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Modification of the microstructure of tef [*Eragrostis tef* (Zucc.) Trotter] flour ultrasonicated at different temperatures. Impact on its techno-functional and rheological properties

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ABSTRACT

Tef flour comes from a nutritionally-rich ancient grain gaining increasing interest in gluten-free market. Gluten-free sources are modified by different means to improve their functionality. Ultrasound treatment (US) alters flours' structure and leads to physically modified flours with a wider application range. The aim of the present work was to evaluate the impact of US treatments of moderate treatment time, 10 min, and high concentration of the aqueous flour dispersion, 25%, on the microstructural, starch damage, apparent amylose content, techno-functional, pasting and rheological properties of two tef flour varieties, white and brown. Temperature was varied (20, 40, 45, 50, and 55 °C) to modulate the impact of sonication. US treatments led to general particle fragmentation which markedly increased starch damage and lightness (L*) values. Apparent amylose content was higher after ultrasonication, as consequence of molecular fragmentation due to cavitation. Increased starch granules' exposed area led to enhanced interaction with water, promoting the water absorption index (WAI) and swelling power (SP) of treated flours. Pasting properties showed increased pasting temperatures as well as decreased viscometric profiles with lower breakdown viscosities, indicative of starch rearrangement improved by increasing temperature. Rheological properties indicated higher consistency in gels after US treatments, with improved ability to withstand stress and lower values of $\tan(\delta)_1$ reflecting a higher solid-like behavior and higher strength of the gel. Temperature was found to be a crucial variable during US treatments, showing an improved degree of modification at higher temperatures in ultrasonicated tef flours, following the same trend in both varieties.

1. Introduction

Gluten-free (GF) market has been growing in recent years due to increasing consumers demanding a wider variety of products. This consumer trend derives from an increasing number of celiac disease patients (and other gluten-related disorders) being diagnosed because of better diagnosis methods, and a fashion of healthy people to remove gluten from their diet following the health improvement that elimination of gluten has in many diseases (Witczak et al., 2016). The elimination of gluten from formulations represents a technological challenge to the food industry given that gluten is responsible for the structure and quality of the product. Such GF products tend to exhibit lower nutritional values in comparison to gluten-containing counterparts, given that the GF sources have much lower levels of nutrients such as proteins, vitamins, minerals, and dietary fiber (Witczak et al., 2016). The strategies adopted by the food industry are the use of novel GF sources with better nutritional profiles in the development of products, and the implementation of technologies to modify the physicochemical properties of the GF sources for a wider range of industrial utilization.

Tef [*Eragrostis tef* (Zucc.) Trotter] is an ancient grain indigenous to Ethiopia known to be drought resilient with plantation in warmer seasons (Barretto et al., 2021). This considerable resistance to harsh environmental conditions has resulted in increased interest in tef as good candidate for human and animal foods in a global climate change scenario, where the availability of water is being reduced (Dueñas et al., 2021). In addition, tef is also gaining global popularity due to its

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attractive nutritional composition. Tef is considered to have a better food value than major grains, namely, wheat, barley, sorghum, and maize, as it is normally used as a whole grain due to the small size of the grain, resulting in flours with a rich carbohydrate and fiber profile, an equilibrated balance of essential amino acids, and a higher amount of iron, calcium, and zinc than the other mentioned cereals (Bultosa, 2016; Ronda et al., 2015). Its production and consumption have heightened in recent years due to the increased interest of its application in gluten-free (GF) food research (Barretto et al., 2021). Tef grain flours are used to improve dietary fiber, starch, protein, and mineral content supply in GF food product and in wheat breads to improve their iron and antioxidant contents (Bultosa, 2016).

Native starches and flours in general usually have poor processing properties and are unstable under various temperatures, pH values, and shear conditions, so they are frequently modified genetically, chemically, physically and/or enzymatically to improve their properties (Chung et al., 2009; Zhang et al., 2021; Zhu, 2015). Among these different approaches, physical modifications are better perceived in food applications for being considered as a natural modification method resulting in highly safe ingredients since they do not require any chemical or biological agent (Acevedo et al., 2022; Kaur and Gill, 2019). Ultrasonication is a physical treatment linked to the emerging concept of "green chemistry and technology" for environmentally friendly applications (Zhu, 2015). Ultrasound (US) is acoustic energy above the frequency audible for humans (18–20 kHz) (Kaur and Gill, 2019). The US treatments are applied in water-flour bi-phase systems, where the acoustic waves generate collapsing bubbles (phenomenon called cavitation) which alter the treated matter's structure and affect their physicochemical and functional properties (Acevedo et al., 2022; Kaur and Gill, 2019; Luo et al., 2008; Yang et al., 2019). The efficiency of the ultrasound treatments depends on many factors, such as sonication power and frequency, time and temperature of the treatment, and conditions of the flour suspension (e.g., concentration in the treated suspension, biological origin) (Kaur and Gill, 2019). Among these parameters, treatment time and temperature have been proved to be determinative variables in the extent of modification caused to the treated matter (Amini et al., 2015; Vela et al., 2021a). Longer sonication times have been determined to reduce the stability of the starch structure which detriments its techno-functionality since starch granules become further disrupted and degraded (Bel Haaj et al., 2013). Given that US treatment requires keeping the flour in an aqueous dispersion, annealing process (ANN) always occurs simultaneously as a lateral effect of flour sonication. ANN refers to the physical modification of starches and flours performed in excess water (>60% w/w) at a temperature below the onset temperature of gelatinization (Chung et al., 2009; Wang et al., 2018). ANN, highly dependent on the dispersion temperature, alters the structural arrangement of starch, improving crystalline perfection and facilitating interactions between starch chains, resulting in the modification of its physicochemical properties (Zavareze and Dias, 2011).

