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Impact of pretreatments and drying methods on techno-functional properties, *in-vitro* protein and starch digestibility, and amino acid composition of avocado (*Persea americana* Mill.) seed flour

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Abstract

Avocado (*Persea americana* Mill.) is a popular plant fruit known for its rich nutritional profile and health benefits. However, their by-product seeds of the avocado, frequently discarded as waste, contain significant amounts of nutrients, mainly proteins and starches. However, there is limited information on the impact of pretreatments and drying methods on the *in-vitro* protein and starch digestibility of avocado seed flour. Avocado seeds sliced were subjected to various pretreatments (ascorbic acid (0.5 %, w/v), blanching, and roasting) and drying methods (fluidized bed, hot air oven, solar, and freeze dryers), and their effects on the techno-functional properties and *in-vitro* protein and starch digestibility of the resulting avocado seed flour were evaluated. In this study, drying methods had a significant influence on *in-vitro* protein digestibility of avocado seed flour ($p < 0.05$), with values ranging from IVPD% (70.79 to 90.53 %). The sample treated with ascorbic acid and dried with a fluidized bed dryer showed the highest 90.53% IVPD, and the lowest protein digestibility in untreated avocado seed flour. The amylose content value ranged from 38.54 to 43.68 % and the amylopectin content from 56.32 to 61.39 %. The highest amylose content (43.68 %) was observed in the hot air dryer-treated with roasting (HAD R_o) avocado seed flour. *In-vitro* starch digestibility with resistant starch value ranged from (35.74 to 43.77%), which promotes gut health. In this instance, unconventional food like avocado seed flour emerges as a sustainable food ingredient, providing functional, nutritional, and textural properties. Thus, optimized pretreatments and drying methods for avocado seed flour enable diverse applications in bakery, noodles, sausages, weaning foods, and functional beverages, unlocking their commercial potential in the food industry.

Keywords: avocado seed, drying methods, functional, *in-vitro* digestibility, pretreatments

Introduction

The growing population and fluctuating dietary habits have driven a momentous rise in the production of food, especially fruit processing, such as fruit juices and frozen products, leading to increased by-product waste from the food industry. For example, fruit by-products, such as seeds, peels, stems, and shells, account for more than 50 % of the fresh fruit weight inevitably generated during processing [1]. A significant portion of these by-products is discarded as waste, leading to environmental pollution, nutritional loss, economic, and social impacts. This waste contributes 6–10% of global food waste and human-induced greenhouse gas emissions, which drive the climate change challenge and food insecurity [2,3].

In Ethiopia, avocado production has seen substantial increases, reaching around 140,000 metric tons annually. The possible reason for the significant surge is attributed to favorable agroecological conditions found in regions such as Sidama and Oromia [4], however, where the agricultural by-products, including avocado seeds, are often underutilized. The avocado seed, which makes up 10-22% of the fruit's weight, contains a kernel that represents 60-80 % of the seeds weight. The avocado seed, comprising 10-22% of the fruit's weight and containing a kernel that accounts for 60-80% of the seeds weight, is rich in polyphenols, flavonoids, and essential fatty acids, highlighting its considerable antioxidant capacity, which is linked to health benefits exhibiting potential anti-inflammatory and reduced risk of metabolic syndrome [5,6]. Hence, it is gaining increasing attention for various applications, like food products, and other products such as cosmetics and pharmaceuticals as ingredients. Once discarded as waste, the avocado seed is now appreciated for its versatility and benefits far beyond the fruit's edible pulp.

However, plant by-products such as avocado seeds have great potential to be transformed into valuable resources, especially as food ingredients, due to their rich nutrient profiles, bioactive compound qualities, and health benefits. Currently, consumers demand natural food products, including diverse plant-based foods, which help address micronutrient deficiencies and prevent non-communicable diseases related to nutritional shortfalls (3,6). Furthermore, utilizing agro-processing by-product waste as an alternative means of converting resources is crucial, as it could contribute to achieving sustainable development goals (SDGs) and meet the increasing demand for eco-friendly and 'zero-waste-level' principles [1]. Therefore, leveraging nutritional preservation technologies, such as green drying technology, is vital, as it supports the

development of sustainable food ingredients to combat malnutrition through innovation, plant-based food solutions.

Recent studies highlight avocado seed flour's potential as a functional food ingredient, offering opportunities for valorization of this by-product into useful resources [7]. Thus, the functional properties of food materials, such as water and oil absorption capacity, and emulsifying properties, including unconventional foods, like avocado seed flour, play a key role in its application in food formulations. Optimizing the application of avocado seed flour is important to take into account the influence of various pretreatments and drying methods on its functional properties and protein digestibility [8]. For instance, pretreatments can enhance the nutritional profiles and functional attributes of the flour, while different drying methods can affect the texture, color, and overall quality of the final product [9].

Understanding these effects can lead to better processing methods that improve the digestibility of proteins and starches in avocado seed flour, thus boosting its potential as a functional food ingredient [10]. Avocado seed flour offers potential nutritional benefits; nevertheless, a comprehensive evaluation of the effects of pretreatments and drying methods on its functional properties and the *in-vitro* digestibility of its protein and starch content remains poorly documented. Thus, the thoughtful application of avocado seed is effective without compromising its nutritional value, which becomes increasingly important, necessitating research into efficient processing methods.

This study aims to evaluate the impact of the pretreatment and drying methods on functional profiles, *in-vitro* starch and protein digestibility of avocado seed flour, and presents indispensable data to enable its use in innovative plant-based food formulations. Based on this purpose, for the first time, a detailed evaluation of the impact of various drying methods on the quality and digestibility of avocado seed flour is conducted. It serves as an important preliminary study to supply data proximately required by the fast-growing food industry for the development of functional foods.

2. Materials and Methods

2.1. Raw materials

This study obtained raw avocado seed samples (Figure 1. Picture taken during the collection of fresh avocado seeds (uncoated) from Sunvado Agro-Processing Plc, a local avocado oil processing company in Yergalem Town Zuriya, Hawassa, Ethiopia.



Figure 1. Picture taken during the collection of fresh avocado seeds (uncoated)

2.2. Preparation of avocado seed flour (ASF)

The avocado seed samples were manually cleaned and washed with distilled water, dried with tissue paper at room temperature, and transported in an ice-box plastic pack to the laboratory. The seeds were then de-coated and sliced into 3.5 ± 0.01 mm thin slices using a laboratory-scale slicer (model: Robot Coupe-CL30, France). The uniformity of the sliced samples was established with a digital vernier caliper (Mitutoyo, Japan). Subsequently, the sliced avocado seeds were separated and subjected to three different pretreatment conditions.

Condition 1: Ascorbic acid was dissolved in distilled water at a 0.5% (w/v) ratio. The sample was fully immersed in this solution at room temperature for 60 minutes. Condition 2: Sliced samples were blanched in boiling water temperature at 71.5 ± 0.01 °C for 3 minutes, then promptly transferred to 25 °C cold water for 2 minutes to stop overcooking. Condition 3: The sliced avocado seeds were roasted at 160°C for 10 minutes in an oven (model: Comet-F3222AERW, China). Meanwhile, untreated samples or those treated with distilled water, serving as controls, were individually labeled with three-digit codes. For subsequent drying process methods, 200 ± 0.5 g of treated or untreated avocado seed slices were weighed using a digital balance (model: WT10001X, China) with ± 0.001 precision and put inside the dryers until a constant weight loss was achieved. The dried samples were ground using a laboratory hammer mill (model: 51WAY, ZN-08, China) at 2500 rpm for 15 minutes and passed through a 300 μ m sieve to produce uniform avocado seed flour. The flow process of ASF is presented in Figure 2. Flow diagram of avocado seed flour (ASF) preparation using various drying methods

