

## Article

# Chemical Composition, Physicochemical and Bioactive Properties of Avocado (*Persea americana*) Seed and Its Potential Use in Functional Food Design

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**Abstract:** The appropriate use of avocado seed waste after industrial processing could reduce the problem of overconsumption and food waste in accordance with the “zero waste” concept. The presented study evaluates the physicochemical and bioactive properties of avocado seed and its possible use in functional food design, for example, cereal snacks in the form of cookies. The profile of polyphenol and lutein content was determined by chromatographic methodology, and the phenolic compounds content and antioxidant properties of the avocado seed powder were determined using spectrophotometric methods. The chemical composition (content of protein, carbohydrates, fiber, fat) and physicochemical properties, i.e., water activity, water holding capacity, and solubility in water of avocado seed powder, were examined. According to the fiber content (21.6 g/100 g) and bioactive compounds present in the avocado seed powder (content of phenolic 62.1 mg GAE/1 g, antioxidant potential (122.4 mmol Trolox/100 g), and low solubility in water (16.2%)), it could be considered a valuable additive to cereal snacks. Our designed cereal products with various amounts of added avocado seed powder (6%, 12%, and 18%) showed that 6% added powder promoted an almost five-fold increase in the polyphenol content and four-fold higher antioxidant potential of the snacks compared to the control samples. In addition, the lowest level addition of avocado seed powder increased the dietary fiber content of the product to 4%; hence, they adhered to the nutrition claim of “source of fiber” in accordance with Regulation (EC) No. 1924/2006.

**Keywords:** avocado seed; dietary fiber content; polyphenol content; antioxidant activity; food design



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## 1. Introduction

Avocado is considered the world’s healthiest fruit due to its nutritional value [1]. Its pulp is a good source of protein, fiber, monounsaturated fatty acids, antioxidants, vitamins, and minerals such as folic acid, pantothenic acid, copper, potassium, sodium, vitamin K, and vitamin B6 [2]. However, ever-growing consumer interest in avocado fruit and the industrial processing of avocado into products such as avocado oil and paste has contributed to a vast amount of waste products, including avocado peel and seeds [3]. The avocado seed accounts for 13–18% of the weight of the whole fruit and usually remains unused [4,5]. The appropriate use of these residues could diminish the issues of overconsumption while counteracting the effects of food waste, in line with the concept of “zero waste” [6]. Accordingly, more and more studies have focused on the valuable properties of avocado seed and the possibility of its further use [7–9].

The lack of commercial use of avocado seeds to date has been linked to the absence of information regarding the antinutritional compounds present therein. The presence of substances such as oxalates, some tannins, hydrocyanic acid, and cyano-genic glycosides—the well-known amygdalin and persin—have been detected in avocado seeds [10,11]. The

majority of these components can be eliminated using culinary processing [9], such as soaking, cooking, roasting, fermentation, and drying [12–16]. Recent studies have indicated that the amygdalin content of the freeze-dried avocado seed is negligible [17,18]. However, concerns remain regarding the content of persin, which is higher in freeze-dried avocado seed than in avocado pulp [18] and is the acetogenin found in the highest amounts in some avocado varieties [19]. Although there is a lack of human studies focused on the determination of the effects of the antinutrients present in avocado seed and their health effects, animal studies on avocado seed ethanol extract have shown to have no genotoxic effects; hence, it could be used as a food, cosmetic or pharmaceutical additive [10,20].

The avocado seed is a good source of carbohydrates, fat, protein, dietary fiber [7,21,22], and bioactive compounds [23–25]. It has higher antioxidant potential and higher content of total phenolic compounds than avocado pulp [4]. In addition, its anti-inflammatory, antihypertensive, hypoglycemic, hypolipidemic, and analgesic effects are described in the literature [26–28]. The possibility of using avocado seeds in food design has been a recent focus of research for scientists. The addition of avocado seeds to cakes [29], candy [30], a beverage alternative to coffee [31], and extruded snacks has been previously explored [9,17].

