Scientific Papers. Series D. Animal Science. Vol. LXVIII, No. 2, 2025
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

QUALITY ASSESSMENT OF BREAD BASED ON COMPOSITE FLOURS FROM AVOCADO SEEDS FLOUR AND WHEAT FLOUR

Daniela STOIN[1], Ioana-Alina POP[1], Mariana-Atena POIANA[1], Călin JIANU[1], Ariana-Bianca VELCIOV[1], Carmen Daniela PETCU[2], Diana MOIGRADEAN[1]

[1]University of Life Sciences "King Mihai I" from Timisoara, Faculty of Food Engineering, 119 Calea Aradului Street, 300645, Timisoara, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Spl Independentei, District 5, 050097, Bucharest, Romania

Corresponding author email: marianapoiana@usvt.ro

Abstract

The main purpose of this study was to explore the potential of avocado seeds flour (ASF) as a novel source of bioactive components and its valorization in the development of bread formulations with enhanced sensory, nutritional, functional and technological properties. ASF was used to replace wheat flour (WF) at percentages of 0% (control sample), 5%, 10%, 15% and 20% (w/w). Standard methods were used to examine the proximate composition, physical and sensory characteristics, total phenolic content and antioxidant activity of the resulting bread formulations. The results of the sensory analysis showed that the bread sample with a 15% ASF incorporation was the most appreciated by the evaluators. The obtained results also showed an improvement in the nutritional profile of the breads, proportional to the increase in the percentage of ASF in the composite flour mixes, as well as a significant increase in functional attributes. These findings provide conclusive evidence of the potential of ASF to be used as a partial replacement of WF in the formulation of innovative flour products with improved functional properties.

***Key words**: avocado seeds flour, functional bread, nutritional profile, sensory evaluation, total phenolic content.*

INTRODUCTION

Today's consumers have changed their food selection criteria. While safety and sensory factors used to predominate, nowadays more and more people are also choosing foods according to their impact on health, their ability to optimize the body's functioning and to prevent or delay the onset of diseases (Mitrea et al., 2003; Petcu et al., 2007; Vicentini et al, 2016; Hadj Saadoun et al., 2021; Stoin et al., 2023). The growing interest in foods with beneficial effects on well-being is the result of the increased nutrition education, an increased concern for maintaining good health through diet, as well as the growing number of people suffering from chronic diseases (cardiovascular disease, diabetes, osteoporosis, cancer, allergies, etc.) who see nutrition as a means of prevention or complementary treatment. Therefore, the intake of supplements, nutraceuticals, bio-functional foods, nutricosmetics and cosmetics rich in bioactives compound from vegetable products (Stoin et al., 2009; Poiana et al., 2009; Galal et al., 2020; Eliopoulos et al., 2022) is a sustainable and environmentally friendly way to be supported.

In this regard, the valorization of vegetable wastes and by-products from the food industry, through the production of functional ingredients (flours) with nutritional value and antioxidant potential, used in the fortification process of food products, represents a sustainable solution for the development of innovative products, waste reduction and resource optimization (Lahiri et al., 2023). The demand for such products is growing, driven by consumer concern for healthy eating, sustainability and efficiency in the use of ingredients. By-products from various sectors of the food industry (such as bran from cereal processing, whey from the dairy industry, pulp or seeds from fruit and vegetables from the canning industry) are increasingly used to create foods with high nutritional value. They can be incorporated into bakery products, snacks, functional drinks or food supplements, helping to enrich their fiber, protein, antioxidants and other health-promoting substances. In addition, current trends in the food industry promote circular economy and sustainability, and by-product recovery companies are succeeding in meeting both environmental requirements and consumer preferences for nutritious and environmentally friendly products (Khorairi et al., 2023; Nirmal et al., 2023).

By-products of fruit processing include avocado pits. The avocado fruit (*Persea americana*), originally from Central America and southern Mexico, belongs to the *Lauraceae* family. Avocado is considered to be one of the most valuable fruits on a global scale, primarily due to its high content of lipids, protein, fiber, vitamins (C, E, K, B complex) and minerals (phosphorus, sodium, magnesium, potassium, iron, zinc). These nutrients are essential for human health, and avocados therefore have the potential to improve health and help treat certain conditions (Mahawan et al., 2015; Ford et al., 2020; Permal et al., 2023; Sanchez-Rosario et al., 2025). However, an increase in consumer interest in avocado fruit and its industrial processing has led to the generation of by-products, primarily avocado peel and seeds, as most of industrial applications use only the pulp (Duarte et al., 2016). Avocado seeds comprise 13-18% of the total fruit weight and are often discarded (Wang et al., 2010). Appropriate utilisation of these by-products has the potential to address issues associated with food waste, in accordance with the zero waste concept. Consequently, there has been an increasing focus in the academic literature on the valuable properties of avocado seeds and the potential for their further utilisation as sources of valuable bioactive compounds (Setyawan et al., 2021; Permal et al., 2023; Sanchez-Rosario et al., 2025). Avocado seeds have been identified as a significant source of carbohydrates, monounsaturated fats, proteins, dietary fiber (Araújo et al., 2018; Salazar-López et al., 2020), and bioactive compounds (Salazar-López et al., 2020; Jiménez et al., 2021; Permal et al., 2023; Sánchez-Rosario et al., 2025). In addition, there are studies describing the anti-inflammatory, anti-hypertensive, hypoglycemic, hypolipidemic and analgesic properties of avocado pits (Kristanti et al., 2017; Alkhalaf et al., 2019).