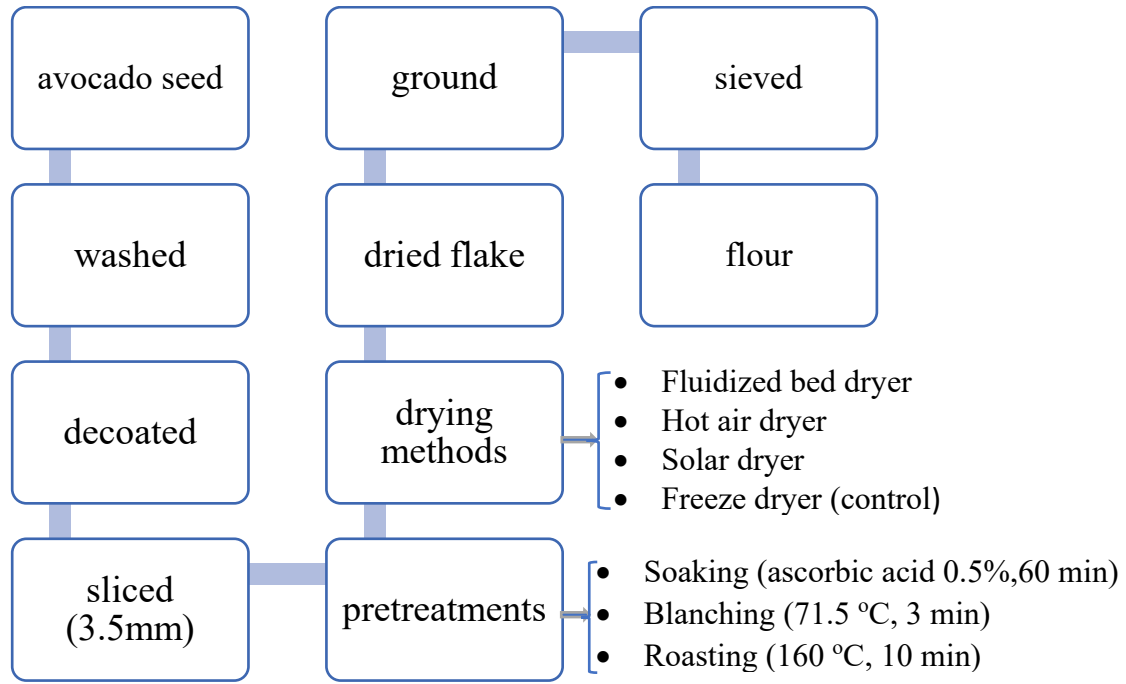


Figure 2. Flow diagram of avocado seed flour (ASF) preparation using various drying methods

2.3. Determination of amino acid compositions and functional properties of avocado seed flour

2.3.1. Amino acid profiles analysis

Amino acid profiles analysis was performed by ultra-high-performance liquid chromatography (UHPLC) using the ortho-phthal aldehyde (OPA) and fluorenyl methyl chloroformate (FMOC) derivatization, as previously described by [11].

2.3.2. Functional and flowability properties of avocado seed flour

2.3.2.1. Bulk and tapped density

Bulk density (BD) and tapped density (TD) were determined by the method [12] with some modifications. Briefly, 2 g of flour samples were placed in the 10 mL measuring cylinder of pre-weighted (W_1) and the volume was recorded as bulk volume. Then, the measuring cylinders containing the samples were tapped 100 times on the flat surface, and the change in the volume was weighed as the tapped volume (W_2). The BD and TD of avocado seeds flour was determined by the following Equations 18 and 19, respectively.

$$BD \left(\frac{g}{mL} \right) = \frac{W_2 - W_1}{\text{Volume of sample after tapping}} \quad (18)$$

$$TD \left(\frac{g}{mL} \right) = \frac{\text{Weight of sample}}{\text{volume of tapped}} \quad (19)$$

2.3.2.2. Flowability indices: Carr's index (CI), Hausmer's ratio (HR)(cohesiveness), and angle of repose (θR)

Carr's index (CI), Hausmer's ratio (HR)(cohesiveness)

The CI and the HR are used to describe the flow indices and fluidity of the powder. The *CI* was determined as the ratio between the tapped and bulk densities and that of bulk density as described by [13] Equations 20 and 21, respectively.

$$\text{Carr's index}(CI\%) = \left(\frac{TD - BD}{BD} \right) 100 \quad (20)$$

$$\text{Hausner's Ratio (HR)} = \left(\frac{TD}{BD} \right) \quad (21)$$

Angle of repose

The measurement of the angle of repose (ϕ) was determined using a static and loose base piling method by [14]. The flour sample was poured into a funnel and placed at a fixed height from a selected base. The flour was then allowed to flow into a selected base. The angle of repose was determined using the inverse tangent from height to diameter of the cone. The experiment was conducted in triplicate for each flour sample, and the result was expressed as degrees ($^{\circ}$) with below (Equation 21).

$$\text{angle of repose}(\phi) = \tan^{-1} \left(\frac{2H(\text{height of pile (cm)})}{(D)\text{diameter of the base of the pile (cm)}} \right) \quad (22)$$

Water and oil absorption capacity

The water and oil absorption capacity of avocado seed flour samples was determined as described by [15] with some modifications. Two grams (2 g) of the flour sample was placed in a pre-weighed conical graduated centrifuge tube, and 10 mL of distilled water or sunflower oil was added to the centrifuge tube and mixed thoroughly using a vortex for 30 seconds. The solution of the sample was allowed to stand at room temperature for 10 minutes, followed by centrifugation at 5000 x g for 15 minutes. The supernatant (free water or oil) was drained out and a re-weighed centrifuge tube sample with absorbed water and oil was used to measure

WAC and OAC. Water absorption capacity (WAC) and oil absorption capacity (OAC) were calculated using the following equations 23 and 24 below. Assume the pre-determined density of water and oil is 1 g/mL, and 0.893g/mL, respectively was used.

$$WAC(\%) = \left(\frac{W_2 - W_1}{W_1} \right) \text{density of water} \quad (23)$$

$$OAC(\%) = \left(\frac{W_2 - W_1}{W_1} \right) \text{density of oil} \quad (24)$$

Whereas, W_1 - is the weight of the dry flour sample (g), W_2 - the weight of the sample with absorbed (g) of water and oil

Solubility and swelling power

The water solubility index and swelling power of the avocado seed flour samples were determined according to the method previously reported by [16]. The supernatant obtained from water absorption capacity (WAC) was dried in the oven dryer at 100 °C until constant weight loss. Then, the dry weight of the dissolved solid was taken to measure solubility power. While the swelling power of the sample was determined by dissolving 0.5 g of flour in 10 mL of distilled water in a screw cap test tube of 15 mL. The slurry was heated in a hot water bath at 60 °C for 30 minutes with constant stirring and air-cool down at room temperature. Then, the paste was centrifuged at 3000 x g for 15 minutes, and the supernatant was poured into a pre-weighed evaporating moisture dish and dried at 100 °C using a hot air oven until constant weight was achieved and the weight of the swollen granules was noted. The solubility and swelling power of the samples were calculated by the following Equations 25 and 26, respectively.

$$\text{Solubility index}(\%) = \frac{W_1 \times 100}{W_s(1 - Mc)} \quad (25)$$

$$\text{Swelling power} \left(\frac{g}{g} \right) = \frac{W_2 \times 100}{W_{dm}(100 - \text{solubility})} \quad (26)$$

Where: W_1 is the weight of supernatant after drying (g), W_s is the weight of the sample (g), Mc is the moisture content of the sample on a dry basis (%db), W_2 is the weight of centrifuged swollen granules, W_{dm} is the weight of dry matter. The weight of dry matter is calculated as, $W_{dm} = W_s(1 - Mc)$.

