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Comparative Evaluation of the Antioxidative and Antimicrobial Nutritive Properties and Potential Bioaccessibility of Plant Seeds and Algae Rich in Protein and Polyphenolic Compounds

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Abstract: Spice plants are not only a source of nutrition compounds but also supply secondary plant metabolites, such as polyphenols. Therefore, their bioaccessibility is an important issue. In order to understand the biological activity of polyphenols present in spice plants, it is necessary to broaden knowledge about the factors influencing their bioaccessibility, including nutritional factors. Therefore, the objective of this research was to determine the antioxidative and antimicrobial nutritive properties and potential bioaccessibility of plant seeds and microalgae rich in protein and polyphenolic compounds. Plant seeds rich in protein—i.e., black cumin, milk thistle, fenugreek, almonds, white sesame, white mustard, eggfruit and the two most popular algae, chlorella and spirulina—were analyzed for total polyphenolic compounds (TPC) and antioxidant properties (ABTS, FRAP), as well as their potential bioaccessibility, antimicrobial activity, basic chemical composition and amino acid profiles. With regard to the TPC, the highest levels were found in star anise, followed by milk thistle, white mustard and fenugreek, whereas the lowest were noted in white sesame, almonds, eggfruit, spirulina and chlorella. White mustard and milk thistle showed the highest antioxidant capacities and almonds, eggfruit, spirulina, and chlorella the lowest according to the ABTS and FRAP assays. The widest spectrum of microbial growth inhibition was detected for fenugreek extract, which showed antimicrobial activity against four analyzed microorganisms: *B. subtilis*, *P. mirabilis*, *V. harveyi* and *C. albicans*. The protein from seeds of black cumin, milk thistle, white mustard and eggfruit and chlorella was not limited by any essential amino acids. Among all analyzed plants, fenugreek seeds were judged to have potential for use in food formulation operations in view of their antioxidant activity and amino acid profile. Based on the results, intake of polyphenols together with protein in plant seeds does not have a major impact on the potential bioaccessibility of a range of polyphenols and phenolic metabolites.

Keywords: plant species; spirulina; chlorella; bioactive compounds; antiradical activity; ferric reducing antioxidative power; potential bioaccessibility; amino acid profile

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1. Introduction

According to the International Organization for Standardization [1], spices are natural plant products used to improve the flavor, aroma, taste and color of food products that may also have additional properties; e.g., antioxidant or bacteriostatic properties. There are about 109 spices grown in different parts of the world, and India is known as the "Land of Spices". Spices are derived from different parts of plants: seeds (e.g., cardamom), leaves

(e.g., bay leaf), flowers buds (e.g., clove), fruits (e.g., pepper), bark (e.g., cinnamon) and rhizome (e.g., ginger). A wide variety of spice plants provide edible seeds. Seeds are the dominant source of human calories and protein [2,3]. Seed protein is a composite of hundreds of different enzymes and structural proteins; however, its protein complement is dominated by a family of storage proteins [4]. The most important and popular seed food sources are cereals, followed by legumes and nuts. Spice plants are used in beverages, liquors and pharmaceutical, cosmetic and perfumery products. Some algae, such as spirulina and chlorella, are also used as color and flavor additives. They can be found in many applications around the world; e.g., powdered algae are used in a wide range of products, such as pasta, soups and sauces [5,6]. Nutritionists at the Food and Nutrition Institute have placed spice plants in a new nutrition pyramid along with updated guidance on the proper behavior and nutrition required to stay healthy. Spice plants, due to their aromatic and taste properties, can help diversify people's diets and, most importantly of all, limit salt consumption [7].

In terms of nutrition, spice plants contain variable amounts of protein, fat and carbohydrates and small quantities of vitamins and inorganic elements. Moreover, they supply secondary plant metabolites, including glucosides, saponins, tannins, alkaloids, essential oils, organic acids and others, that possess medicinal, antioxidant and antimicrobial properties [8,9]. The bioaccessibility of polyphenols—i.e., the extent to which they can be released and absorbed in the digestive tract and used by the body—is an important issue. The affinity and binding of proteins from different sources to phenolic compounds is a widely studied phenomenon [10]. Whether this interaction also impacts the bioaccessibility of polyphenols is still a matter of debate. The content and relative bioaccessibility of antioxidant components from plant raw materials and products (various types of nuts, cereal grains, groats, lupine seeds, etc.) depend on many factors, including the type of raw material used (species, variety, origin), the degree of purification and the type of technological or culinary processing, as well as the composition of the food product [3,11]. Compounds contained in the plant matrix may limit the absorption of antioxidant ingredients in the gastrointestinal tract; for instance, fiber (e.g., hemicellulose), bivalent elements and sticky and protein-rich meals may limit the bioaccessibility of polyphenols, while easily digestible carbohydrates, fats (especially for hydrophobic polyphenols; e.g., curcumin) and antioxidants may increase the availability of these compounds [12]. In order to understand the biological activity of polyphenols, it is necessary to broaden knowledge about the factors influencing their bioaccessibility, including nutritional factors.

The antimicrobial activity of spice plants has been an object of interest for scientists since before the 20th century. Since that time, numerous different plants have been tested for antibacterial and antifungal properties, and many of them have been found to have activities against microorganisms. However, the major antimicrobial components of spices are in their essential oils. The majority of the antimicrobial components of spices are phenol compounds with a hydroxyl group (-OH) [13,14]. The most active are carvacrol, eugenol and thymol [15,16]. Some of these compounds are also toxic to animals, but others may not be toxic. Indeed, many of these compounds have been used in the form of whole plants or plant extracts for food or medical applications for humans [14]. Additionally, plants and their extracts have important potential as manipulators of rumen fermentation, providing productivity and health benefits [17]. They have specific effects on members of the rumen microflora and fauna that can be beneficial for animal productivity and health [14].