Cereal snacks in the form of savory cookies, despite their relatively low nutritional value—high in starch, sugar, fat, and sodium content—are popular worldwide because of their high palatability. They also have the disadvantage of being low in dietary fiber and minerals [32]. World Health Organization (WHO) recommendations indicate that a healthy adult should consume 25 g of dietary fiber per day. However, most Europeans do not meet these recommendations; daily fiber intake is 16–24 g [33].

The purpose of this study was to examine the possibility of designing cereal snacks as a source of dietary fiber and bioactive compounds while considering the nutritional, bioactive, and physicochemical properties of freeze-dried avocado seed powder.

## 2. Materials and Methods

### 2.1. Material

The material used in this study was avocado (*Persea americana*) seed of the Hass variety, purchased from a Polish food market (Warsaw, Poland). Fruits from three independent batches were cleaned, the pulp was separated, and the resulting seeds were subjected to sublimation drying in a single-chamber freeze-dryer (Donserv Freeze-dryer model Espilon) at  $-50\text{ }^{\circ}\text{C}$ , under a pressure of 10 Pa, with a shelf temperature of  $21\text{ }^{\circ}\text{C}$ , after the raw materials were frozen at  $-30\text{ }^{\circ}\text{C}$ . This process required 72 h to complete. The material was then ground into a powder using a grinder with grinding knives (MKM 6003, Bosch, Stuttgart, Germany) and then passed through a sieve to obtain 0.5 nm particles.

The resulting freeze-dried avocado seed powder was used to prepare snacks at 6%, 12%, and 18% additive levels (Table 1).

**Table 1.** Formula composition (%) of snacks with avocado seed powder at 6%, 12%, and 18%.

Formulation Ingredient	Basic Recipe	6% Addition Avocado Seed Powder	12% Addition Avocado Seed Powder	18% Addition Avocado Seed Powder
Wheat flour type 450	53	47	41	35
Water	32	32	32	32
Olive oil	13	13	13	13
Instant yeast	1	1	1	1
Salt	1	1	1	1
Avocado seed powder	-	6	12	18

## 2.2. Methods

### 2.2.1. Water Activity ( $a_w$ ) and pH of Avocado Seed Powder

The water activity ( $a_w$ ) was measured using an AquaLab handheld water activity meter (version 5; Decagon Devices, Inc., Pullman, DC, USA). pH was measured in a prepared 2% aqueous solution of avocado seed powder. pH was determined by a potentiometric method using a laboratory pH meter (Elmetron CP-511).

### 2.2.2. Dry Matter Content of Avocado Seed Powder

Dry matter/moisture content was determined gravimetrically for the studied powders using the AOAC (2002) method [34]. Weighing vessels with the test powder were placed in a dryer (SUP 200W, Wamed, Warsaw, Poland) and dried at  $105 \pm 2$  °C until a constant weight was achieved.

### 2.2.3. Water Holding Capacity (WHC) of the Avocado Seed Powder

The WHC of the avocado seed powder was determined according to the procedure described by Sudha et al. (2007) [35]. An amount of 1 g of each fiber preparation was measured into test tubes, and 50 mL of distilled water was added. They were then centrifuged at 10,000 rpm (centrifuge MPW-380 R, Poland) for 15 min, and the excess water was poured off. The powder with absorbed water was weighed again, and WHC was expressed as g water/100 g powder.

### 2.2.4. Water Solubility Index (WSI) of the Avocado Seed Powder

The WSI was measured using a modified procedure described by Yousf et al. (2017) [36]. After mixing 2.5 g of powder with 30 mL of distilled water, the samples were incubated at  $37 \pm 1$  °C for 30 min and then centrifuged at  $12,076 \times g$  for 20 min (MPW-380 R centrifuge, Poland). Collect the supernatant in a pre-weighed weighing pan and dry to constant weight at  $103 \pm 2$  °C. WSI is expressed as the percentage of powder dissolved.

