.20	28.60 ^{b} ± 0.17	33.50 ^{d} ± 0.36	64.60 ^{f} ± 0.17	31.50 ^{c} ± 0.26	56.20 ^{e} ± 0.17	23.00
Serine	37.70 ^{c} ± 0.17	29.70 ^{b} ± 0.17	28.70 ^{a} ± 0.20	50.30 ^{e} ± 0.26	49.70 ^{e} ± 0.36	42.80 ^{d} ± 0.17	
Glutamic acid	91.60 ^{a} ± 0.10	92.20 ^{a} ± 0.17	104.80 ^{b} ± 0.30	116.50 ^{c} ± 0.26	171.00 ^{e} ± 0.26	149.60 ^{d} ± 0.20	
Proline	2.38 ^{e} ± 0.03	2.16 ^{d} ± 0.01	1.59 ^{a} ± 0.01	3.28 ^{f} ± 0.01	2.11 ^{c} ± 0.02	1.97 ^{b} ± 0.02	
Glycine	35.40 ^{d} ± 0.10	28.50 ^{b} ± 0.17	33.00 ^{c} ± 0.20	35.10 ^{d} ± 0.26	38.00 ^{e} ± 0.10	14.30 ^{a} ± 0.20	
Alanine	59.70 ^{e} ± 0.17	47.10 ^{b} ± 0.26	70.70 ^{f} ± 0.10	55.20 ^{d} ± 0.20	52.90 ^{c} ± 0.17	40.80 ^{a} ± 0.17	
Cysteine *	8.22 ^{b} ± 0.03	8.43 ^{b} ± 0.01	6.69 ^{a} ± 0.02	8.31 ^{b} ± 0.02	23.10 ^{c} ± 0.17	27.90 ^{d} ± 0.10	6.00
Valine *	39.60 ^{b} ± 0.17	35.40 ^{a} ± 0.17	44.60 ^{c} ± 0.17	45.20 ^{d} ± 0.26	51.60 ^{e} ± 0.30	45.20 ^{d} ± 0.17	39.00
Sulf. Methionine *	16.20 ^{a} ± 0.10	19.10 ^{b} ± 0.10	23.60 ^{d} ± 0.17	27.20 ^{e} ± 0.17	27.60 ^{e} ± 0.26	21.90 ^{c} ± 0.17	16.00
Isoleucine *	28.20 ^{a} ± 0.10	30.00 ^{b} ± 0.17	30.70 ^{c} ± 0.17	40.00 ^{e} ± 0.17	34.30 ^{d} ± 0.20	45.30 ^{f} ± 0.20	30.00
Leucine *	53.60 ^{b} ± 0.17	49.80 ^{a} ± 0.17	55.80 ^{c} ± 0.20	80.40 ^{f} ± 0.26	69.10 ^{d} ± 0.17	79.60 ^{e} ± 0.36	59.00
Tyrosine	34.20 ^{c} ± 0.26	25.20 ^{b} ± 0.17	21.50 ^{a} ± 0.10	50.80 ^{e} ± 0.50	44.40 ^{d} ± 0.26	25.40 ^{b} ± 0.10	
Phenylalanine *	26.00 ^{a} ± 0,00	26.50 ^{b} ± 0.17	28.20 ^{c} ± 0.17	52.30 ^{e} ± 0.10	46.60 ^{d} ± 0.20	25.90 ^{a} ± 0.10	30.00
Histidine *	17.60 ^{d} ± 0.05	10.20 ^{a} ± 0.17	16.30 ^{c} ± 0.10	18.50 ^{e} ± 0.20	21.60 ^{f} ± 0.17	14.50 ^{b} ± 0.10	15.00
Lysine *	41.20 ^{c} ± 0.17	27.80 ^{a} ± 0.26	116.20 ^{f} ± 0.26	63.60 ^{d} ± 0.44	30.10 ^{b} ± 0.17	68.40 ^{e} ± 0.10	45.00
Arginine	49.70 ^{e} ± 0.17	40.90 ^{c} ± 0.17	47.00 ^{d} ± 0.10	38.40 ^{b} ± 0.26	80.60 ^{f} ± 0.17	19.60 ^{a} ± 1.82	
Tryptophan *	27.80 ^{c} ± 0.26	26.80 ^{b} ± 0.10	26.00 ^{a} ± 0.17	27.40 ^{c} ± 0.17	31.80 ^{d} ± 0.20	31.50 ^{d} ± 0.10	6.00
Total essential amino acids	286.12	272.63	403.09	478.31	367.30	416.40	269.00