A number of studies have also been conducted on the potential uses of avocado seeds in the form of flour or powder in the design of foods such as cakes (Puspitasari et al., 2020), candies (Ifesan et al., 2015) and extruded snacks (Permal et al., 2023). The valorization of avocado seeds can be achieved through the process of spray-drying, which involves turning avocado seeds into a stable powder (Wong et al., 2017).

The objective of this research was to obtain a functional ingredient with nutritional value and antioxidant potential, a by-product of avocado fruit processing, and to use it to develop a new, healthy and sustainable flour product that meets the requirements of the European market and has a positive impact on consumer health.

MATERIALS AND METHODS

Production of avocado seed flour (ASF)

Avocado seed flour was prepared using method as described by Oktarini et al. (2023) with minor modifications. The flour was obtained from whole kernels, from which no oil extraction was performed. The avocado kernels were skinned and washed with clean water to remove any impurities. Cleaned avocado pits were cut into small 0.5 cm pieces. The resulting pieces were dried in a food dehydrator (Biovita DELUXE) at 60°C for 8 h, then milled using a laboratory mill (PerkinElmer). The resulting flour was then sieved using a sieve in accordance with ISO 5223, subsequently packaged in polyethylene bags and stored at 4°C for a period of approximately 15 days, until further analysis.

Raw material and ingredients

ASF was obtained following the process described above, and the wheat flour (WF) 72% extraction, yeast and salt were purchased from specialty stores in Timisoara, Romania. Four types of composite flours were prepared from WF and ASF, as follows: M1 (95% WF: 5%ASF); M2 (90% WF: 10% ASF); M3 (85% WF: 15% ASF); M4 (80% WF: 20% ASF) and a control sample CS (100% WF + 0% ASF).

Technological process of obtaining bread

Bread samples were prepared according to the recipes shown in Table 1. Samples were prepared using the method described by Peluola-Adeyemi et al. (2021) and Stoin et al. (2023), with minor modifications. The bread formulations were obtained by the direct dough preparation method. From the composite flour mixtures (M1-M4), four bread samples were prepared, as well as a control sample (CB) made exclusively from wheat flour (100% WF:0% ASF). Water (58%), yeast (3%) and salt (2%) were added to each bread sample. Each homogenized flour mixture, emulsified yeast, salt and water were placed in the bowl of a Hauser DM601 mixer. These ingredients were mixed on speed 1 for 7 minutes and on speed 2 for 10 minutes until a homogeneous dough was obtained. The dough was then divided into 200 g pieces, shaped, placed in trays and subjected to fermentation at a temperature of 37°C and a relative humidity of 78% for 60 minutes. Following this, the dough was baked at 220°C for 50 minutes. Subsequently, the bread samples were allowed to cool to room temperature, then packed in polyethylene bags and stored for further analysis (Oktarini et al., 2023; Stoin et al., 2023).

Table 1. Recipe of bread formulas and technological parameters

Ingredients	Bread samples				
	CB	B5ASF	B10ASF	B15ASF	B20ASF
WF (g)	500	450	400	350	450
ASF (g)	0	50	100	150	0
Salt (g)	10	10	10	10	10
Yeast (g)	15	15	15	15	15
Water (mL)	350	350	350	350	350
Technological parameters					
Kneading time (min)	12				
Dough temperature (°C)	23				
Fermentation time (min)	50				
Temperature (°C)	32				
Baking time (min)	40				
Temperature (°C)	210				

WF - Wheat flour; **ASF** - Avocado seeds flour; **CB** – Control bread (100% wheat flour (WF): 0% avocado seeds flour (ASF); **B5ASF** - Bread sample with 95% wheat flour (WF): 5% avocado seeds flour (ASF); **B10ASF** - Bread sample with 90% wheat flour (WF): 10% avocado seeds flour (ASF); **B15ASF** - Bread sample with 85% wheat flour (WF): 15% avocado seeds flour (ASF); **B20ASF** - Bread sample with 80% wheat flour (WF): 20% avocado seeds flour (ASF).

Proximate composition of flours and samples bread

The standard method described by the Association of Official Analytical Chemists (AOAC 2000) was used to assess the proximate composition of WF, ASF and bread formulas. The carbohydrate content (%) was calculated by subtracting the other macronutrients (moisture, fat, protein, fiber, ash) from 100. All samples were measured in triplicate. The energy value was calculated according to the equation described by Dossa et al. (2023):

$$\textit{Energy value (kcal/100 g)} = 9 \times \textit{fat (\%)} + 4 \times \textit{carbohydrates (\%)} + 4 \times \textit{proteins (\%)} \quad (1)$$

Physical characteristics of bread samples

Physical characteristics such as volume, porosity, crumb elasticity and height/diameter ratio were determined for each bread formula according to the methodology described by Cirlincione et al. (2022) and Dossa et al. (2023). The volume of each bread sample was measured using the Fornet apparatus, which operates on the principle of determining the volume of rapeseed displaced by the product analysed. For each bread sample, three consecutive measurements were taken, and the arithmetic mean of the results obtained was taken. The volume of the bread is calculated as the ratio between the volume of rapeseed displaced by the analysed product and the mass of the bread sample, multiplied by 100, and the result is expressed in cm^3/100 g.

The elasticity of the core is based on the principle of compressibility and the subsequent relaxation of the core when subjected to pressure under specific conditions, followed by the measurement of the height to which it returns following the removal of the pressing force. The method entails the compression of a core cylinder with a diameter of 4 centimeters and a height of 6 centimeters, for 1 minute. Subsequent to the cessation of pressure, the degree of return to the original shape is evaluated. The core's elasticity is calculated as the ratio of the cylinder's height before pressing to its height after pressing and its return to its original position, multiplied by 100, with the result expressed in %.