### 2.2.5. Fat Content in the Avocado Seed Powder

The fat content (without hydrolysis) was determined using the weight method after extraction (Soxhlet) according to AOAC Official Method 920.39 (2006) [37].

### 2.2.6. Total Carbohydrate Content in the Avocado Seed Powder

The total carbohydrate content (from calculations) was based on the results of dry matter, total protein, total ash, fat, and whole-wheat dietary fiber.

### 2.2.7. The Content of Dietary Fiber in the Avocado Seed Powder

The total dietary fiber content, including soluble and insoluble fiber fractions, was determined using the weight method after enzymatic hydrolysis according to AOAC 991.43, AACC 32-07 [37].

### 2.2.8. Total Protein Content in the Avocado Seed Powder

The total protein content ( $N \times 6.25$ ) was determined by titration (Kjeldahl) according to AOAC Official Method 945.18-B (2005) [37].

### 2.2.9. Ash Content in the Avocado Seed Powder

The total ash content was determined by weight (after roasting) at 900 °C, according to the official method of AOAC 923.03 (2006) [37]. The roasting process took 10 h to complete.

### 2.2.10. Energy Value of the Avocado Seed Powder

The energy value (from calculations) was based on the energy value of the contained protein, fat, carbohydrates, and fiber according to Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011. (OJ L 304, 22.11.2011, as amended) [38].

#### 2.2.11. Extraction of Bioactive Components of the Avocado Seed Powder and Prepared Snacks

For the extraction of ingredients from the avocado seed powder, 4 g of powder was taken, whereas, for the extraction from the preparation of snacks, 5 g of ground snacks was taken; 30 mL of 70% (*v/v*) methanol (Pol-Aura Chemical Reagents, Zabrze, Poland) solution in water was added to both samples. The samples prepared in this manner were extracted for 30 min at 60 °C in a shaking hothouse (IKA KS 4000 and Control, IKA Ltd., Warsaw, Poland). The samples were then centrifuged at 10,000 rpm for 15 min in a refrigerated centrifuge (MPW-380R, MPW Med. Instruments, Warsaw, Poland) to separate the liquid layer from the precipitate. Determinations were performed on the resulting supernatant.

#### 2.2.12. Total Phenolic Compounds Content in the Avocado Seed Powder and Prepared Snacks

The total content of total phenolic compounds was determined using the spectrophotometric method described by Singelton and Rossi (1965) [39]. The principle of the method is based on the occurrence of a color reaction between the Folin-Ciocalteu (Sigma-Aldrich, Poznań, Polska) reagent and sodium carbonate and compounds of polyphenol nature. The intensity of the resulting coloration was measured using a spectrophotometer (UV-VIS UV-6100A, Metash Instruments Co., Ltd., Shanghai, China) at a wavelength of 750 nm. The content of polyphenolic compounds was expressed as GAE (Gallic Acid Equivalent), i.e., the amount of mg of gallic acid per 1 g of the dry weight of powder.

#### 2.2.13. Polyphenols Profile in the Avocado Seed Powder

The separation of polyphenols was carried out using the method described by Ponder et al. (2021) [40] using a Shimadzu (USA Manufacturing Inc., Tampa, FL, USA) high-performance liquid chromatography (HPLC) set. The identification and separation of phenolic compounds were performed on a Synergi Fusion-RP 80i chromatography column (250 × 4.60 mm) using a two-phase acetonitrile/deionized water (55% and 10%) flow gradient at pH 3.00. Fluka and Sigma-Aldrich 99% purity stand-in standards were used for substance identification.