Recently, there has been a shift in the food industry towards natural compounds. Consumers are increasingly looking for products that meet their requirements not only in terms of taste but also with regard to having a varied composition and beneficial nutritional value and functional. In response to the increase in consumer knowledge about the impact of food on health and well-being, producers are being forced to look for new solutions in food production. In highly developed countries, the market response to such a problem is the production of food with special health values, referred to as functional food. These are foods that, apart from nutrients, also contain compounds that have a beneficial

effect on health, development and well-being [18]. These substances include dietary fiber, oligosaccharides, alcohol derivatives of sugars, amino acids, peptides, proteins, glycosides, alcohols, isoprenoids, vitamins, choline compounds, lactic acid bacteria, minerals and unsaturated fatty acids, as well as antioxidants and phytochemicals. Food referred to as functional must influence processes that significantly reduce the risk of developing certain diseases, the modulation of which enables improvements in health. Consumer expectations prompted our research aimed at determining the antimicrobial and antioxidative properties and amino acid profiles of proteins in selected spice plants and algae widely used in the kitchen. Since the ability to synthesize amino acids in human organisms is limited, it is necessary to analyze seed plants, and in the case of vegetarians and vegans, plants and plant products are the only sources of these valuable constituents indispensable for the normal functioning of the organism.

In the literature, much information on the antimicrobial and antioxidant activities of spice plants is available, but data on the potential bioaccessibility of their phenolic compounds and antioxidant properties are scarce. It is known that the activity of polyphenols may, however, be hampered when consumed together with protein-rich food products due to the interaction between polyphenols and proteins [19,20]. Therefore, the objective of this research was to determine the antiradical capacities, reducing power, antimicrobial properties and amino acid profiles of selected spice plants and microalgae with a view to investigating their potential as food supplements and food additives for use in various systems. Additionally, an experiment was performed to simulate gastrointestinal digestion in an in vitro model in order to determine the potential bioaccessibility of the polyphenols and antioxidant properties in the plants.

2. Materials and Methods

2.1. Plant Samples

Plant seeds rich in protein—namely, black cumin (*Nigella sativa*), milk thistle (*Silybum marianum*), fenugreek (*Trigonella foenum-graecum* L.), almond (*Prunus dulcis*), white sesame (*Sesamum indicum* L.), white mustard (*Sinapis alba*), star anise (*Illicium verum* Hook. f.), powdered biomass from eggfruit (*Pouteria lucuma* spp.)—as well as selected algae—spirulina (powdered cyanobacteria biomass *Arthospira plantensis* spp.) and chlorella (powdered biomass from *Chlorella vulgaris* spp.)—were purchased from the Market Hall in Wrocław (Lower Silesia, Poland). Before analysis, samples (except algae) were milled using a GM 200 mill (Retsch GmbH, Haan, Germany). All plant samples were stored in a freezer at −18 °C till further analysis.

2.2. Polyphenolic Compounds and Antioxidant Properties

2.2.1. Extraction Procedure

The material (~1 g) was mixed with 10 mL of 80% MeOH in deionized water with 1% HCl. Then, samples were sonicated twice (800 W, 40 Hz, Sonic 6D, Polsonic, Warsaw, Poland) for 20 min at 20 °C and left for 24 h at 4 °C. After this procedure, the extract was centrifuged for 10 min at 19,000× *g*, and the supernatant was filtered through a hydrophilic PTFE with a 0.20 µm membrane (Millex Samplicity Filter, Merck, Darmstadt, Germany) and used for tests.

2.2.2. Total Polyphenolic Compounds

Total polyphenols were measured using the Folin–Ciocalteu method [21]. Briefly, the extract (100 µL) was mixed with distilled water (2000 µL), Folin–Ciocalteu phenol reagent (200 µL) and 20% sodium carbonate solution in water (1000 µL). The sample was incubated for 1 h in the dark at 20 °C. The absorbance was measured at 765 nm (UV-2401 PC, Shimadzu Corp., Kyoto, Japan). Total polyphenolics are presented in mg of gallic acid equivalents (GAE)/g dry matter (DM).

2.2.3. Antioxidant Activity

The procedures for the determination of antiradical activity, using 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and ferric reducing antioxidative power (FRAP) tests, have been described by Re et al. [22] and Benzie and Strain [23], respectively. Briefly, 30 µL of the sample was mixed with 3 mL of ABTS reagent, and 100 µL of the sample was mixed with 3 mL of FRAP reagent. After 6 and 10 min of reaction, respectively, the absorbance was measured at 734 nm for ABTS and 593 nm for FRAP (UV-2401 PC spectrophotometer; Shimadzu, Kyoto, Japan). The antioxidant potency is presented in µmol of Trolox/g DM.

2.3. Potential Bioaccessibility

In vitro digestion was carried out as described previously by Minekus et al. [24], with the modifications described by Lachowicz et al. [25]. For gastrointestinal digestion, the spice plants and algae were mixed with PBS buffer (pH 7.4) and simulated salivary fluid. After that, the material was shaken at 37 °C. The pH of the sample was changed to pH 3, and the mixture was shaken at 37 °C. Then, the pH of digests was changed to 7. The mixtures underwent intestinal digestion in vitro for 120 min at 37 °C. After in vitro digestion, the samples were centrifuged at 19,000× *g* per 10 min and used for the polyphenolic compound and antioxidant activity analyses.