Bread porosity is a critical physical parameter in the analysis of sensory attributes of a bakery product. This parameter provides information about the total volume of pores present in the analysed crumb. The analysis of the structure and thickness of the pores, along with their uniformity, is essential in determining porosity. This parameter is expressed as a percentage of the sample volume and is calculated according to the equation outlined by Dossa et al. (2023).

The determination of the H/D ratio is done in order to assess the degree of development of the bread sample under analysis. A low sample height and a larger diameter are an indication that the bread is inadequate (either due to poor-quality flour or over-fermentation), while a too high sample height and a small diameter indicate an insufficiently fermented bread with a too firm dough. If the H/D ratio is 0.40, the product is well developed. A higher H/D ratio indicates a superior product, while an H/D ratio value below 0.40 represents an inadequate, flattened product. The ratio between the maximum height of the unnotched product or the arithmetic mean of the maximum and minimum heights for the notched product, in centimetres, and the arithmetic mean of two perpendicular diameters, in centimetres, must be calculated. The result is expressed in %.

Phytochemical profile of bread samples

The total phenolic content (TPC) of the bread formulas was determined by spectrophotometric analysis, using the Folin-Ciocalteu method. This method was described by Metzner Ungureanu et al. (2020) and Viola et al. (2023), with some modifications. Extracts were prepared by weighing 1 g of sample and mixing it with 5 mL of methanol/water (80:20, *v/v*). Following homogenisation by vortexing for a period of 2 minutes, the mixture was placed in an ultrasonic bath (model RK 510 H, Germany) at room temperature and sonicated for a period of 1 hour. The mixture was then centrifuged at 9000 rpm for 5 minutes at 4°C using a Universal 320R centrifuge (Hettich, Germany). The absorbances of the bread samples and of the blank sample, prepared under the same conditions as the bread samples, were measured at a wavelength of 765 nm using a spectrophotometer (Specord 200; Analytik Jena Inc., Jena, Germany). TPC was expressed in milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g). The calibration curve was performed for the concentration range from 2.5 to 250 μg/mL. All analyses were performed in triplicate, and the results were reported as the mean value ± standard deviation (SD).

The evaluation of the antioxidant activity (AA) of bread samples (DPPH (2,2-diphenyl-1-picrylhydrazyl) was performed according to the procedure described by Viola et al. (2023), with some modifications. Each bread sample (1 g) was extracted with 4 mL of methanol, then combined with 3 mL of DPPH solution (60 μM) and incubated in the dark for 30 min. The free radical scavenging activity was evaluated by UV-VIS spectrophotometry, measuring the absorbance at 517 nm with a Specord 200 spectrophotometer (Analytik Jena Inc., Jena, Germany) using methanol as a blank. The results were expressed as millimoles Trolox equivalent (mmol TE)/100 g sample. The conversion of the signal from the measurement of the absorbance into an antioxidant activity value was performed based on the calibration curve set in the range 5-400 μM with Trolox as the standard. All measurements were performed in triplicate.

Sensory evaluation of bread samples

The evaluation of the bread samples was carried out by a group of 30 untrained panelists (15 men and 15 women), aged between 20 and 45 years, without food allergies and non-smokers. All panel members were trained according to ISO 6658:2017. Bread slices, including crust, 1 cm thick, were presented on cardboard plates, coded and served randomly under normal lighting conditions and at room temperature. A five-point hedonic scale was used to assess consumer acceptability, with the following ratings: 1-dislike extremely; 2-dislike slightly; 3-neither like nor dislike; 4-like slightly; 5-like extremely. The sensory characteristics evaluated by the evaluators were: appearance, texture, flavor, taste and overall acceptability (Borrelli et al., 2023; Stoin et al., 2023).

Data statistical analysis

Determinations were performed in triplicate and then expressed as mean values ± standard deviation (SD). Statistical data processing was performed using analysis of variance (one-way ANOVA). To estimate the statistical significance of variations between means, Tukey's post-hoc comparison of means and Levene's test for equal variance were included.

RESULTS AND DISCUSSIONS

Proximate composition of flours and bread samples

Table 2 presents the results referring to the proximal composition of flour and bread samples.

The comparison of moisture and protein content between ASF and WF showed lower values for ASF, with a moisture content of 8.594% compared to 12.744% in WF and a protein content of 7.674% compared to 11.534% in WF. On the other hand, the fat, fiber and mineral content of ASF was much higher than that of WF, as follows: the fat content of ASF was 6.368% vs. 1.047% in WF, the fiber content of ASF was 14.592% vs. 1.589% in WF and the mineral content of ASF was 2.757% vs. 0.538% in WF, an intake that recommends the use of ASF as a functional matrix in flour products (Eliopoulos et al., 2022; Siol et al., 2023; Viola et al., 2023). The carbohydrate content in ASF (60.015%) was lower than that in WF (72.548%), which will lead to a reduction in carbohydrate content in the bread samples. The findings of this study are consistent with those reported by Siol et al. (2023) and Viola et al. (2023) on the proximate composition of ASF. These authors concluded that ASF is a valuable ingredient with potential for use in various food matrices to improve nutritional value. The obtained bread formulas (B5ASF, B10ASF, B15ASF, B20ASF) have a better nutritional profile compared to CB, a profile that correlates with the percentage of WF substitution by ASF. The results obtained highlight the significant impact ($p<0.05$) of partial substitution of WF with ASF on the proximal composition of bread samples. The moisture content of the bread samples was lower than that of CB (37.721%), decreasing from 36.928% in B5ASF to 36.277% in B20ASF, which is lower than the average content reported by Anjarwati et al. (2021), who reported bread sample moisture values of 39.12% at 5% substitution of WF with ASF. The moisture content of the bread formulas exhibited a downward trend, influenced by the high fiber content of ASF, which possesses the property of reducing the water absorption capacity (Wang et al., 2002). According to the findings of Zghal et al. (2002), the moisture content of bread samples is influenced by the level of starch gelatinization in the dough during the baking process. Consequently, the lower values obtained in this study could be attributed to the low moisture content of the ASF.