#### 2.2.14. Determination of the Carotenoid Content in the Avocado Seed Powder

The carotenoid content was determined in accordance with the method described by Ponder et al. (2021) [40] using a Shimadzu (USA Manufacturing Inc., Tampa, FL, USA) high-performance liquid chromatography (HPLC) set with a Phenomenex Max-RP 80Å column (250 × 4.60 mm). The mobile phase consisted of a mixture of acetone and n-hexane (5:95) and was used as a gradient phase. For the qualitative identification of carotenoid compounds, external standards of substances (Sigma-Aldrich, Warsaw, Poland) with a purity of 99.9% were employed.

#### 2.2.15. Determination of the Antioxidant Properties in the Avocado Seed Powder and Prepared Snacks

Antioxidant activity was determined using ABTS<sup>•+</sup> cation radicals (2,20-azino-bis-(3-ethyl-benzothiazoline-6-sulfonic acid diammonium) salt) (Sigma-Aldrich, Poznan, Poland) according to the method described by Re et al. (1999) [41]. The principle of the method was to measure the ability to deactivate the synthetic ABTS<sup>•+</sup> cation radicals with antioxidant compounds contained in the test material. These compounds, as a result of the reaction, caused a decrease in the color intensity of the radical solution, which was determined spectrophotometrically (UV-VIS UV-6100A, Metash Instruments Co., Ltd., Shanghai, China) at a wavelength of  $\lambda = 734$  nm. Antioxidant activity was expressed as mmol TEAC (Trolox Equivalent Antioxidant Capacity) per 100 g dry weight of the test powder.

#### 2.2.16. Tannin Content in the Avocado Seed Powder

The tannin content was determined according to the method of Ciszewska et al. (1975) [42]. We added 6 g of powder into 250 mL of boiling water, covered and filtered for 10 min, and quantitatively transferred 175 mL of the filtrate to a 250 mL beaker. We heated the filtrate to boiling, added 20 mL of 4% copper (II) acetate solution, quantitatively

transferred it to a 200 mL volumetric flask, cooled it, added distilled water to the volume, and filtered it with an organ. Then we measured 100 mL of the filtrate in a 200 mL Erlenmeyer flask; added 25 mL of 50% acetic acid and 20 mL of potassium iodide solution. The released iodine was titrated with a 0.05 mol/L  $\text{Na}_2\text{S}_2\text{O}_3$  solution in the presence of starch as an indicator ( $1 \text{ cm}^3 \text{ Na}_2\text{S}_2\text{O}_3$  corresponds to 0.01039 g of tannins). The result was expressed as grams of tannins per 100 g of powder.

#### 2.2.17. Determination of the Color of the Prepared Snacks

The color was determined using Color Grab (color detection) computer software, ver. 3.9.2 (Loomatix Ltd., Haifa, Israel) based on the snack photos taken.

#### 2.2.18. Weight Loss of the Prepared Snacks

Weight loss during baking snacks with avocado seed powder was determined by taking into account the difference in the weights of the products before and after baking and cooling.

#### 2.2.19. Chemical Composition of the Prepared Snacks

A determination of the basic composition of the prepared snacks was made based on calculations, taking into account the declaration of the nutritional value of each ingredient found on the product packaging, and labeled in the tested seed (Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011. (OJ L 304, 22.11.2011, as amended) [38].

#### 2.2.20. Statistical Analysis of the Obtained Results

Statistical analysis of the obtained results was performed using Statistica 13.0 software (Tibco Software Inc., Palo Alto, CA, USA). One-way analysis of variance (ANOVA) and Duncan's post hoc test were applied, assuming a significance level of  $p < 0.05$ .