Dispersibility

Dispersibility was determined using the method described by [17]. Briefly, 10 g of avocado seed flour samples were weighed into a 100 mL-measuring cylinder, and followed by 50 mL of distilled water was added and made up a volume. The mixture was stirred vigorously and allowed to stand for three hours at room temperature. The volume of settled particles was recorded and the dispersibility was determined as follows Equation 27.

$$\text{Dispersibility (\%)} = \left(\frac{(50 - \text{volume of settled particle})}{50} \right) 100 \quad (27)$$

Foaming capacity and stability

The foaming capacity (FC) and foaming stability (FS) of avocado seed flour were conducted as described by [18] with some modifications. Briefly, two grams of avocado seed flour was mixed with 50 mL of distilled water in a 100 mL measuring cylinder. The suspension was thoroughly mixed and shaken using an electric blender at room temperature for 5 min to make foam. The foam volume at 30 seconds after whipping the foam was measured as foaming capacity. In contrast, the foaming volume was recorded 1 hour after whipping to determine foaming stability and was expressed with the following Equations 28 and 29, respectively.

$$FC (\%) = \left(\frac{\text{Volume after whipping} - \text{volume before whipping}}{\text{volume before whipping (mL)}} \right) 100 \quad (28)$$

$$FS (\%) = \left(\frac{\text{Volume of foam after 1hr}}{\text{Volume of foam after whipping}} \right) 100 \quad (29)$$

2.3. Determination of starch compositions and *in-vitro* digestibility of avocado seed flour

Starch extraction yield

Starch extraction was performed based on the alkaline extraction method as described by [19] with some modifications. One part of avocado seed flour was dispensed in five parts of 0.05% (w/v) NaOH solution (mL) and allowed to stand for 24 hours under refrigeration at 5 °C. After homogenization, the suspension was screened through a 250-mesh sieve, the sediment was then washed four times using distilled water and the filtrate was centrifuged (3000 x g for 10 minutes). The supernatant was decanted, the sediment was resuspended in five parts of ethanol and filtered through filter paper, which was used to facilitate drying. Finally, the extracted

sediment was dried using a hot air oven dryer (model: Binder-ED53, German) at 38°C for 8 hours and the moisture content on a dry basis (% db). The starch flour was packaged in polyethylene plastic bag and stored at a refrigeration temperature of 5 °C for further analysis. The starch extraction yield (RSA) was determined using the ratio of the weight of dry starch to the weight of a sample of avocado seed flour and expressed as a percentage (%).

Amylose and amylopectin ratio

The amylose and amylopectin content of avocado seed flour was determined as the described procedure by Arueya and Ojesanmi (2019) with slight modifications. Briefly, 0.2 g of starch flour was mixed with 1 mL of 99% ethanol and 9 mL of 1 M NaOH in a 100 mL flask, then heated in a boiling water bath for 10 minutes to gelatinize the starch. The solution was cooled at room temperature and made up to mark with distilled water and thoroughly shaken. Then, 5 mL of the starch solution was treated with 1 mL (1M acetic acid) and 2 mL of iodine solution (0.2 %) and marked with distilled water in the 100 mL flasks. Then, the solution of the sample was read for the absorbance using a spectrophotometer at 620 nm against the blank solution. The amylose content was determined using the following equation, with the difference used to estimate the amylopectin content (%).

$$\text{Amylose content (\%)} = (\text{Absorbance blank} - \text{Absorbance sample}) * 3.06 * 20\%$$

Where: 3.06 is a conversion factor calculated in milligrams of amylose in unit absorbance, and 20 is the dilution factor.

***In-vitro* starch digestibility in avocado seed flour**

The *in vitro* starch digestibility of the avocado seed flour samples was determined using enzymatic hydrolysis (amyloglucosidase/ α -amylase) approved methods of analysis by the American Association of Cereal Chemists [21] Method No.76-11 using Megazyme assay kits K-AMYL and K-RSTAR (Megazyme International Ireland, Bray Co. Wicklow, Ireland). Briefly, a flour sample (0.2 g) was carefully weighed into a 50 mL Falcon centrifuge tube. Then, 20 mL of sodium acetate buffer (100 mM buffer, pH 5.0) was added and thoroughly vortexed. The mixture was incubated and digested in a boiling water bath at 45 °C for 10 minutes with continuous stirring. Freshly prepared enzymatic solutions (10 mL) (thermostable α -amylase 3,000 U/mL on Ceralpha reagent at pH 5.0, and temperature 40 °C contents diluted 1:30 in reagents; 100 mM sodium acetate buffer at pH 5.0 plus 5 mM calcium chloride) was

added, and centrifuged for 20 min at 3000x g, and 0.7 mL of amyloglucosidase were mixed with the supernatant. The mixture was incubated in a water bath at 37 °C with continuous stirring at a constant temperature. After 20 and 120 min of enzymatic digestion, an aliquot (1 mL) was removed and immediately mixed with absolute ethanol to inactivate enzymes. The mixture was centrifuged at 8000x g for 10 min, and the glucose content in the supernatant was determined using a glucose assay kit. The fraction of solubilized starch was determined by multiplying the amount of glucose by a factor of 0.9, which is the molar weight conversion of glucose to the starch monomer unit (anhydroglucose). All samples were analyzed in triplicate. The percentage of rapidly digestible starch (RDS, within 20 min), slowly digestible starch (SDS, weight (%) of the total starch hydrolyzed between 20 and 120 min), and resistant starch (RS, after 120 min) in the injera samples was calculated using equations.

$$RDS(\%) = \left[\frac{G_{20} - G_0}{TS} \right] \times 0.9 \times 100 \quad (1)$$

$$SDS(\%) = \left[\frac{G_{120} - G_{20}}{TS} \right] \times 0.9 \times 100 \quad (2)$$

$$RS(\%) = TS - [(RDS(\%) + SDS(\%))] \quad (3)$$

G_0 , G_{20} , and G_{120} are the amounts of glucose released from free glucose, 20 min and 120 min, respectively; TS indicates the total starch content.

***In-vitro* protein digestibility (IVPD)**

The protein digestibility of avocado seed flour was determined using the pepsin enzyme digestion method, following the procedure described by [22], with slight modifications. Briefly, a 0.2 g defatted flour sample was placed into a 50 mL centrifuged tube, and mixed with 30 mL of pepsin (1.5 g pepsin activity 1:3000 U/mg in 0.1M phosphate buffer KH_2P_0_4 , pH 2.0). The suspension samples were thoroughly vortexed and incubated in a pre-set shaking water bath at 37 °C for 2 hours. The hydrolysis was stopped by adding 5 mL of 10 % trichloroacetic acid (TCA) at pH 7.0 using 0.1 N NaOH. The mixture was allowed to stand for 30 minutes at 5 °C and was then centrifuged at 4,5000 rpm, 4 °C for 20 minutes. The leftover residues were washed with 15 mL of 0.1 M KH_2P_0_4 buffer, centrifuged twice, and dried in an oven dryer at 60 °C. Protein content in the residue (undigested) was determined using the micro-Kjeldahl method by [21]. Digestibility was determined by obtaining the difference between the total protein content (mg) sample before and after digestion (residue) and expressed as a percentage (%) using the following formula (Equation 33):

$$IVPD (\%) = \left(\frac{\text{total protein in sample} - \text{total protein in undigested}}{\text{total protein in sample}} \right) 100 \quad (33)$$

3. Result and Discussion

3.1. Amino acid composition of avocado seed flour

The chemical composition of raw materials like avocado seeds is significant because it determines their nutritional value, safety, and overall quality, which directly influences the chemical composition of the final processed food products. In this context, Table 1. Amino acid compositions of avocado seed flour treated with ascorbic acid and dried using fluidized bed drying in comparison with teff flour on a dry-weight basis. compares the amino acid composition of avocado seed flour, treated with ascorbic acid and dried in a fluidized bed dryer, with teff flour ($p < 0.05$). The potential reason for comparing avocado seed flour with teff flour lies cereal grain teff is widespread application in traditional food processes, such as injera and its role as a staple grain cereal in the Ethiopian context. This study recorded the amino acid profiles of the avocado seeds in the order: asparagine > valine > histidine > threonine > lysine > methionine, with a total amino acid content of 20.91 mg/100 g. However, there is limited information in the literature regarding the amino acid profile of avocado seeds. This could help fill the literature gaps and could be useful for the development of food products that supplement this essential amino acid or limited essential amino acid profiles of existing products. While in the gran teff flour, the most dominant essential amino acids were valine (4.68 mg/100 g) followed by histidine (3.62 mg/100 g), with the total amino acid composition of 22.02 mg/100 g, which was lower than the previously reported [23]. This may be attributed to varietal differences in the environmental conditions at the sampling sites.