In terms of protein content of the bread formulas, it varied from 10.0345% in B5ASF to 8.852% in B20ASF, and the highest value was recorded in CB (10.265%). A decrease in protein content was observed as the percentage of ASF increased, and the differences between the samples were found to be statistically significant ($p < 0.05$). According to Akua (2012), avocado fruit is a poor source of protein and should be consumed in combination with other protein-rich foods. The reduced protein content of the bread samples could be attributed to the low protein content of the ASF.

The bread samples with added ASF were characterized by higher levels of fibre, fat and minerals compared to BC, whose value increased proportionally with the level of substitution of WF with ASF. The bread recipes obtained in this study proved to be a good source of fiber, with fiber contents ranging from 2.877% in B5ASF to 4.780% in B20ASF, compared to 2.231% in BC. Also, in the case of fibres, there were significant differences ($p<0.05$) between the analysed samples. The increase in fiber content of the bread samples can be attributed to the high level of fiber in avocado seed fraction (ASF), indicating that these seeds are a valuable source of dietary fiber. They have been shown to improve the digestive process in the intestinal tract and to have significant potential for use in the bakery industry (Boshra and Tajul, 2013).

The same upward trend was observed for the fat and ash contents, which ranged from 2.788% to 4.894% and from 1.476% to 2.569% respectively, compared to 2.018% and 1.011% in BC, and can be considered as products with high functional potential (Siol et al., 2023; Marra et al., 2024). The increased fat content observed in all samples can be attributed to the high lipid concentration in avocado seeds, which are rich in monounsaturated fatty acids - compounds recognized for their potential health benefits for consumers (Indriyani et al., 2015). Similarly, a trend of increased fat content has been previously reported by other researchers (Indriyani et al., 2015; Peluola-Adeyemi et al., 2021).

The carbohydrate content was also significantly lower in composite flour bread samples compared to the control (BC), ranging from 45.897% in B5ASF to 42.628% in B20ASF, whereas the control sample exhibited a value of 46.754%. The carbohydrate content reduction in the bread samples may be attributed to the low carbohydrate concentration in the avocado seed flour (ASF), as well as the decreased proportion of wheat flour (WF) in the formulation. The values obtained are close to those reported by Peluola-Adeyemi et al. (2021), who obtained a carbohydrate content between 44.03% and 54.69%.

The energy value calculated from the fat, protein and carbohydrate intakes provided by the component raw materials varied in the following order: BC> B5ASF> B10ASF> B15ASF> B20ASF. As the proportion of avocado seed flour (ASF) increased in the samples, the energy value decreased from 246.28 kcal/100 g in the control bread (CB) to 245.124 kcal/100 g in B20ASF. Thus, the use of ASF in bread formulations resulted in a lower calorie product. The results of this study are similar to those of Peluola-Adeyemi et al. (2021), Anjarwati et al. (2023) and Viola et al. (2023), who observed that as the ASF replacement level increased, the fat, fiber and mineral contents increased and the protein and carbohydrate contents decreased.

Table 2. Proximate composition of flours and bread samples

Samples	Chemical Parameters						
	Moisture (%)	Fat (%)	Protein (%)	Fiber (%)	Ash (%)	CRB* (%)	Energy value (kcal/100 g)
Flours							
WF	12.744±0.214^a	1.047±0.033^a	11.534±0.248^a	1.589±0.034^a	0.538±0.015^a	72.548	345.751
ASF	8.594±0.151^b	6.368±0.093^b	7.674±0.227^b	14.592±0.249^b	2.757±0.099^b	60.015	328.068
Bread samples							
CB	37.721±0.275^a	2.018±0.024^a	10.265±0.027^a	2.231±0.065^a	1.011±0.024^a	46.754	246.238
B5ASF	36.928±0.026^b	2.788±0.090^b	10.034±0.046^b	2.877±0.025^b	1.476±0.023^b	45.897	245.816
B10ASF	36.683±0.042^c	3.413±0.085^c	9.756±0.010^c	3.469±0.029^c	1.959±0.027^c	44.720	245.621
B15ASF	36.488±0.070^d	4.037±0.016^d	9.313±0.012^d	4.096±0.011^d	2.254±0.033^d	43.812	245.433
B20ASF	36.277±0.019^e	4.894±0.085^e	8.852±0.025^e	4.780±0.069^e	2.569±0.038^e	42.628	245.124

*CRB – carbohydrates. a-e different letters between samples signify statistical differences according to One-way ANOVA test. Results are expressed as the mean ± standard deviation (SD) of three independent determinations. Values marked with different superscript letters within the same column are statistically significantly different (one-way ANOVA, $p < 0.05$).

Physical properties of bread samples

The bread samples were subjected to physical analysis, including the determination of volume, porosity, elasticity, and height-to-diameter ratio, in accordance with the methodologies described by Cirlincione et al. (2022) and Dossa et al. (2023). The experimental results are presented in Table 3.