Although many studies have investigated the modification of starches by US, there is little literature available on their effect on flours, where other components besides starch (mainly protein, fiber, and fat) play an important role in the effects of US. Flour is a basic ingredient in human nutrition that needs to be deeply studied, particularly those GF flours with high nutritional profiles such as tef, with higher protein and fiber contents than other common gluten-free flours, such as rice or maize. The objective of the present work was to study the modification of hydration, pasting and rheological properties of tef flour by moderate (short-time/high flour concentration) US treatments to better adapt them to meet the required characteristics for the development of GF foods. The impact on microstructure, the apparent amylose content and starch damage of the US-treated flours was also determined to explain the root of the changes observed in the techno-functional properties. Two factors of known relevance in US treatments were studied: the tef variety (white and brown tef) and treatment temperature, varied from 20 °C (room temperature) to higher temperatures (40, 45, 50 and 55 °C), all below the starch gelatinization temperature in the tef flours.

2. Materials and methods

2.1. Tef flours

Two varieties of tef grains were studied (white and brown), kindly supplied by CYLTEF (Villanazar, Zamora, Spain). Whole tef flours were obtained by milling the grains with an LM 3100 hammer mill (Perten Instruments, Sweden) using an opening screen size of 0.5 mm. The proximal composition of the native flours was: 10.2% protein, 6.7% fiber, 2.2% lipids, 65.4% starch, 10.9% moisture, and 2.63% ash for white tef (WT) and 9.0% protein, 7.0% fiber, 2.6% lipids, 67.1% starch, 8.9% moisture, and 2.75% ash for brown tef (BT). Protein content (N x 5.7) was determined by titrimetric method using a Kjeldahl distillation unit according to the AOAC method 960.52–1961 (2010). Fiber content was obtained by enzymatic-gravimetric method following the AOAC method 991.43 (1994). Lipids were determined by gravimetric method using a Soxhlet apparatus with hexane as extraction solvent, following the AOAC method 923.05–1923 (1996). Starch content was determined using an amylose/amylopectin assay kit (Megazyme, Wicklow, Ireland). Moisture and ash contents were determined with the AACC 44-19 and 08-01, respectively, official methods (AACC, 1999). All determinations were performed in duplicate.

2.2. Ultrasound treatment

Ultrasound treatments were carried out using a Hielscher UP400St sonicator (Hielscher Ultrasonics, Germany) coupled with an S24d22D titanium tip. Treatments were performed on 400 g of flour dispersions at a concentration of 25% (w/w) (g dry flour/100 g of dispersion) in distilled water at the following conditions: frequency of 24 kHz, maximum outlet power of 180 W, pulse of 80% (on-off per second), and time of 10 min. The studied treatment temperatures were 20, 40, 45, 50 and 55 °C, which were kept constant during treatments using recirculating water from a LAUDA RA12 water bath (Lauda-Königshofen, Germany). Treated samples were identified as "WT" for white tef and "BT" for brown tef, followed by the number corresponding to the temperature at which treatment was performed, or "C" in the case of the control flours. A magnetic stirrer was used during treatment to avoid flour sedimentation and ensure a homogenous temperature. Excess water was removed after treatments using a Telstar Lyoquest freeze-dryer (Terrassa, Spain), and retrieved flours were sieved to < 250 μm. Native flours were used as the controls in the study. All flours were stored at 4 °C until use.

2.3. Particle size distribution

Granulometry of the samples was determined using a Mastersizer 2000 equipped with a dry dispersion unit (Malvern Instruments Ltd, Malvern, UK). The presented results are median diameter (D_{50}) and span [$(D_{90}$-$D_{10})/D_{50}$] values as a measure of dispersion, according to Abebe et al. (2015). Samples were measured in triplicate.

2.4. Flour color

The color of the samples was determined using a PCE-CSM5 colorimeter (PCE Instruments, Germany), based on CIELAB color space [L* for lightness from black (0) to white (100), a* from green (−) to red (+), and b* from blue (−) to yellow (+)] with 10° standard observer and D65 standard illuminant. The chroma (C*) and hue (h) were obtained from the CIELAB coordinates. Samples were measured five times.

2.5. Starch damage and amylose content

Damaged starch and amylose content were quantified using K-SDAM and K-AMYL assay kits of Megazyme (Wicklow, Ireland), respectively, following the procedures indicated by Solaesa et al. (2020). Results are referred to dry matter (dm). Samples were evaluated in duplicate.

2.6. Confocal laser scanning microscopy

Staining of carbohydrates and proteins was done using the double-labelling method indicated by Ozturk et al. (2021). Carbohydrates were labelled using a periodic acid-Schiff reaction (Vodovotz and Chinachoti, 1998) and proteins were subsequently labelled with fluorescamine (Bantan-Polak et al., 2001). Samples (~20 mg) were oxidized with 0.5% (w/v) periodic acid for 5 min for carbohydrate labeling, followed by rinsing three times with deionized water. Samples were stained with Schiff's reagent for 10 min, followed by rinsing twice with 0.5% (w/v) potassium sulfite and rinsing 5 times with deionized water. Samples were centrifuged at 13000 rpm for 5 min after each step. Protein labeling was done by adding 200 μL 0.1% (w/v) fluorescamine in acetonitrile and 300 μL 0.1 M sodium (tetra)borate buffer (pH 8.0), allowing 5 min for the reaction, followed by centrifugation (1300 rpm for 5 min) and removal of the supernatant. Samples were rinsed 5 times with deionized water. Glycerol solution [0.1–0.2 mL 75% (v/v)] was added, and aliquots (10 μL) were placed in microscope slides and coverslips were adhered using nail varnish.Samples were visualized using a Zeiss LSM 880 confocal laser scanning microscope (Carl Zeiss Microscopy, Oberkochen, Germany) equipped with 10 × , 20 × , 40 × and 63 × objectives, a water-cooled Innova Enterprise 60 mW output Argon ion laser (Coherent Inc., Santa Clara, USA), an air-cooled 10 mW output Krypton/Argon laser (ILT Laser, Bio-Rad Laboratories, Hercules, USA) and three fluorescence detection channels. Carbohydrates were excited at 488 nm, and emission was detected after passing through 590/60 band filter, while proteins were excited at 405 nm and detection occurred at 450/80 nm (Ozturk et al., 2021). For all samples pinhole size was automatically set at 1 AU. Zen Black software (Carl Zeiss imaging, Oberkochen, Germany) was used to record the digital images. Z-stacks were obtained incorporating the visible range of each sample (usually between 20 and 70 μm). The Zen Blue software (Carl Zeiss imaging, Oberkochen, Germany) was used to produce 3D images from Z-stacks.