2.4. Microorganism and Culture Conditions

2.4.1. Preparation

Evaluation of the antimicrobial properties of the analyzed seeds and algae was undertaken for the following strains of microorganisms: (1) the Gram-positive bacteria *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 9538), *Enterococcus hirae* (ATCC 10542) and *Enterococcus faecalis* (ATCC 29212); (2) the Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 10536), *Proteus mirabilis* (ATCC 2011) and *Vibrio harveyi* (ATCC 12126); and (3) the yeast *Candida albicans* (ATCC 10231). All the microorganisms were obtained from the culture collection of the Department of Biotechnology and Food Microbiology (WUELS, Wrocław, Poland). Bacteria were grown in Luria broth (LB) (Sigma-Aldrich, Poznań, Poland; containing 5 g/L yeast extract 10 g/L tryptone and 5 g/L NaCl) at 37 °C; yeast was grown in yeast extract peptone dextrose (YPD) broth (Sigma-Aldrich, Poznań, Poland; containing 20 g/L bacteriological peptone, 10 g/L yeast extract and 20 g/L glucose) at 30 °C. Agar, at a concentration of 2%, was added to the medium when required. To prepare an inoculation culture for the agar diffusion assay, the microorganisms were grown in a 0.1 L flask containing 10 mL of proper medium on a rotary shaker at 37 °C or 30 °C and 180 rpm for 48 h. The cells was washed in sterile distilled water and adjusted to OD600 = 1.

2.4.2. Extraction Procedure

Extracts of plants were prepared by mixing 1 g of sample with 10 mL of ethanol and stirring with a vortex for 2 h at 25 °C. Then, the solution was centrifuged at 6000× *g* for 5 min and the supernatants were sterilized using the filtration method with a 0.22 µm syringe filter. The filtrates were kept in sterilized vials in refrigeration conditions.

2.4.3. Determination of Antimicrobial Properties with Agar Diffusion Assay

Antimicrobial activities of miscellaneous spice ethanol extracts were tested with the agar diffusion assay (the well diffusion assay). On each plate, which contained the proper (LB or YPD) medium, three wells were produced. Wells on vaccinated agar plates (200 µL of bacterial or yeast standardized overnight to OD600 = 1) were produced with a sterile Pasteur pipette (Ø 8.4 mm diameter). Two were filled with 100 µL of the tested extract, and the third contained 100 µL of ethanol as a control sample. Initially, the plates were incubated for 6 h at 4 °C to achieve full diffusion of the solution tested in the agar medium.

Then, the plates were incubated at 30 °C or 37 °C for 24 h and the diameters of the resulting inhibited growth around each well were measured. All tests were carried out in triplicate.

2.5. Chemical Composition

The dry matter (DM), total protein, fat and ash content were evaluated according to the methods described by the Association of Analytical Chemists [26]. The moisture content of the analyzed samples was determined on the basis of weight loss during thermal drying at 105 °C until a constant weight was achieved. Total nitrogen was determined with the Kjeldahl method using a Büchi Distillation Unit K-355 (Athens, Greece). A nitrogen-to-protein conversion factor of 6.25 was used as a standard [27]. Fat content was determined by means of the Soxhlet method in a Büchi B-811 apparatus (Flawil, Switzerland) with the use of diethyl ether after hydrolysis of the sample with 4N HCl. The total ash content was determined by adding 1 g of the protein preparation to a crucible, incinerating it in a muffle furnace at 550 °C and determining the weight of the residue. Total carbohydrates were calculated using a difference calculation (100 – the sum of protein, fat, ash and moisture). Data are reported as mean values ± standard deviation (SD) for two measurements.

2.6. Amino Acid Composition

The analyzed samples were previously degreased with the use of Soxhlet's apparatus. Then, the materials were acid hydrolyzed [28,29] and placed in an AAA400 automatic amino acid analyzer (INGOS s.r.o., Prague, Czech Republic). A two-wavelength photometer (440 and 570 nm) was employed as a detector. The length of the column packed with ion exchanger Ostion LG ANB (INGOS) was 350 × 3.7 mm, and the column temperature was maintained at 40–70 °C and the detector temperature at 121 °C. The amino acids were quantified using the ninhydrin method. Glutamine and asparagine was expressed as glutamic acid and aspartic acid, respectively. No analysis was carried out for tryptophan. Calculations were performed with the computer program Chromulan (Pikron s.r.o., Prague, Czech Republic). All amino acid profiles were analyzed in duplicate.

2.7. Scoring of Amino Acids

The amino acid score (AAS) was calculated for adults using the standard method recommended by the FAO/WHO [30]:

$$\text{AAS} = \frac{\text{essential amino acids contents}}{\text{recommended essential amino acids}} (\%)$$

2.8. Statistics

The data are presented as means ± standard deviation. The results were compared using the one-way Duncan's multiple range test (ANOVA). Statistica version 13.3 (Dell Software Inc., Round Rock, TX, USA) was used for the statistical analyses. Differences were considered significant when $p \leq 0.05$.

3. Results and Discussion

3.1. Polyphenolic Compounds, Antioxidant Activity and Potential Bioaccessibility

The results for total phenolics content (TPC) and for the potential bioaccessibility of polyphenolic compounds after in vitro digestion are presented in Figure 1A. The TPC was statistically significant for the chosen plant seeds and algae (Figure 1A). The TPC ranged from 29.44 to 1635 mg GAE/g DM. In order, TPC was highest in star anise, followed by milk thistle, white mustard and fenugreek, and the lowest content was noted in white sesame, almonds, eggfruit, spirulina and chlorella. The TPC analyzed in all plant seeds and algae was similar to data from previous studies [31–34]. The TPC in fenugreek was around give times higher, and that in white sesame seeds was around eight times lower, than the results obtained by Mashkor [35] and Lin et al. [36]. Generally, differences in the results for TPC were likely due to genotypic and environmental differences, such as temperature, climate, location, pest exposure, diseases within analyzed species and determination methods [37].