Table 3. Physical properties of bread samples

Bread samples	Bread quality indicators			
	Volume (cm^3/100 g)	Porosity (%)	Elasticity (%)	Ratio between high and diameter (H/D)
CB	410±0.064^a	78.696±0.194^a	73.629±0.058^a	0.494±0.015^a
B5ASF	408±0.526^b	76.330±0.145^b	72.283±0.030^b	0.488±0.015^a
B10ASF	406±0.506^c	74.373±0.219^c	71.406±0.076^c	0.485±0.011^a
B15ASF	404±0.559^d	72.256±0.107^d	70.323±0.047^d	0.483±0.011^a
B20ASF	402±0.577^e	70.334±0.077^e	69.368±0.032^e	0.479±0.007^a

$^{a-e}$different letters between samples signify statistical differences according to One-way ANOVA test. Results are expressed as the mean ± standard deviation (SD) of three independent determinations. Values marked with different superscript letters within the same column are statistically significantly different (one-way ANOVA, $p < 0.05$).

The results of the physical properties evaluation of the bread formulas revealed statistically significant differences ($p < 0.05$) between the control sample (CB) and the avocado seed flour (ASF)–substituted samples. The control bread exhibited the highest values for volume (410 cm^3/100 g), porosity (78.696%), elasticity (73.629%), and height-to-diameter (H/D) ratio (0.494), whereas the ASF-containing samples showed significantly lower values for these parameters. For the bread samples with added ASF, the volume ranged from 408 cm^3/100 g for sample B5ASF to 402 cm^3/100 g for sample B20ASF, the porosity ranged from 76.330% for sample B5ASF to 70.334% for the B20ASF sample, the elasticity ranged from 72.283% for the B5ASF sample to 69.368% for the B20ASF sample, and the H/D ratio ranged from 0.488 for the B5ASF sample to 0.479 for the B20ASF sample. The decrease in the values of the physical parameters of the bread recipes could be related to the activity of the enzymes present in the ASF, which may reduce the ability of the dough to retain the gases generated during fermentation. This effect could lead to an increase in the viscosity of the dough, resulting in a lower final volume of the product. A progressive decrease in loaf volume was observed with increasing levels of avocado seed flour (ASF) substitution. The control sample (CB) showed the highest loaf volume, while the lowest volume was recorded in B20ASF, indicating a negative correlation between ASF content and loaf volume. This can be attributed to the reduced gluten network formation due to the low WF content. Porosity values followed a similar decreasing trend, with higher ASF incorporation resulting in denser crumb structures. This could be related to the interference of fiber from ASF with gas retention during fermentation and baking. Elasticity measurements showed a slight decrease with increasing ASF levels, suggesting that ASF may affect the viscoelastic properties of the dough. However, moderate substitutions (e.g. B5ASF and B10ASF) maintained acceptable levels of elasticity for bread texture. The height/diameter (H/D) ratio gradually decreased with SFA fortification. These results are consistent with previous reports that composite flours with higher fiber and lower gluten content produce flatter products. The results are comparable with other studies (Peluola-Adeyemi et al. 2016, 2021), according to which the physical properties of bread samples with added ASF decreased in proportion to the WF/ASF ratio.

Phytochemical profile of bread samples

The phytochemical profiles of the bread samples were assessed in terms of total phenolic content (TPC) and antioxidant activity (AA). These assessments are presented in Table 4.

Table 4. Phytochemical profile of bread samples

Bread samples	Phytochemical Parameters	
	Total phenolic compounds (mg GAE/g)	Antioxidant activity (mmol TEAC/100g)
CB	20.470±0.091 ^{a}	7.217±0.092^{a}
B5ASF	22.844±0.021^{b}	8.187±0.035^{b}
B10ASF	23.176±0.027^{c}	8.490±0.069^{c}
B15ASF	23.478±0.018^{d}	8.808±0.084^{d}
B20ASF	23.937±0.031^{e}	9.187±0.035^{e}

$^{a-e}$different letters between samples signify statistical differences according to One-way ANOVA test. Results are expressed as the mean ± standard deviation (SD) of three independent determinations. Values marked with different superscript letters within the same column are statistically significantly different (one-way ANOVA, $p < 0.05$).

The findings demonstrated that the TPC and AA levels increased in relation to the level of ASF incorporated into the bread samples, with statistically significant differences observed between the various ASF levels and the control sample. Notably, the TPC values of ASF-enriched bread samples were higher than that of the control. The maximum TPC recorded was found in B20ASF (23.937 mg GAE/g), while the minimum was observed in the control bread (CB), with 20.470 mg GAE/g. The study revealed a consistent trend in the antioxidant activity (AA) among various bread samples. The bread sample B20ASF exhibited the highest antioxidant activity (9.187 mmol TEAC/100 g), while the lowest activity was observed in the control sample CB (7.217 mmol TEAC/100 g). These findings suggest that the bioactive compounds present in ASF contribute to the overall antioxidant potential of the final product. The B20ASF sample demonstrated the highest antioxidant activity, underscoring the functional potential of ASF in enhancing the nutritional quality of bread. The relatively low total phenolic content (TPC) observed in the bread samples may be attributed to the high baking temperature (220°C), which could lead to the degradation or denaturation of a substantial proportion of phenolic compounds (Harbourne et al., 2009). Similarly, a tendency of elevated TPC and AA content has been documented by other researchers (Indriyani et al., 2015; Peluola-Adeyemi et al., 2021).

Sensory evaluation of bread samples

The sensory evaluation scores of the bread samples supplemented with different levels of ASF are presented in Table 5.

