International Journal of Biological Macromolecules 253 (2023) 126908

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Microwave radiation induces modifications in the protein fractions of tef flours and modulates their derived techno-functional properties

Grazielle Náthia-Neves [a], Caleb S. Calix-Rivera [a,b], Marina Villanueva [a], Felicidad Ronda [a,*]

[a] Department of Agriculture and Forestry Engineering, Food Technology, College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid, 44, 34004 Palencia, Spain
[b] Department of Agroindustrial Engineering, Pacific Littoral Regional University Center, National Autonomous University of Honduras (UNAH), Choluteca 51101, Honduras

ARTICLE INFO

Keywords:
Tef proteins
Physical modification
Protein fractionation

ABSTRACT

The impact of microwave (MW) treatments on the structure, solubility, and techno-functional properties of the proteins in starchy matrices is still poorly understood. This study aimed to investigate the effects of MW intensity by applying 1, 2, and 6 min of radiation on two tef flour varieties moistened at 15 % and 25 %. The fractionation method recovered ~83 % of the total protein content in untreated flours. The interaction between treatment time and moisture content (MC) significantly influenced the extraction of protein fractions. Samples treated at 25 % MC showed significant reductions in albumins (up to −74 %), globulins (up to −79 %), and prolamins (up to −32 %). The SDS-extractable proteins of both tef flours presented similar molecular weights (12–100 kDa). SDS-PAGE analysis revealed decreased band intensity in MW-treated samples compared to untreated flours, and confocal analysis showed changes in the native state of proteins in treated samples. Shorter treatments at low MC significantly improved the emulsifying stability of tef flours, particularly in brown tef flour, with an enhancement of up to 203 %. The hydration properties significantly increased in flours treated at 25 %MC for 6 min. Pearson correlation analysis demonstrated the influence of treatment time and MC on protein recovery and functional properties of tef flours.

1. Introduction

Cereal grains and derived products have historically been a significant source of dietary energy and nutrients of mankind, in both developed and developing countries [1,2]. However, celiac gluten-sensitive individuals and those with wheat allergies must adhere to a gluten-free diet [3]. In this context, tef [*Eragrostis tef* (Zucc.) Trotter], an ancient Ethiopian gluten-free staple grain, has gained interest due to its valuable nutritional value, which sets it apart from other gluten-free alternatives [4,5]. The grains are characterized by their oval shape, measuring from 0.9 to 1.7 mm in length and 0.7 to 1.0 mm in diameter [6]. Commercially, tef grains are classified into different cultivars, which exhibit a range of colors from ivory white to dark brown [7]. From a nutritional point of view, tef has a higher protein and soluble fiber content than sorghum, corn, and rice [4,8]. This cereal also offers a complete profile of essential amino acids and a high lysine content when compared to pearl millet and sorghum [6]. Additionally, tef contains higher levels of minerals such as iron, calcium, and zinc when compared to other cereal grains like wheat, barley, and sorghum [9]. Regarding functionality, tef demonstrates a lower water absorption capacity and a higher foaming capacity when compared to other gluten-free flours such as rice, oat, maize, chickpea, quinoa, and buckwheat [10].

According to the literature, gluten-free products show some deficits in technological (negative impact on viscosity and elasticity of the bread doughs), nutritional (lack of proteins and dietary fiber), and sensory (inadequate/poor taste and texture, with detection of particles in the mouth and dry mouth feeling) aspects when compared to traditional cereal-based goods [11,12]. In recent years, the PROCEREALtech research group has focused on enhancing the quality of gluten-free ingredients and their derived products by employing microwave (MW) treatments [13–16]. During the MW treatment, heat is generated through the interaction of microwaves (frequency range of 300–300,000 MHz) with charged and polar molecules (e.g., water and mineral salts), which are present in the food matrices [17]. This interaction allows MW energy to uniformly distribute heat throughout the food, resulting in volumetrically distributed heat sources [18]. In

* Corresponding author at: Av. Madrid, s/n, 34004 Palencia, Spain.
E-mail address: mfronda@uva.es (F. Ronda).

https://doi.org/10.1016/j.ijbiomac.2023.126908
Received 30 June 2023; Received in revised form 29 August 2023; Accepted 13 September 2023
Available online 14 September 2023

contrast to conventional heating techniques, MW heating achieves faster heating rates and shorter processing times. MW heating induces more pronounced surface evaporation in comparison to traditional methods, due to the heightened movement of moisture from the interior of the food [19]. Consequently, specific non-covalent bonds in proteins, such as disulfide and hydrogen bonds, may break during heating, promoting the unfolding of protein structures [20]. These changes in the protein's native state due to heating may result in modifications in hydrophobicity properties and susceptibility to the activity of proteolytic enzymes [17]. These modifications, in turn, impact the extractability of proteins and their ability to form emulsions and foams, significantly impacting the potential applications of proteins within food systems [21,22].

Recently, there has been a remarkable industrial interest in food processing using MW treatments because this technology is simple, fast, and cheap, maintains the high nutritional value of the treated samples, has greater penetration depth, prevents Maillard browning reactions, and is an energy-saving treatment [17]. Some studies have investigated the effect of MW treatment on the chemical, physical and functional properties of cereal and legumes starches [15,16,23,24]. However, there is a relative scarcity of studies that specifically address the effects of MW treatment on the proteins present in gluten-free flours. To the best of our knowledge, there are currently no available studies that evaluate the effect of MW radiation on the protein fractions of tef flours, considering the intensity of the treatment, and its consequent impact on the techno-functional properties of the flours. Therefore, the aim of this study was to evaluate the effect of MW treatment depending on the treatment time (1, 2, and 6 min) and the maximum temperature reached by the flour during the treatment (62, 75, 110 °C), on Osborne protein fractions and techno-functional properties of two tef flour varieties moistened at 15 % and 25 %. Confocal microscope and electrophoretic analysis (SDS-PAGE) were employed to investigate the changes in the polymeric protein distribution in the treated flours. The findings from this research will contribute valuable insights into the modulation of protein content and functionality through hydrothermal treatment assisted by MW. Furthermore, the results obtained from this study are expected to contribute to a better understanding of the relationship between the structural and functional properties of MW-treated flours.

2. Materials and methods

2.1. Flour preparation and characterization

Two tef (*Eragrostis tef* Zucc.) flour varieties, Boset white (DZ-CR-409) and Felagot brown (DZ-CR-442), were kindly provided by the Ethiopian Institute of Agricultural Research (EIAR). To obtain the flours, the tef grains were manually cleaned by winnowing, sifting, and sorting to remove all chaff, dust, and other impurities. Then, the grains were ground using a laboratory mill (Perten Instruments, Stockholm, Sweden) fitted with a 0.5 mm screen size. The flour samples were stored in impermeable plastic bags at 4 °C until further analysis and MW treatment.

The moisture (method 44–19), crude fat (method 30–25, using hexane as solvent), and ash (method 8–01) content were determined according to methodologies recommended by AACC [25]. Total nitrogen (N) was quantified using an automated combustion method employing a carbon, nitrogen, and sulfur analyzer (LECO CNS 928, UK). The total protein content was calculated using a conversion factor of 6.25 (N x 6.25). Total starch content and amylose content were determined using the K-AMYL assay kit from Megazyme (Megazyme Bray, Ireland). All analyses were performed in triplicate.

2.2. Microwave treatment

Before MW treatment, the moisture content (MC) of tef flours was adjusted to 15 % and 25 % by adding distilled water. Subsequently, the moistened samples were placed in a hermetically sealed polyethylene bag and stored for 24 h at 4 ± 2 °C to achieve moisture equilibrium. Following this, 50 g of the moisturized tef flours were accurately weighed and introduced into a 1000-mL hermetically sealed cylindrical Teflon® container and treated (900 W, 2450 MHz) in an adapted commercial MW oven SHARP R-342 (Osaka, Japan) for 1, 2, and 6 min of irradiation in cycles of 10 s of radiation and 50 s of rest. To ensure uniform radiation distribution, the containers underwent constant longitudinal rotation at a rate of 60–70 rpm. During each treatment, temperature control was carried out using Testoterm® temperature strips from TESTO (Madrid, Spain). Any agglomerates formed during treatment were manually disintegrated using a laboratory mortar and subsequently sieved to a particle size of <500 μm. Finally, the flours were dried at 35 °C in an incubation chamber (Memmert ICP260, Schwabach, Germany) until they returned to the initial moisture content of the two native (untreated) tef flours. Untreated tef flours were used as control. The selection of time intervals was based on preliminary testing to effectively cover the temperature range to be studied (62, 75, and 110 °C). Table 1 presents the treatment conditions used in this study, along with the corresponding maximum temperatures achieved in each condition and the identification of the samples referenced throughout the text.

