



Article

Improvement of Functional Properties and In Vitro Digestibility of Durum Wheat, Chickpea, and Amaranth Flours Using Power Ultrasound

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Abstract

Ultrasound technology has emerged as a promising non-thermal approach for modifying the structural and functional properties of food matrices. However, its impact on starch digestibility remains insufficiently understood, particularly in complex systems such as cereal, legume, and pseudocereal flours. This study evaluated the effect of power ultrasound on the functional properties and in vitro digestibility of durum wheat (DWF), chickpea (CF), and amaranth (AF) flours. Flours were treated using an ultrasonic probe (20 kHz, 60% amplitude) for 10 and 20 min while maintaining the sample temperature at 15 °C. Structural and functional properties were assessed, including color, morphology, water absorption, FT-IR spectra, pasting behavior, thermal properties, and starch fractions. Ultrasound induced structural modifications, including starch granule disruption and increased surface roughness. Lightness increased in DWF, CF, and AF, although the magnitude of the response differed among flour types. Water absorption improved in DWF but decreased in CF and AF. FT-IR spectra suggested molecular rearrangements, while viscoelastic and thermal analyses showed increased viscosity in DWF and CF and reduced viscosity in AF. Ultrasound also modified starch fractions and in vitro starch digestibility in a flour-dependent manner, reflecting the distinct structural responses of cereal, legume, and pseudocereal starches to acoustic cavitation. Overall, ultrasound modified the functionality of flour and starch digestibility in a matrix-dependent manner, supporting its potential as a clean-label technology for tailoring the functional properties of different flour matrices.

Keywords: acoustic cavitation; flour functionality; clean-label technology

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1. Introduction

Flour is a fundamental component of human nutrition, serving as a primary ingredient in staple foods consumed worldwide. Derived mainly from cereals such as wheat, maize, and rice, flour provides essential nutrients including carbohydrates, proteins, and micronutrients, and plays a key role in global food security [1,2]. According to the Food and Agriculture Organization (FAO), wheat-based products account for approximately 20% of global dietary energy intake [3], highlighting the strategic role of wheat in ensuring food and nutrition security.

Driven by the increasing global demand for flour-based products, research has focused on developing technologies that enhance both the functional performance and nutritional quality of flours. Conventional approaches such as enzymatic modification, fermentation, and fortification have been widely explored; however, these methods may involve additional processing steps or chemical inputs [4,5]. Consequently, there is a need for alternative, sustainable, and clean-label technologies that improve flour functionality while preserving nutritional value.

Against this background, ultrasound has emerged as a promising non-thermal processing technology in the food industry. High-intensity ultrasound generates acoustic cavitation, producing localized shear forces, microstreaming, and transient pressure variations that can disrupt and reorganize biopolymer structures, particularly starch and proteins [6,7]. These effects have been shown to modify key functional properties such as water absorption, rheology, and thermal behavior in a variety of food systems, including corn, potato, rice, and teff flours and starches [8–16].

Despite the growing body of research on ultrasound-treated flours and starches, most studies have focused on structural and physicochemical modifications, with limited attention to their effects on starch digestibility and nutritional functionality. In particular, the relationship between ultrasound-induced structural changes and the enzymatic susceptibility of starch remains insufficiently understood, especially in complex matrices such as legume and pseudocereal flours. Understanding these effects is essential, as modifications in starch digestibility can directly influence the glycemic response and nutritional quality of flour-based foods. Therefore, integrating functional and digestibility analyses is crucial for developing next-generation clean-label ingredients that offer both technological and health-related benefits.

Durum wheat, chickpea, and amaranth flours were selected because they represent cereal, legume, and pseudocereal matrices with contrasting physicochemical compositions and starch architectures. Durum wheat flour is rich in gluten-forming proteins and contains A-type starch granules; chickpea flour is characterized by higher protein and amylose contents together with abundant dietary fiber; and amaranth flour contains very small starch granules embedded within a protein-rich matrix. These compositional and structural differences provide an excellent model for investigating whether ultrasound-induced modifications depend on the botanical origin of the flour, thereby expanding the potential applications of this green technology across different food systems.

Building on this rationale, this study aimed to evaluate the effect of power ultrasound on the functional properties and *in vitro* digestibility of durum wheat, chickpea, and amaranth flours, with particular emphasis on the relationship between structural modifications and enzymatic susceptibility of starch.

2. Materials and Methods

2.1. Experimental Design

The study followed a completely randomized experimental design. Three flour matrices (durum wheat, chickpea, and amaranth) were evaluated independently. For each

flour type, three treatments were considered: untreated flour (control), ultrasound treatment for 10 min, and ultrasound treatment for 20 min. Each treatment was processed under identical experimental conditions.

Unless otherwise indicated, all analytical determinations were performed in triplicate (technical replicates), and results are expressed as mean $\pm$ standard deviation.

2.2. Preparation of Flour Samples

Durum wheat grains (Cirno C2008 variety) were obtained from Munsa, S.A. de C.V. (Ciudad Obregón, Sonora, Mexico). Chickpea and amaranth grains were purchased from a local market in Santiago de Querétaro, México.

The chickpea, amaranth, and wheat grains were tempered overnight at room temperature (25 ± 1 °C) for 16 h and ground into flour using a Buhler semolina mill with six corrugated roller passage systems. The remaining coarse grains were passed through a Buhler mill (Buhler Holding AG, Switzerland) to reduce particle size. Particle size was determined on a vibrating sieve using standard EE sieves (150 mm), following the established methods of AACC 26-21.02 and 26-41.01 [17]. Representative photographs of the untreated durum wheat, chickpea, and amaranth flours are provided in Supplementary Figure S1.

2.3. Ultrasonic Exposure

Flour suspensions were prepared at 10% (*w/w*) by dispersing 50 g of flour in 450 g of distilled water, resulting in 500 g of suspension. The suspensions were treated using a UIP500hdT ultrasonic processor (Hielscher Ultrasonics GmbH, Teltow, Germany) equipped with a 22 mm diameter titanium sonotrode, operating at 20 kHz and 60% amplitude. Sonication was applied for 10 and 20 min. During ultrasound treatment, the sample temperature was maintained at 15 ± 1 °C using an external ice-water bath to minimize heating caused by acoustic cavitation. Since calorimetric measurements were not performed, the effective acoustic power and acoustic energy density delivered to the samples were not determined.

After sonication, the flour suspensions were spread onto aluminum trays and dried in a 30 L forced-convection oven (Luzeren, model DHG-9070A, Beijing, China) at 40 °C for 24 h. The dried material was subsequently milled and stored in hermetically sealed glass containers at room temperature until further analyses.

2.4. Physical Properties

2.4.1. Flour Color

Flour color was measured using a portable spectrophotometer (Ci62, X-Rite Inc., Grandville, MI, USA). The hue angle (H°) was calculated from CIE L, a, and b* coordinates as $H^\circ = \tan^{-1} (b^*/a^*)$. Measurements were performed under illuminant D65 with a 10° standard observer.

2.4.2. Morphology

Flour morphology was examined by environmental scanning electron microscopy (ESEM; Philips XL30) at an accelerating voltage of 20 kV using a gaseous secondary electron detector (SEI). Images were acquired at 1500 $\times$ under a chamber pressure of 1 Torr. Samples were sputter-coated with gold/palladium (JFC-1100, JEOL, Akishima, Japan).

2.4.3. X-RAY Diffraction

X-ray diffraction (XRD) patterns were obtained using a Siemens D-500 diffractometer (Bragg–Brentano $\theta/2\theta$ geometry) with Cu K α radiation ($\lambda = 1.5418$ Å) generated at 40 kV

and 30 mA. A graphite monochromator was used, with a divergence slit of 0.3° and a receiving slit of 0.05° . Scans were recorded over a 2θ range of $3\text{--}60^\circ$, with a step size of 0.05° and a counting time of 10 s per step.