Table 5. Quality attributes scored in sensory assessment of bread samples

Bread samples	Sensory evaluation				
	Appearance	Taste	Flavor	Texture	Overall acceptability
CB	4.328±0.033^{a}	4.038±0.031^{a}	4.037±0.021^{a}	4.235±0.111^{a}	4.105±0.025^{a}
B5ASF	4.274±0.024^{a}	4.120±0.073^{a}	4.054±0.080^{a}	4.220±0.119^{a}	4.144±0.023^{a}
B10ASF	4.256±0.013^{b}	4.223±0.069^{b}	4.156±0.013^{b}	4.203±0.059^{a}	4.190±0.016^{a}
B15ASF	4.243±0.029^{b}	4.283±0.017^{c}	4.296±0.008^{c}	4.180±0.050^{a}	4.240±0.013^{b}
B20ASF	4.211±0.008^{c}	4.134±0.026^{a}	4.101±0.053^{a}	4.048±0.022^{a}	4.101±0.073^{a}

$^{a-c}$different letters between samples signify statistical differences according to One-way ANOVA test. Results are expressed as the mean ± standard deviation (SD) of three independent determinations. Values marked with different superscript letters within the same column are statistically significantly different (one-way ANOVA, $p < 0.05$).

For all sensory attributes evaluated by the panelists (appearance, taste, flavor, texture, and overall acceptability) no statistically significant differences ($p<0.05$) were observed among the bread samples. The bread sample supplemented with 15% ASF was rated as having the most favourable sensory characteristics. The sensory evaluation of bread appearance showed that the control sample (CB) received the highest score (4.328), followed by B5ASF (4.274), while the highest score among the ASF-enriched samples was recorded for B15ASF (4.243). Variations in the scores were also recorded for the taste of the bread samples, with the scores increasing proportionally with the percentage of replacement of WF by ASF, up to sample B15ASF, which received a score of 4.238, which then decreased to 4.134 in sample B20ASF, compared with CB, which received a score of 4.038. There were also variations in the flavour scores of the bread samples, with the scores increasing proportionally with the percentage of WF replaced by ASF up to sample B15ASF, which scored 4.296, and then decreasing to 4.101 in sample B20ASF, compared to CB, which scored 4.037. The texture of the bread samples with added ASF was well accepted by the panel members, with sample B20ASF receiving a score of 4.048, compared to CB, which however received the highest score of 4.235.
The same increasing trend, proportional to the increasing rate of WF substitution with ASF, up to sample B15ASF, which received a score of 4.240, was also observed for the overall acceptance scores of the bread recipes compared to the CB sample, which received a score of 4.105. The addition of ASF affected the sensory characteristics of the bread samples analysed as follows: crust and crumb appearance was different from that of CB, crust crispier, crumb denser; slightly astringent, slightly bitter taste; strange aroma. Sensory evaluation of breads based on WF and ASF shows that the use of up to 15% ASF in the recipe results in an increase in consumer acceptance. In previous studies (Peluola-Adeyemi et al. 2016, 2021; Viola et al., 2023) a similar increase in sensory attributes of bread samples was observed when WF was replaced by increasing levels of ASF.

CONCLUSIONS

The results of the present study demonstrate the considerable potential of avocado seed flour (ASF) in the development of functional foods, thus supporting its use as a functional ingredient in the food industry. Partial replacement of WF with ASF in the bread recipe resulted in an increase in the nutritional value of the bread as reflected by increased fibre, healthy fat and mineral content. These changes highlight the potential health benefits of the product. Sensory evaluation of WF and ASF based bread shows that the use of up to 15% ASF in the recipe, results in increased consumer acceptance. The comprehensive nutritional, physical, phytochemical, and sensory evaluation of the bread samples demonstrated that avocado seed flour (ASF) may serve as a valuable plant-based source for the development of new food products with enhanced functionality. Given these considerations, the industrial implementation of these bread formulations is recommended to facilitate their availability as a high-quality food product with high nutritional value.

REFERENCES

Akua, A.O. (2012). *Formulation and shelf life evaluation of avocado (Persea americana) fruit spread.*
MSc Thesis in Food Science and Technology.

Alkhalaf, M.I., Alansari, W.S., Ibrahim, E.A., ELhalwagy & M. E. (2019). Anti-oxidant, anti-inflammatory and anti-cancer activities of avocado (*Persea americana*) fruit and seed extract. *J. King Saud Univ. Sci.*, *31*, 1358–1362.

Anjarwati, D., Oktarini, W. D., Setiawan, A., Ulhaq, N. D., & Putri, D. N. (2023). Effect of substitution ratio of wheat flour with coconut dregs and avocado seed flour as gluten-free flour on the chemical characteristics of white bread. *Agrointek: Jurnal Teknologi Industri Pertanian*, 17(4), 915-926.

Araújo, R. G., Rodriguez-Jasso, R. M., Ruiz, H. A., Pintado, M. M. E. & Aguilar, C.N. (2018). Avocado By-Products: Nutritional and Functional Properties. *Trends Food Sci. Technol*., *80,* 51–60.

AOAC, (2005). *Methods of the Association of Official Analytical Chemists*. Official methods of analysis 18th ed., Airlington Virginia Association, 806 – 842.

Borrelli G. M., Menga V., Giovanniello V. & Ficco, D. B. M. (2023). Antioxidants and phenolic acid composition of whole meal and refined-flour, and related biscuits in old and modern cultivars belonging to three cereal species. *Foods*, 12(13), 2551.

Boshra, V., & Tajul, A.Y. (2013). Tropical fruits: A new frontier in the bakery industry. *International Journal of Medical Sciences and Biotechnology*, *1*, 51-60.

Cirlincione, F., Venturella, G., Gargano, M. L., Ferraro, V., Gaglio, R., Francesca, N., Rizzo, B. A., Russo, G., Moschetti, G. & Settanni, L. (2022). Functional bread supplemented with *Pleurotus eryngii* powder: a potential new food for human health. *Int. J. Gastron. Food Sci*., 27, 100449.