Moreover, each plant seed and algae could generally contain different mixtures of polyphenols, which influenced the TPC [38]. After the in vitro analysis in a simulated digestive system, the highest potential bioaccessibility for the TPC among the tested plant seeds and algae was noted in white sesame, almonds, spirulina and chlorella. The TPC determined in fenugreek, white mustard, black cumin and eggfruit was also potentially bioaccessible, as their values were above 1 [25]. Among all the analyzed materials, star anise and milk thistle presented the lowest potential bioaccessibility for TPC after in vitro digestion. Thus, the best plant seeds for the production of new functional food were found to be white sesame, almond, spirulina and chlorella. However, fenugreek, white mustard, black cumin and eggfruit can also be added to new nutraceutical food, but their potential bioaccessibility indexes will depend on various factors; e.g., the type of food, the enriched matrix food, the interaction of polyphenolic compounds with their chemical compounds and behavior of the ingredients in the digestive system [39]. The differences in the obtained values for the potential bioaccessibility of polyphenolic compounds may have resulted from the quality of the polyphenolic compounds. It has been previously found that the process of digestion itself can enhance the release of bioactive compounds, especially polyphenols [40–42], whereas the low relative bioaccessibility for the ingredients could suggest interactions with other components of the research material, such as proteins and/or enzymes in the digestive system [39]. The formation of protein–phenolic complexes can significantly affect protein hydrophobicity, structure and solubility, as well as the isoelectric point and thermal stability. Additionally, the combination of phenolic compounds with proteins may affect the blocking of various amino acid residues. Their complexation can also impact the bioaccessibility and antioxidant activity of phenolics [43].

The results for antioxidant activities (ABTS and FRAP assays), as well as the potential bioaccessibility of these activities after in vitro digestion, are presented in Figure 1B,C, respectively. The antioxidant capacity of the analyzed plant seeds and algae ranged from 0.01 (white sesame) to 1.79 μmol/g DM (star anise) in the ABTS assay (Figure 1B) and from 0.24 (fenugreek) to 71.75 μmol/g DM (star anise) in the FRAP assay (Figure 1C). Among the plants and algae, star anise, milk thistle and white mustard seeds showed the highest antioxidant capacity, whereas almond, spirulina and white sesame had the lowest in both tests. The high antioxidant activity in star anise, milk thistle and white mustard seeds was likely due to their having the highest TPC, as a positive linear correlation between TPC and antioxidant capacity in plant seeds and algae was observed. Additionally, these plants contained high amino acid and total proteins contents. The exception was eggfruit, which had the lowest concentrations of total proteins and low TPC. The antioxidant activities (ABTS and FRAP assays) measured in the milk thistle and fenugreek were similar to the results presented by Wojdyło et al. [34]. The antioxidant activity measured in the other samples was similar to the results from other authors [31–33]. According to the bioaccessibility index calculated for the analyzed materials, the antiradical ingredients, especially in fenugreek, white sesame and eggfruit, and the reducing compounds in white sesame, chlorella, almond and spirulina were highly bioaccessible. These plants with high relative accessibility indexes for polyphenols and antioxidant ingredients could be good functional additions for phenolics and protein-rich foods. The lowest potential bioaccessibility (below 1) for antioxidant activity was noted in the milk thistle and star anise. The low relative accessibility index for polyphenols could have resulted from complexes with proteins, limiting their absorption and also their ability to exert antioxidant activity [43]. Antioxidant properties mainly depend on the levels of bioactive compounds, including polyphenolic compounds, but also on the interactions with other compounds in the research material, such as proteins. The bioaccessibility of polyphenols depends on several factors, which include their release from the food matrix, their molecular size, their hydrophilic/lipophilic balance as related to their glycosylation and their different pH-dependent transformations (degradation, hydrolysis, epimerization and oxidation within the gastrointestinal tract), as well as their solubility and the interactions between polyphenols and food components [44]. In contrast, in research conducted on soy and dairy drinks, the bioaccessibility of polyphenols was

not significantly affected when polyphenols were consumed in protein-rich products [19]. Therefore, the interactions of proteins with polyphenols and the formation of complexes are important factors influencing the functional quality of products and their pro-health properties and should be taken into account during the design of functional foods [43].