Table 1. Amino acid compositions of avocado seed flour treated with ascorbic acid and dried using fluidized bed drying in comparison with teff flour on a dry-weight basis.

Amino acid compositions	Amino acid content value (mg/100 g)		
	Avocado seed flour	Teff flour	Recommended value ¹
Histidine*	3.24± 0.09 ^b	3.62± 0.01 ^a	1.6
Isoleucine*	0.13± 0.01 ^b	0.15± 0.01 ^a	1.3
Lysine*	0.88± 0.01 ^b	0.93± 0.03 ^a	1.6
Phenylalanine*	0.06± 0.01	ND	1.9
Methionine*	0.77±0.02	ND	1.7
Threonine*	0.98± 0.03 ^a	0.79± 0.01 ^b	0.9

Tryptophan*	0.19± 0.01 ^a	0.19± 0.03 ^a	
Valine*	5.23± 0.46 ^a	4.68± 0.19 ^b	1.3
Cysteine	ND	0.02± 0.01	
Aspartic acid	0.13±0.01 ^b	1.39±0.02 ^a	
Arginine	0.24 ± 0.01 ^b	0.39 ± 0.01 ^a	
Alanine	0.17 ± 0.01 ^a	0.08±0.01 ^b	
Serine	0.28±0.02 ^a	0.29± 0.01 ^a	
Asparagine	8.61±0.91 ^b	9.49±1.06 ^a	
<i>Total**</i>	20.91± 0.65 ^b	22.02± 0.89 ^a	

Where, the result is expressed in (Mean ± standard deviation, n=3), **sum of essential, and non-essential amino acids, ND-not detected,¹recommended value for adult amino requirements in human nutrition (WHO & FAO, 2007), *essential amino acid. means followed by different superscript letters in the same row are significantly different by Tukey's post-hoc test at 5 % probability ($p < 0.05$).

3.2. Flowability and functional properties of avocado seeds dried using various drying methods

3.2.1. Flowability and density properties of avocado seed flour

The flowability of food flour is a dynamic basis in food processing as it impacts the processing efficiency, consistency, and final product quality [25]. The results of the bulk density, tapped density, Carr's index, Hausner ratio, and angle of repose of avocado seeds flour are presented in Table 2. Interaction effect of pretreatments with drying methods on density and flowability properties of avocado seed flour

Bulk and tapped density: are key physical property of food flour, representing the weight-to-volume ratio. It influences packaging design and the choice of packaging material required [26]. Bulk density is significantly impacted by numerous aspects such as particle shape, size, surface characteristics, and density of the solid. However, it can be increased by reducing particle size, compacting, and using suitable packaging materials. Higher bulk density requires denser packaging and indicates product porosity, influencing packaging design and material selection [27]. In the present study, the bulk density was significantly ($p < 0.05$) affected by the interaction of the pretreatment and drying methods. The bulk and tapped density of avocado seeds flour ranges from 0.29 to 0.56 g/mL and 0.60 g/mL to 0.71 g/mL, respectively. The highest bulk density of avocado seed flour (0.56 g/mL) was found in avocado seeds that were roasted and dried in a hot air oven (HAD*R₀), while the lowest bulk density (0.29 g/mL) was observed in the seeds treated by blanched and dried using a freeze dryer (FD*B₁). The bulk density of avocado seeds is slightly similar to previous studies, with the bulk density of potato

peel flour dried using cabinet and sun-drying were 0.64 g/mL and 0.36 g/mL, respectively [28]. However, it is lower than anchote tuber flour (0.71 to 0.83 g/mL reported by [29], and pumpkin seed- based wheat-sorghum composite flour muffins (0.53 to 0.63 g/mL) [30]. The possible reason for the variation in bulk density results was because of moisture content, particle size, gelatinization of starch, and type of flour. Furthermore, the low bulk density content of the food flour sample reveals a better formulation of the weaning food [31,32]. In this sense, the low bulk density of avocado seed flour dried under various drying methods suggests that it may be a good choice for weaning food formulation. This implies lower bulk density in food flour samples promotes digestibility and increases dietary energy content because more flour particles can bind together. As a result, more food can be made with less water while still achieving the desired energy and nutrient density [33].

In this result, the tapped density of the avocado seed flour ranges from 0.60 to 0.71 g/mL. Previous studies found that cereal-based flour has a higher tapped density of varied between 0.76 and 0.93 g/mL [34–36]. The fact that the higher bulk and tapped density measurement values suggests that the particles were close to one another and successfully filled the vacant space, which indicates less volume is required for packaging, and less trapped air within the flour, reducing the potential for product oxidation and enhance storage stability, and also the higher bulk density, as it helps to reduce transport and packaging cost, and suitability for application in food preparation.

Carr's index and Hausner ratio: are two important quality attributes used to evaluate the product's ability to settle and also provide information about the flowability behavior of flour samples and compressibility, particularly in the processing industries. The scale flowability of flour with $CI < 15$ is categorized as very good, $15 < CI < 20$ as good (accepted for CI is 20), $20 < CI < 35$ as fair, $35 < CI < 45$ as poor, and CI is greater than 45 noted as very poor [37]. Powders classed as low cohesive are those with HR less than 1.2, moderate cohesive are those with HR between 1.2 and 1.4, and high cohesive are those with HR greater than 1.4 [38]. In this finding, the pretreatment and drying methods significantly ($p < 0.05$) impacted the flowability of Carr's index (CI) and Hausner ratio (HR) of avocado seed flour. The highest flowability CI (%) value of avocado seed flour was observed in the avocado seed treated under various pretreatments and dried in a freeze-dryer. This indicates that $CI > 45$ % has inferior flow properties and higher cohesiveness based on the standard scale ranges [37]. On the other hand, avocado seeds treated, blanched and dried in a hot air oven and roasted dried in fluidized bed dryers had a low CI (%) of avocado seed flour 11.15 %, and 11.18 %, respectively, with

statistically no significant ($p > 0.05$) difference. It reveals that better flowability ranges within the flowability scale range Carr's index (CI %) values (5 to 15) are excellent for free-flow. The flowability of flour samples is affected by their moisture content, as increased moisture helps agglomerate formation due to strong inter-particle liquid bridges. This leads to stickiness formation that reduces the flour product's ability to flow smoothly. The flour samples with better flow properties depict consistent flow without any external force support [39].

Angle of repose (AoR, degrees): is another important physical property used for the description of bulk particulates such as food flours, grains, seeds, and grits. The scale flowability for the angle of repose of degrees is: the angle of repose ($^{\circ}$) 25-30 $^{\circ}$ is excellent flowability, 31-35 $^{\circ}$ as good, 36-40 $^{\circ}$ as fair, 41-45 $^{\circ}$ as passable, 46 -55 $^{\circ}$ as poor, 56-65 $^{\circ}$ as very poor, >66 as extremely very poor flowability flour [40].