2.3. Protein fractionation and quantification

First, the untreated and treated flours were defatted with hexane (1:8 w/v) for 21 h in a shaker operating at 30 °C and 270 rpm. The mixture was centrifuged (1500 ×*g* for 15 min) and the flour was kept in a fume hood for 24 h to ensure a complete evaporation of the hexane. The protein fractionation of the defatted flours was carried out in duplicate, following the method of Osborne [26], with some modifications described by Ronda, et al. [27]. Briefly, 75 mg of each flour and 1.5 mL of distilled water were placed into an Eppendorf tube and vortexed vigorously. The water-soluble albumins fractions were extracted twice by shaking (270 rpm and 30 °C) for 3 h each time. The extracts were centrifuged at 21100 ×*g* for 5 min at 20 °C to obtain a clear supernatant, and all supernatants were combined as albumins fraction. After albumins extraction, the residue was used to extract globulins using 1.5 mL of 50 mM Tris HCl (pH 7.8) in a similar procedure for albumins. Similarly, prolamins and glutelins were successively extracted using 50 % aqueous 1-propanol and 50 % aqueous 1-propanol containing 1 % of DTT (Dithiothreitol), respectively. After each fraction extraction, the

Table 1
Microwave treatment conditions and identification of samples used in the study.

Sample	Moisture content (%)	Treatment time (min)	Treatment temperature (°C)
WT-C	–	–	–
BT-C	–	–	–
WT15-1	15	1	62 ± 3
WT15-2	15	2	75 ± 5
WT15-6	15	6	110 ± 10
WT25-1	25	1	62 ± 3
WT25-2	25	2	75 ± 5
WT25-6	25	6	110 ± 10
BT15-1	15	1	62 ± 3
BT15-2	15	2	75 ± 5
BT15-6	15	6	110 ± 10
BT25-1	25	1	62 ± 3
BT25-2	25	2	75 ± 5
BT25-6	25	6	110 ± 10

Where WT and BT refer to white and brown tef flours, respectively. Control samples (represented by the letter C) refer to untreated samples.

residue was washed with the respective extraction solvent to prevent cross-contamination between fractions. Each extracted fraction was quantified in triplicate according to the Bradford [28] method. Albumins and globulins fractions were quantified using an external calibration curve obtained with bovine serum albumin (BSA) standard dissolved in distilled water, which concentration ranged from 43 to 426 μg / mL ($R^2 = 0.992$). Prolamins and glutelins fractions were quantified using an external calibration curve obtained with wheat gliadins standard dissolved in 50 % aqueous 1-propanol, covering concentrations from 102 to 810 μg/mL ($R^2 = 0.997$). The protein content of each fraction extracted from the tef flours was expressed as milligrams (mg) of the protein fraction per gram (g) of tef flour.

2.4. Protein molecular weight (MW) distribution by SDS-PAGE

All flours were analyzed by SDS-PAGE according to Laemmli [29], with some modifications. The protein fractions were run in 12 % separating gel and 5 % stacking gel. The samples were analyzed under a reducing medium. The same amount of protein (~45 μg) was dispersed in 1 mL of loading buffer ((Tris-HCl, 0.3 M, pH 6.5), glycerol (50 %, v/v), β-mercaptoethanol (25 %, w/v), SDS (10 %, w/v) and bromophenol blue (2 %, w/v)). The mixture was kept under continuous stirring (6 °C and 67 rpm) overnight to extract the protein fractions. After that, the samples were boiled at 100 °C for 5 min, and the blended slurry was centrifuged at 20000 ×*g* for 5 min. Then, 15 μL of the supernatant fraction was loaded into each well and run using a Mini-Protean Cell system (Bio-Rad Laboratories, USA) at a constant amperage of 25 mA/gel for 70 min. Protein bands were then visualized after staining with 0.1 % (w/v) Coomassie Blue R-250 (Sigma Aldrich, Germany) in a methanol/acetic acid/water (40:10:50, volume) (Merck) and distaining in the solvent mixture. The molecular weight of prominent bands was estimated by comparing them with NZYBlue Protein Marker (NZytech, Lisbon, Portugal), which consists of a mixture of 11 highly purified prestained proteins ranging from 10 kDa to 180 kDa. SDS-PAGE electropherograms were generated using a Gel Doc™ EZ Imager (Bio-Rad, Mississauga, ON, Canada) that was operated by Image Lab (version 4.1, Bio-Rad).

2.5. Confocal laser scanning microscopy

A double-labeling technique was used to stain and monitor the carbohydrates and protein phases in tef flours. Carbohydrates were labeled using periodic acid-Schiff (PAS) and proteins were labeled using fluorescamine dye, following the protocol described by Ozturk, et al. [30]. 0.1–0.2 mL 75 % (v/v) glycerol solution was added to the microtubes to keep the labeling stable until confocal analysis. After samples staining and before the analysis, 10 μL aliquots were dispersed onto microscope slides and coverslips were adhered using nail varnish. The samples were imaged with a confocal Leica laser scanning microscope TCS SP5X (Leica TCS SP5X, Mannheim, Germany). The images were taken using HCX PL Apo CS 63 × 1.40 NA oil immersion objective lens, using a 405 nm blue diode laser for excitation of fluorescamine-labeled proteins and detected between 440 and 520 nm, and an Argon laser at 488 nm excitation and 580–680 nm detection for PAS-labeled starches. 3-D images were obtained by collecting around 20 laser-generated optical planes separated ~1–2 μm that were presented as z maximum projections. Leica Application Suite Advanced Fluorescence software was used for the capture, and ImageJ was used for image presentation.

2.6. Techno-functional properties

Water absorption capacity (WAC), water solubility index (WSI), water absorption index (WAI), swelling power (SP), emulsifying activity (EA), emulsification stability (ES), foaming capacity (FC) and foam stability (FS) were determined according to the methodology proposed by Abebe, et al. [31]. For WAC determination, 2 g (dry matter, d.m.) of the sample was stirred into 20 mL of distilled water for 30 s, undergoing three stirring cycles with a 10-min interval between each. The samples were then centrifuged at 3000 ×*g* for 30 min at 25 °C, and the released water was drained. WAC was quantified as g of retained water/g of flour d.m. For WAI, WSI, and SP, aqueous dispersions (1 g/100 mL dispersion) of the flour were vortexed for 30 s and then boiled for 15 min. After cooling to room temperature, the dispersions were centrifuged at 3000 ×*g* for 10 min. The supernatant was collected and brought to dryness for WSI determination (expressed as g of dissolved solids in supernatant/100 g of flour d.m.), while the sediment was weighed to determine WAI (g of sediment/g of flour d.m) and SP (g of sediment/g of insoluble solids in flour d.m.). For FC determination, aqueous dispersions (5 g/100 mL dispersions) of the flours were manually stirred for 5 min to produce foam. FC was calculated as the increment in volume of the flour dispersion, expressed in milliliters (mL). The FS was determined by measuring the foam volume after 60 min and expressed as a percentage of the initial foam volume. For EA and ES measurements, a mixture of flours (7 g), distilled water (100 mL), and corn oil (100 mL) was homogenized using an Ultra-Turrax T25 homogenizer (IKA, Staufen, Germany) at 1000 rpm for 1 min, followed by centrifugation at 1300 ×*g* for 5 min. EA was determined as the ratio of the emulsion volume to the initial total volume, expressed as a percentage. ES was determined after subjecting the emulsion to 80 °C for 30 min in a water bath, cooling to room temperature, and centrifuging at 1300 × g for 5 min. ES was expressed as the ratio of the emulsified layer to the total initial volume and expressed as percentage. All functional properties were performed in triplicate.

2.7. Statistical analysis

All measurements were carried out with a minimum of two replicates. ANOVA analyses were conducted using the Statgraphics Centurion v. 16 software (Bitstream, Cambridge, MN, USA). To determine significant differences ($p < 0.05$) among samples, the Fisher's least significant difference (LSD) test was applied. Pearson correlation coefficients (r) were also calculated to describe the relationship between the MW treatment parameters and both the total protein recovery and functional properties.