Relative crystallinity was estimated from the X-ray diffractograms using JADE software (Materials Data Inc., Livermore, CA, USA). Before analysis, baseline correction was performed to separate the crystalline and amorphous contributions. The areas corresponding to the crystalline peaks and the amorphous halo were integrated, and the relative crystallinity (%) was calculated according to Equation (1):

$$\text{Relative crystallinity (\%)} = \frac{A_c}{A_c + A_a} \times 100. \quad (1)$$

where A_c is the integrated area of the crystalline peaks and A_a is the integrated area of the amorphous region.

2.5. Physicochemical Properties

2.5.1. Water Activity and Absorption Capacity

Water activity (a_w) was evaluated at 25°C using an Aqualab® 4te dew point water activity meter (Decagon Devices, Inc., Pullman, WA, USA). The water sorption capacity of the flours was measured according to the American Society for Testing and Materials (ASTM) standard method (2005).

2.5.2. Proximate Composition

Protein, fat, crude fiber, ash, and moisture contents were determined, and the carbohydrate content of the flour from each treatment was calculated by difference using official methods of the American Association of Cereal Chemists [17]. All proximate composition components (protein, lipids, dietary fiber, ash, and carbohydrates) were expressed on a dry weight basis. Carbohydrate content was calculated by difference after correcting the samples for moisture content, whereas moisture was reported separately on a wet basis to characterize the flour samples.

2.5.3. Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier transform infrared (FT-IR) spectra were obtained to evaluate the vibrational bands of bonds and functional groups in each sample using a Bruker Alpha Platinum ATR spectrometer (Bruker Co., Billerica, MA, USA) according to the conditions reported by Santiago-Ramos et al. [18].

2.6. Thermal and Pasting Properties

2.6.1. Viscoamylographic Profile (RVA)

A Rapid Viscoanalyzer (3D Newport Scientific Pty., Ltd., Warriewood, NSW, Australia) was used to measure viscosity as described by Calderón-Peralta et al. [19]. The amylographic profile of the flours was determined using a suspension of 4 g of flour in 24 g of water (dry weight), according to the 70-10 methodology described in AACC [17]. The parameters evaluated were: initial gelatinization temperature, maximum viscosity, final viscosity, and setback.

2.6.2. Differential Scanning Calorimetry (DSC)

DSC equipment (Mettler-Toledo®, model 821e, Schwerzenbach, Switzerland) controlled by STARe version 10.0 software was used. A 3 mg sample was weighed into an aluminum crucible and heated from 40 to 100°C at a rate of $10^\circ\text{C}/\text{min}$.

2.7. In Vitro Digestibility

The in vitro digestibility of the flours was assessed by quantifying starch fractions and their susceptibility to enzymatic hydrolysis.

Total starch, amylose, amylopectin, and resistant starch contents were determined using commercially available enzymatic assay kits from Megazyme (Bray, Ireland), following the manufacturer's validated procedures and the corresponding AOAC/AACC Official Methods [17,20].

2.7.1. Total Starch

Total starch was determined using the Total Starch Assay Kit (K-TSTA) according to AOAC Method 996.11 and AACC Method 76-13.01 [17,20]. Briefly, samples were hydrolyzed with thermostable α -amylase and amyloglucosidase, converting starch into glucose. The released glucose was subsequently quantified using the glucose oxidase/peroxidase (GOPOD) colorimetric method, and total starch content was calculated from the measured glucose concentration.

2.7.2. Amylose and Amylopectin

Amylose and amylopectin contents were determined using the Amylose/Amylopectin Assay Kit (K-AMYL) based on the selective precipitation of amylopectin with Concanavalin A (Con A) followed by enzymatic hydrolysis of the remaining amylose fraction. Glucose released after enzymatic hydrolysis was quantified using the GOPOD reagent, and amylopectin content was calculated by difference.

2.7.3. Resistant Starch

Resistant starch was quantified using the Resistant Starch Assay Kit (K-RSTAR) following AOAC Method 2002.02 and AACC Method 32-40.01 [17,20]. Samples were incubated with pancreatic α -amylase and amyloglucosidase under controlled conditions to hydrolyze digestible starch, while resistant starch remained undigested. The resistant starch fraction was subsequently recovered, solubilized in potassium hydroxide, enzymatically hydrolyzed to glucose, and quantified by the GOPOD colorimetric assay.

All determinations were performed in triplicate and expressed as g/100 g dry weight.

2.8. Statistical Analysis

For each flour type and ultrasound treatment (Control, US10, and US20), three independent experimental batches were prepared and analyzed. Data were expressed as mean $\pm$ standard deviation (Mean $\pm$ SD). For each flour type, data were analyzed using a one-way analysis of variance (ANOVA) considering ultrasound treatment as the fixed factor, according to the following statistical model (Equation (2)):

$$Y_{ij} = \mu + T_i + \varepsilon_{ij} \quad (2)$$

where Y_{ij} is the observed response variable, μ is the overall mean, T_i is the fixed effect of the ultrasound treatment (Control, US10, or US20), and ε_{ij} is the random experimental error associated with each observation, assumed to be independently and normally distributed with constant variance.

When significant differences were detected ($p < 0.05$), Dunnett's multiple comparisons test was applied, with the untreated flour as the reference treatment, using GraphPad Prism (Version 9.0). Dunnett's post hoc test was selected because the primary objective was to compare each ultrasound-treated flour with its corresponding untreated control, which served as the experimental reference.

3. Results

3.1. Physical Properties of Flours

Table 1 shows the color properties of the flours after US treatment. In all treatments, lightness (L^*) tends to increase with increasing sonication time. In DWF and CF treatments, a decrease in chroma (C) is observed, indicating more opaque/grayish colors in the flour; the opposite is observed in AF, where this value increases, indicating purer color. Finally, the hue (Hue°) shows a tendency toward yellow located at 90° in all treatments. The table describes the changes in percentage crystallinity between wheat, chickpea, and amaranth flour treatments. The percentage crystallinity of the flours was influenced by ultrasound treatment in a flour-dependent manner (Table 1), which can be attributed to the cavitation-induced structural rearrangements of starch granules. In durum wheat flour, ultrasound treatment resulted in a significant increase in crystallinity, suggesting a partial reorganization of starch chains into more ordered domains following disruption of the native polymeric matrix. This behavior is consistent with ultrasound's ability to weaken amorphous regions and promote molecular realignment. In chickpea flour, ultrasound initially reduced crystallinity at short exposure times, likely due to disruption of ordered structures, followed by a significant increase after prolonged treatment, indicating a recrystallization process driven by starch chain mobility. In contrast, amaranth flour exhibited negligible changes in crystallinity regardless of ultrasound exposure, reflecting the greater structural stability of its starch and the presence of non-starch polysaccharides that limit molecular rearrangement. Overall, these results indicate that ultrasound modifies the balance between amorphous and crystalline regions of starch depending on the botanical origin and macromolecular organization of the flour matrix.

Table 1. Physical color properties of wheat, chickpea and amaranth flours treated with ultrasound.

