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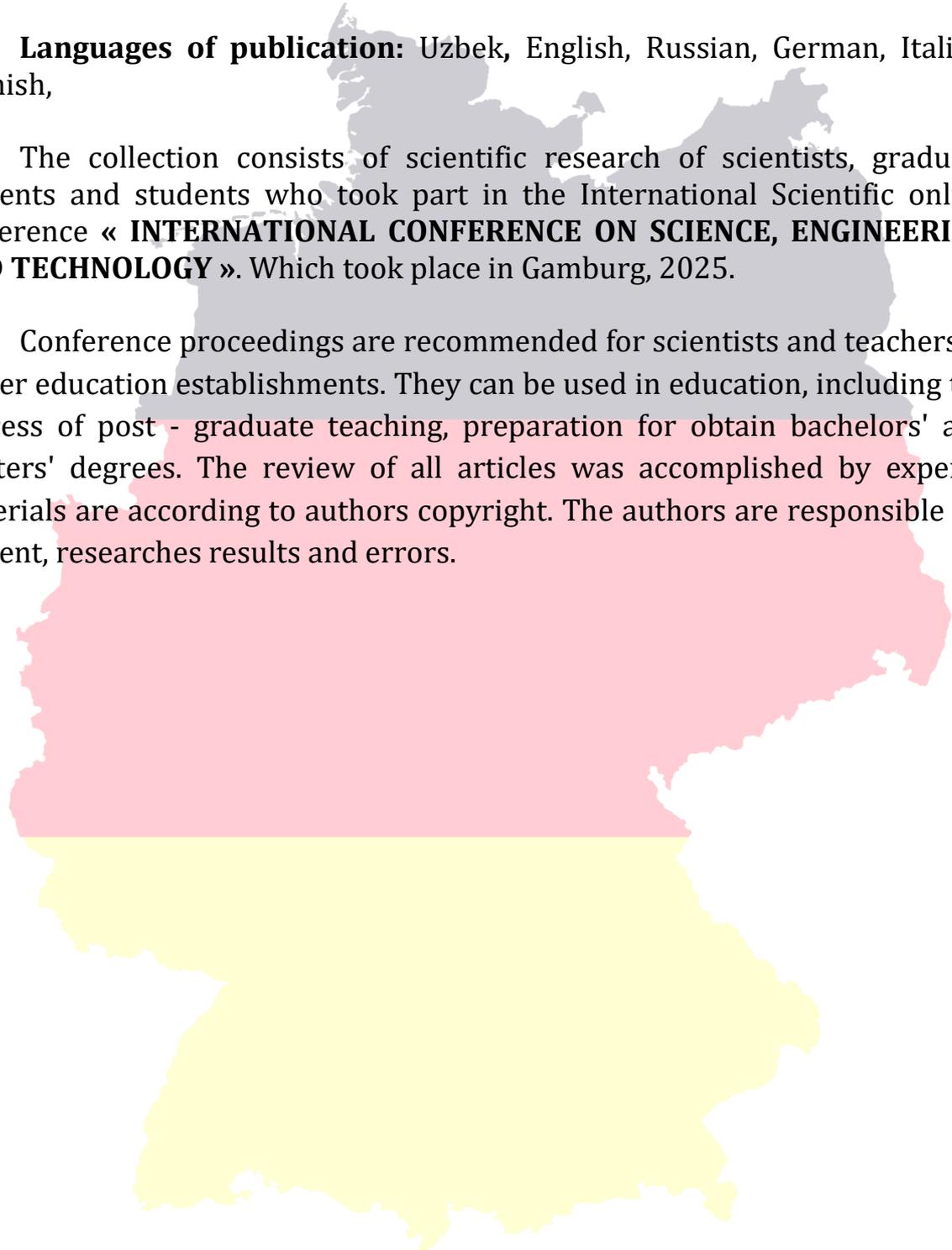


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ANTIOXIDANT PROPERTIES AND PROTEIN CONTENT OF WATERMELON

Khojiyeva Guzal Uktamovna

Assistant of the Department of " Agricultural and food technology " of Jizzakh Polytechnic Institute. E-mail: xojiyeva.guzal@mail.ru.

Shoyiqulova Bibisora Shavkat qizi

Student of Jizzakh Polytechnic Institute.

E-mail: guzal.jpi@mail.ru Phone number +998 90 495 15 00

Abstract: The growing interest in a plant-based diet leads to the search for new sources of protein in the human diet as an alternative to animal proteins. Plant materials that can supplement protein as additives in food products are being studied. Watermelon seeds (*Citrillus lanatus* L.) are rich in proteins and waste from the food industry; however, their extraction is not completely cost-free, and the flour production process may involve additional costs related to their extraction and processing. The studies showed that watermelon seed protein concentrate, obtained using the alkaline extraction method, contained 82.52 g/100 g of protein and 1.51 g/100 g of fat. The polyphenol content in the protein preparation from defatted watermelon seeds was 1.9 mg gallic acid/g, and the antioxidant activity of the concentrate was 29.26 $\mu\text{mol Trolox/g}$ (by the ABTS+). The obtained watermelon seed protein concentrate was characterised by solubility of more than 80% (at pH = 10), water absorption at the level of 2.46 (g water/g) and oil absorption equal to 2.1 (ml oil/g), showed poor foaming properties (1.51%), and was characterised by low emulsification.