3. Results and discussion

3.1. Proximal composition of tef flours

The proximal composition of the studied flours (Table 2) revealed significant ($p < 0.05$) differences between the white and brown tef varieties. The brown tef presented more proteins, lipids an ash content than white tef. The amylose and total starch content did not show any statistical difference among the studied varieties. The values found in this study were in close agreement with those reported in literature for different tef varieties ranging from 10.3 % to 11 % for moisture, 8.9 % to 13.33 % for proteins, 2.0 % to 3.3 % for lipids, 21.1 % to 30 % for amylose content, and 66 % to 76 % for total starch [9,32–34]. Only the

Table 2
Proximal composition of tef flours expressed as g/100 g.

Flours	Moisture	Proteins*	Lipids*	Ash*	AC**	Total starch*
White Tef	11.05 ± 0.02[a]	9.56 ± 0.30[a]	2.68 ± 0.03[a]	2.60 ± 0.04[a]	20.28 ± 1.92[a]	68.60 ± 0.43[a]
Brown Tef	10.49 ± 0.05[b]	12.06 ± 0.31[b]	3.27 ± 0.05[b]	4.05 ± 0.01[b]	20.48 ± 0.57[a]	66.06 ± 3.54[a]

* Results are referred as g/100 g flour on a dry matter.
** AC: Amylose content, referred to the total starch content. Values within a column with different letters are significantly different ($p < 0.05$).

ash content of brown tef was higher than the values reported by Alemneh, et al. [33] and Abebe and Ronda [9] (1.7–3.5 %). Minor variations to this work might be attributed to the harvesting time, variety, climate and soil conditions, sun exposure, post-harvest management and partly to analysis method differences.

3.2. Protein fractions quantification

The total protein content (Table 2) showed significant ($p < 0.05$) differences between the white (9.56 g/100 g dry matter, d.m.) and brown (12.06 g/100 g flour d.m.) tef varieties. This contributed to a higher amount of total protein extracted from brown tef (10.1 %) compared to white tef (7.9 %) after Osborne fractionation. As shown in Table 3, the Osborne fractionation method performed in this study can be considered satisfactory, since >83 % of total protein content was recovered from the two untreated flours. A smaller amount (18–25 %) was recovered by Shumoy, et al. [5] from seven tef varieties, while Adebowale, et al. [35] recovered >90 % of the total protein content of three tef varieties. From the extracted fractions, albumins accounted for 12.5 %, globulins 5 %, prolamins 28 % and glutelins 54.6 % of the total protein content of native (untreated) white tef flour, while for untreated brown tef, albumins accounted for 11.9 %, globulins 3.7 %, prolamins 24.4 % and glutelins 60 % of the total protein content. Commonly, prolamins and glutelins families account for most of the protein content in most cereals, with the exception of rye, triticale and oats, where albumins and glutelins represent the main protein fractions [36]. In agreement to these results, glutelins have been reported to be the major storage protein of tef by Assefa [37] and Gebru, et al. [38]. On the other hand, Adebowale, et al. [35] reported prolamins (38–43 %) as the major protein fraction present in tef while Shumoy, et al. [5] gave the major quantitative importance to albumins and globulins (86–90 %). These reported inconsistencies about the composition of different tef protein fractions have also been observed by other authors for tef and other cereals and pseudocereals as a result of differences in the extraction conditions especially in the solvent used for the extraction [39–41]. Adebowale, et al. [35] used tert-butanol 60 % (v/v) with DTT as reducing agent to extract the prolamins fraction while Shumoy, et al. [5] used 0.5 M sodium chloride to extract albumins and globulins fractions. Here, were used water, 50 mM Tris HCl and only 50 % aqueous 1-propanol to extract albumins, globulins and prolamins, respectively.

Analysis of variance (Table 3) showed that the time of MW radiation and MC of the flour during treatment, as well as their double interaction, significantly affected the extraction of all protein fractions (with the exception of glutelins) in white tef flour. However, for the brown tef variety, the MC did not affect the extraction of globulins and treatment time did not affect the extraction of prolamins and glutelins, and the double interaction of these factors only affected the extractions of albumins and globulins (see Table 3 and Fig. S1 and Fig. S2). The longest treatment time (6 min) led to important changes in the solubility of the proteins of the four Osborne fractions. The WT15–6 sample showed a reduction in the extracted amount of albumins (47 %) and an increase in the amount of globulins (19 %) and glutelins (9 %) while non-significant changes were observed in the prolamins fraction. When the same treatment time was performed at 25 % MC (WT25–6 sample) a more prominent reduction in the content of albumins (72 %), globulins (60 %) and prolamins (21 %) with respect to the untreated flour was obtained. Similarly, greater reduction in albumins (75 %), globulins (78 %) and prolamins (32 %) fractions were observed for the BT25–6 sample. These results suggest that 6 min of MW radiation (maximum temperature reached 110 ± 10 °C) modified the solubility of tef proteins, mainly when the treatment was performed at 25 % MC. Short MW treatments of 1 and 2 min, led to slight changes in proteins solubility, particularly when they were performed at 15%MC.

The slightly increase in the glutelins fraction found in the microwaved-treated samples under the mildest conditions (short times and low MC) could be related to the decrease in the extraction efficiency of albumins, globulins, and prolamins fractions. In other words, the treatment may have reduced the solubilization of these fractions in their respective solvents, that could be extracted with the reducing agent used for the extraction of the glutelins. Additionally, the treatment applied to

Table 3
Effect of MW treatment on proteins fractions of tef flours.

Sample	Albumins		Globulins		Prolamins		Glutelins			
	Content (mg/g)	Ratio (%)	Content (mg/g)	Ratio (%)	Content (mg/g)	Ratio (%)	Content (mg/g)	Ratio (%)	Total (g/100 g)	Recovery (%)
WT-C	9.8 ± 0.4^{de}	12.5	3.9 ± 0.3^{b}	5.0	22 ± 2^{bc}	28.0	43 ± 2^{a}	54.6	7.9 ± 0.2^{bc}	83 ± 2^{bc}
WT15–1	10.0 ± 0.8^{e}	12.3	4.8 ± 0.5^{c}	5.9	19 ± 3^{ab}	23.3	48 ± 2^{bcd}	58.5	8.1 ± 0.3^{cd}	85 ± 3^{cd}
WT15–2	9.2 ± 0.4^{d}	11.1	4.9 ± 0.1^{c}	5.8	20 ± 1^{ab}	23.9	49 ± 1^{d}	59.2	8.4 ± 0.1^{d}	87 ± 1^{d}
WT15–6	5.2 ± 0.1^{b}	6.7	4.6 ± 0.3^{c}	6.0	20 ± 2^{ab}	26.2	47 ± 2^{bcd}	61.0	7.6 ± 0.3^{b}	80 ± 4^{b}
WT25–1	6.8 ± 0.3^{c}	8.6	3.8 ± 0.1^{b}	4.8	23 ± 2^{c}	29.7	45 ± 1^{ab}	57.0	7.9 ± 0.3^{bc}	82 ± 3^{bc}
WT25–2	4.6 ± 0.2^{b}	5.5	3.9 ± 0.4^{b}	4.7	27 ± 1^{d}	32.6	48 ± 3^{cd}	57.2	8.4 ± 0.3^{d}	87 ± 3^{d}
WT25–6	2.7 ± 0.3^{a}	4.1	1.6 ± 0.2^{a}	2.3	17 ± 1^{a}	25.9	45 ± 2^{abc}	67.7	6.7 ± 0.3^{a}	70 ± 3^{a}
Analysis of variance and significance (p-values)										
F1: Moisture content	***		***		*		ns		ns	ns
F2: Treatment time	**		*		*		ns		***	***
(F1) x (F2)	***		***		***		ns		**	**
BT-C	12.1 ± 0.1^{f}	11.9	3.8 ± 0.1^{c}	3.7	25 ± 2^{cd}	24.4	61 ± 3^{ab}	60.0	10.1 ± 0.4^{c}	84 ± 4^{c}
BT15–1	10.8 ± 0.2^{e}	10.3	3.8 ± 0.1^{c}	3.6	26 ± 2^{c}	24.8	64 ± 3^{b}	61.3	10.5 ± 0.4^{c}	87 ± 4^{c}
BT15–2	10.4 ± 0.3^{e}	10.0	4.2 ± 0.2^{d}	4.1	25 ± 2^{cd}	23.9	64 ± 1^{b}	62.1	10.3 ± 0.2^{c}	85 ± 2^{c}
BT15–6	4.1 ± 0.6^{b}	4.5	2.6 ± 0.1^{b}	2.9	22 ± 3^{bc}	24.3	62 ± 1^{ab}	68.3	9.1 ± 0.4^{b}	76 ± 3^{b}
BT25–1	8.0 ± 0.2^{d}	8.6	4.4 ± 0.3^{d}	4.7	18 ± 2^{a}	19.6	62 ± 3^{ab}	67.1	9.2 ± 0.1^{b}	76 ± 1^{b}
BT25–2	5.7 ± 0.3^{c}	6.1	4.3 ± 0.5^{d}	4.6	20 ± 2^{ab}	21.8	62 ± 2^{ab}	67.4	9.3 ± 0.2^{b}	77 ± 2^{b}
BT25–6	3.1 ± 0.7^{a}	3.8	0.8 ± 0.1^{a}	1.0	17 ± 1^{a}	21.1	59 ± 2^{a}	74.0	8.1 ± 0.1^{a}	67 ± 1^{a}
Analysis of variance and significance (p-values)										
F1: Moisture content	*		ns		***		*		**	**
F2: Treatment time	***		***		ns		ns		**	**
(F1) x (F2)	***		***		ns		ns		ns	ns