Physical Properties				
Treatments	Luminosity (L^*)	Chromaticity (C)	Hue $^\circ$	Crystallinity (%)
DWF	69.74 ± 0.61	17.65 ± 0.55	85.92 ± 0.033	11.32 ± 0.5
DWUF 20/10	77.09 ± 0.35 ****	10.50 ± 0.055 ****	86.48 ± 0.062	16.27 ± 0.4 *
DWUF 20/20	78.91 ± 0.11 ****	10.87 ± 0.065 ****	85.37 ± 0.04 *	16.89 ± 0.7 *
CF	63.77 ± 0.09	17.20 ± 0.071	85.01 ± 0.088	15.45 ± 0.3
CFU 20/10	71.70 ± 0.15 ****	16.96 ± 0.016	82.33 ± 0.024 ****	11.26 ± 0.1 *
CFU 20/20	76.16 ± 0.16 ****	15.68 ± 0.3 ****	83.04 ± 0.19 ****	19.80 ± 0.9 *
AF	64.36 ± 0.11	13.20 ± 0.057	81.69 ± 0.12	11.34 ± 0.1
AFU 20/10	68.59 ± 0.015 ****	15.27 ± 0.033 ****	81.08 ± 0.04	11.45 ± 0.2
AFU 20/20	72.37 ± 0.155 ****	16.21 ± 0.071 ****	81.34 ± 0.031	11.69 ± 0.2

DWF: Durum wheat flour; DWUF: Durum wheat flour + ultrasound; CF: Chickpea flour; CFU: Chickpea flour + ultrasound; AF: Amaranth flour; AFU: Amaranth flour + ultrasound; 20/10: 20 kHz for 10 min; 20/20: 20 kHz for 20 min. Data are reported as mean ± standard deviation. Values with an asterisk (*) within the same column indicate a significant difference ($p < 0.05$), using Dunnett's test independently for each type of flour. * ($p < 0.05$); **** ($p < 0.0001$).

3.2. Morphology of Flours

Figure 1 shows the micrographs of starch granules from durum wheat, chickpea, and amaranth flours subjected to ultrasound treatment at 1500× magnification. In the control wheat flour (Figure 1A1), starch granules appeared embedded within a continuous polymeric matrix, with limited granule separation. After ultrasound treatment (Figure 1A2,A3), starch granules were more clearly individualized and dispersed, indicating disruption of the surrounding matrix and partial disaggregation with increasing sonication time.

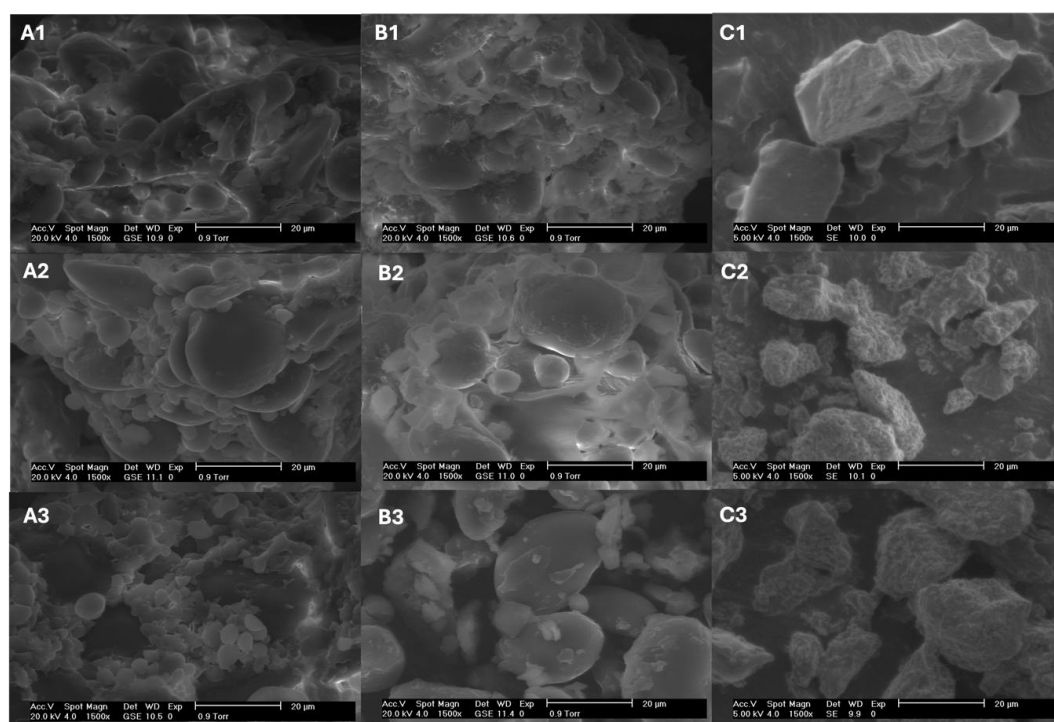


Figure 1. Micrographs of wheat, chickpea and amaranth flours treated with US at 1500 X. (A1) Control wheat flour; (A2) Wheat flour + ultrasound (20 kHz/10 min); (A3) Wheat flour + ultrasound (20 kHz/20 min); (B1) Control chickpea flour; (B2) Chickpea flour + ultrasound (20 kHz/10 min); (B3) Chickpea flour + ultrasound (20 kHz/20 min); (C1) Control amaranth flour; (C2) Amaranth flour + ultrasound (20 kHz/10 min); (C3) Amaranth flour + ultrasound (20 kHz/20 min).

In chickpea flour, control samples (Figure 1B1) exhibited starch granules partially covered by non-starch components. In contrast, ultrasound-treated samples (Figure 1B2,B3) showed improved granule separation and a more heterogeneous distribution, with evidence of surface irregularities and fragmentation at longer exposure times.

Amaranth flour micrographs revealed small starch granules with relatively smooth surfaces in the control sample (Figure 1C1). Following ultrasound treatment (Figure 1C2,C3), granules appeared more dispersed, with increased surface roughness and a higher proportion of granules with diameters $\leq 20 \mu\text{m}$, suggesting structural modification induced by cavitation. Overall, the morphological changes observed were more pronounced with increasing ultrasound exposure time and varied by flour type.

3.3. Physicochemical Properties of Flours

Table 2 shows the water absorption parameters obtained in the wheat, chickpea, and amaranth flour treatments after exposure to US. In the WSI in the DWF treatments, no significant differences were observed when applying US. In the case of the CF treatments, the application of US decreased the percentage of soluble compounds, whereas for AF, this percentage increased with increasing exposure time. On the other hand, with the WAI in the DWF treatments, a significant increase in their absorbent capacity was observed as exposure time to US increased. In the CF and AF treatments, however, a significant decrease was observed compared to their control treatments. Table 2 also shows the water activity (aw) of the flours. In DWF, the US treatment decreased this parameter, while in the CF and AF treatments, it increased.

Table 2. Water absorption properties of wheat, chickpea and amaranth flours treated with ultrasound.

Treatments	Water Absorption Properties		
	WSI (g Solids/100 g Sample)	WAI (g gel/g Sample)	Water Activity (a_w)
DWF	0.055 ± 0.006	1.98 ± 0.025	0.48 ± 0.0025
DWFU	0.056 ± 0.002	2.25 ± 0.024 ***	0.34 ± 0.001 ***
20/10			
DWFU	0.056 ± 0.001	2.28 ± 0.032 ***	0.303 ± 0.002 ***
20/20			
CF	0.27 ± 0.01	3.91 ± 0.093	0.383 ± 0.003
CFU 20/10	0.21 ± 0.002 **	2.81 ± 0.013 ****	0.48 ± 0.001 ***
CFU 20/20	0.24 ± 0.002 *	2.93 ± 0.039 ****	0.39 ± 0.003 *
AF	0.24 ± 0.001	7.69 ± 0.025	0.281 ± 0.002
AFU 20/10	0.46 ± 0.012 ***	3.88 ± 0.36 ****	0.357 ± 0.005 ***
AFU 20/20	0.49 ± 0.007 ***	4.13 ± 0.124 ****	0.405 ± 0.004 ****

WSI: Water Solubility Index; WAI: Water Absorption Index; DWF: Durum wheat flour; DWFU: Durum wheat flour + ultrasound; CF: Chickpea flour; CFU: Chickpea flour + ultrasound; AF: Amaranth flour; AFU: Amaranth flour + ultrasound; 20/10: 20 kHz for 10 min; 20/20: 20 kHz for 20 min. Data are reported as mean ± standard deviation. Values with an asterisk (*) within the same column indicate a significant difference ($p < 0.05$), using Dunnett's test independently for each type of flour. * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$); **** ($p < 0.0001$).