* Essential Amino Acids.

3.3. Fatty Acid Composition

Table 3 shows the fatty acid composition determined in the protein preparations. The most relevant data such as the sum of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and n-3, n-6, n-9 fatty acids are placed at the bottom of the table in bold.

Table 3. Fatty acid composition of alternative and unconventional protein sources compared to the whey protein concentrate (WPC80—control sample).

Fatty Acid	Edible Insects	Algae	SCP	Potato	Rice	WPC80 (Control)
	[g/100 g]					
C6:0		0.0004	0.0004	0.000002	0.0003	0.0005
C8:0		0.001	0.001	0.00003	0.001	0.001
C10:0		0.0004	0.002	0.00003	0.0003	0.002
C12:0	0.015	0.001	0.002	0.00002	0.0002	0.002
C14:0	0.109	0.016	0.018	0.0001	0.002	0.007
C14:1n5	0.004					0.0004
C15:0	0.017	0.001	0.002		0.0002	0.001
C16:0	5.515	0.397	0.389	0.001	0.054	0.032
C16:1n7	0.111	0.042			0.001	0.001
C17:0	0.048	0.003				0.0002
C17:1n7			0.013			
C18:0	2.449	0.044	0.031	0.0004	0.012	
C18:1n9c + C18:1n9t	4.735	0.034	0.383	0.001	0.039	0.0226
C18:2n6c + C18:2n6t	6.742	0.119	0.107	0.0001	0.014	
C18:3n6 (gamma)	0.006	0.258				
C18:3n3 (alpha)	0.375	0.0002	0.012			
C20:0	0.071	0.001	0.001		0.001	
C20:1n15	0.008					
C20:2n6	0.006	0.002				
C20:3n6		0.003				
C20:4n6		0.001		0.001		
C22:0	0.013	0.001			0.001	
C22:1n9	0.034					
C22:2n6	0.027	0.001	0.002		0.0005	
C23:0		0.0004	0.002			
C24:0	0.004	0.001	0.003		0.001	
SFA	**8.241**	**0.466**	**0.452**	**0.002**	**0.072**	**0.0453**
MUFA	**4.892**	**0.076**	**0.396**	**0.001**	**0.040**	**0.0243**
PUFA	**7.157**	**0.384**	**0.122**	**0.001**	**0.015**	**0.0000**
n-3	**0.375**	**0.0002**	**0.012**	**0.000**	**0.000**	**0.0000**
n-6	**6.782**	**0.384**	**0.110**	**0.001**	**0.015**	**0.0000**
n-9	**4.769**	**0.034**	**0.383**	**0.001**	**0.039**	**0.0226**

