Impact of heat treatment of sorghum grains on flour properties

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Abstract

Introduction. This paper aimed to evaluate the nutritional and rheological properties of heat-treated and untreated sorghum flour with medium size fraction (M) and integral.

Materials and methods. Amino acids were determined by high-performance liquid chromatography, fatty acids and volatiles by gas chromatography, minerals by atomic absorption spectrophotometry, and rheology by dynamic testing methods.

Results and discussion. The application of dry heat treatment on sorghum flour led to a reduction in the content of essential amino acids. However, a higher lysine content was observed in the treated M fraction compared to the control fraction. The content of non-essential amino acids significantly decreased after the application of heat treatment and grinding. The dry heat treatment temperature of 133 °C applied to the red sorghum grains, along with fractionation, resulted in an increase in the content of mono- and polyunsaturated fatty acids and a decrease in the saturated fatty acid content in the treated M fraction compared to the control fraction. There were considerable differences between samples regarding sodium, iron, zinc, and copper content. Heat treatment led to a decrease in the percentage of 2,4-pentadienenitrile volatile compound and the identification of 2,4-hexadienenitrile in the treated M fraction compared to the control fraction.

The obtained elastic modulus values showed that the dough prepared from the treated fraction M red sorghum flour possessed slightly higher elastic energy compared to the sample prepared from whole red sorghum flour treated at 133 °C. The maximum gelatinization temperature for the sample with the treated M fraction of red sorghum flour was lower than that of the control sample. The dough from treated M fraction of red sorghum flour showed higher compliance for both creep and recovery compared to that made from the control fraction.

Conclusion. Sorghum dry heat treatment and fractionation led to significant differences in nutritional and rheological profile.

Introduction

Sorghum (*Sorghum bicolor* L. Moench) originates from East Africa (Sudan and Ethiopia) and has come to be cultivated worldwide, being the fifth most cultivated cereal after corn, wheat, rice, and barley (Ratnavathi et al., 2016), due to its agronomic advantages, as it is resistant to drought, pests, and diseases (Pérez et al., 2010). The cultivation of sorghum is significant for semi-arid regions of the world, especially in Africa and Asia, due to its agronomic benefits in harsh environments, drought resistance, high productivity, and low production costs (Adebowale et al., 2020). Since the year 2000, the area allocated to this crop has come to represent approximately 3% of the total area occupied by cereal crops. Global production reached around 59 million tons in 2018 (Cabrera-Ramírez et al., 2020; Pezzali et al., 2020). The world's top five sorghum producers are the United States (25%), India (21.5%), Mexico (almost 11%), China (9%), and Nigeria (almost 7%). Together, these five countries account for 73% of total global production. Based on the region of cultivation and/or use by different populations, sorghum has various names. In Africa, it is known as large millet, milo, durra, and orshallu, while in China it is called gaoliang, and Indians call it jowar, cholam, or jonna (Djameh et al., 2015; Rashwan, 2017). Globally, the largest consumer of sorghum is China, and within the European Union, it is Spain. In the European Union, the most significant sorghum-cultivating countries are France and Italy.

In Romania, the surface used for sorghum cultivation in 2020 was 8.4 million hectares, with a production of 26.6 thousand tons (National Institute of Statistics: Bucharest, Romania, 2022), followed by Spain, Hungary, and Bulgaria. In Romania, both red and white sorghum hybrids are cultivated. Sorghum is an excellent source of starch and protein, containing phenolic compounds, such as flavonoids (Shahidi and Naczk, 1995), phenolic acids, and tannins. Starch and sugar are released more slowly from sorghum than from other cereals (Klopfenstein and Hoseney, 1995), which is beneficial for people with diabetes. As a potential source of resistant starch and β-glucans (Niba and Hoffman, 2003), sorghum can be used as a prebiotic food ingredient in value-added food products. Processed sorghum is consumed worldwide in various forms: bread, biscuits, cakes, couscous, tortillas, porridge, alcoholic and non-alcoholic beverages, extruded products, and pasta.