2.7. Hydration properties

Water absorption capacity (WAC), water absorption index (WAI), water solubility index (WSI) and swelling power (SP) were measured at 10% flour dispersion (w/v) following the method described at Abebe et al. (2015). WAC was determined by adding 2 g of the sample with 20 mL of distilled water in centrifuge tubes, which were vortexed for 30 s followed by 10 min of resting at room temperature (this procedure was done 3 times). The tubes were then centrifuged for 30 min at 3000×*g*, the supernatant removed, and the sediment weighted and used to express the results as g H_2O/g flour. For WAI, WSI and SP, 2 g of the sample (w_0) were dispersed in 20 mL of distilled water using tared centrifuged tubed. Samples were cooked at 95 °C for 15 min, cooled down to room temperature, and centrifuged at 3000×*g* for 10 min. The supernatant was poured into a pre-weighed evaporating dish to determine its solid content by evaporation overnight at 110 °C (w_{ds}), and the sediment in the centrifuge tube was weighted (w_{ss}). WAI, WSI and SP were calculated using the equations:

$$WAI\left(\frac{g\ sediment}{g\ flour}\right)=\frac{w_{ss}}{w_0}$$

$$WSI\left(\frac{g\ soluble\ solids}{100\ g\ flour}\right)=\frac{w_{ds}}{w_0}\times 100$$

$$SP\left(\frac{g\ sediment}{g\ insoluble\ solids\ in\ flour}\right)=\frac{w_{ss}}{w_0-w_{ds}}$$

All results are referred to dry matter (dm). Samples were evaluated in triplicate.

2.8. Pasting properties

Pasting properties were measured using a Kinexus Pro+ rheometer (Malvern Instruments Ltd, Malvern, UK) equipped with a starch pasting cell geometry as described by Vela et al. (2021b). A flour sample of 3.5 g (14% moisture basis) was mixed with 25.0 ± 0.1 mL of distilled water in the test canister. Pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV) and setback viscosity (SV) were established using the rSpace software (Malvern Instruments Ltd, UK). Samples were measured in duplicate.

2.9. Gel's rheological properties

Dynamic oscillatory tests were performed on gels prepared as indicated in section 2.8 using Kinexus Pro+ rheometer (Malvern Instruments Ltd, Malvern, UK) as described by Vela et al. (2021b). The tests consisted of strain sweeps from 0.1 to 1000% strain at constant frequency of 1 Hz to determine the linear viscoelastic region (LVR) and frequency sweeps from 10 to 1 Hz at 1% strain within LVR. Frequency sweeps data were adjusted to the potential equations as described by Ronda et al. (2014). Samples were measured in duplicate.

2.10. Statistical analysis

The statistical analysis was performed by analysis of variance (ANOVA) and Least Significant Difference (LSD) test (p-value ≤0.05) using Statgraphics 19-X64 software (Cambridge, MN, USA). Significant differences between samples were reported with different letters.

3. Results and discussion

3.1. Morphology and color characteristics

The values determined for particle size distribution are presented in Table 1 and the graphs are presented in Fig. 1. The median size (D_{50}) of treated samples was significantly reduced after sonication. Said reduction was seen to be improved by increasing temperature in both varieties, showing the smallest result for the samples treated at 55 °C. In WT, however, no significant differences were found for the samples treated at or above 40 °C. Consequently, samples' dispersion (span values) increased after treatment in both studied varieties, indicative of less uniform size distributions. Similar results have been reported by Monroy et al. (2018) upon sonication of cassava starch. The particle fragmentation is a consequence of shear forces leading to cavitation and breaking macromolecules (Luo et al., 2008), which has been reported to happen even at sonication times as short as 2 min (Vela et al., 2021b). Previous studies have reported signs of partial gelatinization after US treatments at high temperatures (Vela et al., 2021a), leading to higher D_{50} values than samples treated at lower temperatures. However, in this study this behavior was not observed, probably because the highest studied temperature was far enough from the onset gelatinization temperature of the native flour (62.42 ± 0.09 °C for WT-C and 64.80 ± 0.04 °C for BT-C, vs 55 °C in the highest studied treatment), and that the sonication time was short (10 min), reducing the susceptibility of small particles gelatinization.

Color parameters (L*, a*, b*, C* and h) are given in Table 1. Different values were obtained for each variety given their natural differences, with BT showing more marked changes after treatments which could be expected due to its natural darker color being more susceptible to be

Table 1
Physical properties, content of damaged starch and amylose, and hydration properties of the studied tef flours.