The highest content of saturated fatty acids was determined in the preparation of edible insects. It was followed by a much lower amount determined in algae, SCP, and WPC80. The amount of SFA, in this case, was almost identical. Lower values were determined in rice protein, and the amount of SFA in potato protein was negligible. MUFA were again determined in the highest amount in the edible insect preparation (4.892 g/100 g); in the other preparations, the amounts of MUFA are marginally low, in all cases, below 0.4 g/100 g. In the case of PUFA, considered essential and most desirable for health reasons, the highest content was again determined in the edible insect preparation (7157 g/100 g). The other preparations contained meager amounts of PUFA. Potato protein was at the limit of detection, and no PUFA were detected in WPC80. The n-3 fatty acids were detected in the edible insect preparation at 0.375 g/100 g, while in the other preparations, the amounts determined were marginal or not detected at all. The same is true for n-6 and n-9 fatty acids, where the only higher value was determined in the edible insect preparation, while in the other samples, the content is trace or zero. The higher contents of individual fatty acids determined in the edible insect preparation are due to the overall decidedly higher fat content of this preparation than the others because the insects were not subjected to a defatting process. In such a form as in this study, the powder from *Acheta domesticus* can act as a nutritional enrichment ingredient for food products, not only through protein but also through its fatty acid content. After the defatting process, a formulation targeting

only the protein source can be obtained. Zielińska (2022), in her work, showed that the defatting process can result in a significant reduction in total fat content (18.54 mg vs. 3.43 mg/100 g) [42]. The higher SFA content may be because insect fat is a fat of animal origin. In addition to insects, only SCP and algae can act as an ingredient providing n-3 fatty acids, as they have not been labeled in the other formulations. However, the amounts of SCP and algae added to food would be too low to provide at least a similar amount as insects. In the case of algae, the main obstacle limiting the amounts that can be used in food is the unfavorable sensory properties [24]. The formulations studied are considered a potential source of protein, so the fat content and individual fatty acids may be an additional nutritional and health-promoting benefit, but it is not the most critical feature; in this case, the lower the fatty acid content, the better. The results show that all preparations have low fatty acid content except insects, but when they undergo defatting, they also become a good high-protein product.

3.4. Element Content

Besides macronutrients, an essential element of any food product, are elements that significantly affect health by taking part in many metabolic transformations. Table 4 shows the results of determining the content of nine health-relevant elements in protein preparations.

Table 4. Element content in alternative and unconventional protein sources compared to the whey protein concentrate (WPC80—control sample). Different letters (a–f) in the same row indicate a significant difference at $p < 0.05$.

Element	Edible Insect	Algae	SCP	Potato	Rice	WPC80 (Control)
	[mg/kg]					
Ca	1590 ^{c} ± 46	1820 ^{d} ± 4.0	376 ^{b} ± 4.0	14.4 ^{a} ± 0.3	48.9 ^{a} ± 1.9	4180 ^{e} ± 22
Mg	1090 ^{e} ± 26	2980 ^{f} ± 7.0	573 ^{c} ± 3.0	106 ^{b} ± 4.0	68.2 ^{a} ± 0.2	618 ^{d} ± 1.0
K	11900 ^{e} ± 44	18,700 ^{f} ± 137	794 ^{b} ± 3.0	2180 ^{c} ± 4.0	94.7 ^{a} ± 0.4	4230 ^{d} ± 4.0
Na	3780 ^{d} ± 22	34,300 ^{f} ± 235	196 ^{a} ± 3.0	7600 ^{e} ± 70	591 ^{b} ± 2.0	1490 ^{c} ± 3.0
Fe	59.2 ^{c} ± 0.2	257 ^{f} ± 2.0	77.7 ^{e} ± 0.4	72.7 ^{d} ± 0.5	8.49 ^{a} ± 0.2	19.3 ^{b} ± 0.3
Cu	29.7 ^{f} ± 0.3	0.750 ^{a} ± 0.01	7.44 ^{e} ± 0.03	2.29 ^{c} ± 0.04	6.26 ^{d} ± 0.02	1.15 ^{b} ± 0.02
Mn	43.5 ^{f} ± 0.5	14.3 ^{d} ± 0.2	36.3 ^{e} ± 0.3	2.92 ^{c} ± 0.03	2.12 ^{b} ± 0.03	<0.1 ^{a}
Se	0.150 ^{c} ± 0.01	0.441 ^{e} ± 0.01	0.135 ^{b} ± 0.01	<0.001 ^{a}	0.170 ^{d} ± 0.01	0.168 ^{d} ± 0.01
P	9000 ^{d} ± 143	8000 ^{c} ± 265	10,000 ^{e} ± 300	600 ^{a} ± 17	3000 ^{b} ± 96	3000 ^{b} ± 20