It has been asserted that sorghum has low protein quality due to the structural properties of kafirin, the predominant protein, a deficiency of amino acids such as threonine, tryptophan, and lysine, and interactions with phenolic molecules, such as condensed tannins, which form complexes with proteins and reduce their digestibility (Martinez et al., 2020). However, the gluten-free bakery industry is challenging, and currently, many researchers are focusing on composite flour formulations. Current research on heat-treated sorghum flour is limited, and a gap in food product development using this flour has been identified.

Understanding how certain physical treatments applied to sorghum grains affect the physicochemical composition of whole meal flour and different-sized sorghum flour particles, as well as their functionality, is important for managing the quality of finished products. The heat generated during the dry heat treatment process, as well as the frictional heat and mechanical energy required for grinding, can affect the structural and molecular properties of starch as well as other properties of sorghum grains. There is limited information on the characteristics of different-sized sorghum flour particles when sorghum grains are subjected to physical treatments. Additionally, knowledge of the nutritional and rheological properties of sorghum flour plays an essential role in explaining the complex interactions between components, along with the nature of the environment in which they are associated and investigated. Thus, this paper aimed to explore the characteristics of integral and fractionated red sorghum flour with or without heat treatment applied to the sorghum grains. For this purpose, the fatty acids, amino acids, volatile compounds, minerals content, and rheological behavior were determined.

Materials and methods

Materials

For the experimental research, red sorghum grains from a hybrid sorghum variety cultivated in Romania, specifically in the northern region of Moldova, were used. The red sorghum grains (ES Alize) were sourced from a retail vendor in Fălticeni (Suceava, Romania).

The sorghum grains were processed using two methods: first, a dry heat treatment was applied, and then they were milled. The optimal conditions were established before and they are presented in our previous paper (Batariuc et al., 2023). The dry heat treatment was applied to the sorghum grains spread in a uniform 10 mm layer on a tray, and placed in a convection oven (Binder ED53 L, Tuttlingen, Germany) for 15 minutes at 133°C. For milling the sorghum grains, a KitchenAid grain mill (model 1065 KGM, Whirlpool Corporation, Benton Harbor, MI, USA) was used. The whole sorghum flour was then separated using a vibrating sieve system (Retsch AS, Haan, Germany) to obtain the M particle size (200 µm < M < 250 µm). Samples codifications were the following: I_TOM – integral treated sample, OM – treated sample with M particle size, CM – control untreated sample with M particle size.

Amino acids profile

The amino acids were determined using a Vanquish UHPLC high-performance liquid chromatograph with a fluorescence detector, Thermo Fisher, equipped with a chromatographic column (Hypersil Gold 150 x 4.65 µm, with a flow rate of 0.80 mL/min). A sample of 5 g was hydrolyzed with 20 mL of 4 M hydrochloric acid at a temperature of 95°C for 24 hours. After filtering through filter paper, the obtained extract was neutralized with 15 mL of 10% potassium hydroxide solution. The samples were then re-filtered through 0.45 µm pore size filters and injected into the equipment. Derivatization was performed on a pre-column with ortho-phthalaldehyde (OPA) at a 1:2 ratio, using the automatic sample injection program, with an injection volume of 1 µL.

Fatty acids profile

The fatty acid analysis was performed using a gas chromatograph CG-FID (Agilent Technologies, 6890N GC, Wilmington, DE, USA) equipped with a DB-WAX capillary column with a polyethylene glycol stationary phase (30 m × 0.25 mm × 0.25 µm) and a flame ionization detector (FID). Approximately 30 ± 0.1 mg of flour sample was dissolved in 2 mL of iso-octane. The mixture was subjected to transesterification with 200 µL of methanolic potassium hydroxide solution (2 mol/L) under vigorous stirring. The resulting organic phase was mixed with sodium hydrogen sulfate. After collecting the supernatant, 1 µL of the sample was directly injected into the gas chromatograph (GC). The gases used for FID analysis were hydrogen at 40 mL/min, air at 450 mL/min, and helium at 30 mL/min. The injection volume was 1 µL in a 1:20 split mode. The temperature program was as follows: the initial oven temperature was set to 60°C, held for 1 min, increased from 60 to 200°C at a rate of 10°C/min, held for 2 min, then from 200 to 220°C at a rate of 5°C/min, and held for 20 min. The injector and detector temperatures were set at 250°C. Fatty acid identification in the samples was completed by comparing their retention times with those of a standard mixture (Senila et al., 2020).