	Particle size		Color properties					Chemical composition			Hydration properties			
Sample	D_{50} (μm)	$(D_{90}-D_{10})/D_{50}$	L*	a*	b*	C*	h	Starch damage (g/100g)	Starch damage increase (%)	Amylose content (%)	WAC (g/g)	WAI (g/g)	WSI (g/100g)	SP (g/g)
WT-C	139c	2.20a	73.4a	4.49d	13.22e	13.96e	71.3a	2.55a	–	20.3a	1.15c	5.4a	6.0bc	5.7a
WT-20	108b	2.77b	76.4b	3.35c	11.8d	12.27d	74.1b	2.88b	13a	22.0b	1.02 ab	7.6b	6.2c	8.1b
WT-40	103 ab	2.83bc	77.5c	3.10b	11.01c	11.44c	74.3b	3.66c	44b	22.0b	1.00 ab	8.6c	5.8bc	9.1c
WT-45	102 ab	2.80bc	78.3d	2.95a	10.46b	10.87b	74.3b	3.81c	49b	22.1b	1.02 ab	8.3c	5.4 ab	8.8c
WT-50	100 ab	2.88bc	78.4d	2.82a	10.17a	10.55a	74.5b	4.56d	79c	22.1b	0.98a	8.5c	6.0bc	9.0c
WT-55	95a	2.96c	78.3d	2.84a	9.98a	10.37a	74.1b	5.07e	99d	22.1b	1.03b	8.5c	4.6a	9.0c
SE	4	0.08	0.2	0.05	0.07	0.08	0.2	0.06	3	0.2	0.01	0.1	0.3	0.1
Analysis of variance and significance (p-values)														
	***	***	***	***	***	***	***	***	***	**	***	***	*	***
BT-C	121e	2.44a	59.1a	8.09d	11.80e	14.3e	55.6a	2.30a	–	21.5a	1.13c	5.4a	5.4c	5.7a
BT-20	93c	3.04d	65.6b	5.66c	10.08d	11.6d	60.7b	2.63b	14a	22.5b	1.02a	7.2b	4.5b	7.5b
BT-40	109d	2.76c	69.2d	4.62a	8.71a	9.9a	62.1c	3.62c	57b	22.5b	1.08b	6.9b	4.7b	7.3b
BT-45	107d	2.58b	68.6cd	5.19b	9.03bc	10.4c	60.1b	3.59c	56b	22.9b	1.01a	8.0c	3.7a	8.3c
BT-50	87b	3.03d	68.0c	4.81a	9.20c	10.4bc	62.4c	4.12d	80c	22.5b	1.16d	7.8c	4.4 ab	8.1c
BT-55	82a	3.04d	69.0d	4.70a	8.93 ab	10.1 ab	62.3c	4.95e	115d	22.8b	1.16d	8.2c	4.5b	8.6c
SE	1	0.02	0.3	0.08	0.08	0.1	0.3	0.08	4	0.2	0.01	0.2	0.2	0.2
Analysis of variance and significance (p-values)														
	***	***	***	***	***	***	***	***	***	*	***	***	**	***

D_{50}: median diameter; $(D_{90}-D_{10})/D_{50}$: size dispersion. L*, a*, b*: CIELAB color coordinates; C*: Chroma; h: hue. WAC = Water absorption capacity. WAI = Water absorption index. WSI = Water solubility index. SP = Swelling power. Starch damage, amylose content, WAC, WAI, WSI, SP are referred to dry matter.
SE: Pooled standard error from ANOVA. Different letters in the same column within each studied variety indicate statistically significant differences between means at $p < 0.05$. Analysis of variance and significance: ***$p < 0.001$. **$p < 0.01$. *$p < 0.05$. ns: not significant.

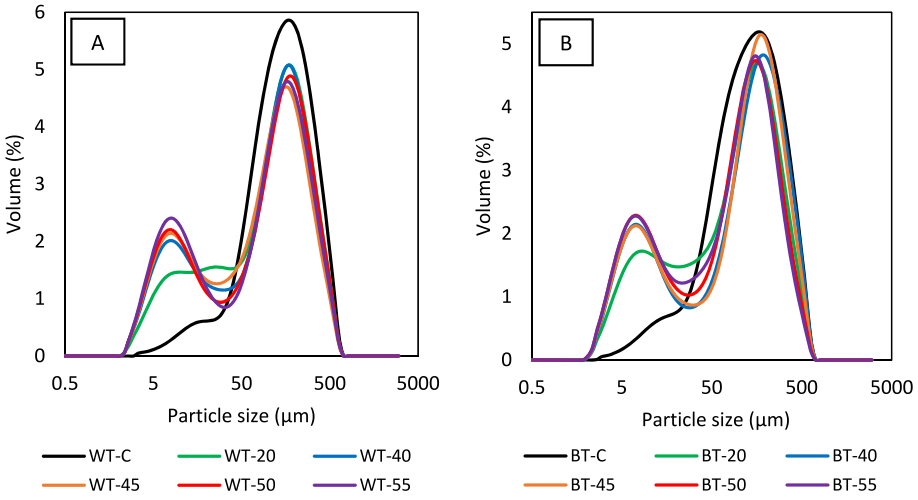

Fig. 1. Particle size distribution of the (A) white tef and (B) brown tef studied flours. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

altered by sonication. An increase in lightness (L*), ranging between 4.1 and 6.8% in WT samples and between 11.0 and 17.1% in BT samples, was detected after ultrasonication. The increased L* after ultrasonication could be a positive effect of treatments, particularly in tef flours, where white tef is preferred over brown tef by consumers, making white tef flours more expensive (Barretto et al., 2021; Dueñas et al., 2021). This rise in lightness value was slightly promoted by higher temperatures and could be associated with the particle size reduction, and consequent increased surface area allowing more light reflection (Solaesa et al., 2020). The values of a*, b*, and the derived hue angle (h) indicated that yellowish tones increasingly faded with increasing treatment temperature. The chroma (C*) determined for control samples were significantly higher than those determined for sonicated flours, indicative of more vivid colors before ultrasonication.