Table 3 presents the proximate composition of durum wheat, chickpea, and amaranth flours subjected to ultrasound treatment. In all flours, ultrasound application resulted in a significant reduction in moisture content compared to their respective controls, with a more pronounced decrease observed as sonication time increased. Lipid content showed a significant reduction in durum wheat and chickpea flours after ultrasound treatment, whereas no significant changes were detected in amaranth flour. Protein content slightly decreased in durum wheat and chickpea flours following ultrasound exposure, while amaranth flour exhibited a small but significant increase in protein content at both treatment times. Fiber content was significantly reduced in chickpea flour with increasing ultrasound exposure, whereas only minor variations were observed in wheat and amaranth flours. Ash content showed a slight but significant decrease in all flours after ultrasound treatment. Because of these compositional changes, the calculated carbohydrate content increased significantly in all ultrasound-treated samples, particularly at longer exposure times.

Table 3. Proximate chemical analysis of durum wheat, chickpea and amaranth flours treated with ultrasound.

Treatment	Moisture	Lipids	Protein	Fiber	Ash	Carbohydrates
DWF	10.21 ± 0.002	1.72 ± 0.04	10.51 ± 0.03	0.36 ± 0.01	1.07 ± 0.003	86.35 ± 0.01
DWFU 20/10	7.11 ± 0.003 ****	1.03 ± 0.08 **	9.53 ± 0.001 **	0.44 ± 0.16	1.02 ± 0.006 *	87.98 ± 0.01 *
DWFU 20/20	7.02 ± 0.001 ****	1.11 ± 0.03 **	9.57 ± 0.001 **	0.60 ± 0.01 *	1.02 ± 0.01 *	87.7 ± 0.01 *
CF	12.20 ± 0.004	6.41 ± 0.27	22.42 ± 0.65	7.57 ± 1.10	1.09 ± 0.001	62.51 ± 0.01
CFU 20/10	8.98 ± 0.001 ***	5.78 ± 0.08 **	20.39 ± 0.03 ***	4.91 ± 0.43 **	1.04 ± 0.009 *	67.88 ± 0.01 **
CFU 20/20	7.09 ± 0.002 ****	4.21 ± 0.55 ***	19.79 ± 0.43 ***	3.45 ± 0.71 ***	1.02 ± 0.001 *	71.53 ± 0.01 **
AF	11.20 ± 0.002	7.55 ± 0.1	15.86 ± 0.04	3.59 ± 0.60	1.06 ± 0.001	71.94 ± 0.01
AFU 20/10	8.87 ± 0.001 ***	7.22 ± 0.31	16.08 ± 0.06 *	3.11 ± 0.64	1.04 ± 0.003 *	72.55 ± 0.01 **
AFU 20/20	7.35 ± 0.002 ***	7.05 ± 0.44	16.21 ± 0.24 *	3.45 ± 0.38	1.02 ± 0.002 *	72.27 ± 0.01 **

Protein, lipids, fiber, ash, and carbohydrate contents are expressed on a dry weight basis (g/100 g dry matter). Moisture content is reported separately on a wet basis and was not included in the calculation of carbohydrate content by difference. DWF: Durum wheat flour; DWFU: Durum wheat flour + ultrasound; CF: Chickpea flour; CFU: Chickpea flour + ultrasound; AF: Amaranth flour; AFU: Amaranth flour + ultrasound; 20/10: 20 kHz for 10 min; 20/20: 20 kHz for 20 min. Data are reported as mean $\pm$ standard deviation. Values with an asterisk (*) within the same column indicate a significant difference ($p < 0.05$), using Dunnett's test independently for each type of flour. * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$); **** ($p < 0.0001$).

Figure 2 shows the Fourier-transform infrared (FT-IR) spectra of durum wheat, chickpea, and amaranth flours subjected to ultrasound treatment. All spectra exhibited the characteristic absorption bands associated with carbohydrates, proteins, and lipids, with no new peaks detected after ultrasound application, indicating that the treatment did not induce chemical modifications but rather affected molecular organization. In durum wheat flour (Figure 2A), ultrasound treatment resulted in noticeable changes in band intensity, particularly in the region between 2800 and 1800 cm^{-1} , which is commonly associated with C–H stretching vibrations and carbonyl groups related to lipids and proteins. A decrease in band intensity was observed as sonication time increased.

In chickpea and amaranth flours (Figure 2B,C), ultrasound treatment led to an overall increase in the intensity of several absorption bands across the studied spectral range. Notably, changes were observed in the region between 1500 and 900 cm^{-1} , corresponding to the fingerprint region of carbohydrates, where C–O, C–C, and C–O–C stretching vibrations are predominant. The increase in band intensity was more pronounced at longer ultrasound exposure times. Additionally, slight variations were observed in the broad band around 3300 cm^{-1} , attributed to O–H stretching vibrations, suggesting modifications in hydrogen-bonding interactions within the flour matrix.

Overall, the FT-IR spectra indicate that ultrasound treatment alters the intensity and distribution of vibrational bands in a flour-dependent manner, reflecting changes in molecular interactions and structural arrangement rather than the formation of new chemical bonds.