The interaction of pretreatments and drying methods significantly exhibited ($p < 0.05$) effect on the angle of repose of the avocado seed flour sample. The angle of repose of avocado seed flour ranges from 25.74 to 57.76 $^{\circ}$ as displayed in Table 2. Interaction effect of pretreatments with drying methods on density and flowability properties of avocado seed flour According to Jan et al. (2015), commercial rice flour has a higher angle of repose (66.57 $^{\circ}$), which has extremely poor flowability. In this instance, the avocado seed sample that was blanched and dried in solar-drying had the highest angle of repose of avocado seed flour (57.76 $^{\circ}$), which was designated as poor flowability. In contrast, the sample treated with 0.5 % ascorbic acid and dried in a fluidized bed dryer had the lowest value of degrees (25.74 $^{\circ}$), indicating excellent flowability with a significant variation between interplays. The potential cause of the variation could be the high moisture content of the solar-dried avocado seed, which causes samples dried at lower temperatures to have an increasing tendency of the angle of repose, which leads to more moisture and a tendency for the surface layer of moisture surrounding the particles to aggregate due to surface tension.

Table 2. Interaction effect of pretreatments with drying methods on density and flowability properties of avocado seed flour

Pretreatment* drying		Bulk density (g/mL)	Tapped density (g/mL)	Flowability properties		
				Carr's index (%)	Hausner ratio	Angle of repose ($^{\circ}$)
FBD	C ₀	0.51±0.01 ^e	0.60±0.06 ^f	16.68±0.09 ⁿ	1.17±0.01 ⁿ	40.57±0.50 ^{ef}
FBD	AA _{0.5}	0.52±0.02 ^d	0.64±0.04 ^e	23.76±0.06 ^l	1.24±0.03 ^l	25.77±0.11 ^h
FBD	B ₁	0.53±0.01 ^c	0.69±0.1 ^b	31.61±0.18 ^j	1.32±0.02 ^j	37.24±0.21 ^{fg}
FBD	R ₀	0.55±0.03 ^b	0.61±0.03 ^f	11.18±0.05 ^o	1.12±0.01 ^o	46.57±0.22 ^{cd}

HAD	C _o	0.5±0.01 ^f	0.64±0.02 ^e	26.72±0.08 ^k	1.27±0.06 ^k	44.93±1.02 ^{de}
HAD	AA _{0.5}	0.44±0.05 ^j	0.64±0.05 ^e	45.35±0.09 ^f	1.45±0.01 ^f	36.65±0.10 ^{fg}
HAD	B ₁	0.55±0.01 ^b	0.61±0.06 ^f	11.15±0.11 ^o	1.11±0.03 ^o	51.13±0.86 ^{bc}
HAD	R _o	0.56±0.02 ^a	0.68±0.01 ^c	23.48±0.10 ^m	1.23±0.02 ^m	55.66±0.91 ^{ab}
SD	C _o	0.47±0.02 ⁱ	0.68±0.03 ^c	>45	1.47±0.01 ^e	52.65±1.02 ^b
SD	AA _{0.5}	0.49±0.01 ^g	0.66±0.04 ^d	37.29±0.11 ⁱ	1.37±0.02 ⁱ	37.98±0.11 ^{fg}
SD	B ₁	0.48±0.01 ^h	0.68±0.12 ^c	42.45±0.13 ^g	1.42±0.05 ^g	57.76 ± 1.01 ^a
SD	R _o	0.48±0.03 ^h	0.71±0.11 ^a	>45	1.48±0.03 ^d	47.64±0.11 ^{cd}
FD	C _o	0.32±0.01 ^l	0.64±0.01 ^e	>45	1.98±0.01 ^c	34.39±0.71 ^g
FD	AA _{0.5}	0.33±0.03 ^k	0.71±0.10 ^a	>45	2.51±0.02 ^b	25.74±0.90 ^h
FD	B ₁	0.29±0.06 ^m	0.71±0.2 ^a	>45	2.52±0.06 ^a	37.51±1.01 ^{fg}
FD	R _o	0.47±0.01 ⁱ	0.66±0.05 ^d	40.11±0.27 ^h	1.40±0.01 ^h	41.07±0.90 ^{ef}

Tukey's post-hoc test with ($p < 0.05$) indicates that means in the same column that is followed by different superscript letters are significantly different from one another. The results were shown as means $\pm$ standard deviation (SD) ($n=3$).

3.2.2. Functional properties of avocado seed flour

Functional properties are basic physicochemical characteristics of food ingredients that influence their behavior during preparation, cooking, and ultimately determine the end-user product, including sensory attributes. It includes water/oil absorption capacity, solubility index, swelling cap, foam capacity and foaming stability [41]. In this present study, the significance ($p < 0.05$) interaction effect of pretreatments and drying methods on functional properties of avocado seed flour is depicted in Table 3.

Water absorption capacity or oil absorption (WAC/OAC): is the amount of water/moisture or oil that food flour can take up to achieve the desired consistency and quality product. It shows the optimal amount of water or oil required to be added to a dough to prevent it from becoming excessively sticky during processing. A low or high water/oil level can negatively affect the final quality of a food product. The WAC/OAC of various foods or flours can be attributed to variations in hydrophilic/hydrophobic constituents, including protein, starch, fat, and the level of interaction with water molecules and also the possibility of the conformational properties [42]. Our findings showed that the water absorption capacity (WAC) of avocado seed flour ranged from 164.69 to 258.44 %, while the oil absorption capacity (OAC) varied between 75.41 % and 199.93 %. The highest water absorption of avocado flour (258.44 %) was observed in avocado seed flour treated with ascorbic acid and dried in a freeze-dryer, while the highest OAC (199.93 %) of avocado seed flour occurred with a fluidized bed drying. Hence, the high oil absorption capacity suggests its potential use in food formulations such as bakery products and sausages.

The higher water absorption of the flour sample can be attributed to increased starch solubility, amylose leaching, and crystalline structure breakdown. A previous study reported that the increase in WAC increases swelling power, leading to improved solubility of barnyard grass seed flour treated by gamma-irradiation [43]. In this case, the increase in WAC reduced the swelling power of avocado seed flour dried using freeze and solar drying with various treatments. A possible explanation may be that the starch granules retain their structure and absorb water within their matrix, leading to higher swelling power but lower free water absorption. In contrast, avocado seed flour dried in a fluidized bed and a hot air oven dryer showed an increase in WAC and swelling power, resulting in enhanced solubility by reducing the granule swelling. This implies that the avocado seed flour dried under a fluidized bed dryer and a hot air oven dryer with pretreated ascorbic acid could be more suitable for application in the food system requiring more swelling, such as noodle flours.

Swelling power or swelling capacity (SC): measures the amount of water (in milliliters) absorbed by one gram of food material as it swells under specific conditions. Hence, swelling capacity reflects the starch's ability to absorb water and expand, indicating the strength of associative forces within starch granules. Thus, the swelling power of food flour is influenced by particle size, type of flour used, processing methods, and chemical compositions such as starch structure, other polymers present in the flour, and protein molecules that bind to water by hydrogen bonding [44]. In this study, avocado seed flour exhibited a swelling power ranging from 3.34 to 8.74 g/g and a solubility index of 24.19 to 45.06 %. These values were somewhat similar to the swelling power of sweet potato flour (6.30 to 9.50 g/g) [45] but lower than anchote tuber flour dried under different pretreatments (9.39 to 12.60 g/g) reported by [46]. The potential variation may be due to the source of raw materials, chemical composition, and variation in drying methods.

On the other hand, dispersibility is the tendency of a flour substance can be evenly distributed within a bulk liquid phase through mechanical stirring. The dispersibility of the avocado seed flour was significantly influenced ($p < 0.05$) by the interaction between pretreatment and drying methods. The dispersibility of the avocado seed flour varied from 83.62 to 93.02 %. The highest dispersibility of avocado seed flour (93.02 %) was observed when dried in a freeze and treated with ascorbic acid, whereas the lowest dispersibility (82.62 %) was noted for avocado seed flour dried using solar drying with ascorbic acid. This result demonstrated a higher dispersibility range of 60 to 65% compared to what was reported in the black rice flour-based instant beverage cereal mix [47]. This difference in dispersibility may be attributed to factors

such as flowability, ingredient variety, and processing techniques. Improved dispersibility facilitates the proper dissolution of powder by reducing lump formation and agglomerations. In another study, products with a Hausner ratio (HR) ranging from 1.19 to 1.25 exhibited enhanced flowability and increased dispersibility [48]. This suggests a strong correlation between the dispersibility and flowability of a flour product.