Albumins, globulins, prolamins and glutelins contents were expressed as mg of protein fraction per gram of tef flour. The ratio refers to the percentage of the protein fraction with respect to total protein extracted. The different letters in the corresponding column within each tef 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.

the white tef flour at 25 % for 6 min negatively affected the total protein recovery. The proteins from brown tef flour were shown to be more sensitive to MW radiation, since the treatment at 15 % MC for 6 min and all treatments performed at 25 % MC negatively affected the recovery of the total proteins. This could be attributed to the differences in the proximate composition of the studied flours (Section 3.1). Differences in moisture, ash and lipids may have an influence on the response of proteins to MW treatment, resulting in variations in their recovery. The temperature reached in the longest treatment (Table 1) could promote thermal denaturation of tef proteins, which involves firstly a dissociation of subunits followed by a re-association of only partially unfolded molecules, that would result to the formation of either soluble or insoluble proteins [42]. Moreover, MW heating may induce the formation of large polymeric aggregates, which are reported to decrease the extractability of the proteins [43,44]. This was confirmed by the confocal microscopy images (see 3.4 Section). To date, no publication has determined the effects of MW treatments on protein fractions from tef, however the results obtained in this study are in agreement to those reported by Nugdallah and El Tinay [45] and Sashikala, et al. [42] who indicated that the albumins and globulins fractions of cowpea and green gram seeds, respectively, decreased as a result of thermal (cooking in excess water) processing. For Nugdallah and El Tinay [45], the prolamins fractions slightly increased after heat treatment, while no significant difference in these fractions was observed by Sashikala, et al. [42] after processing. Both authors also reported that the reduction of some protein fractions was accompanied by an increase in the glutelins fractions. From a technological point of view, MW treatment employed here may be a good tool to produce tef-based gods as it has a positive effect on glutelins, a family of proteins known for favors elasticity and dough strength properties in bakery products [6].

3.3. Protein characterization by SDS-PAGE

SDS-PAGE was performed to evaluate the changes in protein profiles of white and brown tef flours induced by the MW treatments. Fig. 1 shows the SDS–PAGE protein molecular weight distribution profile of the untreated and treated white and brown tef flours under reducing conditions. The SDS-extractable proteins of both tef flours appear within a similar molecular-weight range in the region from approximately 12 to 100 kDa, with major bands approximately at 12–15 and 16–22 KDa (low molecular weight region (LMW)) and 45–60 (high molecular weight region (HMW)); faint signals above 60 kDa were also observed. Similarly, Moroni, et al. [46] and Shumoy, et al. [5] observed most significant bands of tef flour in the region of 14.4–66.2 kDa. Moreover, according to Moroni, et al. [46], albumins, globulins, prolamins and glutelins components appear at 15, 20 and 35 kDa, and glutelin polypeptides may be present around 60 and 110 kDa.

Electrophoresis revealed that brown tef had more intense polypeptide bands in the LMW region (15–22 KDa) than white tef. These proteins comprise the prolamins named alpha-eragrostins and their aggregates (α3.1, α2.3, α4.2, α5.1, α1.1) and delta-eragrostins and their aggregates (δ.1.3, δ.1.4 and δ.2), according to the nomenclature reported by Zhang, et al. [47]. Using the same extraction buffer, the banding pattern reported by Moroni, et al. [46] showed in tef two main components in the LMW region (17–22 kDa) and additional ones in the HMW region (40–60 kDa), which were also suggested as prolamin proteins. Adebowale, et al. [35] reported two major prolamin bands with molecular weights of ~20.3 and ~ 22.8 kDa and found no bands above 23 KDa in tef protein extract under reducing conditions. These contradictory results could be related to SDS-PAGE experimental conditions and extraction buffers, as well as to the genotype and growing conditions of the studied tef varieties.

As displayed in Fig. 1, only the MW-treated sample for 6 min showed fewer visible bands and remarkably decreased band intensity compared to the untreated flours. Some soluble polypeptides with LMW (<45 kDa) and HMW (>60 kDa) disappeared after 6 min of MW treatment (mainly at 25 % MC). This confirms the high reduction in total protein recovery from samples with 25 % of MC treated for 6 min in both studied varieties (Fig. 1) and suggests that this effect on the proteins solubility could be associated with heat-induced denaturation of the proteins and formation of large polymeric aggregates or even due to some interaction of the proteins with other compounds of tef flours [44]. The disappeared fractions could be attributed to albumins and globulins as they were the fractions that had the solubility most reduced by the treatment (Section 3.2); in addition, albumins are reported as the most heat-sensitive protein fraction [48]. The results obtained in this study show that not only of the temperature reached during the treatment (Table 1), but also of both the heating time applied and the sample MC can affect the protein solubility profile of tef flour [44].

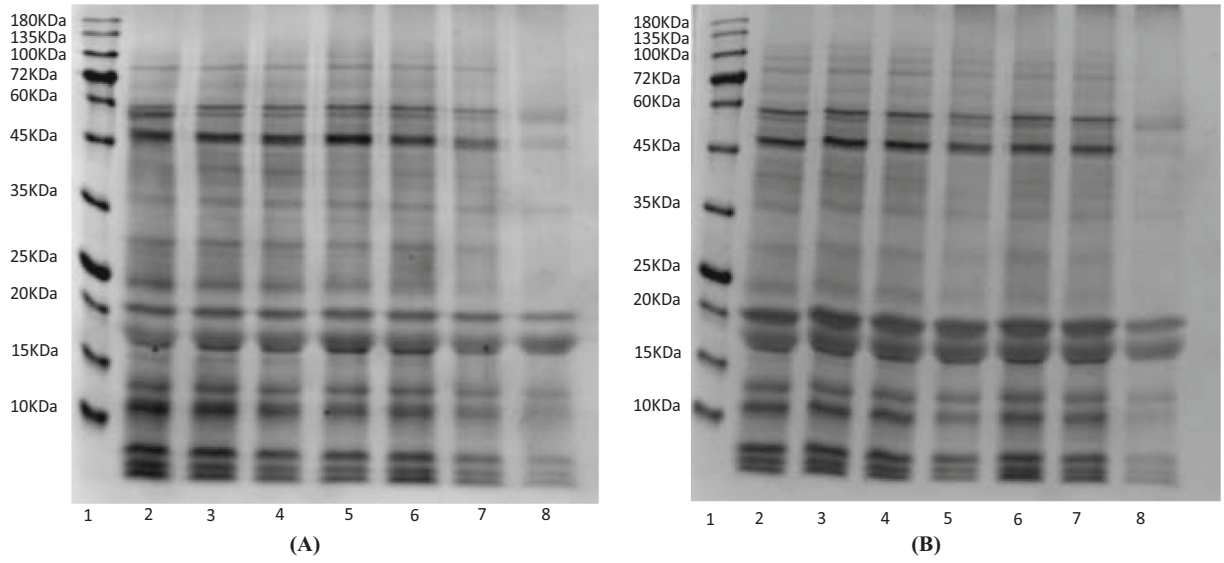


Fig. 1. S–PAGE profiles of white (A) and brown (B) tef proteins under reducing conditions. Lane 1, molecular weight markers; lane 2, untreated tef flour; lane 3, treated flour with 15 % moisture content (MC) for 1 min; lane 4, treated flour with 15 % MC for 2 min; lane 5, treated flour with 15 % MC for 6 min; lane 6, treated flour with 25 % MC for 1 min; lane 7, treated flour with 25 % MC for 2 min; lane 8, treated flour with 25 % MC for 6 min.