3.2. Starch damage

Damaged starch content followed an increasing trend with an increase in treatment temperature (Table 1), reaching values up to 99% (WT-55) and 115% (BT-55) higher than their corresponding control samples. Despite the well-known disruptive effect of ultrasonication on macromolecules, US did not cause any sharp increase in starch damage amount on their own (WT-20 and BT-20). However, combining US with higher temperatures drastically increased starch damage amount. Treatment temperature is a key parameter determining the extent of modification achieved by ultrasonication (Amini et al., 2015; Vela et al., 2021a). Results also indicate that starch in tef flours might be more susceptible to being damaged depending on their botanical origin, given that by applying the same treatment conditions, the determined percentual increases were always higher for BT than for WT.

3.3. Amylose content

Amylose content showed a uniform increase after US treatment, regardless of the treatment temperature (Table 1). This reported increase is believed to derive from degradation of the α-1,6-glucosidic bonds on the molecular chain of amylopectin by the shear forces induced by US, increasing the amount of linear chains by generation of some short amylose-like chains (Babu et al., 2019; Zhang et al., 2021). Ultrasonication has been said to lead to polymer fragmentation in a non-random manner, having a definite chain length limiting the degradation process (Czechowska-Biskup et al., 2005), which could explain the similar results obtained in all treatments. The fact that no significant differences were found among different temperatures seems to indicate that the chain rupture was provoked by ultrasonication conditions (e.g., time, power, and frequency), and was mainly influenced by the nature of the flour and its concentration in the dispersion, rather than the temperature during treatment.

3.4. Confocal laser scanning microscopy

To have a clearer vision of the impact of US treatments on carbohydrates and proteins in the flours, individual staining of these structures was done using periodic acid-Schiff's method and fluorescamine, respectively (Ozturk et al., 2021). Confocal laser scanning micrographs are presented in Fig. 2. Control samples showed large protein aggregates, while carbohydrates had a much smaller size. In agreement with what has been discussed in previous sections, it can be seen that US treatments led to general fragmentation of both carbohydrate and protein structures, which seems to have an increased effect at higher temperatures. It has been indicated by previous authors that ultrasonication caused fragmentation of starches and flours by evaluating morphological changes by scattering electron microscopy and laser light particle size analyses (Amini et al., 2015; Kaur and Gill, 2019; Luo et al., 2008; Vela et al., 2021a, 2021b), indicative that US treatments were causing a general fragmentation of the components present in the treated sample. The sonic energy of US treatments is trapped by the dispersed granules, producing high-frequency vibrations that eventually break the starch granules (Monroy et al., 2018). Size reduction of proteins has been reported in vegetable protein isolates after sonication at a frequency of 20 kHz, at exposure times as short as 15 s with greater results for longer times (Mir et al., 2019; O'Sullivan et al., 2016). The decrease in protein size was attributed to disruption of the hydrophobic and electrostatic interactions due to the high hydrodynamic shear forces associated with cavitation (O'Sullivan et al., 2016). In the present study, protein size reduction was particularly marked in treatments performed at 50 and 55 °C. This protein size reduction along with the decrease in non-covalent bonding and aggregation are attributed to the disruption of the hydrophobic and electrostatic interactions, and hydrogen bonds that maintain protein aggregates in the native flours, by the shear forces of US cavitation (O'Sullivan et al., 2016; Zhu and Li, 2019).

3.5. Hydration properties

The results obtained for WAC, WAI, WSI and SP are presented in Table 1. A significant decrease in WAC of flours was observed after sonication, except for BT-50 and BT-55. Changes in WAC might be consequence of US treatments through its effect on the hydrophilic parts of proteins and carbohydrates, which were found to be lessened by ultrasonication at room temperature (Vela et al., 2021b) but amplified when performed at higher temperature (Vela et al., 2021a). Higher temperatures allow a higher degree of starch chain mobility, which facilitates the ordering of double helices leading to reorganization of starch molecules with increased water binding sites (Zavareze and Dias, 2011). The increases obtained for BT-50 and BT-55, even if statistically significant, were moderate, so it is believed that the reorganization was limited by the short treatment time. The extent of starch chain mobility and realignment of double helices may differ between cultivars (Zavareze and Dias, 2011), which suggests that the BT variety was more susceptible to be influenced by the effect of ANN during treatment, while the WT variety was mainly affected by ultrasonication, given that their WAC values were lower than the control at all studied temperatures.

WAI and SP, on the other hand, significantly increased after treatments, at all studied temperatures. In WT flours no significant differences were found among treatments carried out at temperatures of 40 °C and above, while 45 °C was the limit temperature for BT flours. Ultrasonication induces an increase in swelling capacity by breaking intermolecular bonds of starches and crystalline molecular structure, resulting in morphological changes and less compact granular arrangements (Amini et al., 2015; Luo et al., 2008). These increased results are associated with the particle size reductions due to cavitation (see section 3.1 and 3.4), since smaller structures (both carbohydrates and proteins) have an increased surface area in contact with water that facilitates solid-liquid phase interaction during gelatinization. Similar results have been reported by Amini et al. (2015) when treating corn starch by ultrasonication for short periods ($\leq$ 15 min), finding that temperature is the most important parameter influencing swelling power.