Foaming capacity and stability: are the indices of whipping characteristics of protein-based materials or behavioral proteins at air-water interfaces [42]. The foaming capacity of avocado seed flour ranges from 6.28 to 11.14 % and the foaming stability of 11.79 to 20.04 %. Avocado seed flour treated with ascorbic acid and dried in a fluidized bed drier exhibited the highest foaming capacity (11.14%), while the lowest value (6.28 %) was recorded in a blanched and dried with solar drier, with a significant ($p < 0.05$) difference between the pretreatment and drying techniques. These values were higher than those of mango seed flour, which had a foaming capacity of 5.15 to 5.94 % [49]. The variation in foaming capacity values may be due to the presence of fat and protein molecules. Hence, one alternative insight reason may be due to this process can disrupt the structure of the starch granules, which could result in their swelling and possibly changing their foaming properties.

Total soluble solids (TSS) and water activity (a_w): TSS and a_w of avocado seed flour dried at various pretreatment and drying methods are displayed in Table 3. Interaction effect of pretreatments with drying methods on functional properties, total soluble solids, and a_w of avocado seeds flour TSS mentions a combined content of all dissolved substances present in sample materials, including sugar, organic-inorganic acids, amino acids, vitamins, minerals, proteins, fatty acids, carbohydrates, hormones and salts [50]. In this study, the TSS and water activity (a_w) of avocado seed flour were significantly influenced by the interaction between various pretreatments and drying methods. The TSS and a_w of avocado seed flour varied from 3.48 to 5.01 °Brix and 0.38 to 0.55, respectively. The greater TSS value (5.01 °Brix) was observed in a treated sample with roasted dried in a hot air oven as compared untreated (control) sample dried in a freeze-dryer (3.48 °Brix). The possible reason may be because of the breakdown of starch into simple sugar and other soluble solids molecules during a thermal process [51]. This implies that a relatively high TSS value could be used to promote food ingredients and applications in the food system to modify the texture and thickness of food products. Compared to other studies, the result of this study is relatively lower than the custard apple bagasse flour of TSS (7.55 °Brix) [52].

On the other hand, the high-water activity (0.55) of the untreated avocado seed flour samples dried in a freeze-dryer was recorded with a significant difference ($p < 0.05$) of the treated samples. In this study, all results of the water activities of avocado seed flour treated under various drying methods are within standard ranges. A low water activity of avocado seed flour was found in all samples ($a_w < 0.6$), suggesting that less growth of food-borne bacteria ($a_w > 0.75$), moulds for growth ($a_w > 0.6$), and yeasts needed for growth ($a_w > 0.61$) [53].

Table 3. Interaction effect of pretreatments with drying methods on functional properties, total soluble solids, and a_w of avocado seeds flour

Pretreatment *drying		Functional properties							Total soluble solids (°Brix)	Water activity (a_w)
		Water absorption (%)	Oil absorption(%)	Solubility index (%)	Swelling power (g/g)	Dispersibilit y (%)	Foaming capacity (%)	Foaming stability (%)		
FBD	C _o	189.69±1.23 ^{de}	131.59±0.32 ^c	32.34±1.11 ^{defg}	4.02±0.11 ^f	87.8±0.01 ^{ef}	10.07±0.06 ^{ab}	18.27±0.11 ^{ab}	4.07±0.01 ^j	0.45±0.01 ^{fg}
FBD	AA _{0.5}	208.41±1.15 ^{bcd}	199.93±0.28 ^a	34.16±1.01 ^{cdef}	5.55±0.43 ^c	90.01±0.01 ^c	11.14±0.10 ^a	20.04±0.10 ^a	4.55±0.02 ^f	0.38±0.01 ⁱ
FBD	B _l	208.34±1.18 ^{bcd}	155.51±0.31 ^b	33.76±1.72 ^{cdef}	4.59±0.22 ^{de}	87.8±0.04 ^{ef}	9.14±0.02 ^{bc}	16.72±0.12 ^{bc}	4.49±0.01 ^g	0.44±0.01 ^g
FBD	R _o	168.56±1.21 ^e	110.71±0.23 ^c	26.92±1.16 ^{ghi}	4.63±0.19 ^{de}	87.01±0.01 ^f	7.47±0.01 ^{def}	13.89±0.01 ^{def}	4.86±0.03 ^b	0.41±0.10 ^h
HAD	C _o	164.69±1.17 ^e	99.81±0.31 ^f	38.94±1.01 ^{bc}	5.44±0.32 ^c	83.21±0.02 ^{hi}	8.14±0.03 ^{cde}	15.04±0.02 ^{cde}	4.62±0.01 ^{ef}	0.49±0.10 ^{cd}
HAD	AA _{0.5}	183.44±1.10 ^{de}	155.53±0.26 ^b	45.06±1.76 ^a	8.74±0.62 ^a	87.8±0.01 ^{ef}	6.82±0.02 ^f	12.74±0.01 ^f	4.63±0.03 ^e	0.44±0.03 ^g
HAD	B _l	183.47±1.08 ^{de}	111.26±0.24 ^e	34.42±1.17 ^{cde}	4.74±0.50 ^d	87.9±0.06 ^{ef}	8.54±0.03 ^{cd}	15.72±0.01 ^{cd}	4.74±0.10 ^{cd}	0.46±0.08 ^{def}
HAD	R _o	193.31±1.19 ^{cde}	111.08±0.29 ^e	41.84±1.12 ^{ab}	8.73±0.21 ^a	88.61±0.02 ^{de}	9.14±0.01 ^{bc}	16.74±0.11 ^{bc}	5.01±0.01 ^a	0.46±0.05 ^{def}
SD	C _o	239.44±1.89 ^{ab}	75.41±0.41 ^j	28.77±1.18 ^{fghi}	3.34±0.16 ^h	83.01±0.02 ^{hi}	6.95±0.02 ^{ef}	12.97±0.03 ^{ef}	4.16±0.04 ⁱ	0.52±0.02 ^b
SD	AA _{0.5}	183.78±1.57 ^{de}	97.27±0.22 ^g	27.05±1.10 ^{ghi}	3.99±0.10 ^f	86.01±0.05 ^g	6.88±0.02 ^f	12.85±0.01 ^f	4.17±0.02 ⁱ	0.54±0.08 ^a
SD	B _l	182.61±1.78 ^{de}	84.55±0.15 ⁱ	28.65±1.12 ^{efghi}	3.58±0.11 ^g	83.74±0.03 ^h	6.28±0.03 ^f	11.79±0.01 ^f	3.81±0.10 ^g	0.51±0.06 ^b
SD	R _o	243.46±1.81 ^{ab}	88.72±0.17 ^h	26.15±1.10 ^{hi}	4.47±0.17 ^e	82.62±0.01 ⁱ	6.94±0.01 ^{ef}	12.06±0.02 ^{ef}	4.78±0.11 ^c	0.47±0.09 ^{de}
FD	C _o	239.56±1.67 ^{ab}	99.97±0.11 ^f	30.42±1.67 ^{defgh}	3.65±0.13 ^g	89.03±0.05 ^d	6.48±0.02 ^f	12.16±0.02 ^f	3.48±0.01 ^k	0.55±0.11 ^a
FD	AA _{0.5}	258.44±1.43 ^a	133.29±0.13 ^c	24.19±1.14 ⁱ	3.46±11 ^{gh}	93.02±0.01 ^a	6.55±0.01 ^f	12.27±0.01 ^f	4.27±0.03 ^h	0.53±0.05 ^b
FD	B _l	256.25±1.59 ^a	155.28±0.27 ^b	33.63±1.17 ^{cdef}	4.57±0.11 ^{de}	91.01±0.01 ^b	8.21±0.02 ^{cd}	15.17±0.13 ^{cd}	4.35±0.02 ^g	0.48±0.01 ^d
FD	R _o	228.45±1.67 ^{abc}	128.21±0.25 ^d	35.54±1.23 ^{bcd}	6.75±0.55 ^b	90.00±0.05 ^c	8.54±0.01 ^{cd}	15.73±0.01 ^{cd}	4.67±0.01 ^{de}	0.48±0.04 ^d