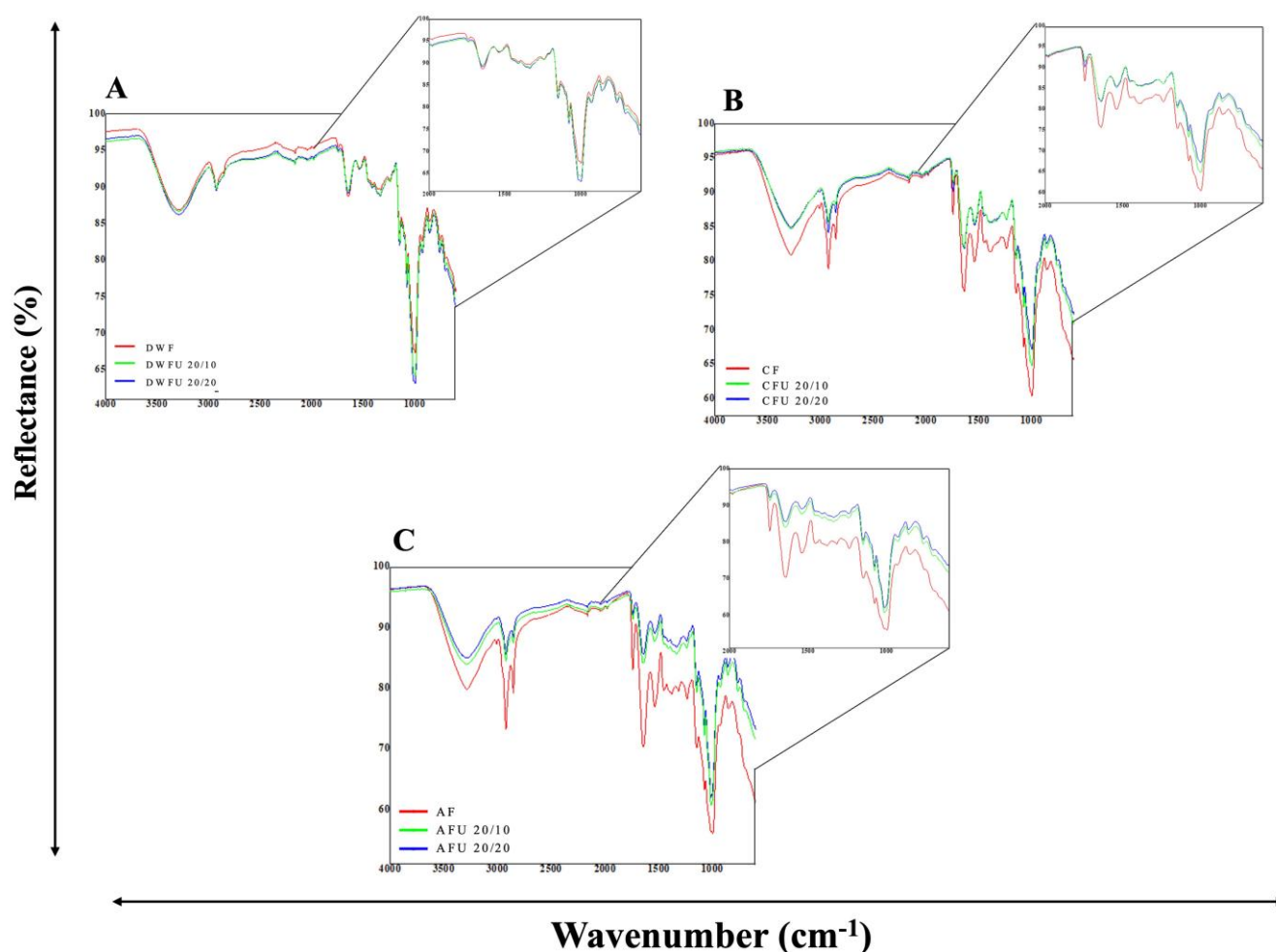


Figure 2. Infrared spectra of wheat, chickpea, and amaranth flours treated with ultrasound. (A) Wheat flour; (B) Chickpea flour; (C) Amaranth flour. DWF: Durum wheat flour; DWFU: Durum wheat flour + ultrasound; CF: Chickpea flour; CFU: Chickpea flour + ultrasound; AF: Amaranth flour; AFU: Amaranth flour + ultrasound; 20/10: 20 kHz for 10 min; 20/20: 20 kHz for 20 min.

3.4. Thermal and Pasting Properties of Flours

Figure 3 shows the viscoamylographic profile of wheat (DWF), chickpea (CF), and amaranth (AF) flours treated with US (+U), respectively. Control treatments are shown in red, 20 kHz/10 min (20/10) conditions in green, and 20 kHz/20 min (20/20) in blue. The figure shows changes in the gelatinization phenomena of each flour when US was applied. Panel A shows the treatments to which the wheat flour was subjected; differences are observed in the maximum viscosity peaks located at a temperature of 95 °C (isothermal stage) as well as in the final viscosity (final straight located at the end of the cooling stage). Panel B shows the same behavior in chickpea flour at maximum viscosity; however, only the final viscosity of CFU 20/20 differs from that of the control treatment. Finally, Panel C shows the US treatments to which the amaranth flour was subjected, which exhibited different behavior from wheat and chickpea flours, with a decrease in the maximum viscosity peak and a decrease in the final viscosity in the AFU 20/20 treatment.

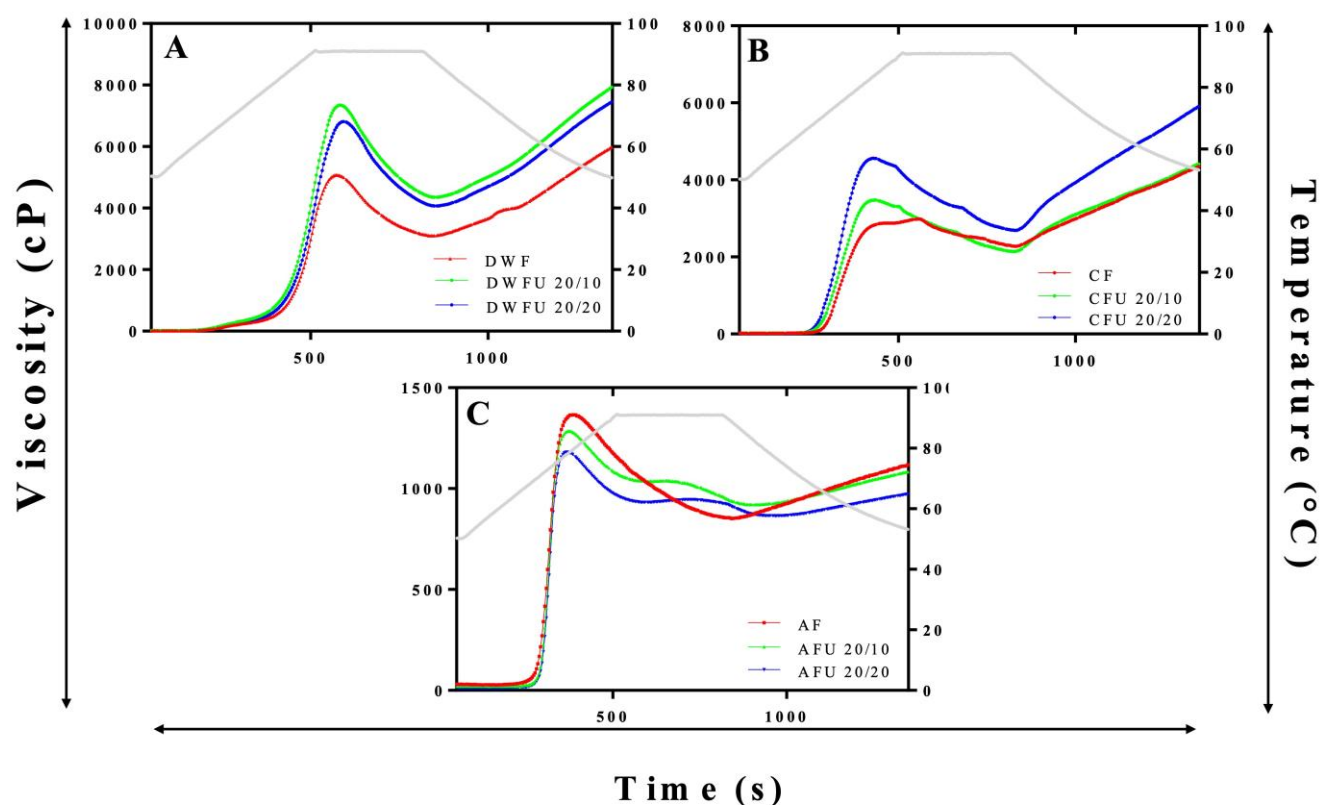


Figure 3. Viscoamylographic profile of wheat, chickpea and amaranth flours treated with ultrasound. (A) Wheat flour; (B) Chickpea flour; (C) Amaranth flour. DWF: Durum wheat flour; DWFU: Durum wheat flour + ultrasound; CF: Chickpea flour; CFU: Chickpea flour + ultrasound; AF: Amaranth flour; AFU: Amaranth flour + ultrasound; 20/10: 20 kHz for 10 min; 20/20: 20 kHz for 20 min. The gray line represents the heating program from 50 to 95 °C. Each line represents the average of three repetitions per treatment.