WSI was significantly lower in BT treated samples than BT-C, while in WT flours only WT-55 was found to be significantly different than WT-C. The extent of modification caused to solubility depends not only on

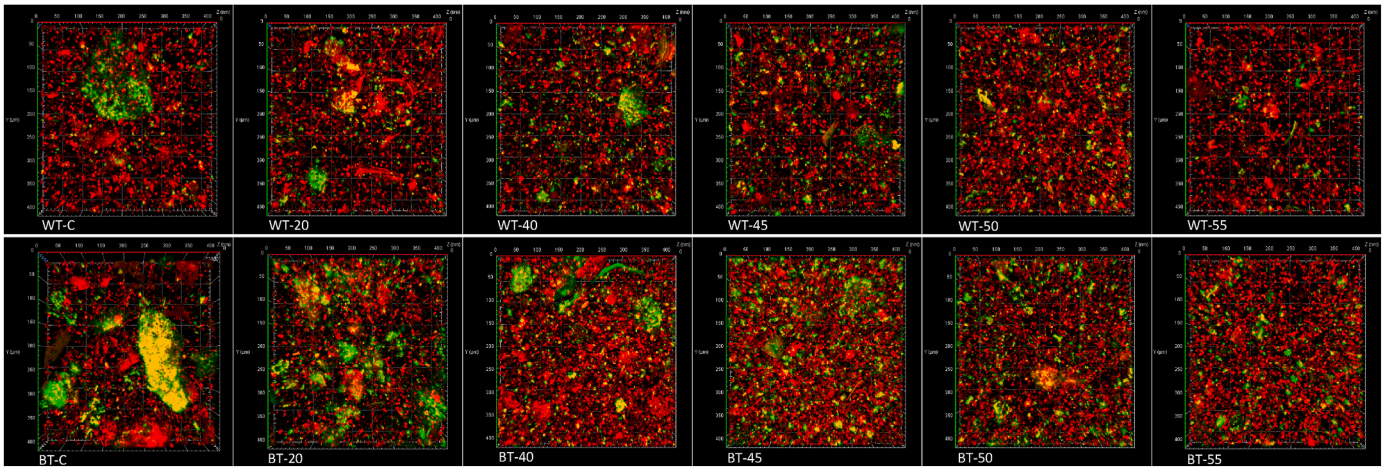

Fig. 2. 3D confocal laser scanning micrographs of the studied samples. Carbohydrates are shown in red (Schiff's reagent) and proteins in green (fluorescamine). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

ultrasonication parameters but also on the type and composition of the treated matter (Zhu, 2015). It has been reported that solubility of starches increased after ultrasonication (Amini et al., 2015; Luo et al., 2008; Zhu, 2015), while studies on flours have found that solubility is mainly decreased by US (Vela et al., 2021a, 2021b). This different effect could be explained by the more complex composition of flours, and how the treatments impact those components [particularly proteins which in the studied flours represent 10.2% (WT) and 9.0% (BT)], and the different interactions among them after being sonicated. Results seem to indicate that increasing temperature contributed to solubility reduction. In annealed starch, a reduction of solubility is due to the strengthening of the bonds between amylose and amylopectin or between amylopectin molecules, preventing leaching of amylose out of the granule (Zavareze and Dias, 2011). In the studied conditions, an increased proportion of amylose chains due to the US may have facilitated the formation of the aforementioned bonds during treatment. A similar solubility decrease has been reported by Wang et al. (2018) in annealing treatments of rice starch.

3.6. Pasting properties

The pasting properties determined for the studied flours are presented in Table 2, and the viscometric profiles are shown in Fig. 3. Results showed a significant delay of the gelatinization process and a general reduction of the viscometric profiles after US treatments. The pasting temperatures (PT) in ultrasonicated samples showed an increasing trend with increasing treatment temperature, reaching 4.9 °C (WT-55) and 4.2 °C (BT-55) higher than their corresponding control flours. It has been said by Tester et al. (2000) that starch rearrangement after ANN makes it harder for the amorphous regions to hydrate as temperature increases due to the strengthening of intragranular bonded forces. However, in annealed cassava and corn starches, no alteration of PT was reported after treatments (Chung et al., 2009; Gomes et al., 2005), indicating that the degree of rearrangement depends greatly on

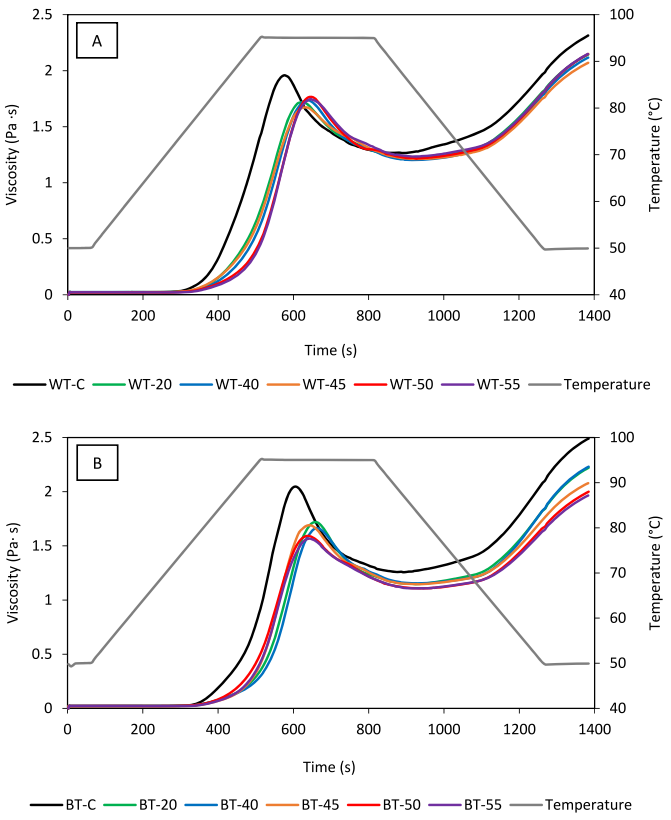

Fig. 3. Viscometric profiles of the studied (A) white tef and (B) brown tef flours. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

Table 2
Pasting properties of the studied flours and rheological properties of the gels made with them.