Keywords: watermelon seed flour, protein concentrate, nutritional properties, antioxidant properties, functional properties.

Introduction: Watermelon (*Citrullus lanatus* L.), a member of the Cucurbitaceae family, is one of the plant species commonly used as human food [1]. Watermelon originates from South Africa, and its cultivation has been widespread in regions around the world [2]. According to available data, the annual global production of watermelon is about 100 million tons [3]. The world leaders in watermelon production are China, Iran, Turkey, and Brazil [3]. Watermelon is a horticultural plant grown mainly for its sweet and juicy fruit, which consists of 68% pulp, about 2% seeds, and 30% peel. In terms of chemical composition, watermelon fruit is a source of many nutrients, i.e., proteins, fats, carbohydrates, minerals (potassium, magnesium), and vitamins (mainly vitamin C) [4,5]. However, more than 90% of the total mass of the fruit is water [6].

Watermelon is used in the food industry to produce juices, cocktails and nectars, and its processing generates significant amounts of unused peel and



seeds, which are generally treated as waste. The peel is used to produce products, such as pickles and preserves and to extract pectin [7]. Taking into account FAO data from 2022 [3], the estimated waste from watermelons in the form of seeds is from two to five million tons. Watermelon seeds may have potential use in the food industry because when pressing fruit juices, they remain intact after removing the pulp and peel. In recent years, watermelon seeds have become an innovative raw material used in the oil industry [8]. However, they are most often an additive used to feed farm animals due to their rich chemical composition [9]. Watermelon seeds contain an average of 42% fibre, 27% fat, 17% protein, 12% carbohydrates, and 2% ash, and are also a source of minerals, including calcium, phosphorus, potassium, magnesium, sodium, and zinc [10]. In addition, watermelon seeds are a source of polyphenols and, therefore, have antioxidant properties, and their addition to baked goods can increase their health benefits [11] or flour obtained from them can be a substitute for wheat flour in bakery products, including in cookies [12] or bread [13].

Proteins from seeds are among the top 10 global markets for functional food production, valued for their essential amino acids and health-promoting properties [25]. Watermelon seed proteins, with their rich amino acid profile and nutritional value, are an attractive raw material for food products such as bread, cakes, protein bars, and beverages. As a plant-based protein sourced from waste, it offers an alternative to animal proteins, especially for vegan and vegetarian diets. However, factors like pH, temperature, ionic strength, solvent type, extraction time, and the solid-liquid ratio can complicate industrial-scale extraction [21]. Polyphenols in watermelon seeds may also enhance their antioxidant properties, making them valuable in functional foods [21]. This study aimed to isolate watermelon seed proteins and assess their nutritional, antioxidant, and functional properties.

Chemical Composition

The chemical composition of the analysed watermelon seed flour and protein concentrate are presented in [Table 1](#). The defatting process affected the content of dry matter, total protein, fat and carbohydrates in the flour obtained from watermelon seeds. An increase in the total protein content by 8% and a decrease in the fat content by 88% were noted. The increase in the protein content in the flour resulted from the defatting process, which concentrates the protein by reducing other components, such as fatty acids. The presented data were similar to the results obtained by El-Adawy and Taha [10], analysing defatted watermelon seed kernels. The protein content of defatted watermelon seed flour is lower than in soy and sunflower flours (50–53%) [20]. However, watermelon seeds and the protein preparations obtained from them can be a source of protein in some food recipes. The ash



content in both analysed flours was statistically insignificant and ranged from 3.53 to 3.67 g/100 g.

Table 1.

Chemical compositions (g/100 g) of watermelon seed flour and protein concentrate.

Analysed Material	Dry Matter	Total Protein	Ash	Fat	Carbohydrates
Non-defatted flour	95.75 ± 0.02 ^a	32.08 ± 0.01 ^c	3.53 ± 0.04 ^a	6.83 ± 0.07 ^a	53.31 ± 0.06 ^b
Defatted flour	95.26 ± 0.05 ^a	34.71 ± 0.02 ^b	3.67 ± 0.05 ^a	0.86 ± 0.08 ^c	56.02 ± 0.10 ^a
Protein concentrate	95.16 ± 0.05 ^a	82.52 ± 0.29 ^a	2.50 ± 0.2 ^b	1.51 ± 0.03 ^b	8.63 ± 0.41 ^c

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Values are means ± standard deviation. n = 3; ^{a-c} the same letters in the columns mean homogeneous groups (Duncan's test $p \leq 0.05$).

The protein preparation obtained from defatted watermelon seed flour contained over 82 g/100 g of protein in dry matter, 2.50 g/100 g of ash, and 1.51 g/100 g of fat. In the study conducted by Gadalkar and Rathod [19], the protein preparation obtained from defatted watermelon seed flour contained 54.48 g/100 g protein, which is a much lower value compared to that obtained by the authors in the present study but was characterised by a higher content of both fat (4.6 g/100 g) and ash (5.9 g/100 g).