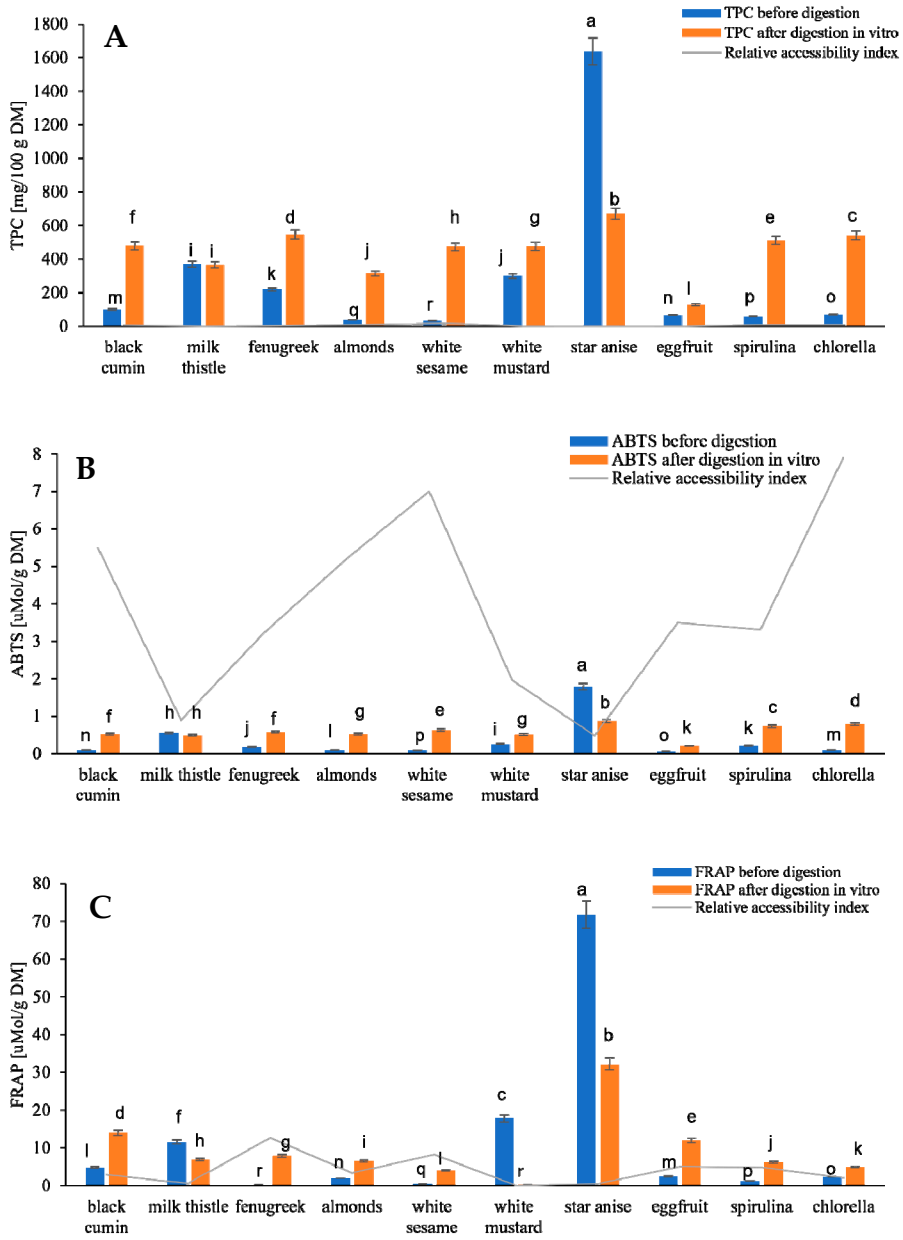

Figure 1. Total polyphenols content (**A**), antioxidant activity (ABTS+ (**B**); FRAP (**C**)) and their potential bioaccessibility in the analyzed plant materials.

Antioxidant activity tests are commonly used to assess the redox potential of compounds. Thus, the Person correlations between the antioxidant results (from the ABTS and FRAP assays) and the TPC were 0.992 and 0.986, respectively. As in the study by Wojdyło et al. [34] a strong correlation between antioxidant capacity and TPC in the analyzed plants was noted.

3.2. Antimicrobial Activity

The zones of inhibition of the growth of microorganisms in the analyzed plant materials are shown in Table 1. This study found that various bacteria and yeast were sensitive

to several plant seed and algae ethanol extracts. Milk thistle extract was found to show activity against both Gram-positive (*B. subtilis*, *E. faecalis)* and Gram-negative bacteria (*P. mirabilis).* Growth of *E. faecalis* was also inhibited by cold-pressed milk thistle seed flours [45]. Food-borne *E. faecalis* is connected with common dental diseases and is able to colonize oral biofilm [46]. Accordingly, milk thistle extract may have beneficial properties as a food additive. Almond ethanol extract showed activity against *B. subtilis* and *V. harveyi.* The presented results expand the spectrum of plant seeds, with the ethanol extracts of fenugreek, white sesame, star anise and, as mentioned before, milk thistle, as well as almond, showing anti-*Bacillus* activity. Star anise extract achieved the highest ratings for inhibition of *B. subtilis* growth—approximately 9 mm. In 2009, there was a report about a novel toxin produced by *B. subtilis* strains isolated from foods that could be related to food poisoning outbreaks [47]. *V. harveyi* is the prevalent bacterial pathogen in farmed shrimp [48]. Activity against this Gram-negative bacteria was also shown by fenugreek, white sesame and eggfruit ethanol extracts. The World Health Organization identified *P. aeruginosa* as a quality indicator for drinking water [49]. Effective growth inhibition of *P. aeruginosa* was observed for white mustard seed extract. Previous studies have shown similar activity in extracts from basil, clove, cumin, dill, garlic and thyme [2]. In August 2008, *P. mirabilis* was identified as responsible for an outbreak of food poisoning in Beijing [50]. Eggfruit ethanol extracts may be useful to reduce the growth *P. mirabilis*—the extract revealed significant inhibition zones (4.18 mm). Activity against this Gram-negative bacteria was also shown by milk thistle, fenugreek, white mustard seed and star anise extracts. Previous research only pointed to anti-*Proteus* activity in garlic [2]. Fenugreek and white sesame extracts indicated minor antifungal activities against *C. albicans.* Likewise, *C. albicans* has been found to be sensitive to garlic and clove extracts [51]. Regarding the current and previous results, there is a need to evaluate the usefulness of spices in terms of their antimicrobial activities against bacteria and fungi. Among the selected plant seeds and algae, black cumin, spirulina and chlorella showed no inhibition of the chosen microorganisms. However, cumin oil has demonstrated antimicrobial and antifungal activities in laboratory tests against Gram-positive and Gram-negative bacteria species, proving to be effective against the genera *Clavibacter*, *Curtobacterium*, *Rhodococcus*, *Erwinia*, *Xanthomonas*, *Ralstonia* and *Agrobacterium* isolated from clinical isolates and foods [8], as well as against the fungi *Penicillium notatum*, *Aspergillus niger*, *A. fumigatus*, *Microsporum canis* [8]. The strong inhibition of the growth of microorganisms by the plant materials, especially milk thistle and fenugreek, resulted from the strong positive correlation between TPC and antimicrobial activity against bacteria and yeast (the Person correlation was higher than 0.6). This could be correlated with active constituents, such as isomeric flavonolignans—namely, silibinin (silybin), silychristin and silidianin, collectively known as silymarin, which is extracted from dried milk thistle seeds [52,53], and catechin, epicatechin, gallic acid, coumaric acid, cinnamic acid and vanillic acid, found in fenugreek ethanolic extract [35].

Table 1. Zones of inhibition (mm) of the growth of microorganisms in the analyzed plant materials.