	Pasting properties						Gels' rheological properties							
Sample	PT (°C)	PV (Pa · s)	TV (Pa · s)	FV (Pa · s)	BV (Pa · s)	SV (Pa · s)	G₁' (Pa)	a	G₁'' (Pa)	b	tan(δ)₁	c	τ_{max} (Pa)	Cross over (Pa)
WT-C	76.8a	2.05b	1.32c	2.43b	0.73b	1.10c	327a	0.003a	45c	0.292b	0.138c	0.289a	390a	531a
WT-20	79.4b	1.73a	1.22a	2.16a	0.51a	0.94b	453d	0.023c	42.1b	0.265a	0.100a	0.301b	522c	645c
WT-40	79.9b	1.75a	1.24 ab	2.20a	0.51a	0.96b	479e	0.002a	40.4 ab	0.291b	0.100a	0.325cd	549d	636bc
WT-45	79.5b	1.73a	1.20a	2.14a	0.52a	0.93b	414c	0.012b	41.9b	0.264a	0.105 ab	0.320c	479b	617b
WT-50	80.8c	1.76a	1.23 ab	2.16a	0.53a	0.93b	399c	0.072d	39.1a	0.273a	0.109 ab	0.333d	490b	626b
WT-55	81.7d	1.78a	1.27b	2.15a	0.51a	0.88a	365b	0.018bc	39.6a	0.285b	0.112b	0.332d	568e	619b
SE	0.2	0.02	0.01	0.03	0.01	0.02	6	0.002	0.6	0.002	0.003	0.003	4	11
Analysis of variance and significance (p-values)														
	***	***	**	**	***	***	***	***	**	***	**	***	***	***
BT-C	79.2a	2.04e	1.25d	2.49e	0.78d	1.241e	254a	0.084d	44.7d	0.314c	0.176d	0.230a	227a	314a
BT-20	81.3b	1.57a	1.07a	1.98a	0.50b	0.914b	289cd	0.067c	37.3b	0.299a	0.109a	0.314c	281b	374b
BT-40	82.1bc	1.66c	1.14b	2.22c	0.52bc	1.086c	291cd	0.068c	36.9b	0.311bc	0.127b	0.247b	236a	379b
BT-45	82.7cd	1.73d	1.19c	2.30d	0.54c	1.108d	277b	0.062b	35.7a	0.317c	0.145c	0.256b	238a	338a
BT-50	82.9cd	1.61b	1.11b	2.02b	0.50b	0.912b	294d	0.030a	39.9c	0.309bc	0.137bc	0.311c	355d	470d
BT-55	83.4d	1.57a	1.11b	1.98a	0.46a	0.866a	281bc	0.068c	36.6 ab	0.304 ab	0.129b	0.322c	318c	414c
SE	0.3	0.01	0.01	0.01	0.01	0.003	7	0.002	0.3	0.002	0.004	0.005	6	10
Analysis of variance and significance (p-values)														
	***	***	***	***	***	***	**	***	***	*	***	***	***	***

PT = Pasting Temperature. PV = Peak Viscosity. TV = Trough Viscosity. FV = Final Viscosity. BV = Breakdown Viscosity. SV = Setback Viscosity. Power law model was fitted to frequency sweeps experimental data [$G' = G_1' \cdot \omega^a$; $G'' = G_1'' \cdot \omega^b$; $\tan\delta = (\tan\delta)_1 \cdot \omega^c$] where G_1', G_1'' and $\tan(\delta)_1$ are the coefficients obtained from the fitting and represent the elastic and viscous moduli and loss tangent respectively, at a frequency of 1 Hz. The *a*, *b* and *c* exponents quantify the dependence degree of dynamic moduli and the loss tangent with the oscillation frequency. τ_{max} represents the maximum stress tolerated by the sample in the LVR.
SE: Pooled standard error from ANOVA. Different letters in the same column within each studied variety indicate statistically significant differences between means at $p < 0.05$. Analysis of variance and significance: ***$p < 0.001$. **$p < 0.01$. *$p < 0.05$. ns: not significant.

ANN conditions (time, temperature, and water content) and the modified starch source. In the studied samples, it is believed that the PT increase was consequence of the increased proportion of short amylose chains derived from the US treatment, which allowed an improved starch rearrangement by ANN. This synergistic effect of ultrasonication joined with ANN has been previously reported in US-modified rice flour (Vela et al., 2021a).

A notable reduction in peak (PV), trough (TV), final (FV), breakdown (BV) and setback (SV) viscosities was observed in both varieties. Mechanical oscillation and cavitation during ultrasound treatments could cause the breakage of long chains and reduce the interaction among starch granules, leading to a viscosity reduction (Yang et al., 2019; Yang et al., 2019; Zhu and Li, 2019). Similar behavior has been reported in US-treated starches (Amini et al., 2015; Luo et al., 2008) and rice and quinoa flours (Vela et al., 2021b; Zhu and Li, 2019). Treatment temperature could also have influenced this reduction, given that lower viscosity values may be caused by the increased amylose content and degree of crystallinity after molecular rearrangement of starch granules due to ANN (Wang et al., 2018). PV was determined later in the test in US-treated flours (see Fig. 3), indicating that ultrasonication led to a thermodynamically more stable arrangements that required longer exposure to 95 °C for starch granules to fully hydrate and gelatinize before collapsing. It has been indicated by Li et al. (2020) that PV is positively correlated with the amount of amylopectin branches, prone to absorb more water during heating, reflecting an impact of US on amylopectin branches. Amylopectin branches with medium chains are involved in the short-term starch retrogradation process (Li et al., 2020), which are believed to be degraded during treatments, explaining why FV and SV are reduced in ultrasonicated samples. SV is related to short-term starch retrogradation, dominantly controlled by amylose component (Li et al., 2020). The significant reduction of SV values reflects the preferential damage caused to the amorphous regions by ultrasonication (Yang et al., 2019), in agreement with the results determined in section 3.3. Viscosity development in flours depends on the water-binding capacity of its components (mainly starch but also protein and fiber and the interactions among them). Water binding capacity of quinoa protein isolates was found to be improved by ultrasonication possibly due to particle size reduction and improvement in the solubility (Mir et al., 2019). The higher proteins-water interaction may restrict the water available for starch and thus, contribute to limiting viscosity development.