3.4. Confocal microscopy

To study the effect of MW treatment on the microstructure of tef flours, confocal laser scanning microscopy was used. Fig. 2 shows 3D confocal micrographs showing the distribution of protein matrix and carbohydrates in the untreated and treated tef flours. In the samples submitted to the MW treatment, agglomerated protein bodies were observed, which suggest that the employed treatment changed the native state of tef proteins. According to Adebowale, et al. [35], thermal denaturation of tef proteins occurs at 70 °C, which accounts for aggregation when tef flours were treated for >2 min ($T > 70$ °C, Table 1). In this regard, the present study suggests that MW treatment performed in both tef flours for longer than 2 min (for both MC) causes proteinaceous material to aggregate due to denaturation. Aggregates are clearly distinguishable as spots of higher fluorescence intensity than the background (black). This partial unfolded state of tef proteins induced by MW can affect their solubility and functional properties in different ways. For example, Zhao, et al. [49] observed an increase in the solubility and foaming ability of glutelins after thermal treatment as a result of the formation of protein aggregates, while Moisio, et al. [50] reported that extrusion process decreased protein solubility due to partial denaturation of oat globulins. Here, the presence of the agglomerated material in the tef flours after the MW treatment could be used to explain the low protein recovery in the samples treated for 6 min (Table 3). Under these treatment time, the temperature was above 100 °C. Moreover, these results also confirm the low solubility of the proteins in the electropherograms obtained from the flours treated for 6 min, which suggested that the treatment led to the formation of insoluble protein aggregates. In addition, a collapse of the proteins was localized in the white and brown tef flours treated at 25 % MC for 2 min ($T = 78$ °C). This unfolded protein state reinforces that the treatment can promote different changes in the native state of proteins depending on the employed conditions.

3.5. Functional properties

Hydration properties (WAC, WAI, WSI and SP) and surfactant activity properties (emulsifying activity and stability –EA, ES–, foaming capacity and stability –FC, FS–) of the control (untreated flours) and treated flours are presented in Table 4. The WAC values were 0.94 and 0.95 g/g for WT-C and BT-C, respectively. The interaction between treatment time and MC significantly ($p < 0.001$) affected the WAC of both tef flours. The flours treated by short heating times at 15 % MC did not show any significant change in WAC, however, WAC increased significantly in the white (1.02 g/g at 15 % MC and 1.50 g/g at 25 % MC) and brown (0.99 g/g at 15 % MC and 1.56 g/g at 25 % MC) tef flours when treated for 6 min. Higher WAC values could be related to the extent of starch gelatinization and damaged starch, as well as changes in the protein structures [51]. In general, the WAC increases with increasing heating time, because generated heat can lead to a rupture of the hydrogen bonds between the amorphous and crystalline regions followed by slight expansion of the amorphous region, which results in a more exposure of the hydrophillic domains [52]. These changes also seem to be favored by a higher sample MC during thermal treatment [52].

The WAI, WSI and SP parameters measured in white and brown tef flours treated for short times (1 and 2 min) at 15 % MC did not show any statistical difference among then and with respect to their respective control samples. However, these same parameters measured for the white tef treated at 25 % MC showed a significant increase at all evaluated times (except for WSI treated for 1 min) when compared to the untreated sample. A similar effect was observed in the WAI and SP values of white tef samples treated at 15 % MC for 6 min. The highest increases in WAI (29 %), WSI (90 %) and SP (34 %) were observed in the white tef flour treated at 25 % for 6 min. WAI and SP are related to interaction between starch chains within the amorphous and crystalline domains and are affected by amylose and amylopectin content, molecular weight distribution and branching length and degree, phosphate groups and starch molecule conformation [13]. The increase in WSI parameter observed in the samples treated for the longest time may be a result of shrinkage and/or disintegration of the starch granules, which leads to a weakening of amylose-amylopectin bonds and increasing amylose-water interactions [52]. Surprisingly, no statistical difference was observed in WAI values from brown tef flours at all studied treatment conditions. A significant increase in WSI was observed in the BT15-6 (increased 86 %) and BT25-6 (increased 176 %), while SP showed a significantly increase only in BT25-6, from 5.9 g/g (control flour) to 7.0 g/g. These findings agreed with Bashir and Aggarwal [53] and Kamble, et al. [54], who found a positive effect of increasing the treatment time (1–2 min) and temperature (50–90 °C) in the WSI and SP parameters measured from chickpea flour and durum wheat semolina treated samples, respectively. According to these authors, the increase in the

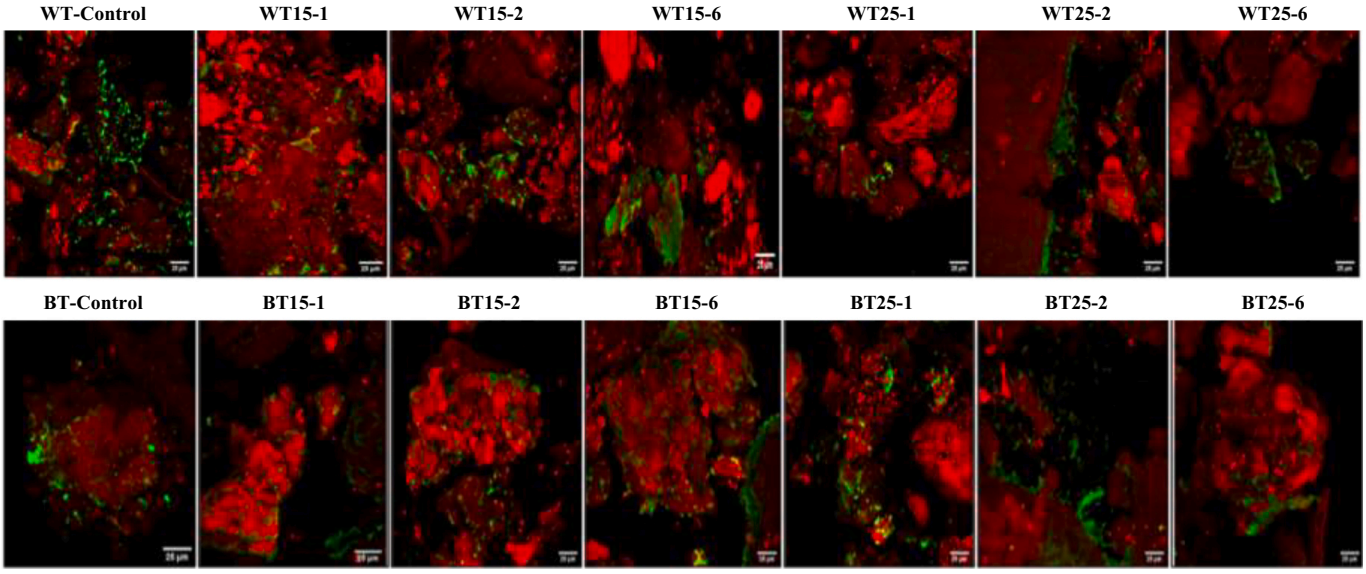


Fig. 2. Confocal images of tef flours before and after MW treatments. Red and green colors indicate carbohydrates labeled using periodic acid-Schiff (PAS) and proteins labeled using fluorescamine dye, respectively. Scale bar: 25 μm.

Table 4
Effect of microwave treatment on functional properties of tef flours.