Figure 4 shows the differential scanning calorimetry (DSC) thermograms of durum wheat, chickpea, and amaranth flours subjected to ultrasound treatment. All samples exhibited a single endothermic transition associated with starch gelatinization. In durum wheat flour (Figure 4A), ultrasound-treated samples exhibited a slight shift in the gelatinization peak toward lower temperatures relative to the control, along with an increase in peak area, indicating changes in thermal behavior with sonication time. Chickpea flour (Figure 4B) showed a similar trend, with ultrasound treatments modifying the shape and intensity of the endothermic peak, particularly at longer exposure times. In contrast, amaranth flour (Figure 4C) exhibited a shift in the gelatinization peak toward higher temperatures after ultrasound application, accompanied by noticeable changes in peak intensity. Overall, these thermograms indicate that ultrasound treatment alters the gelatinization behavior of the flours in a manner dependent on botanical origin and exposure time.

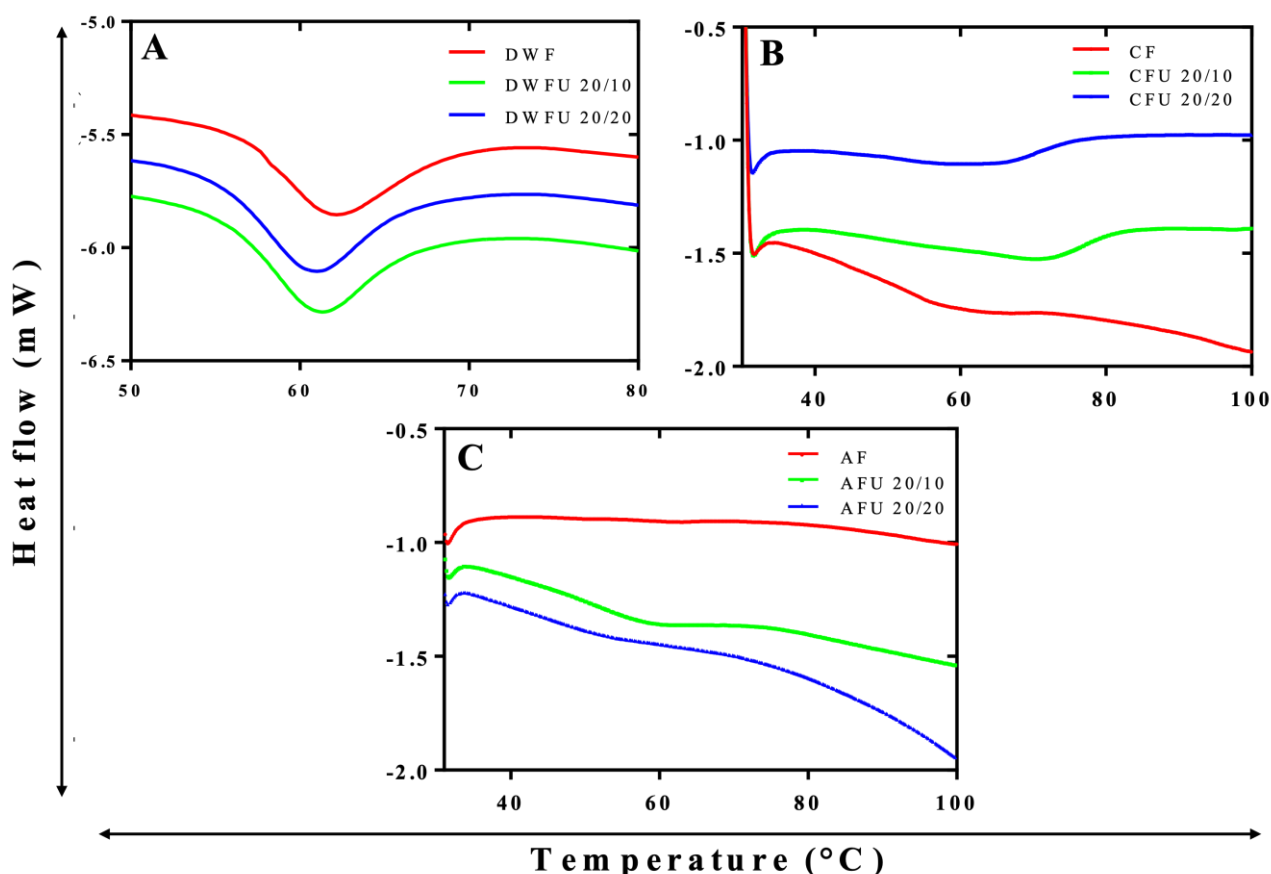


Figure 4. Differential scanning calorimetry of durum wheat, chickpea, and amaranth flours treated with US. (A) Wheat flour; (B) Chickpea flour; (C) Amaranth flour. DWF: Durum wheat flour; DWFU: Durum wheat flour + ultrasound; CF: Chickpea flour; CFU: Chickpea flour + ultrasound; AF: Amaranth flour; AFU: Amaranth flour + ultrasound; 20/10: 20 kHz for 10 min; 20/20: 20 kHz for 20 min. Each line represents the average of three replicates per treatment.

Table 4 shows the thermal and pasting properties of the flours measured in the RVA. Significant differences in peak viscosity were observed when US was applied across all treatments. For final viscosity, significant differences were observed in the DWF treatments compared to the control; however, in the CF and AF treatments, there were no significant differences between the control and the 10 min US application. Furthermore, the setback viscosity and pasting temperature differed significantly among all treatments compared with the control. The gelatinization enthalpy also showed significant differences with respect to the control.

Table 4. Thermal and pasting properties of wheat, chickpea and amaranth flours treated with ultrasound.

Thermal and Pasting Properties					
Treatments	Peak Viscosity	Final Viscosity	Setback	Pasting Temperature (°C)	Enthalpy of Gelatinization ΔH (J/g)
		(cP)			
DWF	5073 ± 32	6158 ± 26	3055 ± 0.1	66.30 ± 0.002	6.13 ± 0.002
DWFU 20/10	7348 ± 14 ***	8143 ± 23 ***	3790 ± 0.2 ***	64.90 ± 0.002 *	6.53 ± 0.002 *
DWFU 20/20	6814 ± 11 ***	7647 ± 28 **	3577 ± 0.5 **	65.95 ± 0.005 *	6.36 ± 0.002 *
CF	2985 ± 13	4490 ± 11	2372 ± 0.3	69.90 ± 0.001	0.99 ± 0.002
CFU 20/10	3475 ± 18.5 ***	4506 ± 29	3244 ± 2.1 ***	68.15 ± 0.005 *	1.82 ± 0.002 *

CFU 20/20	4561 ± 17 ***	6065 ± 24 **	3407 ± 1.4 ***	67.75 ± 0.002 *	1.45 ± 0.002 *
AF	1366 ± 22	1131 ± 30	279 ± 1.8	69.95 ± 0.001	0.01 ± 0.002
AFU 20/10	1286 ± 23 *	1093 ± 21	173 ± 1.2 **	71.05 ± 0.003 *	0.86 ± 0.002 *
AFU 20/20	1181 ± 14 **	982 ± 9*	118 ± 1.4 **	71.45 ± 0.001 *	0.24 ± 0.002 *

DWF: Durum wheat flour; DWFU: Durum wheat flour + ultrasound; CF: Chickpea flour; CFU: Chickpea flour + ultrasound; AF: Amaranth flour; AFU: Amaranth flour + ultrasound; 20/10: 20 kHz for 10 min; 20/20: 20 kHz for 20 min. Data are reported as mean ± standard deviation. Values with an asterisk (*) within the same column indicate a significant difference ($p < 0.05$), using Dunnett's test independently for each type of flour. * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$); **** ($p < 0.0001$).