Calcium is an important element in terms of bone health and the functioning of the neuromuscular system. The highest amount of calcium was determined in WPC80 (4180 mg/kg), presumably because dairy is one of the best sources of calcium, and WPC80 comes specifically from milk [43]. In contrast, edible insects (1590 mg/kg) and algae (1820 mg/kg) have noticeably higher calcium content than the other sources, so that they can provide an additional source of this element in the human diet. As for magnesium, which also plays an important role in the functioning of the neuromuscular system, the highest amounts are found in algae (2980 mg/kg) and edible insects (1090 mg/kg). The least magnesium is found in rice protein (68.2 mg/kg). Magnesium is vital, especially for athletes who often consume high-protein products. Athletes have a higher need for magnesium, which is often not covered by a normal diet, so an additional source of magnesium in the form of protein from algae or insects can be helpful [44]. Potassium is an element to which special attention should be paid for hypertension prevention. The highest potassium content, several times higher than the others, was determined in algae protein (18,700 mg/kg) and insect protein (11,900 mg/kg). A much smaller but also significant potassium content was found in WPC80 (4230 mg/kg), while the lowest potassium content was determined in rice protein (94.7 mg/kg). An important element to watch out for is

sodium, as excess in the diet is not advisable and can lead to hypertension [45]. By far, the highest sodium content was determined in algae as high as 34,300 mg/kg, which means that even small amounts of algae can cause the recommended sodium intake per day to be exceeded, as the RDA (recommended dietary allowance) for sodium is 1500 mg/day in adults [46]. Next in terms of sodium content is potato protein (7600 mg/kg), edible insects (3780 mg/kg), and WPC80 (1490 mg/kg), while the lowest sodium content is found in SCP and rice protein (196 and 591 mg/kg). Therefore, the last two sources may be useful in people with hypertension and cardiovascular disease. Another element is iron, which is responsible for, among others, oxygen transport in the body and is essential for physically active people, pregnant women, and people on plant-based diets [47,48]. Algae preparation has the highest iron content (257 mg/kg), followed by SCP and potato protein (77.7 and 72.7 mg/kg). In insects, the iron content is considerably lower than in algae (59.2 mg/kg), but it is still high. The fact that edible insects can be a good source of iron, calcium, magnesium, potassium, and phosphorus has already been written about by Kouřimská and Adámková (2016) and Zielińska et al. (2015) [19,49]. The insects also had higher iron content than the WPC80 and rice protein preparations (19.3 and 8.49 mg/kg). However, algae stands out as an important source of iron. A study by Üstün-Aytekin et al. (2022) demonstrated the suitability of using *Arthrospira platensis* to enrich traditional kefir at 0.05% and 0.1%. Kefir with such an addition performed favorably in terms of sensory qualities and had four times the iron content of regular kefir. There was also a slight increase in the calcium content [50]. In the case of copper content, only edible insect protein stands out among the tested preparations (29.7 mg/kg), while the other preparations have a significantly lower content of this element (<7.5 mg/kg). The same is true for manganese, as edible insects have the highest manganese content (43.5 mg/kg). In addition, SCP contains slightly less manganese (36.3 mg/kg), while the other preparations have a low manganese content. The selenium content in all samples is low except algae and ranges between 0.441 mg/kg in algae and <0.001 mg/kg in potato protein. The RDA for selenium is 0.055 mg/day for adults [46]. So, to meet the daily requirement for selenium, it would be necessary to consume about 125 g of algae per day, which can be difficult due to the unfavorable sensory properties of algae [24]. Almost all tested preparations show a high phosphorus content, and only potato protein has significantly less phosphorus than the others (600 mg/kg). The most phosphorus was determined in SCP (10,000 mg/kg), followed by insect (9000 mg/kg) and algae (8000 mg/kg). WPC80 and rice protein contained an identical amount of phosphorus (3000 mg/kg). An adult's RDA for phosphorus is 700 mg/day [46]. Phosphorus plays an important role in many metabolic processes. It is essential for the body's energy metabolism and plays an important role in bone health and the thyroid gland. Children with an increased need for phosphorus, athletes, and pregnant women are mainly at risk of deficiency [51]. All of the preparations examined, except for potato protein, can be classified as rich sources of phosphorus.