Di Stefano, V., Buzzanca, C., Melilli, M. G., Indelicato, S., Mauro, M., Vazzana, M., Arizza, V., Lucarini, M., Durazzo, A. & Bongiorno, D. (2022). Polyphenol characterization and antioxidant activity of grape seeds and skins from Sicily: a preliminary study. *Sustainability*, 14, 6702

Dossa, S., Negrea, M., Cocan, I., Berbecea, A., Obistioiu, D., Dragomir, C., Alexa, E. & Rivis, A. (2023). Nutritional, physico-chemical, phytochemical, and rheological characteristics of composite flour substituted by baobab pulp flour (*Adansonia digitata* L.) for bread making. *Foods*, *12*(14), 2697.

Duarte, P.F., Chaves, M.A., Borges, C.D. & Mendonça, C.R.B. (2016). Avocado: characteristics, health benefits and uses. *Cienc. Rural*, 46, 747–754.

Eliopoulos, C., Markou, G., Langousi, I. & Arapoglou, D. (2022). Reintegration of Food Industry By-Products: Potential Applications. *Foods*, *11*, 3743.

Ford, N. A., Liu, A.G. (2020). The Forgotten Fruit: A Case for Consuming Avocado within the Traditional Mediterranean Diet. *Front. Nutr.*, *7*, 78.

Galali, Y., Omar, Z.A. & Sajadi, S.M. (2020). Biologically active components in by-products of food processing. *Food Sci. Nutr.*, *8*, 3004–3022.

Hadj Saadoun, J., Bertani, G., Levante, A., Vezzosi, F., Ricci, A., Bernini, V. & Lazzi, C. (2021). Fermentation of agri-food waste: a promising route for the production of aroma compounds. *Foods*, *10*, 707.

Harbourne, N., Jacquier, J. C., & O'Riordan, D. (2009). Optimisation of the aqueous extraction conditions of phenols from meadowsweet (*Filipendula ulmaria* L.) for incorporation into beverages. *Food Chemistry*, *116*(3), 722-727.

Association of Official Analytical Chemists (AOAC). (2000). Official Methods of Analysis, 17th ed.; Washington, DC, USA: AOAC.

Ifesan, B., Olorunsola, B. & Ifesan, B.T. (2015). Nutritional composition and acceptability of candy from avocado seed (*Persea americana*). *Int. J. Agric. Innov. Res.*, *3*, 1732–1735.

Indriyani, L., Rohman, A., & Riyanto, S. (2015). Physico-chemical characterization of avocado (*Persea americana mill.*) oil from three Indonesia avocado cultivars. *Research Journal of medicinal Plant*, 10, 67-78.

ISO 6658:2017. (2017). Sensory Analysis –Methodology - Overall Guidelines. ISO: Geneva, Switzerland.

Jimenez, P., Garcia, P., Quitral, V., Vasquez, K., Parra-Ruiz, C., Reyes-Farias, M., Garcia-Diaz, D. F., Robert, P., Encina, C. & Soto-Covasich, J. (2021). Pulp, leaf, peel and seed of avocado fruit: a review of bioactive compounds and healthy benefits. *Food Rev. Int.*, *37*, 619–655.

Khorairi, A., Sofian-Seng, A.N.S., Othaman N.S., Abdul Rahman, R., Mohd Razali, H., Lim, N.S. & Wan Mustapha, W.A. (2023). A review on agro-industrial waste as cellulose and nanocellulose source and their potentials in food applications. *Food Reviews International*, *39*(2), 663-688.

Kotilainen, L., Rajalahti, R., Ragasa, C. & Pehu, E. (2006). Health Enhancing foods. Opportunities for Strengthening. Sector în Developing Countries, World Bank. *Agriculture and Rural Development*, www.worldbank.org/rural, 82.

Kristanti, C. D., Simanjuntak, F. P. J., Dewi, N. K. P. A., Tianri, S. V. & Hendra, P. (2017). Anti-inflammatory and Analgesic Activities of Avocado Seed (*Persea americana* Mill.). *J. Pharm. Sci. Community*, *14*, 104–111.

Lahiri, A., Daniel, S., Kanthapazham, R., Vanaraj, R., Thambidurai, A., & Peter, L. S. (2023). A critical review on food waste management for the production of materials and biofuel. *Journal of Hazardous Materials Advances*, *10*, 100266.

Mahawan, M. A., Tenorio, M. F. N., Gomez, J. A. & Bronce, A. R. (2015). Characterization of Flour from Avocado Seed Kernel. *Asia Pac. J. Multidiscip. Res.*, *3*, 4.

Marra, A., Manousakis, V., Zervas, G. P., Koutis, N., Finos, M. A., Adamantidi, T. & Tsoupras, A. (2024). Avocado and its by-products as natural sources of valuable anti-inflammatory and antioxidant bioactives for functional foods and cosmetics with health-promoting properties. *Applied Sciences*, *14*(14), 5978.

Metzner Ungureanu, C.R., Lupitu, A.I., Moisa, C., Rivis, A., Copolovici, L.O. & Poiana, M.-A. (2020). Investigation on High-Value Bioactive Compounds and Antioxidant Properties of Blackberries and Their Fractions Obtained by Home-Scale Juice Processing. *Sustainability,* 12, 5681.

Mitrea, S.I., Petcu, D.C. & Savu, G. (2003). *Food safety through the application of the HACCP system*. Bucharest, RO: Bogdana Publsihing House.