### 3. Results and Discussions

#### 3.1. The Properties of the Avocado Seed Powder

##### 3.1.1. Physicochemical Properties of the Avocado Seed Powder

Table 2 shows the physicochemical test results of the powder obtained from the freeze-dried avocado seed. In general, the sublimation drying process employs a low temperature and an absence of oxygen, which affects the preservation of the high nutritional and bioactive content of the raw material [43,44]. Additionally, the drying method influences the physicochemical properties of the avocado powder, including water activity. Water present in raw materials includes free water and water bound to the matrix [45]. In our case, the utilized freeze-drying process removed both types of water [46]; hence, low water activity (0.03) was obtained in the tested avocado powder. The low water activity of the avocado seed powder was essential to maintain microbiological safety during storage and its subsequent applications [47]. Physicochemical properties such as water holding capacity (WHC) and water solubility index (WSI) of the powder demonstrate its ultimate potential use in food design. The freeze-dried avocado seed powder had a low WHC (16.2%) and a low WSI (1.61 g  $\text{H}_2\text{O}$ /100 g powder). The water holding index describes the physical and chemical absorption of water. Soluble fractions of dietary fiber and protein are the main components responsible for water absorption [48,49]. The ratio of soluble components such as pectin, neutral hemicellulases, plant mucilages, and polysaccharides to insoluble components such as cellulose, hemicellulose, and lignins is a factor that significantly influences the WSI of the formulations [50]. The fiber present in the avocado seed powder was mostly insoluble fractions, hence, exhibiting a low WHC and WSI. This suggested that the powder could be utilized as an additive in products for which the palpability of the powder particles is desirable, such as in solid products (cakes, cookies, bread, and cereal snacks) [51].



**Table 2.** Physical and chemical properties of freeze-dried avocado seed powder.

Material	Water Activity [ $a_w$ ]	Dry Matter [g H <sub>2</sub> O/ 100 g of Powder]	pH	WHC [g H <sub>2</sub> O/ 100 g of Powder]	WSI [%]
Avocado seed powder	0.03 ± 0.01	2.6 ± 0.01	6.32 ± 0.01	1.61 ± 0.11	16.2 ± 0.06

### 3.1.2. Nutritional Value of Avocado Seed

Table 3 shows the nutritional value of the freeze-dried avocado seed powder. Carbohydrates accounted for a significant part (67.5%) of the avocado seed powder, whereas for protein, it was 3.4%, and for fat, it was 3.2%. However, according to the literature data, the basic composition of avocado seeds is inconclusive. Reports have shown that a substantial portion of the macromolecules present in the seed is carbohydrates, in the range of 44.7% [27] to 79.5% [22], of which approx. 91% of all carbohydrates are starch [52]. Additionally, the amount of protein determined in the avocado seed ranges from 2.64% [53] to as much as 23% [30], whereas the fat content ranges from 0.71% [1] to 14.1% [30]. An examination of the fatty acid profile of avocado seeds revealed that they were rich in fatty acids, mainly oleic acid, linoleic acid, and palmitic acid [54]. The avocado seed is also a good source of dietary fiber, consisting of 21.6 g of fiber/100 g of powder, of which 18.7 g are insoluble fractions. Barbosa-Martin et al. (2016) described that the fiber content of the avocado seed is 47.84 g/100 g, where insoluble fractions were 36.39 g/100 g [49]. Our results showed that the ash content of freeze-dried avocado seed was 1.6%, which was lower than that reported by Mahawan et al. [1] (2.83%). The avocado seed has been shown to contain minerals such as calcium, potassium, phosphorus, zinc, sodium, iron, copper [30,55] and vitamins: A, C, E, thiamin (B1), riboflavin (B2), and niacin (B3) [38]. The estimated energy value of the avocado seed is 1498 kJ/100 g or 356 kcal/100 g.

**Table 3.** Nutritional value of freeze-dried avocado seed powder.

Material	Energy Value [kcal/100 g].	Fat (Including Saturated Fatty Acids) [g/100 g].	Carbohydrates (Including Sugars) [g/100 g]	Fiber (Including Insoluble Fractions) [g/100 g]	Protein [g/100 g]	Ash [g/100 g]
Avocado seed powder	356	3.2 ± 0.01	67.5 ± 0.01	21.6 ± 0.01 (18.7 ± 0.01)	3.4 ± 0.01	1.6 ± 0.01

### 3.1.3. Bioactive Compounds Present in the Avocado Seed

The presented study showed that the avocado seed powder was a source of bioactive compounds (Table 4). Sublimation-dried avocado seed powder of the Hass variety had a total polyphenol content of 62.1 mg GAE/1 g (dry matter). However, the literature data on the polyphenol content of freeze-dried avocado seed varies from 51 mg GAE/1 g (dry matter) [56] to 88 mg GAE/1 g (dry matter) [6].