3.5. Toxins Content

A crucial issue in food products is their safety, so protein preparations were checked for the possible presence of selected common mycotoxins. The results are included in Table 5.

Levels of individual mycotoxins were below the detection threshold in most cases. aflatoxin B2, G1, and G2 were not detected in any preparation tested. In the case of aflatoxin B1, it was detected only in the rice preparation, while the level can be considered safe as it does not exceed the threshold of 5 µg/kg, which was set as a maximum by the European Commission [52]. Contamination in rice often results from fungal infections, which can be reduced through various crop protection methods. There are also opportunities to reduce aflatoxin contamination through, e.g., ozonation [53]. In the case of ochratoxin A, it was detected only in the preparation of edible insects, but the amount of the toxin detected was very low and did not exceed the threshold set by the European Commission for various foods of 2–10 µg/kg (only infant formulae has a set limit of 0.5 µg/kg) [52]. It should

be added that the safety of edible insects can depend on the manner and control of their breeding and the quality of the feed they are fed [54]. The above data shows that almost all formulas are safe and do not contain dangerous toxins. Only edible insects and rice protein may not be suitable in foods for infants.

Table 5. Mycotoxin content in alternative and unconventional protein sources compared to the whey protein concentrate (WPC80—control sample).

Toxin	Edible Insect	Algae	SCP	Potato	Rice	WPC80 (Control)
	[µg/kg]					
Ochratoxin A	1.60	<1.50 *	<1.50 *	<1.50 *	<1.50 *	<1.50 *
Aflatoxin B_1	<0.500 *	<0.500 *	<1.00 *	<0.500 *	2.09	<0.500 *
Aflatoxin B_2	<0.500 *	<0.500 *	<0.500 *	<0.500 *	<0.500 *	<0.500 *
Aflatoxin G_1	<0.500 *	<0.500 *	<0.500 *	<0.500 *	<0.500 *	<0.500 *
Aflatoxin G_2	<0.500 *	<0.500 *	<1.00 *	<0.500 *	<0.500 *	<0.500 *
Total aflatoxins	g.o.	g.o.	g.o.	g.o.	2.1	g.o.

* Below detection.

4. Conclusions

The results of the work above show that all the unconventional protein sources tested have high nutritional value and can be considered for designing new high-protein food products, especially fermented food products. In particular, it is worth considering the addition of edible insects, algae, SCP, potato, or rice preparations to fermented products, as fermentation can further enhance the nutritional value by increasing protein digestibility. Each should be treated individually for the best and optimal use of protein preparations. All are characterized by a high protein content per 100 g of product but vary in the content of other components such as fatty acids, carbohydrates, or the presence of particular elements. For this reason, each of the studied preparations may find different applications in the food industry and play a different role as a high-protein superfood. Potato protein, rice protein, and SCP performed best in terms of protein content compared to WPC80, while edible insect and algae protein had lower overall protein content. Nonetheless, insects and algae can also be comparable sources of protein if subjected to technological processes that reduce fat and carbohydrate content and increase protein digestibility through fermentation. For a complete view of the quality of the proteins discussed in the paper, further studies should be conducted to test the suitability of these preparations after implementation into food products, including fermented ones.

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