Seeds and Algae	Gram-Positive Bacteria (G+)				Gram-Negative Bacteria (G−)				Yeast
	B. subtilis	*S. aureus*	*E. hirae*	*E. faecalis*	*P. aeruginosa*	*E. coli*	*P. mirabilis*	*V. harveyi*	*C. albicans*
Black cumin	-	-	-	-	-	-	-	-	-
Milk thistle	1.56 ± 0.02	-	-	6.83 ± 0.63	-	-	3.85 ± 0.76	-	-
Fenugreek	3.05 ± 0.64	-	-	-	-	-	3.75 ± 0.68	3.73 ± 0.53	0.28 ± 0.06
Almond	3.42 ± 0.77	-	-	-	-	-	-	2.89 ± 0.49	-
White sesame	2.98 ± 0.01	-	-	-	-	-	-	0.53 ± 0.01	1.25 ± 0.01
White mustard	-	-	-	-	6.95 ± 1.38	-	3.09 ± 0.01	-	-
Star anise	8.89 ± 0.03	-	-	-	-	-	2.71 ± 0.01	-	-
Eggfruit	-	-	-	-	-	-	4.18 ± 0.26	3.05 ± 0.50	-
Spirulina	-	-	-	-	-	-	-	-	-
Chlorella	-	-	-	-	-	-	-	-	-

-, Not identified (trace or % < 0.1).

3.3. Proximate Composition

Results regarding the chemical composition of the tested seeds and algae are presented in Table 2. The tested plant materials showed similar levels of humidity not exceeding 11%. Dry matter content in the seeds ranged from 91.17% (fenugreek) to 96.38% (white sesame). Across all analyzed seed samples, the protein content ranged from 16.33 g/100 g (milk thistle) to 24.58 g/100 g (almond). However, its content was almost three times lower compared to the studied algae, where it ranged from 55.40 g/100 g (chlorella) to 69.35 g/100 g (spirulina). The results of our study show the same range of protein content as in the report by Gonçalvesa et al. [51], and Barreca et al. [54] noted that almond presents similar overall nutrient profiles even when comparing different varieties, years of production and growing regions. Furthermore, the protein content in milk thistle seeds in this study was lower than the data obtained by El-Haak et al. [55]. This varied protein content may be related to seed maturity stage and environmental conditions. The key ingredients plants need for protein production are glucose and nitrates, which are taken up from the soil by the roots. When glucose and nitrates are joined, they produce amino acids. During protein synthesis, multiple amino acids are bound together to make proteins [56]. The fat content in the analyzed samples differed, ranging between 5.13 g/100 g (fenugreek) and 52.25 g/100 g (almond).

Table 2. Chemical compositions (g/100 g) of the analyzed plant materials.

Raw Material	Dry Matter	Total Protein	Fat	Ash	Carbohydrates
Black cumin	94.43 ± 0.08 ^{d}	20.39 ± 1.22 ^{d}	36.85 ± 1.14 ^{c}	4.54 ± 0.02 ^{e}	32.65 ± 3.11 ^{d}
Milk thistle	92.83 ± 0.02 ^{f}	16.33 ± 0.08 ^{e}	23.47 ± 0.87 ^{d}	6.51 ± 0.01 ^{c}	46.53 ± 4.52 ^{c}
Fenugreek	91.17 ± 0.03 ^{g}	23.70 ± 1.05 ^{c}	5.13 ± 0.22 ^{f}	3.47 ± 0.03 ^{f}	58.87 ± 3.85 ^{b}
Almonds	95.58 ± 1.02 ^{b}	24.58 ± 0.07 ^{c}	52.25± 4.27 ^{b}	4.81 ± 0.02 ^{e}	13.94 ± 1.75 ^{f}
White sesame	96.38 ^{a} ± 0.07 ^{a}	24.06 ± 1.01 ^{c}	51.02 ± 1.89 ^{b}	5.12 ± 0.02 ^{d}	16.18 ± 2.13 ^{e}
White mustard	95.08 ± 0.02 ^{c}	20.89 ± 1.52 ^{d}	36.24 ± 0.96 ^{b}	3.72 ± 0.01 ^{f}	34.23 ± 4.74 ^{d}
Star anise	89.19 ± 1.58 ^{h}	5.92 ± 0.18 ^{f}	68.75 ± 3.69 ^{a}	3.11 ± 0.03 ^{f}	11.41 ± 1.85 ^{f}
Eggfruit	92.75 ± 2.35 ^{f}	1.72 ± 0.98 ^{g}	2.23 ± 0.87 ^{g}	0.52 ± 0.01 ^{g}	88.28 ± 9.55 ^{a}
Spirulina	94.37 ± 3.12 ^{d}	69.35 ± 4.05 ^{a}	7.52 ± 1.45 ^{e}	11.47 ± 0.05 ^{b}	6.03 ± 0.15 ^{g}
Chlorella	94.07 ± 2.01 ^{e}	55.40 ± 3.21 ^{b}	8.15 ± 1.74 ^{e}	14.13 ± 0.07 ^{a}	16.39 ± 1.45 ^{e}

Values are means ± SD of two determinations; a–h, the same letters in columns mean homogenous groups.

The chosen plant seeds and algae were characterized by very diverse carbohydrate contents, ranging from 13.94 g/100 g (almond) to 88.28 g/100 g (eggfruit). Similar carbohydrate contents were observed by Kochhar et al. [56], who analyzed fenugreek seeds. Moreover, based on data presented by Lunn and Buttriss [57], presoluble carbohydrates constitute a major part of the total carbohydrate content, whereas, in higher plants, insoluble carbohydrates are a major constituent of total carbohydrates.