Nirmal, N. P., Khanashyam, A. C., Mundanat, A. S., Shah, K., Babu, K. S., Thorakkattu, P & Pandiselvam, R. (2023). Valorization of fruit waste for bioactive compounds and their applications in the food industry. *Foods*, *12*(3), 556.

Nyakang'i, C. O., Marete, E., Ebere, R., & Arimi, J. M. (2023). Physicochemical properties of avocado seed extract model beverages and baked products incorporated with avocado seed powder. *International Journal of Food Science*, *2023*(1), 6860806.

Oktarini, W. D., Anjarwati, D., Setiawan, A., Ulhaq, N. D., Putri, D. N. (2023). Physical Properties and Consumer Acceptance of White Bread with The Substitution of Coconut Dregs and Avocado Seed Flour. *agriTECH*, *43*(3), 278-287.

Peluola-Adeyemi, O.A, Bolaji, O.T., Adepoju, P.A, Adeyeye, S.A.O., & Bello, I.E (2020): Quality significance and assessement of bread spiced with

chili pepper (*Capsicum annuum*). *Journal of Food Stability*, 3, 81-91.
Peluola-Adeyemi, O. A., Abdus-Salaam, R. B., Obi, T. E., & Toyiemedo, N. C. (2021). Quality evaluation of Bread produced from Wheat flour using Avocado (*Persea americana*) paste as substitute. *Journal of Food Stability*, *4*(1), 1-12.
Petcu, C.D., Savu, C., Mitrănescu, E., & Chirilă, S., (2007). The implementation of the integrated quality and food safety management system in the food industry units. *Lucrări Ştiinţifice Medicină Veterinară,* XL, 545-51.
Permal, R., Chia, T., Arena, G., Fleming, C., Chen, J., Chen, T., Chang,W.L., Seale, B., Hamid, N. & Kam, R. (2023). Converting Avocado Seeds into a Ready to Eat Snack and Analysing for Persin and Amygdalin. *Food Chem.*, *399*, 134011.
Poiana, M. A., Alexa, E., & Bragea, M. (2009). Studies concerning the phosphorus bioavailability improvement of some cereals used in nourishment, *Romanian Biotechnological Letters*, *14*(3), 4470–4476.
Puspitasari, M., Hasri, R. N. & Wahyuni, T. (2020). The Substitution of Avocado Seed Flour to Rice Flour in the Manufacture of Traditional Palembang Food Gandus Cake. *In Proceedings of the 1st International Conference on Health, Social Sciences and Technology Palembang, Indonesia*, 37–40.
Salazar-López, N.J., Domínguez-Avila, J. A., Yahia, E.M., Belmonte-Herrera, B. H., Wall-Medrano, A., Montalvo-González, E. & González-Aguilar, G.A. (2020). Avocado Fruit and By-Products as Potential Sources of Bioactive Compounds. *Food Res. Int.*, *138*, 109774.
Sanchez-Rosario, R., Castillo, L., Féliz-Jiménez, A., Vargas, S., Pérez-Romero, R., Aquino, M. & Abutokaikah, M. T. (2025). Characterization of Avocado (*Persea americana* Mill) Seed Extract from the Variety Semil 34 Cultivated in the Dominican Republic. *Applied Sciences*, *15*(2), 922.
Setyawan, H.Y., Sukardi, S. & Puriwangi, C.A. (2021). Phytochemicals Properties of Avocado Seed: A Review. *IOP Conf. Ser. Earth Environ. Sci.*, *733*, 012090.
Siol, M. & Sadowska, A. (2023). Chemical Composition, Physicochemical and Bioactive Properties of Avocado (*Persea americana*) Seed and Its Potential Use in Functional Food Design. *Agriculture*, *13*, 316.
Stoin, D., Pirsan, P., Radu, F., Poiana, M. A., Alexa, E. & Dogaru, D. (2009). Studies regarding the myrosinase enzymatic activity from black mustard (*Brassica nigra*) seeds. *J. Food Agric. Environ*, *7*, 44–47.
Stoin, D., Jianu, C., Velciov, A., Negrea, M. & Poiana, M.A. (2023). Quality evaluation of functional bread developed from wheat flour and malted barley flour mixtures, *International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management, SGEM*, 23(6.2), 345–352.
Vicentini, A., Liberatore, L. & Mastrocola, D. (2016). Functional Foods: Trends And Development Of The Global Market*. Ital. J. Food Sci.*, *28*, 338-351.
Viola, E., Buzzanca, C., Tinebra, I., Settanni, L., Farina, V., Gaglio, R. & Di Stefano, V. (2023). A functional end-use of avocado (cv. Hass) waste through traditional semolina sourdough bread production. *Foods*, *12*(20), 3743.
Wang, J., Rosell, C. M. & Benedito de Barber, C. (2002). Effect of the addition of different fibres on wheat dough performance and bread quality, *Food Chemistry*, *79*, 221–226.
Wang,W., Bostic, T.R. & Gu, L. (2010). Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars. *Food Chem*., *122*, 1193–1198.
Wong, C. W., Teoh, C. & Putri, C. (2017). Effect of Enzymatic Processing, Inlet Temperature, and Maltodextrin Concentration on the Rheological and Physicochemical Properties of Spray-dried Banana (*Musa Acuminata*) Powder. *J. Food Process. Preserv.*, 42.
Zghal, M.C., Scanlon, M.G., & Sapirstein, H.D. (2002). Cellular Structure of Bread Crumb and its Influence on Mechanical Properties. *Journal of Cereal Science*, 36, 167-176.
Zero Waste International Allince. (2018). Available online: https://zwia.org/zero-waste-definition (accessed on 10 October 2024).