**Table 4.** Content of individual and total phenolic, carotenoids, tannins and antioxidant activity of freeze-dried avocado seed powder.

Compounds	
Carotenoids [mg/100 g]	
Lutein	0.323 ± 0.001
Phenolic compounds [mg/100 g]	
Gallic acid	8.82 ± 0.4
Chlorogenic acid	33.65 ± 1.31
P-hydrobenzoic acid	10.74 ± 0.45

Table 4. Cont.

Compounds	
Caffeic acid	4.42 ± 0.11
Benzoic acid	138.12 ± 9.61
Catechin	2.60 ± 0.02
Epigallocatechin	0.82 ± 0.00
Rutinoside-3-O-quercetin	0.40 ± 0.05
Glycoside-3-O-kaempferol	0.90 ± 0.03
Quercetin	2.81 ± 0.09
Total Phenolic [mg GAE/1 g dry matter]	62.10 ± 0.02
Antioxidant activity [mmol Troloxu/100 g dry matter]	122.40 ± 0.01
Tannins [g/100 g]	0.16 ± 0.01

In freeze-dried avocado seeds of the Hass variety, the presence of such polyphenol compounds such as gallic acid, chlorogenic acid, *p*-hydroxybenzoic acid, benzoic acid, coffee acid, catechin, epigallocatechin, rutinoside-3-O-quercetin, glycoside-3-O-kaempferol, quercetin, and lutein, which belong to the carotenoid group, have been identified (Table 3). Other studies [7,57–62] also detected the presence of coumaric acid, ferulic acid, procyanidins, kaempferol, hydroxycinnamic acid vanillin, and vanillic acid. Those detected in the avocado seed from the carotenoids, in addition to lutein, were  $\beta$ -carotene [61]. Hence, the composition and amount of individual phenolic compounds in the avocado fluctuated [57].

The antioxidant activity of the avocado seed powder was determined as approx. 122 mmol TEAC/100 g (dry matter). A higher value of 173.3 mmol TEAC/100 g (dry matter) in the freeze-dried avocado seed was reported by Pahua-Ramos et al. (2012) [62]. The available data on both the nutritional value and bioactive compounds (total polyphenol content, antioxidant potential, and tannin content) in avocado seed vary due to differences in the form of the seeds tested (usually fresh or convection-dried seed were tested), the variety of assay methods used, extraction methods, or the solvent used for extraction [63,64]. In addition, the content of bioactive compounds in avocado seeds differs depending on the maturity, growth conditions, and avocado variety [65,66]. The obtained results can also be influenced by the method and temperature of storage of samples, the parameters of the processes used, the time of exposure to light, and the contact of the powders with atmospheric oxygen [67]. Reports have shown that the aqueous-ethanol extract of avocado seeds inhibits oxidation processes in raw pork chops [56], while avocado seed powder prevents oxidation of meat fat and positively affects the overall quality of meat via inhibition of the rate of oxidative damage [68,69]. Plant tannins are phenolic derivatives that are responsible for the bitter and astringent taste of fruits [70]. Their presence has also been detected in avocado seeds [12]. The level of tannins in the tested avocado seed powder was 0.16 g/100 g, whereas Oluwaniya et al. reported 0.76 mg/100 g [71]. Furthermore, avocado seed powder, compared to other fruit seeds tested, has a low tannin content, almost two-fold lower compared to mango seed powder [72].