3.5. In Vitro Digestibility of Flours

Table 5 presents the in vitro digestibility values of durum wheat, chickpea, and amaranth flours subjected to ultrasound treatment. The results demonstrate the effect of ultrasonic processing on the enzymatic digestibility and structural fractions of starch in each flour. Ultrasound significantly modified starch fractions in all flours; however, the response depended on the botanical origin of the matrix. Durum wheat and chickpea flours showed increased total starch availability accompanied by reductions in amylose content and increases in resistant starch. In contrast, amaranth flour exhibited a decrease in resistant starch and an increase in the proportion of amylopectin. Damaged starch content also increased significantly after ultrasound treatment in all three flour matrices. The most pronounced increases were observed in durum wheat and chickpea flours, whereas amaranth flour, despite presenting the lowest initial damaged starch content, also exhibited a marked increase following sonication. These results indicate that acoustic cavitation partially disrupted starch granules, increasing the proportion of mechanically and structurally damaged starch in a flour-dependent manner. These findings indicate that ultrasound-induced cavitation affected starch molecular organization differently depending on flour composition, rather than producing a uniform effect on starch digestibility. The differences observed among durum wheat, chickpea, and amaranth reflect the influence of amylose content, starch granule size and organization, and interactions with the protein matrix. These results suggest that ultrasound-induced cavitation altered the molecular structure of flour components, facilitating enzyme accessibility, as further supported by the increase in damaged starch observed after ultrasound treatment.

Table 5. In vitro digestibility of durum wheat, chickpea and amaranth flours exposed to US.

Treatments	Total Starch (g/100 g of Product)	Amylose (%)	Amylopectin (%)	Resistant Starch (g/100 g of Product)	Damaged Starch (%)
DWF	81.97 ± 2.5	28.05 ± 0.6	70.51 ± 0.1	0.41 ± 0.3	33.5 ± 1.9
DWFU 20/10	88.98 ± 4.6 **	24.24 ± 1.2 **	75.28 ± 0.2 **	2.18 ± 0.1 **	39.9 ± 2.4 **
DWFU 20/20	87.34 ± 5.6 **	23.54 ± 2.1 **	76.29 ± 0.9 **	2.01 ± 0.1 **	46.3 ± 3.5 **
CF	58.59 ± 6.7	34.75 ± 0.3	68.85 ± 0.3	1.55 ± 0.6	10.3 ± 2.8
CFU 20/10	66.46 ± 4.5 **	29.56 ± 3.4 ***	71.45 ± 0.2 ***	3.12 ± 0.1 **	25.6 ± 2.1 **
CFU 20/20	68.89 ± 3.9 **	28.45 ± 4.5 ***	72.04 ± 2.0 ***	3.23 ± 0.1 **	28.5 ± 3.2 **
AF	69.02 ± 0.6	2.51 ± 0.4	91.14 ± 0.3	4.76 ± 0.6	7.4 ± 1.2
AFU 20/10	71.78 ± 2.5 *	1.02 ± 0.1 **	94.45 ± 2.6 **	0.78 ± 0.2 *	22.98 ± 4.5 **
AFU 20/20	72.36 ± 0.9 *	1.86 ± 0.1 **	95.35 ± 5.8 **	1.36 ± 0.3 *	23.8 ± 1.5 **

DWF: Durum wheat flour; DWFU: Durum wheat flour + ultrasound; CF: Chickpea flour; CFU: Chickpea flour + ultrasound; AF: Amaranth flour; AFU: Amaranth flour + ultrasound; 20/10: 20 kHz for 10 min; 20/20: 20 kHz for 20 min. Data are reported as mean ± standard deviation. Values with an asterisk (*) within the same column indicate a significant difference ($p < 0.05$) when tested using Dunnett's test for each type of flour. * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$).

4. Discussion

Ultrasound is recognized as an emerging non-thermal processing technology capable of inducing physical and structural modifications in food matrices through acoustic cavitation phenomena. The collapse of cavitation bubbles generates localized high shear forces, microstreaming, and transient pressure and temperature gradients, which collectively disrupt supramolecular structures and promote molecular rearrangements [8,21]. In the present study, the application of ultrasound resulted in pronounced modifications in the physical, physicochemical, thermal, and pasting properties of durum wheat, chickpea, and amaranth flours, with the magnitude and direction of these changes being strongly influenced by the botanical origin and compositional characteristics of each flour.

The changes observed in color parameters following ultrasound treatment provide indirect evidence of structural rearrangements within the flour matrix. The increase in lightness (L^*) across all flours suggests enhanced light scattering due to particle size reduction, redistribution of surface components, and increased surface roughness of starch granules, as observed in the microscopic analysis. Similar trends have been reported in ultrasound-treated cereal and pseudocereal flours, where cavitation-induced fragmentation and disaggregation altered optical properties [12,22]. The decrease in chromaticity observed in durum wheat and chickpea flours may be associated with the separation of lipids and proteins from the starch matrix, leading to a more heterogeneous surface composition. In contrast, the increase in chromaticity in amaranth flour suggests the exposure of intrinsic pigments or colored compounds following the disruption of the surrounding matrix, a phenomenon previously reported in teff and quinoa flours subjected to mechanical or ultrasonic treatments [11,23].

Water absorption and solubility properties were markedly affected by ultrasound treatment and closely linked to changes in starch granule morphology and molecular organization. The significant increase in the water absorption index (WAI) observed in durum wheat flour indicates greater accessibility of hydroxyl groups within starch chains, thereby facilitating hydrogen bonding with water molecules. This effect can be attributed to the disruption of amorphous regions within the starch granule, as well as the partial fragmentation of polymer chains caused by cavitation forces [7,8]. Additionally, the improved dispersion of starch granules observed under the microscope increases the effective surface area available for hydration, further enhancing water uptake.

In contrast, the decrease in WAI observed in chickpea and amaranth flours suggests that ultrasound affects these matrices through different mechanisms. Legume and pseudocereal flours contain higher levels of proteins, lipids, and non-starch polysaccharides, which may undergo denaturation, aggregation, or depolymerization under ultrasonic conditions. Ultrasound-induced protein aggregation has been reported to reduce the availability of hydrophilic sites, thereby limiting water binding capacity [15]. In amaranth flour, the presence of β -glucans and other non-starch polysaccharides likely plays a critical role, as ultrasound can depolymerize these components, reducing their water-holding and gel-forming capacities [24]. The variations observed in WSI are associated with the balance between polymer fragmentation and molecular reassociation induced by acoustic cavitation. In wheat flour, the limited changes suggest that starch depolymerization was minimal under the applied ultrasonic conditions. Conversely, the reduction observed in chickpea flour may be due to protein aggregation and reduced macromolecular solubilization. In contrast, the increase in amaranth flour indicates greater release of soluble carbohydrates and low-molecular-weight compounds following cavitation-induced disruption of the flour matrix.

The observed reductions in moisture content across all ultrasound-treated flours can be attributed to enhanced mass transfer during sonication, followed by more efficient moisture removal during drying. Ultrasound is known to create microchannels and

disrupt cellular structures, facilitating water diffusion and evaporation [8]. The decrease in lipid content observed in durum wheat and chickpea flours may result from cavitation-induced lipid oxidation or from the physical separation of lipids from the starch–protein matrix, as previously reported for ultrasound-treated wheat gluten and cereal flours [6]. Changes in protein and fiber content, particularly in chickpea flour, further indicate that ultrasound affects the integrity of storage proteins and cell wall components, potentially leading to downstream effects on hydration, thermal stability, and pasting behavior.

X-ray diffraction analysis revealed that ultrasound treatment modified starch crystallinity in a flour-dependent manner, highlighting the complex balance between structural disruption and molecular reorganization. In durum wheat flour, the increase in crystallinity following ultrasound exposure suggests that cavitation initially disrupts amorphous regions, increasing chain mobility and allowing starch molecules to rearrange into more ordered crystalline domains during subsequent drying. Similar recrystallization phenomena have been reported in ultrasound-treated corn and rice starches, where increased crystallinity was associated with enhanced molecular order and stability [12,25]. In chickpea flour, the initial decrease in crystallinity at shorter sonication times likely reflects the dominance of structural disruption, whereas the increase observed at longer exposure times indicates chain reassociation and recrystallization. This behavior underscores the time-dependent nature of ultrasound-induced structural transformations.