Sample	WAC (g/g)	WAI (g/g)	WSI (g/100 g)	SP (g/g)	EA (g/100 g)	ES (g/100 g)	FC (mL)	FS (g/100 g)
WT-C	0.94 ± 0.01^{ab}	5.6 ± 0.4^{a}	4.2 ± 0.3^{a}	5.8 ± 0.4^{a}	42.1 ± 0.3^{e}	12.3 ± 0.2^{d}	8.5 ± 0.1^{d}	47 ± 8^{b}
WT15-1	0.93 ± 0.01^{a}	5.7 ± 0.2^{ab}	4.1 ± 0.2^{a}	5.9 ± 0.2^{a}	43.9 ± 0.1^{f}	14.6 ± 0.4^{e}	8.0 ± 0.9^{d}	48 ± 7^{b}
WT15-2	0.95 ± 0.02^{b}	5.9 ± 0.4^{ab}	3.9 ± 0.1^{a}	6.2 ± 0.4^{ab}	38.0 ± 0.1^{d}	10.7 ± 0.9^{c}	9.0 ± 0.7^{d}	45 ± 11^{b}
WT15-6	1.02 ± 0.01^{d}	6.3 ± 0.2^{bc}	4.3 ± 0.4^{a}	6.6 ± 0.3^{bc}	1.1 ± 0.1^{b}	0 ± 0^{a}	4.0 ± 0.7^{bc}	0 ± 0^{a}
WT25-1	0.96 ± 0.02^{c}	6.3 ± 0.6^{bc}	4.1 ± 0.5^{a}	6.9 ± 0.3^{c}	4.4 ± 0.1^{c}	4.3 ± 0.3^{b}	5.3 ± 0.4^{c}	0 ± 0^{a}
WT25-2	1.03 ± 0.01^{d}	6.8 ± 0.5^{cd}	6 ± 1^{b}	7.2 ± 0.6^{cd}	0 ± 0^{a}	0 ± 0^{a}	3 ± 0^{b}	0 ± 0^{a}
WT25-6	1.51 ± 0.01^{e}	7.2 ± 0.1^{d}	8 ± 1^{c}	7.8 ± 0.1^{d}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
Analysis of variance and significance (p-values)								
F1: Moisture content	*	**	**	***	**	**	*	*
F2: Treatment time	**	ns	ns	ns	ns	*	ns	ns
(F1) x (F2)	***	ns	*	ns	***	***	*	**
BT-C	0.95 ± 0.01^{b}	5.7 ± 0.2^{abc}	5.0 ± 0.3^{a}	5.9 ± 0.2^{ab}	45.3 ± 0.5^{d}	5.6 ± 0.4^{b}	7 ± 2^{d}	51 ± 2^{b}
BT15-1	0.96 ± 0.02^{bc}	5.3 ± 0.3^{a}	4.5 ± 0.3^{a}	5.5 ± 0.3^{a}	48.1 ± 0.5^{e}	17 ± 1^{d}	6.3 ± 0.4^{cd}	53 ± 20^{b}
BT15-2	0.97 ± 0.01^{cd}	5.4 ± 0.1^{ab}	4.7 ± 0.2^{a}	5.6 ± 0.1^{a}	45.1 ± 0.6^{d}	13.5 ± 0.1^{c}	5.0 ± 0.7^{c}	51 ± 7^{b}
BT15-6	0.99 ± 0.01^{d}	6.2 ± 0.7^{c}	9.3 ± 0.8^{b}	6.7 ± 0.9^{bc}	1.1 ± 0.1	0 ± 0^{a}	2.0 ± 0.1^{ab}	0 ± 0^{a}
BT25-1	0.89 ± 0.02^{a}	5.4 ± 0.1^{ab}	4.3 ± 0.1^{a}	5.6 ± 0.1^{a}	7.3 ± 0.5^{c}	5.8 ± 0.1^{b}	4.5 ± 0.7^{c}	0 ± 0^{a}
BT25-2	0.95 ± 0.03^{bc}	5.9 ± 0.5^{bc}	5.0 ± 1.9^{a}	6.2 ± 0.7^{abc}	0 ± 0^{a}	0 ± 0^{a}	2.3 ± 0.4^{b}	0 ± 0^{a}
BT25-6	1.56 ± 0.01^{e}	6.2 ± 0.1^{c}	13.8 ± 0.4^{c}	7.0 ± 0.2^{c}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
Analysis of variance and significance (p-values)								
F1: Moisture content	ns	ns	ns	ns	**	**	ns	*
F2: Treatment time	**	**	***	**	ns	*	**	ns
(F1) x (F2)	***	ns	**	ns	***	***	ns	**

WAC: water absorption capacity, WAI: water absorption index, WSI: water solubility index, SP: swelling power, EA: emulsion activity, ES: emulsion stability, FC: foaming capacity, and FS: foaming stability. All values refer to sample dry matter. The different letters in the corresponding column within each tef 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.

WSI and SP in the treated flours could be attributed to the formation of monomeric unit of proteins and radiation induced depolymerisation of the starch (amylopectin branches) and formation of simple sugars that have higher tendency for water as compared to untreated samples.

EA, ES, FC and FS properties are mainly related to the proteins fractions and their structure. A positive effect of treatment ($p < 0.05$) on EA and ES was observed for both flours treated for 1 min at 15 % (4 % increase in EA and 19 % in ES for white tef flour and 6 % increase in EA and 203 % in ES for brown tef flour). Similarly, previous report indicated that MW treatment improved the EA and ES of wheat gluten by applying short treatment times (<3 min) [55]. These results emphasize that short-term treatments performed under low MC are an effective approach to enhance the emulsifying capacity of flours, thereby expanding their potential as emulsifying agents in food products. However, a significant and remarkable reduction on these properties was observed in the samples treated for 6 min at 15 % MC and in all samples treated at 25 % MC (except for ES in brown tef treated for 1 min). A similar trend was found in the FC and FS of tested samples, as a significant reduction on these properties was observed in both flours treated for 6 min at 15 % and for all evaluated samples treated at 25 %MC. These important losses of emulsifying and foaming properties in the samples treated at these conditions could be explained by changes in the solubility and structural conformation of proteins that occurred during treatment. Moreover, the reduction on these properties of the treated tef flours may be attributed to a reduction in some polar amino acids, change in their polarity or denaturation, and dissociation of the constituent protein [56]. As presented in Table 1, the increase in the treatment time and the MC of the samples promoted an increase in the temperature of the treated flours (up to 110 °C). This may have altered the native state of the proteins, leading to the formation of insoluble aggregates, which increased the loss of surface adsorption properties. This results corroborates the findings in the Sections 3.2 and 3.3 that indicate a decrease in the proteins solubility of flours treated under these conditions. Moreover, the confocal analysis (Section 3.4) also showed remarkable differences in the protein structure of the samples treated at 25 % MC. From the results found in this section, it can be inferred that MW treatment could be a useful technology for food processing since, depending on the employed conditions, it allows to improve or reduce some functional properties required to produce desirable changes in food systems.

3.6. Correlation analysis

Pearson correlation coefficient was used to analyze the relationship among treatment time, MC, protein recovery and functional properties of tef flours treated with MW at different times and MC. All the correlation coefficients are displayed in Table 5. According to the results, the treatment time was positively correlated with WAC and WSI in both studied tef flours. In addition, the treatment time was significantly ($p < 0.05$) correlated with the WAI in white tef flour and with the SP and FC in brown tef flour. It suggested that the foregoing indicators were increased as the treatment time values increased. However, there were significant negative correlations between treatment time and protein recovery, EA and ES (in both studied tef flours) and FC (in brown tef flour). The MC showed a significant positive correlation with WAC and WAI only for the treated white tef flours. However, negative correlations between MC and protein recovery, EA, ES and FS were observed in both treated flours, which were significant ($p < 0.05$).

These findings show that treatment time and MC effectively influenced the protein recovery and the functional properties of tef flours treated by MW. As mentioned previously (Section 3.5), EA, ES, FC and FS properties are mainly related to the proteins fractions and structure, which was confirmed by the Pearson correlation analysis. As presented in Table 5, the protein recovery was positively correlated with EA and ES (p < 0.05) in treated white tef flours and it appeared to be extremely significant correlated ($p < 0.01$) with EA, ES, FC and FS in the treated brown tef flours.

4. Conclusions

The MW treatment had a significant impact on the fractionation, solubility, and native state of tef proteins. Shorter MW treatments (1 and 2 min) resulted in minor changes in protein recovery, protein solubility

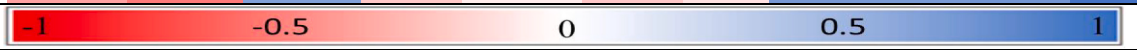
Table 5
Pearson correlation coefficients.