3.4. Amino Acid Composition

The basis for determining the nutritional value of products is the analysis of the amino acid composition. The amino acid scores (AASs) according to the FAO/WHO reference standard [30] are presented in Table 3. Among all the analyzed samples, the limiting amino acids were lysine and methionine with cysteine. Lysine was present in an insufficient amount in white sesame and star anise seeds and in spirulina (AAS = 35.33, 30.34 and 42.31%, respectively). However, it is worth mentioning that white sesame seeds were found to be a good source of aromatic amino acids (phenyloalanine and tyrosine), histidine, glutamic acid and arginine compared to the other analyzed plant seeds (Table S1). However, the opposite results were presented by Makinde and Akinoso [58], who found that the dominant amino acid in white sesame seeds was leucine. The star anise seeds' isoleucine, leucine, sulfur amino acids and valine values were lower than those recommended by the FAO/WHO/UNU [30]. This plant is also not a good source of non-essential amino acids (Table S2). Based on the present data, it can be concluded that protein in spirulina is also

poor in histidine and sulfur amino acids. However, according to various authors [59–62], spirulina belongs to the group of products in which the protein contains sufficient amounts of essential amino acids except for methionine, cysteine and lysine.

Among the analyzed materials, fenugreek and almond were limited in sulfur amino acids (AAS = 19.87 and 16.67%, respectively). However, fenugreek seeds were found to have good quantities of isoleucine, lysine and aromatic amino acids as compared to the requirements for essential amino acids for schoolchildren. Similar data were also reported by Feyzi et al. [63], who analyzed fenugreek seeds. Therefore, this plant seed can be suggested for use in cereals and snack foods, including bread, biscuits and cakes, since it is rich in lysine and poor in histidine and methionine, in contrast to cereals. With regard to the almond seeds, the lysine value (38.33%) was lower than that recommended by the FAO/WHO/UNU [30]. In general, the protein in *Fabaceae* family crops is limited in sulfur amino acids [64]. On the other hand, these materials were found to be a good source of asparagine, glutamine, arginine and glycine compared to the other samples. Similar findings were presented by Barreca et al. [54], who analyzed almond proteins and found them to have good arginine content.

The rest of the analyzed plant seeds—i.e.: black cumin, milk thistle, white mustard, eggfruit and chlorella—demonstrated complete proteins (Table 3). Black cumin seeds were found to be a good source of aromatic amino acids (phenyloalanine and tyrosine) and glutamine. Furthermore, the amino acid composition of milk thistle protein was dominated by aromatic amino acids, but also threonine, asparagine, glutamine, arginine and serine, which was also confirmed by Sadowska et al. [65]. It is worth emphasizing that white mustard seeds were found to be good source of histidine, lysine and threonine. With regard to non-essential amino acids, there were significant amounts of aspartic acid in white mustard seeds. Powdered eggfruit was characterized by only low amounts of essential amino acids (i.e., leucine, lysine and arginine) compared to the other analyzed seeds. With regard to the non-essential amino acids, the analyzed material was found to be a good source of glutamic acid. For chlorella, the dominant essential amino acids were threonine, valine and leucine. Other authors [66] have also confirmed that chlorella's protein has the right amount of essential amino acids.

To sum up, the protein from the seeds of black cumin, milk thistle, white mustard, eggfruit and chlorella was not limited in any essential amino acids in comparison to the requirements for essential amino acids for schoolchildren (FAO/WHO/UNU [30]). Therefore, these plants can be considered a good sources of amino acids in the diet.

Table 3. Amino acid scores (AASs) for adults according to the FAO/WHO/UNU standards [30].