3.3. Starch composition and in vitro digestibility of avocado seed flour

The results highlight significant interactions between drying methods and pretreatments on *in-vitro* starch digestibility and amylose content in avocado seed flour, presented in Table 4. Amylose and in-vitro starch digestibility of avocado seed flour dried under different drying methods and pretreatments

Amylose and amylopectin content of avocado seed flour

In this study, the amylose content value ranged from 38.54 to 43.68 % and the amylopectin content from 56.32 to 61.39 %. The highest amylose content (43.68 %) was observed in the hot air dryer with roasting (HAD R₀) treatment, while the lowest (38.54 %) occurred in the fluidized bed dryer with control (FBD C₀). The higher amylose content in the avocado seed flour sample dried with hot air with roasting indicates that thermal processing promotes amylose leaching and induces structural change in starch granules. This is consistent with findings by [54], who reported that high-temperature drying disrupts starch granule integrity, thereby increasing amylose content, which aligns with the HAD R₀ results. On the contrary, amylopectin content was highest in the FBD C₀ sample (61.39 %) and lowest in HAD R₀ (56.32 %). This inverse relationship reflects the dynamic balance between amylose and amylopectin, where more intense drying method conditions, like hot air dryer (HAD), appear to reduce amylopectin content. Supporting this [55] found that prolonged heat exposure leads to the degradation of amylopectin chains, which helps explain the lower amylopectin content observed in the HAD-treated samples. In this context, amylose and amylopectin are complementary in food processing, with amylose driving gel strength and structure, and amylopectin ensuring viscosity, clarity, and stability. Therefore, avocado seed starch, as a by-product, is a cost-effective and sustainable alternative, but its amylose-amylopectin profile needs optimization conditions, including temperature, and enzymatic treatment for versatile applications in food and non-food materials.

In-vitro starch digestibility of avocado seed flour

The total starch content and its digestibility fractions (RDS, SDS, RS) indicate how processing influences the nutritional value and functional characteristics of avocado seed flour. The mean value of starch yield and moisture content of the avocado seed flour was 27.54 % and 6.31 % on a dry weight basis (db), respectively. The total starch of the avocado seed flour ranged from 75.86 to 88.77 %. The highest total starch value (88.77 %) was observed in avocado seed flour dried using fluidized bed dryer with ascorbic acid (FBD AA_{0.5}). This indicates that the use of

ascorbic acid likely helps preserve starch granule integrity during fluidized bed drying, reducing gelatinization and enhancing starch retention. This supports findings by [56], indicating that antioxidants like ascorbic acid can limit oxidative damage and starch degradation during drying. Avocado seed flour sample dried using fluidized bed dryer with ascorbic acid (FBD AA_{0.5}) exhibited the highest rapidly digestible starch (RDS) and resistant starch (RS) 23.76 % and 43.77 %, respectively, indicating enhanced starch hydrolysis. In contrast, HAD C₀ and FD C₀ showed lower RDS values of 15.72 % and 15.49 %, respectively, likely due to denser starch structures formed without pretreatments. This increased digestibility may result from ascorbic acid weakening the starch granule structure, thereby increasing enzyme accessibility, as reported by [57]. A high resistant starch (RS) content is ideal for functional foods aimed at promoting gut health or supporting low glycemic index diets. Meanwhile, the HAD R₀ sample, with its higher slow-digestible starch (SDS) content (26.23%), is well-suited for applications that require sustained energy release, such as in sports nutrition.

Table 4. Amylose and in-vitro starch digestibility of avocado seed flour dried under different drying methods and pretreatments

Pretreatment *drying	Amylose (%)	Amylopecti n (%)	<i>In-vitro</i> starch digestibility (%)			
			RDS	SDS	RS	TS
FBD C ₀	38.61±0.16 ⁱ	61.39±0.02 ^a	16.68±0.09 ^g	22.17±0.01 ^{de}	38.57±0.50 ^{ef}	77.42±0.02 ^g
FBD AA _{0.5}	38.54±0.10 ⁱ	61.46±0.07 ^a	23.76±0.06 ^a	21.24±0.03 ^e	43.77±0.11 ^a	88.77±0.01 ^a
FBD B ₁	39.69±0.11 ^h	60.31±0.04 ^b	21.61±0.08 ^b	25.32±0.02 ^b	41.24±0.21 ^c	88.17±0.02 ^a
FBD R ₀	40.16±0.13 ^g	59.84±0.02 ^c	19.18±0.05 ^d	21.12±0.01 ^e	39.57±0.22 ^{cd}	79.87±0.01 ^e
HAD C ₀	41.57±0.08 ^{de}	58.43±0.06 ^d	15.72±0.08 ^h	19.27±0.06 ^g	40.93±1.02 ^d	75.92±0.07 ^h
HAD AA _{0.5}	42.74±0.15 ^c	57.26±0.01 ^e	15.35±0.09 ^h	21.45±0.01 ^e	39.65±0.10 ^{cd}	76.45±0.04 ^{hg}
HAD B ₁	42.81±0.06 ^b	57.19±0.02 ^e	17.15±0.11 ^f	19.11±0.03 ^g	41.13±0.86 ^c	77.39±0.01 ^g
HAD R ₀	43.68±0.10 ^a	56.32±0.09 ^f	21.48±0.10 ^{bc}	26.23±0.02 ^a	37.66±0.91 ^{ab}	85.37±0.03 ^b
SD C ₀	40.68±0.13 ^f	59.32±0.02 ^c	17.57±0.11 ^f	22.47±0.01 ^d	42.65±1.02 ^b	82.69±0.09 ^c
SD AA _{0.5}	40.66±0.04 ^g	59.34±0.10 ^c	17.29±0.01 ^f	25.37±0.02 ^b	37.98±0.11 ^f	80.64±0.01 ^d
SD B ₁	41.08±0.12 ^f	58.92±0.08 ^d	20.45±0.03 ^d	20.42±0.05 ^f	37.76±1.01 ^f	78.63±0.02 ^f
SD R ₀	41.71±0.11 ^d	58.29±0.04 ^d	18.21±0.01 ^e	21.48±0.03 ^e	37.64±0.11 ^f	77.33±0.08 ^g
FD C ₀	39.64±0.10 ^h	60.36±0.02 ^b	15.49±0.01 ^h	21.98±0.01 ^e	38.39±0.71 ^{ef}	75.86±0.03 ^h
FD AA _{0.5}	39.71±0.11 ^h	60.29±0.05 ^b	18.55±0.01 ^e	22.51±0.02 ^d	35.74±0.90 ^g	76.80±0.01 ^{hg}

FD	B ₁	40.76±0.12 ^g	59.24±0.00 ^c	15.89±0.01 ^h	24.52±0.06 ^c	37.51±1.01 ^f	77.92±0.04 ^g
FD	R _o	40.63±0.15 ^g	59.37±0.01 ^c	16.91±0.07 ^g	21.40±0.01 ^e	40.07±0.90 ^{cd}	78.38±0.02 ^f