White tef

	Time	*MC*	*PR*	*WAC*	*WAI*	*WSI*	*SP*	*EA*	*ES*	*FC*	*FS*
Time	1.000										
MC	0.000	1.000									
PR	-0.724***	-0.396*	1.000								
WAC	0.698***	0.505*	-0.910***	1.000							
WAI	0.509*	0.651*	-0.603**	0.727***	1.000						
WSI	0.537*	0.349	-0.615**	0.777***	0.782***	1.000					
SP	0.400	0.088	-0.211	0.436	0.681*	0.818***	1.000				
EA	-0.539*	-0.697***	0.519*	-0.491*	-0.696***	-0.332	-0.187	1.000			
ES	-0.646**	-0.606**	0.533*	-0.535*	-0.731***	-0.409*	-0.272	0.974***	1.000		
FC	-0.399	-0.414	0.453	-0.495*	-0.735***	-0.443	-0.403	0.507*	0.541*	1.000	
FS	-0.370	-0.527*	0.407	-0.335	-0.612*	-0.196	-0.147	0.743***	0.740***	0.806***	1.000

Brown tef

	Time	*MC*	*PR*	*WAC*	*WAI*	*WSI*	*SP*	*EA*	*ES*	*FC*	*FS*
Time	1.000										
MC	0.000	1.000									
PR	-0.696***	-0.657***	1.000								
WAC	0.688**	0.350	-0.702***	1.000							
WAI	0.441	-0.131	-0.239	0.318	1.000						
WSI	0.662**	0.272	-0.710***	0.851***	0.579**	1.000					
SP	0.528*	-0.077	-0.341	0.429	0.991***	0.678**	1.000				
EA	-0.557**	-0.685***	0.848***	-0.331	-0.122	-0.341	-0.183	1.000			
ES	-0.659**	-0.598**	0.858***	-0.391	-0.243	-0.413	-0.305	0.976	1.000		
FC	-0.529*	-0.282	0.655**	-0.454	-0.451	-0.478*	-0.492*	0.515*	0.584**	1.000	
FS	-0.364	-0.520*	0.675***	-0.211	-0.069	-0.213	-0.111	0.731***	0.719***	0.753***	1.000



Time: Treatment time; MC: moisture content of flours during treatment; PR: Protein recovery; WAC: water absorption capacity; WAI: water absorption index, WSI; water solubility index, SP; swelling power, EA; emulsion activity, ES: emulsion stability, FC: foaming capacity and FS: foaming stability. * significant correlation ($p < 0.05$); ** and *** extremely significant correlation ($p < 0.01$) and ($p < 0.001$), respectively.

and hydration properties, while significantly improving the emulsifying stability of tef flours, particularly when carried out at 15 % MC. However, when the MC was increased to 25 %, more unfavorable changes were observed in the surfactant properties. Therefore, the treatment applied in this study offers an opportunity to modulate the techno-functional properties of tef flours, thereby broadening its potential applications in several food products.

The potential application of these treated flours in food systems requires further analysis. However, considering the positive correlation between treatment time and MC with WAC in both flours, we believe that these flours could be valuable as thickeners or to improve the consistency of shakes or doughs prepared with them. Moreover, treatments carried out for shorter times at low MC resulted in enhanced emulsion stability, indicating that tef flours treated under such conditions could be suitable for the production of desserts, sauces, or aerated products.

CRediT authorship contribution statement

Grazielle Náthia-Neves: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft. **Caleb S. Calix-Rivera:** Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft. **Marina Villanueva:** Conceptualization, Methodology, Investigation, Visualization. **Felicidad Ronda:** Funding acquisition, Supervision, Conceptualization, Resources, Methodology, Investigation, Data curation, Visualization, 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.

Acknowledgments

The authors thank the financial support of the Ministerio de Ciencia e Innovación (PID2019-110809RB-I00/AEI/10.1303/501100011033), the Junta de Castilla y León/FEDER (VA195P20). The authors also thank Francisco Javier Arias (2Smart Biodevices for NanoMed Research Group) for the valuable support provided during the SDS-PAGE analysis and the Ethiopian Institute of Agricultural Research (EIAR) for providing the raw materials. Caleb S. Calix-Rivera thanks the University of Valladolid for the doctorate grant.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2023.126908.

References

[1] K. Poutanen, Past and future of cereal grains as food for health, Trends Food Sci. Technol. 25 (2) (2012) 58–62.
[2] W. Laskowski, H. Górska-Warsewicz, K. Rejman, M. Czeczotko, J. Zwolińska, How important are cereals and cereal products in the average polish diet? Nutrients 11 (3) (2019).