Amino Acid	Black Cumin	Milk Thistle	Fenugreek	Almond	White Sesame	White Mustard	Star Anise	Eggfruit	Spirulina	Chlorella	Standard
Essential											
His	31.70 ± 0.23 ^{d}	51.81 ± 0.13 ^{b}	31.35 ± 0.11 ^{d}	33.80 ± 0.12 ^{d}	35.73 ± 0.03 ^{d}	43.62 ± 0.11 ^{c}	26.12 ± 0.12 ^{e}	100.90 ± 1.98 ^{a}	15.20 ± 0.02 ^{f}	18.66 ± 0.08 ^{f}	16
Ile	39.64 ± 0.11 ^{e}	79.12 ± 0.14 ^{b}	53.37 ± 0.23 ^{c}	44.64 ± 0.15 ^{d}	47.45 ± 0.04 ^{d}	53.21 ± 0.15 ^{c}	28.60 ± 0.14 ^{f}	95.46 ± 1.65 ^{a}	44.94 ± 0.54 ^{d}	45.61 ± 0.07 ^{d}	30
Leu	65.68 ± 0.14 ^{f}	119.91 ± 0.15 ^{b}	75.08 ± 0.14 ^{e}	82.50 ± 0.08 ^{d}	84.17 ± 1.11 ^{d}	97.48 ± 1.17 ^{c}	45.05 ± 0.17 ^{g}	132.41 ± 1.77 ^{a}	73.28 ± 0.78 ^{e}	82.61 ± 0.15 ^{d}	61
Lys	48.64 ± 1.18 ^{d}	92.52 ± 0.09 ^{a}	75.67 ± 0.11 ^{c}	38.33 ± 0.03 ^{e}	**35.33** ± 0.03 ^{e}	88.62 ± 0.18 ^{b}	**30.34** ± 0.09 ^{f}	83.21 ± 0.88 ^{b}	**42.31** ± 0.17 ^{e}	50.40 ± 0.26 ^{d}	48
Met + Cys	26.19 ± 2.01 ^{d}	42.80 ± 0.07 ^{b}	**19.87** ± 0.05 ^{e}	**16.67** ± 0.01 ^{f}	35.83 ± 0.03 ^{c}	38.39 ± 0.02 ^{b}	16.69 ± 0.08 ^{f}	65.15 ± 0.77 ^{a}	20.46 ± 0.08 ^{e}	18.45 ± 0.01 ^{e}	23
Phe + Tyr	82.30 ± 0.22 ^{e}	154.02 ± 1.12 ^{b}	79.91 ± 1.11 ^{e}	106.68 ± 2.04 ^{c}	102.77 ± 1.56 ^{c}	96.98 ± 0.09 ^{d}	59.85 ± 1.15 ^{g}	224.11 ± 2.12 ^{a}	69.62 ± 0.88 ^{f}	75.43 ± 0.58 ^{f}	41
Thr	46.99 ± 0.13 ^{d}	78.82 ± 1.13 ^{b}	41.88 ± 1.12 ^{d}	37.96 ± 1.04 ^{e}	49.76 ± 0.42 ^{d}	65.54 ± 1.02 ^{c}	25.77 ± 0.05 ^{f}	92.48 ± 0.88 ^{a}	43.45 ± 0.54 ^{d}	46.62 ± 0.23 ^{d}	25
Val	53.08 ± 1.15 ^{c}	100.78 ± 0.08 ^{a}	44.35 ± 0.08 ^{d}	53.92 ± 1.20 ^{c}	61.09 ± 0.91 ^{c}	71.37 ± 0.94 ^{b}	32.80 ± 0.19 ^{e}	112.81 ± 1.55 ^{a}	56.71 ± 0.74 ^{c}	58.74 ± 0.45 ^{c}	40
AAS (%)	101.3 ± 1.17 ^{c}	178.3 ± 0.05 ^{a}	82.8 ± 0.06 ^{d}	69.5 ± 1.17 ^{e}	73.6 ± 0.08 ^{e}	159.8 ± 2.01 ^{b}	63.3 ± 1.19 ^{f}	173.5 ± 1.44 ^{a}	88.1 ± 1.15 ^{d}	105.0 ± 1.25 ^{c}	100
Non-essential											
Asp	102.78 ± 2.22 ^{e}	190.59 ± 0.04 ^{b}	120.31 ± 2.06 ^{d}	145.02 ± 2.33 ^{c}	107.76 ± 1.17 ^{e}	96.57 ± 0.15 ^{f}	55.44 ± 1.23 ^{g}	296.71 ± 2.12 ^{a}	85.85 ± 1.19 ^{f}	88.98 ± 0.45 ^{f}	-
Glu	264.27 ± 0.11 ^{c}	417.83 ± 1.10 ^{a}	197.64 ± 2.11 ^{d}	371.79 ± 3.01 ^{b}	257.21 ± 2.21 ^{c}	260.87 ± 2.15 ^{c}	86.14 ± 1.78 ^{f}	204.03 ± 2.03 ^{d}	122.40 ± 1.42 ^{e}	126.93 ± 1.29 ^{e}	-
Ala	48.34 ± 0.08 ^{e}	85.58 ± 0.88 ^{b}	44.02 ± 1.14 ^{f}	56.80 ± 2.12 ^{d}	60.12 ± 1.15 ^{d}	62.27 ± 0.13 ^{d}	33.29 ± 0.33 ^{g}	150.37 ± 1.98 ^{a}	70.90 ± 0.58 ^{c}	70.20 ± 0.57 ^{c}	-
Arg	95.30 ± 1.16 ^{d}	208.01 ± 0.51 ^{a}	116.47 ± 0.14 ^{c}	161.82 ± 0.15 ^{b}	167.48 ± 2.03 ^{b}	85.90 ± 0.87 ^{e}	47.71 ± 0.77 ^{g}	86.75 ± 0.19 ^{e}	72.00 ± 0.95 ^{f}	66.05 ± 0.56 ^{f}	-
Gly	70.60 ± 0.09 ^{c}	109.81 ± 1.56 ^{a}	54.88 ± 0.13 ^{e}	84.67 ± 0.09 ^{b}	65.74 ± 0.08 ^{d}	71.99 ± 0.22 ^{c}	36.60 ± 0.55 ^{g}	109.68 ± 1.11 ^{a}	43.94 ± 0.49 ^{f}	49.51 ± 0.96 ^{f}	-
Pro	41.21 ± 1.13 ^{e}	79.94 ± 0.34 ^{c}	48.63 ± 0.01 ^{d}	54.96 ± 0.07 ^{d}	34.56 ± 0.17 ^{f}	86.85 ± 0.23 ^{b}	33.51 ± 0.21 ^{f}	118.33 ± 1.16 ^{a}	29.22 ± 0.09 ^{g}	30.07 ± 0.03 ^{f}	-
Ser	52.45 ± 2.02 ^{c}	102.55 ± 2.13 ^{a}	55.04 ± 0.14 ^{b}	50.75 ± 0.07 ^{c}	59.38 ± 0.26 ^{b}	59.50 ± 0.15 ^{b}	32.63 ± 0.11 ^{e}	109.29 ± 1.84 ^{a}	41.67 ± 0.23 ^{d}	41.93 ± 0.05 ^{d}	-

Data are means of two replicates. a–g, the same letters in columns mean homogenous groups; bold values indicate first limiting amino acids.

4. Conclusions

As shown by the present results, among the analyzed plants and algae, white mustard and milk thistle showed the highest antioxidant capacities in the ABTS and FRAP assays. The widest spectrum of microbial growth inhibition was indicated for fenugreek extract, which showed antimicrobial activity against four of the analyzed microorganisms, including *B. subtilis*, *P. mirabilis*, *V. harveyi* and *C. albicans*. Among all the analyzed plants, fenugreek seeds showed potential for use in food formulation operations due to their antioxidant activity and amino acid profile. Based on the results, the intake of polyphenols together with protein in plant seeds does not have a major impact on the potential bioaccessibility of a range of different polyphenols and phenolic metabolites.

This work has shown that fenugreek seeds have potential for use in food formulation operations due to their antioxidant activity and amino acid profile. The observed antibacterial activity in this plant suggests that it could play a dual role in food and in non-food systems, where it may also find uses. Bringing out its full potential for utilization in these systems is, however, dependent on the full characterization of biologically active components in the plant.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12168136/s1. Table S1. Concentrations of essential and relatively essential amino acids (g/100 g protein) in the analyzed raw materials; Table S2. Concentrations of non-essential and relatively non-essential amino acids (g/100 g protein) in the analyzed raw materials and accepted patterns.

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