### 3.2. Possibility of Using Avocado Seed Powder in Functional Food Design

For the preparation of snacks, in addition to wheat flour and other ingredients, avocado seed powder was used as a substitute for wheat flour at 6%, 12%, and 18% (*w/w*). The addition of the freeze-dried avocado seed powder was used to ensure that the final products had a level of dietary fiber content that allowed the use of the nutrition claim “source” or “high content” of dietary fiber per 100 g of product (Regulation (EC) No. 1924/2006) [73]. It was observed that as the amount of avocado seed powder added increased, the viscosity

decreased, which gave a more compact texture and lower volume during the rising of the “dough” compared to the control sample. This may be related to the lower water-holding properties of the avocado seed powder compared to the gluten present in wheat flour.

The designed snacks differed in color, water content, and weight loss depending on the amount of avocado seed powder added (Table 5). Replacing wheat flour with avocado seed powder significantly ( $p < 0.05$ ) affected the color of the finished snacks due to the promoted orange color upon contact of the powder with water. Hence, it could be used as a natural colorant [74]. The determined  $a^*$  and  $b^*$  color parameters were positive, owing to the presence of red and yellow colors. Color parameters  $a^*$  and  $b^*$  increased significantly ( $p < 0.05$ ) with increasing avocado seed powder added, but color parameter  $L^*$  showed a dramatic decrease ( $p < 0.05$ ). The observed lower value of the  $L^*$  color parameter indicated that the color of the products was darker after the addition of the avocado seed powder. The higher values of the  $a^*$  and  $b^*$  parameters indicated the production of a more intense orange-red color of the snacks, visible to the naked eye.

**Table 5.** Color, water activity and weight loss of designed snacks.

Material	External Appearance	Color	Water Activity	Weight Loss [%]
Control sample		$L^* 58.1 \pm 2.4^b$ $a^* 4.7 \pm 0.8^a$ $b^* 26.7 \pm 7.0^a$	$0.51 \pm 0.01^d$	$24.1 \pm 0.4^a$
Snack with 6% avocado seed powder added		$L^* 56.3 \pm 2.2^b$ $a^* 8.5 \pm 4.6^{ab}$ $b^* 37.3 \pm 5.2^b$	$0.48 \pm 0.02^c$	$24.1 \pm 0.6^a$
Snack with 12% avocado seed powder added		$L^* 56.0 \pm 3.0^{ab}$ $a^* 13.6 \pm 3.4^b$ $b^* 35.5 \pm 3.8^b$	$0.34 \pm 0.02^b$	$25.2 \pm 0.5^b$
Snack with 18% avocado seed powder added		$L^* 49.6 \pm 5.1^a$ $a^* 20.5 \pm 1.9^c$ $b^* 37.1 \pm 1.4^b$	$0.23 \pm 0.01^a$	$25.2 \pm 0.4^b$

Values after different lowercase letters (a, b, c, d) in the series are significantly different ( $p \leq 0.05$ ). Values are mean  $\pm$  standard error of triplicates.

The water activity ( $a_w$ ) of the product was 0.3, which is desirable in terms of microbiological safety. It has been shown that low water activity protects the product from lipid oxidation, non-enzymatic browning, and the growth of microorganisms, the proliferation



of which stopped at  $a_w < 0.62$  [75]. The addition of avocado seed powder significantly ( $p < 0.05$ ) affected the  $a_w$  of the prepared snacks. The  $a_w$  was significantly ( $p < 0.05$ ) reduced with the increased addition of avocado seed powder to the prepared snacks. Furthermore, increased avocado seed powder elevated the weight loss of the products ( $p < 0.05$ ). The lowered  $a_w$  and higher weight loss were probably related to the low degree of water binding by the powder from the tested seed.