[3] U. Krupa-Kozak, N. Bączek, V.D. Capriles, Ł. Łopusiewicz, Novel gluten-free bread with an extract from flaxseed by-product: the relationship between water replacement level and nutritional value, antioxidant properties, and sensory quality, Molecules 27 (9) (2022).
[4] K. Paff, S. Asseng, A review of tef physiology for developing a tef crop model, Eur. J. Agron. 94 (2018) 54–66.
[5] H. Shumoy, S. Pattyn, K. Raes, Tef protein: solubility characterization, in-vitro digestibility and its suitability as a gluten free ingredient, LWT 89 (2018) 697–703.
[6] M.M. Gebremariam, M. Zarnkow, T. Becker, Teff (Eragrostis tef) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: a review, J. Food Sci. Technol. 51 (11) (2014) 2881–2895.
[7] A. Girija, H. Jifar, C. Jones, R. Yadav, J. Doonan, L.A.J. Mur, Tef: a tiny grain with enormous potential, Trends Plant Sci. 27 (3) (2022) 220–223.
[8] D. Rico, F. Ronda, M. Villanueva, C. Perez Montero, A.B. Martin-Diana, Development of healthy gluten-free crackers from white and brown tef (*Eragrostis tef* Zucc.) flours, Heliyon 5 (10) (2019), e02598.
[9] W. Abebe, F. Ronda, Rheological and textural properties of tef [Eragrostis tef (Zucc.) trotter] grain flour gels, J. Cereal Sci. 60 (1) (2014) 122–130.
[10] A. Culetu, I.E. Susman, D.E. Duta, N. Belc, Nutritional and functional properties of gluten-free flours, Appl. Sci. 11 (14) (2021).
[11] L. Padalino, M.A. Del Nobile, B. la Gatta, M. Rutigliano, A. Di Luccia, A. Conte, Effects of microwave treatment of durum wheat kernels on quality characteristics of flour and pasta, Food Chem. 283 (2019) 454–461.
[12] P. Conte, C. Fadda, N. Drabińska, U. Krupa-Kozak, Technological and nutritional challenges, and novelty in gluten-free breadmaking - a review, Pol. J. Food Nutr. Sci. 69 (1) (2019) 5–21.
[13] M. Villanueva, B. De Lamo, J. Harasym, F. Ronda, Microwave radiation and protein addition modulate hydration, pasting and gel rheological characteristics of rice and potato starches, Carbohydr. Polym. 201 (2018) 374–381.
[14] M. Villanueva, J. Harasym, J.M. Muñoz, F. Ronda, Rice flour physically modified by microwave radiation improves viscoelastic behavior of doughs and its bread-making performance, Food Hydrocoll. 90 (2019) 472–481.
[15] M. Villanueva, J. Harasym, J.M. Muñoz, F. Ronda, Microwave absorption capacity of rice flour. Impact of the radiation on rice flour microstructure, thermal and viscometric properties, J. Food Eng. 224 (2018) 156–164.
[16] C.S. Calix-Rivera, M. Villanueva, G. Náthia-Neves, F. Ronda, Changes on Techno-Functional, Thermal, Rheological, and Microstructural Properties of Tef Flours Induced by Microwave Radiation—Development of New Improved Gluten-Free Ingredients, Foods, 2023.
[17] J. Leszczynska, A. Łącka, J. Szemraj, J. Lukamowicz, H. Zegota, The effect of microwave treatment on the immunoreactivity of gliadin and wheat flour, Eur. Food Res. Technol. 217 (5) (2003) 387–391.
[18] A.A. Sadeghi, P. Shawrang, Effects of microwave irradiation on ruminal protein and starch degradation of corn grain, Anim. Feed Sci. Technol. 127 (1) (2006) 113–123.
[19] T. De Pilli, O. Alessandrino, Effects of different cooking technologies on biopolymers modifications of cereal-based foods: impact on nutritional and quality characteristics review, Crit. Rev. Food Sci. Nutr. 60 (4) (2020) 556–565.
[20] X. Kan, G. Chen, W. Zhou, X. Zeng, Application of protein-polysaccharide Maillard conjugates as emulsifiers: source, preparation and functional properties, Food Res. Int. 150 (2021) 110740.
[21] Y. Zhang, S. Sharan, Survey on Methods for Investigating Protein Functionality and Related Molecular Characteristics 10(11), 2021.
[22] J.E. Kinsella, Functional properties of soy proteins, J. Am. Oil Chem. Soc. 56 (3Part1) (1979) 242–258.
[23] S. Ashraf, S.M.G. Saeed, S.A. Sayeed, R. Ali, Impact of microwave treatment on the functionality of cereals and legumes, Int. J. Agric. Biol. 14 (3) (2012).
[24] A.K. Anderson, H.S. Guraya, Effects of microwave heat-moisture treatment on properties of waxy and non-waxy rice starches, Food Chem. 97 (2) (2006) 318–323.
[25] AACC, Approved Methods of the AACC, AACC St. Paul, 2000.
[26] T.B. Osborne, The Vegetable Proteins, Longmans, Green and Company, 1924.
[27] F. Ronda, J.M. Rodríguez-Nogales, D. Sancho, Multivariate optimisation of a capillary electrophoretic method for the separation of glutenins. Application to quantitative analysis of the endosperm storage proteins in wheat, Food Chem. 108 (1) (2008) 287–296.
[28] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1–2) (1976) 248–254.
[29] U. Laemmli, SDS-page Laemmli method, Nature 227 (1970) 680–685.
[30] O.K. Ozturk, S.G. Kaasgaard, L.G. Palmén, B.C. Vidal, B.R. Hamaker, Enzyme treatments on corn fiber from wet-milling process for increased starch and protein extraction, Ind. Crop. Prod. 168 (2021) 113622.
[31] W. Abebe, C. Collar, F. Ronda, Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours, Carbohydr. Polym. 115 (2015) 260–268.
[32] S.D. Urigacha, The effect of wheat, tef and soybean flours blending ratio and baking temperature on nutritional quality of bread, Int. J. Food Sci. Biotechnol. 5 (4) (2020) 72.
[33] S.T. Alemneh, S.A. Emire, B. Hitzmann, V. Zettel, Comparative study of chemical composition, pasting, thermal and functional properties of Teff (Eragrostis tef) flours grown in Ethiopia and South Africa, Int. J. Food Prop. 25 (1) (2022) 144–158.
[34] H. Shumoy, K. Raes, In vitro starch hydrolysis and estimated glycemic index of tef porridge and injera, Food Chem. 229 (2017) 381–387.
[35] A.-R.A. Adebowale, M.N. Emmambux, M. Beukes, J.R.N. Taylor, Fractionation and characterization of teff proteins, J. Cereal Sci. 54 (3) (2011) 380–386.
[36] A.G.B. Wouters, J.A. Delcour, Cereal protein-based nanoparticles as agents stabilizing air–water and oil–water interfaces in food systems, Curr. Opin. Food Sci. 25 (2019) 19–27.
[37] M. Assefa, Floral morphogenesis, temperature effect on growth and development, and variation in nutritional composition and distribution among cultivars in eragrostis tef (zucc.), Trotter, in: PhD Thesis, University of Wisconsin, Madison, Wisconsin, 1978.
[38] Y.A. Gebru, J. Hyun-Ii, K. Young-Soo, K. Myung-Kon, K. Kwang-Pyo, Variations in Amino Acid and Protein Profiles in White Versus Brown Teff (*Eragrostis Tef*) Seeds, and Effect of Extraction Methods on Protein Yields, Foods, 2019.
[39] X. Guo, H. Yao, Fractionation and characterization of tartary buckwheat flour proteins, Food Chem. 98 (1) (2006) 90–94.
[40] F. Janssen, A. Pauly, I. Rombouts, K.J.A. Jansens, L.J. Deleu, J.A. Delcour, Proteins of amaranth (Amaranthus spp.), buckwheat (Fagopyrum spp.), and quinoa (Chenopodium spp.): a food science and technology perspective, Compr. Rev. Food Sci. Food Saf. 16 (1) (2017) 39–58.
[41] H. Shumoy, K. Raes, Tef: the rising ancient cereal: what do we know about its nutritional and health benefits? Plant Foods Hum. Nutr. 72 (4) (2017) 335–344.
[42] V.B. Sashikala, Y.N. Sreerama, V.M. Pratape, H.V. Narasimha, Effect of thermal processing on protein solubility of green gram (Phaseolus aureus) legume cultivars, J. Food Sci. Technol. 52 (3) (2015) 1552–1560.
[43] C. Daniel, E. Triboï, Changes in wheat protein aggregation during grain development: effects of temperatures and water stress, Eur. J. Agron. 16 (1) (2002) 1–12.
[44] C. Lamacchia, L. Landriscina, P. D'Agnello, Changes in wheat kernel proteins induced by microwave treatment, Food Chem. 197 (2016) 634–640.
[45] G.A. Nugdallah, A.H. El Tinay, Effect of cooking on cowpea protein fractions, Plant Foods Hum. Nutr. 51 (3) (1997) 277–282.
[46] A.V. Moroni, S. Iametti, F. Bonomi, E.K. Arendt, F. Dal Bello, Solubility of proteins from non-gluten cereals: a comparative study on combinations of solubilising agents, Food Chem. 121 (4) (2010) 1225–1230.
[47] W. Zhang, J. Xu, J.L. Bennetzen, J. Messing, Teff, an orphan cereal in the Chloridoideae, provides insights into the evolution of storage proteins in grasses, Genom. Biol. Evol. 8 (6) (2016) 1712–1721.
[48] S.P. Meriles, M.E. Steffolani, A.E. León, M.C. Penci, P.D. Ribotta, Physico-chemical characterization of protein fraction from stabilized wheat germ, Food Sci. Biotechnol. 28 (5) (2019) 1327–1335.
[49] M. Zhao, W. Xiong, B. Chen, J. Zhu, L. Wang, Enhancing the solubility and foam ability of rice glutelin by heat treatment at pH12: insight into protein structure, Food Hydrocoll. 103 (2020) 105626.
[50] T. Moisio, P. Forssell, R. Partanen, A. Damerau, S.E. Hill, Reorganisation of starch, proteins and lipids in extrusion of oats, J. Cereal Sci. 64 (2015) 48–55.
[51] V. Kumar, H.K. Sharma, K. Singh, Effect of precooking on drying kinetics of taro (Colocasia esculenta) slices and quality of its flours, Food Biosci. 20 (2017) 178–186.
[52] Á.G. Solaesa, M. Villanueva, J.M. Muñoz, F. Ronda, Dry-heat treatment vs. heat-moisture treatment assisted by microwave radiation: techno-functional and rheological modifications of rice flour, LWT 141 (2021) 110851.
[53] K. Bashir, M. Aggarwal, Effects of gamma irradiation on the physicochemical, thermal and functional properties of chickpea flour, LWT Food Sci. Technol. 69 (2016) 614–622.
[54] D.B. Kamble, R. Singh, B. Pal Kaur, S. Rani, A. Upadhyay, Effect of microwave processing on physicothermal properties, antioxidant potential, in vitro protein digestibility and microstructure of durum wheat semolina, J. Food Measur. Charact. 14 (2) (2020) 761–769.
[55] E. Yalcin, O. Sakiyan, G. Sumnu, S. Celik, H. Koksel, Functional properties of microwave-treated wheat gluten, Eur. Food Res. Technol. 227 (5) (2008) 1411–1417.
[56] S.A. Almaiman, N.A. Albadr, S. Alsulaim, H.F. Alhuthayli, M.A. Osman, A. B. Hassan, Effects of microwave heat treatment on fungal growth, functional properties, total phenolic content, and antioxidant activity of sorghum (*Sorghum bicolor* L.) grain, Food Chem. 348 (2021), 128979.