3.7. Gel's rheological properties

The rheological properties of gels made with the studied flours are presented in Table 2. Strain sweep measurements were performed to evaluate the consistency of the gels. Two different regions are observed, the linear viscoelastic region (LVR) where the elastic (G′) and viscous (G″) moduli, and the loss tangent [tan(δ) = G''/G'] are constant, and the non-linear region where the gels can no longer withstand the stress and lose their integrity, reaching the cross over point (G' = G″). The maximum stress that gels can resist without losing their structure is denoted by τ_{max} and marks the end of the LVR. The values determined for τ_{max} and the cross over point were significantly increased by US treatments, indicative of gels with higher stability. The increase of τ_{max} did not follow a trend with temperature, however, the highest values were determined for higher temperatures, representing an increase of 46% and 56% (WT-55 and BT-50, respectively) with respect to their corresponding controls. The effect of treatment temperature over this property seems to depend greatly on sonication time, given that a previous study on rice flour treated at different temperatures for 60 min (Vela et al., 2021a) showed a significant decrease of τ_{max} resulting from extensive damage to starch granules that lead to weaker structures. At shorter times and lower temperatures, the effect of US led to an increase in τ_{max} (Vela et al., 2021b). In the present study, the short treatment time (10 min) avoided the damaging effect of high temperature on the starch granules and did not result in decreased gel consistency. The cross over point, or stress at which G' = G″, results were consistent with the τ_{max} results, indicating that gels made with ultrasonicated flours withstood higher stresses before the inversion from elastic-like (G'> G″) to viscous-like (G'< G″) behavior.

The values determined in the frequency sweeps showed that G′ exceeded G″ in the entire range for all studied samples. Higher values of G′ than G″ show the predominance of solid/elastic behavior (Kaur and Gill, 2019), classifying their rheological behavior as "true" gels (Villanueva et al., 2018). Ultrasound treatments led to a significant increase in G'_1, while G''_1 was significantly reduced. It is believed that this increase was mainly caused by the action of cavitation, given that lower temperatures (20 and 40 °C) showed the highest values. The increase in G'_1 reveals disruption of starch granules, weakening the crystalline structure and causing the molecules to catch more water (Kaur and Gill, 2019; Zhang et al., 2021). The short polymeric chains obtained after US treatments due to starch macromolecules fragmentation are capable to reassociate through hydrogen-bonds and thus to form a more elastic three-dimensional network (Monroy et al., 2018). The reduction of G''_1 could be due to the straightening of amylose molecules out of the starch granules, reducing the shear action within the fluid layers (Kaur and Gill, 2019). Treatments led to a loss tangent [$\tan(\delta)_1$] reduction in all studied temperatures, presenting lower values for samples treated at 20 °C, indicating a higher solid-like behavior (i.e., higher elasticity) and higher strength (Kaur and Gill, 2019). It is believed that this reduction was mainly a consequence of cavitation and was not particularly influenced by temperature since a previous study determined that ANN alone led to higher values of $\tan(\delta)_1$ compared to US + ANN treatments at different temperatures (Vela et al., 2021a). Ultrasound waves generate structural disorganization that can allow a better polymeric chain association resulting in a strengthened viscoelastic network (Monroy et al., 2018).

4. Conclusion

Ultrasound treatments showed a noticeable effect on the modification of tef flours' morphology, hydration, pasting, and rheological properties, even at treatments performed at high flour concentration (25% w/w) and short/moderate time (10 min), where treatment temperature had a determinative influence in the extent of modification achieved. Cavitation led to a general particle fragmentation (verified in both polysaccharides and proteins) that resulted in a marked increase of lightness (L*), which could be an advantage for highly pigmented flours such as brown tef. Starch damage increased after treatments, particularly in samples treated at 55 °C, reaching values up to 99% (WT-55) and 115% (BT-55) higher than their corresponding control samples. Amylose content showed a uniform increase after treatments regardless of the applied temperature, probably resulting from degradation of the α-1,6-glucosidic bonds of amylopectin, generating some short amylose-like chains. Increased exposed area in starch granules in US-treated flours improved their interaction with water, which promoted the water absorption index (WAI) and swelling power (SP), and caused a reduction of their viscometric profiles. Pasting temperatures showed an increasing trend with increasing temperature, due to starch rearrangement promoted by ANN. The rheological properties of gels made with the studied flours indicated that ultrasonication led to higher consistency reflected by an increase of up to 46% (WT-55) and 56% (BT-55) in τ_{max}, and a higher solid-like behavior and higher strength shown by the reduction of $\tan(\delta)_1$. A moderately higher effect was determined in US-treated brown tef samples, probably derived from its higher amylose content, more susceptible to be modified by ultrasonication. The improved hydration properties, increased pasting temperatures, and higher gel consistencies after ultrasonication demonstrate improved flour functionality and better adaptation of their processing properties, which can potentially expand the range of utilization for industrial applications of these high-nutritional ancient gluten-free grains.

CRediT authorship contribution statement

Antonio J. Vela: conceived and designed the experiments, analyzed and interpreted the data, contributed reagents, materials, Formal analysis, tools or data, wrote the paper, performed the experiments and, wrote the, Writing – original draft. **Marina Villanueva:** analyzed and interpreted the data, Writing – review & editing. **Oguz K. Ozturk:** analyzed and interpreted the data, Writing – review & editing. **Bruce Hamaker:** analyzed and interpreted the data, Writing – review & editing. **Felicidad Ronda:** Conceptualization, and designed the experiments, analyzed and interpreted the data, contributed reagents, materials, Formal analysis, tools or data, wrote the paper, Funding acquisition, Conceptualization, Methodology, Resources, Investigation, Visualization, Supervision, Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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