Amino Acid Composition

Based on the amino acid profile of the tested samples (Table 2), it can be stated that the defatting process of watermelon seed flour did not statistically affect the amino acid content. Among the exogenous amino acids, leucine, phenylalanine, and valine were found in the largest amounts (on average 13.92 mg/g, 10.44 mg/g, and 9.12 mg/g, respectively). On the other hand, aspartic acid, glutamic acid, and arginine were found in the largest amounts among all the amino acids analysed (18.86 mg/g, 54.87 mg/g and 25.53 mg/g, respectively). The high content of arginine in watermelon seed flour indicates the therapeutic value of this raw material, and its content is higher than in most other oil seeds, including soybeans and peanuts [20]. The obtained amino acid profiles are consistent with the data presented in the literature. The dominant amino acids in watermelon seeds are mainly arginine, glutamic acid, aspartic acid, and serine [10,21].



Table 2.

Amino acid profile (mg/g) of watermelon seed flour and protein concentrate.

Amino Acid	Non-Defatted Flour	Defatted Flour	Protein Concentrate
IAA *			
Leucine	13.89 ± 0.07 ^b	13.94 ± 0.29 ^b	50.52 ± 0.61 ^a
Isoleucine	7.65 ± 0.07 ^b	7.61 ± 0.23 ^b	29.00 ± 0.56 ^a
Methionine	0.87 ± 0.02 ^b	0.62 ± 0.01 ^b	19.64 ± 0.30 ^a
Cysteine	1.18 ± 0.03 ^b	1.19 ± 0.08 ^b	1.65 ± 0.21 ^a
Phenylalanine	10.47 ± 0.16 ^b	10.41 ± 0.27 ^b	43.01 ± 0.54 ^a
Threonine	6.94 ± 0.15 ^b	7.11 ± 0.27 ^b	24.39 ± 0.47 ^a
Lysine	6.31 ± 0.08 ^b	6.28 ± 0.21 ^b	18.00 ± 0.30 ^a
Tyrosine	3.38 ± 0.06 ^b	3.00 ± 0.07 ^b	22.17 ± 0.42 ^a
Valine	9.04 ± 0.04 ^b	9.19 ± 0.23 ^b	33.42 ± 0.52 ^a
DAA **			
Aspartic acid	18.57 ± 0.11 ^b	19.14 ± 0.15 ^b	71.71 g ± 1.22 ^a
Glutamic acid	54.76 ± 1.21 ^b	54.98 ± 1.66 ^b	184.83 ± 4.75 ^a
Serine	9.51 ± 0.01 ^b	9.56 ± 0.41 ^b	33.20 ± 0.90 ^a
Glycine	12.38 ± 0.08 ^b	12.27 ± 0.27 ^b	33.99 ± 0.44 ^a
Alanine	9.69 ± 0.04 ^b	9.73 ± 0.35 ^b	32.42 ± 0.45 ^a
Histidine	4.79 ± 0.28 ^b	4.65 ± 0.42 ^b	16.17 ± 0.37 ^a
Arginine	26.04 ± 0.17 ^b	25.01 ± 0.35 ^b	99.73 ± 1.63 ^a
Proline	6.86 ± 0.30 ^a	7.65 ± 0.69 ^a	16.89 ± 7.65 ^a
Total amino acids	202.29 ± 1.82^b	202.06 ± 5.94^b	730.69 ± 21.34^a

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Values are means ± standard deviation. n = 2; ^{a-b} the same letters in verse mean homogenous groups (Duncan's test $p \leq 0.05$); IAA *— indispensable amino acids; DAA **—dispensable amino acids.



More than three times more amino acids, compared to the analysed flours, were found in the obtained protein concentrate (730.69 mg/g). Among the indispensable amino acids, the dominant ones were leucine (50.52 mg/g), phenylalanine (43.01 mg/g), and valine (33.42 mg/g). In turn, almost 50% of all analysed amino acids were aspartic acid (71.71 mg/g), arginine (99.73 mg/g), and glutamic acid (184.83 mg/g). Wani et al. [21] also reported the dominance of glutamic, aspartic acid, and arginine in watermelon protein isolates.

Compared with the FAO-recommended amino acid formula (Table 3), the analysis of the chemical score indices (CS) and essential amino acid index (EAAI) for watermelon seed flour and protein concentrate indicates clear differences in the amino acid profile of the tested samples. The protein concentrate was characterised by higher CS values for most amino acids, including the leucine (103.73%), isoleucine (117.00%), and phenylalanine-tyrosine pair (215.00%), resulting in the highest EAAI (109.46%) compared to non-defatted flour (63.18%) and defatted flour (56.69%). Lysine was the most limiting amino acid (48.44%) in the analysed protein concentrate. Longe et al. [26] also reported lysine and threonine as limiting amino acids in pumpkin (*Telfaria occidentalis*) based on amino acid scoring. Low levels of lysine have been reported previously in melon seed proteins.

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