In contrast, amaranth flour exhibited minimal changes in crystallinity regardless of ultrasound exposure time, suggesting a greater resistance of its starch structure to ultrasonic energy. This resistance may be attributed to the small granule size of amaranth starch and the protective effect of surrounding non-starch polysaccharides and proteins, which limit the extent of molecular rearrangement. These findings are consistent with previous studies reporting lower susceptibility of pseudocereal starches to physical modification due to their unique structural organization [24].

The structural changes induced by ultrasound were directly reflected in the pasting behavior of the flours. The increase in peak and final viscosity observed in ultrasound-treated durum wheat and chickpea flours indicates enhanced swelling capacity and greater molecular reassociation during cooling, respectively. Ultrasound-induced surface roughness and increased porosity of starch granules facilitate water penetration and swelling, leading to higher viscosity development during heating [25,26]. The higher setback viscosity observed in these flours further suggests increased amylose leaching and a greater tendency toward retrogradation, consistent with the increased crystallinity detected by XRD.

In contrast, amaranth flour exhibited decreased peak and final viscosities after ultrasound treatment, accompanied by an increase in pasting temperature. This behavior is likely associated with the depolymerization of β -glucans and other non-starch polysaccharides, which contribute significantly to viscosity and gel formation in pseudocereals. Ultrasound-induced depolymerization of β -glucans has been shown to reduce apparent viscosity and alter thermal behavior in quinoa and related matrices [24]. The increase in pasting temperature suggests that greater thermal energy is required to induce gelatinization, reflecting a more thermally stable starch structure after ultrasound treatment.

Differential scanning calorimetry further corroborated these findings, revealing that ultrasound alters the gelatinization behavior of the flours in a matrix-dependent manner. The shift in gelatinization peaks toward lower temperatures in durum wheat and chickpea flours indicates reduced thermal energy requirements for gelatinization, likely due to enhanced water accessibility and partial disruption of amorphous regions. The increase in gelatinization enthalpy observed in some treatments suggests the formation of more ordered crystalline structures that require additional energy to melt, consistent with XRD results [27,28]. Conversely, the increase in gelatinization temperature observed in

amaranth flour suggests a more resistant starch structure, consistent with its stable crystallinity and altered pasting behavior.

The FT-IR results obtained in the present study are consistent with previous reports showing that physical processing can modify the molecular organization of chickpea proteins. Cheng and Langrish (2023) demonstrated that processing-induced structural rearrangements detected in the amide regions were closely associated with changes in protein conformation [29]. In the present work, ultrasound produced comparable spectral modifications, although through cavitation rather than thermal drying, supporting the hypothesis that different physical technologies can alter flour structure through distinct mechanisms.

The modifications observed in starch digestibility further support the structural changes induced by ultrasound treatment. The increase in total starch and amylopectin content, along with the reduction in amylose in ultrasound-treated samples, suggests partial depolymerization and molecular rearrangement of starch components. These changes enhance enzymatic susceptibility, as amylopectin is more readily hydrolyzed than amylose due to its branched structure [30]. The increase in damaged starch content observed after ultrasound treatment provides additional evidence of cavitation-induced disruption of starch granules. Damaged starch is characterized by the loss of granular integrity, which increases water absorption and enhances enzyme accessibility by exposing more of the internal amorphous regions [31]. The marked increase observed in durum wheat and chickpea flours is consistent with the higher susceptibility of their larger starch granules to mechanical disruption by acoustic cavitation. Although amaranth flour also exhibited a significant increase in damaged starch, the absolute values remained lower than those of wheat because of its naturally smaller starch granules and more compact starch–protein organization. These observations support the hypothesis that ultrasound modifies starch organization differently depending on the structural characteristics of each flour matrix and help explain the matrix-dependent changes observed in functional properties and starch digestibility [32]. The increase in resistant starch observed in durum wheat and chickpea flours may be associated with retrogradation phenomena and the formation of more ordered structures during drying, as supported by XRD and DSC results. Conversely, the decrease in resistant starch in amaranth flour indicates a disruption of crystalline regions and increased accessibility to digestive enzymes. These findings confirm that ultrasound-induced cavitation alters starch organization at multiple scales, promoting enzyme accessibility and improving *in vitro* digestibility in a flour-dependent manner. This behavior highlights the potential of ultrasound processing not only to tailor functional properties but also to modulate nutritional quality and glycemic response of flour-based products. Future studies should evaluate starch hydrolysis kinetics and estimate the predicted glycemic index of ultrasound-treated flours to understand better the nutritional implications of the structural modifications induced by acoustic cavitation.

Collectively, these results demonstrate that ultrasound induces complex, interrelated structural transformations in flours, affecting starch–protein interactions, hydration behavior, crystallinity, and thermal stability. The combined analysis of color, morphology, proximate composition, crystallinity, pasting, and thermal properties highlights the highly matrix-dependent nature of ultrasound processing. From a technological perspective, these findings support the use of ultrasound as a versatile and sustainable tool to tailor flour functionality without chemical additives, with potential applications in developing clean-label cereal-, legume-, and pseudocereal-based ingredients with customized performance for food processing and formulation.

5. Conclusions

Power ultrasound proved to be an effective non-thermal strategy for tailoring the structural, functional, and nutritional properties of durum wheat, chickpea, and amaranth flours. The results demonstrated that ultrasound-induced cavitation promotes significant modifications in starch–protein interactions, granule morphology, hydration behavior, crystallinity, and thermal transitions, with responses strongly dependent on flour composition and botanical origin. Durum wheat flour exhibited enhanced water absorption and viscosity, reflecting improved functional performance, while chickpea flour showed a balance between structural disruption and molecular reassociation. In contrast, amaranth flour exhibited greater structural resistance, with reduced viscosity and increased thermal stability.

The present results suggest that ultrasound exerts a dual effect on starch digestibility. Depending on the botanical origin of the flour and the extent of structural modification, cavitation may either facilitate enzyme accessibility by disrupting starch organization or promote molecular reassociation and changes in resistant starch formation. Therefore, the nutritional consequences of ultrasound treatment should be interpreted in light of the specific characteristics of each flour rather than as a universal response.

Power ultrasound effectively modified the structural, physicochemical, thermal, and pasting properties of durum wheat, chickpea, and amaranth flours. Its effects on starch fractions and in vitro digestibility were strongly dependent on the botanical origin and compositional characteristics of each flour, demonstrating that acoustic cavitation does not produce a uniform nutritional response across different starch-based matrices. These findings support the potential of ultrasound as a green, clean-label technology for tailoring flour functionality while highlighting the importance of matrix-specific optimization.

A limitation of the present study is that only one representative flour from each botanical group (cereal, legume, and pseudocereal) was evaluated. Therefore, although the results demonstrate that the effects of power ultrasound depend on the structural and compositional characteristics of the flour matrix, caution is warranted when extrapolating these findings to other species within the same botanical group. Future studies that include a broader range of cereal, legume, and pseudocereal flours are necessary to validate the observed matrix-dependent trends and to establish more generalizable structure–function relationships.

Overall, these findings establish power ultrasound as a versatile and clean-label technology for designing flours with tailored functionality and controlled digestibility, offering significant potential for the development of advanced cereal-, legume-, and pseudocereal-based food systems. Future research should focus on process scale-up and validation in real food matrices to fully exploit its industrial applicability.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/applbiosci5030067/s1>, Figure S1: Representative photographs of untreated durum wheat, chickpea, and amaranth flours used in this study.

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