Where, the result is expressed in (Mean ± standard deviation, n=3), FBD C_o - fluidized bed dryer with control, FBD AA_{0.5} - fluidized bed dryer with ascorbic acid (0.5%, w/v), FBD B₁ - fluidized bed dryer with blanching, FBD R_o - fluidized bed dryer with roasting, HAD C_o - heat air dryer with control, HAD AA_{0.5} - hot air dryer with ascorbic acid (0.5%, w/v), HAD B₁ - hot air dryer with blanching, HAD R_o - hot air dryer with roasting, SD C_o - solar dryer with control, SD AA_{0.5} - solar dryer with ascorbic acid (0.5%, w/v), SD B₁ - solar dryer with blanching, SD R_o - solar dryer with roasting, FD C_o - freeze dryer with control, FD AA_{0.5} - freeze dryer with ascorbic acid (0.5%, w/v), FDB₁ - freeze dryer with blanching, FD R_o - freeze dryer with roasting, pretreatment *drying- interaction between pretreatment and drying method, RDS -rapidly digested starch, SDS -slowly digested starch, and RS -resistant starch are major compositions of starch, TS-total starch

3.4. *In-vitro* protein digestibility (IVPD) of avocado seed flour

IVPD is a valuable tool and information for assessing the quantity and quality of protein needed to meet human nutritional requirements [58]. The *in-vitro* protein digestibility (IVPD) of avocado seed flour dried using various drying methods and pretreatments is shown in Figure 12. The methods include FBDA (fluidized bed dryer with soaking in ascorbic acid), FBDCO (fluidized bed dryer without treatment), FDA (freeze dryer with soaking in ascorbic acid), FDB (freeze dryer with blanching), freeze dryer without treatment, HAA (hot air dryer with soaking in ascorbic acid), HAB (hot air dryer with blanching), HADCO (hot air dryer without treatment), and SDCO (solar dryer without treatment) of the avocado seed flour sample. The results indicate that the protein digestibility of avocado seed flour varied significantly ($p < 0.05$) based on drying methods and pretreatments, with values ranging from 70.79 to 90.53 %. The study revealed that the sample dried using a fluidized bed dryer with soaking in ascorbic acid (FBDA) exhibited the highest in IVPD% (90.53 %), whereas the untreated control sample dried by a fluidized bed dryer (FBDCO) displayed the lowest IVPD% (70.79 %) shown in Figure 3. In-vitro protein digestibility (IVPD) of avocado seed flour dried using various drying methods This difference may be attributed to the physical treatments, such as soaking with ascorbic acid, which likely reduced anti-nutritional factors, thereby enhancing the protein digestibility in food.

Previous studies have consistently shown that processing methods and physical treatments can enhance the *in-vitro* protein digestibility of various crop food materials. For example, soaking periods and germination conditions have been found to improve the IVPD % of different cereal grains and legumes: oats (65.16 to 77.33%), wheat (63.18 to 70.21 %) and beans (62.85 to 73.22 %), , chickpeas (64.67 to 80.07 %), peas (81.66 to 91.01 %), lentils (66.18 to 76.32%) [59]. Additionally, studies have demonstrated the impact of different treatments on the IVPD percentage of foods like finger millet (raw: 79.0%, cooked: 84.7%, germinated at 30°C for 48

hours: 92.0%) [60], pumpkin seed meal using conventional thermal methods (83.76% to 94.08%) [61], and sweet potatoes (raw: 52.8%, cooked at 100°C for 60 minutes: 85.7%, dried at 130°C for 60 minutes: 54.7%) was reported by [62]. The possible variations in in vitro protein digestibility percentages for each food material could be attributed to factors such as the source of the plant protein, environmental conditions, chemical compositions, as well as the structure and biochemistry of the protein. In this case, the utilization of unconventional food by-products like avocado seeds shows promise for sustainable protein production. However, successful application depends on selecting appropriate processing methods and optimizing various conditions, such as time, temperature, and pH are vital.

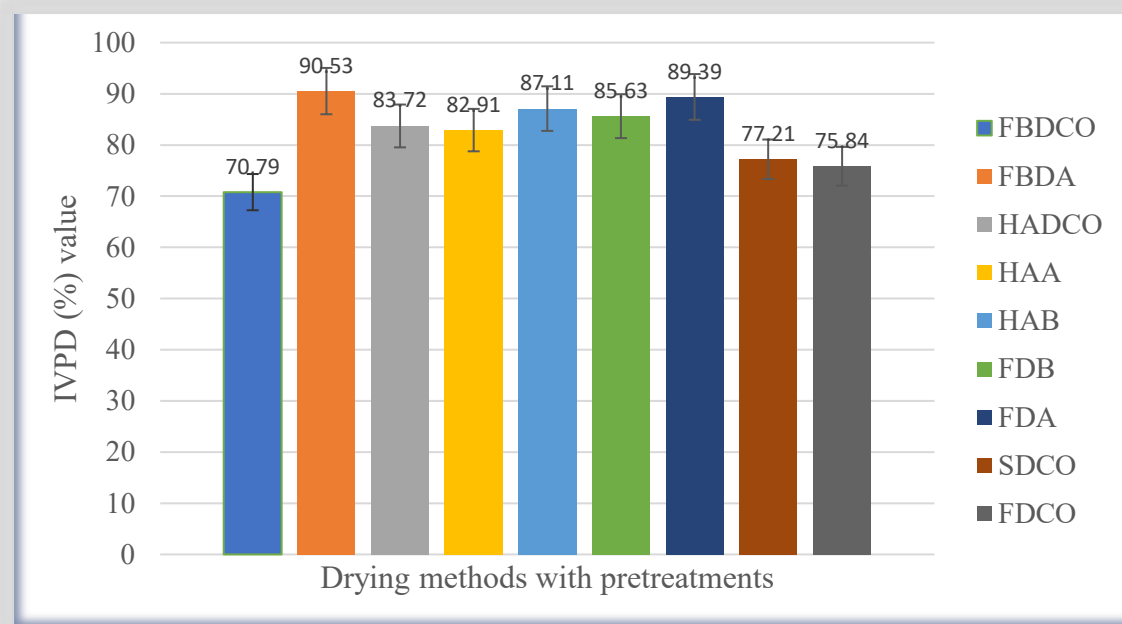


Figure 3. In-vitro protein digestibility (IVPD) of avocado seed flour dried using various drying methods

4. Conclusion

In conclusion, pretreatments and drying methods interact significantly to influence the flowability, functional properties, starch profile, and in-vitro protein digestibility of avocado seed flour. Roasted samples dried in a hot air oven produce the highest bulk density, suitable for compact packaging and lower transport costs, whereas blanched, freeze-dried modifications exhibit lower density, ideal for digestible, energy-dense weaning foods. Ascorbic acid pretreatment combined with fluidized bed drying ensures superior flowability (per Carr's index, Hausner ratio, and angle of repose), compared with the higher moisture content dried sample in the solar dryer. Increased water and oil absorption capacities enhance applications in bakery products, noodles, and sausages, while high amylose and resistant starch content support viscosity control, gut health, and the development of functional foods. Fluidized bed-dried, ascorbic acid-treated flour reaches the highest value of protein digestibility by reducing anti-nutritional factors, highlighting its value as a sustainable protein source. In summary, avocado seed flour is positioned as a versatile and economical by-product with wide applications in food processing, driving innovation as a valorized health ingredient across the industry.

CRedit authorship statement

Desta Dugassa: Writing-original draft, Methodology, Data curation, Formal analysis. **Shure**

Soboka: Reviewing, Editing, Investigation, Data curation

Declaration of competing interest

The authors declare that none of the work reported in this study could have been influenced by any known competing financial interests or personal relationships that any of the authors have disclosed in this study.

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Data availability statement

Data will be provided upon request

Ethical Statement

This research experiment did not involve a human or animal study.

Clinical trial number

Not applicable

Consent to publish

Not applicable

Consent to publish

Not applicable

Declaration for permissions to collect the plants

A permission letter to collect the plant sample was secured from the relevant body.

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