The partial replacement of flour with avocado seed powder in the designed snacks significantly ( $p < 0.05$ ) increased the total polyphenol content and the antioxidant potential of the products (Table 6). The addition of 6% avocado seed powder resulted in a nearly five-fold increase in polyphenol content and almost four-fold higher antioxidant potential of the snacks compared with the control sample. In comparison, the product with 18% avocado seed powder had a 13-fold higher total polyphenol content and 10-fold higher antioxidant potential. Novelin et al. (2022) [76] observed the beneficial effect of the partial replacement of flour with avocado seed powder on the content of bioactive compounds in the designed products. They designed cakes with 2.5%, 5%, 7.3%, and 10% share of seed powder possessed an antioxidant activity range of 4.85% to 35.72%, in which 10% avocado seed powder increased the antioxidant potential by 30% compared to the control sample.

**Table 6.** Total polyphenol content and antioxidant activity of prepared snacks.

Material	Total PHENOLIC [mg GAE/100 g s.m]	Antioxidant Activity [mmol Troloxu/100 g s.m]
Control sample	12.43 ± 0.01 <sup>a</sup>	1.49 ± 0.01 <sup>a</sup>
Snack with 6% avocado seed powder added	60.00 ± 0.01 <sup>b</sup>	5.56 ± 0.01 <sup>b</sup>
Snack with 12% avocado seed powder added	123.40 ± 0.02 <sup>c</sup>	10.80 ± 0.02 <sup>c</sup>
Snack with 18% avocado seed powder added	167.78 ± 0.02 <sup>d</sup>	14.62 ± 0.01 <sup>d</sup>

Values after different lowercase letters (a, b, c, d) in the series are significantly different ( $p \leq 0.05$ ). Values are mean ± standard error of triplicates.

Additionally, the partial replacement of flour with avocado seed powder influenced the nutritional content of the designed snacks. Table 7 shows the energy and nutritional value of the snack products, which differed slightly. The products with added avocado seed powder displayed a reduction in carbohydrate and protein content, while a minimal increase in fat content was observed. Nevertheless, the addition of avocado seed powder positively affected their nutritional value, where 6% powder increased the fiber content (3.8 g) compared to the control sample. Therefore, the designed products could be considered a “source of fiber” [73]. In the case of the product with 18% powder, the fiber content increased more than two-fold (6.5 g) compared to the control sample. Hence, the nutrition label “high fiber content” could apply to this product [73].

**Table 7.** Energy value (kcal/100 g) and nutrient and salt content of designed snacks.

Type of Snack	Energy Value	Fat (Including Saturated Fatty Acids)	Carbohydrates (Including Sugars)	Fiber	Protein	Salt
Control sample	353.77	12.73	51.26	2.49	7.10	0.99
Snack with 6% avocado seed powder added	354.90	12.90	50.94	3.84	6.61	0.99
Snack with 12% avocado seed powder added	356.02	13.06	50.63	5.20	6.11	0.99
Snack with 18% avocado seed powder added	357.15	13.23	50.31	6.56	5.62	0.99

#### 4. Conclusions

Freeze-dried avocado seed powder is a source of nutritional compounds and dietary fiber. It also possesses compounds with high antioxidant potential. The high content of insoluble fiber fractions contributes to the low water solubility and low water holding index of avocado seed powder. Therefore, the studied powder has considerable potential applications in the production of functional foods—especially in products where particle sensitivity has been highlighted, such as cakes, mousses, yogurts, and smoothies. The presented work investigated the potential use of avocado seeds in snacks as a source of fiber. The addition of avocado seed powder positively affected the nutritional value of the designed snacks. The addition of 6%, 12%, and 18% powder significantly increased the fiber content, polyphenolic compounds, and antioxidant activity of the designed products. The 6% replacement of wheat flour with avocado seed powder was labeled with the nutritional claim of “source of fiber”, whereas 18% powder was labeled as a product with “high fiber content.” Our study showed that it was possible to design cereal snacks with 6–18% avocado seed powder. Both snacks with the lowest and highest additions of avocado seed powder had appropriate technological characteristics (ease of molding, correct dough compactness). The amygdalin content of the freeze-dried avocado seed did not give rise to safety issues for consumption, but since the effects of persin on the human body are still unknown, further research is required.

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