The desirable fatty acid content (DFA) was determined as the sum of MUFA, PUFA, and stearic acid (Medeiros et al., 2014). The thrombogenic index was calculated as the sum of C14:0, C16:0 and C18:0 divided by (0.5 x MUFA + 0.5 x n6 + 3 x n3 + n3/n6) (Renna et al., 2012).

Minerals content

The determination of mineral content was carried out using a Shimadzu AA7000 atomic absorption spectrophotometer, ASC-7000 (air-acetylene), equipped with an ASC-6100 autosampler. The mineral content profile was determined for Ca, Na, Fe, Cu, and Zn, elements predominant in sorghum flour. The samples were prepared according to the method described in the technical manual of the Shimadzu AA7000, ASC-7000 spectrophotometer. Five grams of sample were placed in a crucible and calcined at 550°C for 12 hours. If calcination was incomplete, 1 mL of 65% nitric acid was added to the crucible, and after the evaporation of nitric acid, the process was repeated until the ash turned white. After cooling, 1 mL of 65% nitric acid was added to the crucible, and the contents were transferred to a 50 mL volumetric flask and brought to the mark with deionized water (Avramia and Amariei, 2021). The concentrations of macro- and microelements were expressed in mg/kg of analyzed sorghum flour.

Volatile compounds

The determination of volatile organic compounds (VOCs) was performed using a gas chromatograph coupled with a mass spectrometer (GC-MS). A sample amount of 3 g was transferred into a 20 mL headspace vial, and 3 g of sodium chloride was added to enhance VOCs in the headspace and inhibit any enzymatic reactions. The headspace vials were sealed with crimp-top caps with TFE-silicone headspace septa. Identification criteria for compounds required a mass spectrum match score of ≥ 80%. Results were expressed as the percentage of relative peak area (% RPA) for each peak in each sample, calculated by dividing the peak area by the total area of all identified peaks in each chromatogram. The total ion chromatogram (TIC) of each sample was used to integrate peak areas. All measurements were performed in triplicate, and data are presented as mean ± standard deviation.

The GC oven temperature was set at 35 °C (held for 1 min), increased to 100 °C (held for 1 min) at a rate of 5 °C/min, then to 250 °C (held for 3 min) at a rate of 7 °C/min, and finally increased to 250 °C (held for 1 min) at a rate of 10 °C/min. The transfer line temperature was set at 280 °C, and the ion source temperature was set at 250 °C. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, following the method reported by Dippong et al. (2023).

Rheological properties

The rheological properties of the sorghum flour dough were determined using dynamic testing methods, including oscillatory testing and creep-recovery testing. A preliminary test was also conducted to identify the boundaries of the linear viscoelastic region of the samples, with a constant oscillatory frequency of 1 Hz and increasing the stress from 0 to 100 Pa. Dynamic rheological measurements were performed with a Thermo-HAAKE MARS 40 dynamic rheometer (Karlsruhe, Germany) with parallel plates, equipped with a Peltier heating system that maintained a constant sample temperature of 21 °C during testing.

Dough samples, prepared from 50 g of sorghum flour and 50 mL of water, were rested for 5 minutes before testing to reach equilibrium. During testing, the gap between the plates was set to 2 mm, and any excess dough was removed. To prevent moisture loss from the sample during testing, a layer of petroleum jelly was applied around the sample edges. Data processing was carried out using the RheoWin Data Manager software, version 4.85 (Thermo Scientific).

Frequency sweep test. The determination of the variation in elastic (G') and viscous (G") modulus with frequency was carried out by applying a constant stress of 10 Pa, positioned within the linear viscoelastic region, over a frequency range from 0.01 to 20 Hz.

Temperature sweep test. By applying the oscillatory test at a constant frequency of 1 Hz, where the dough was heated at a rate of 4 ± 0.1 °C/min from 20 to 100 °C, values for the elasticity and viscosity moduli were determined.

Creep and recovery. The low-stress oscillatory test was complemented by a creep and recovery test. This involved the sudden application of a 50 Pa stress (within the linear viscoelastic range) for 60 seconds, followed by a 180-second recovery period after the stress was removed, allowing the dough to relax (Iuga et al., 2020).

Statistics

The data were reported at least in triplicate. XL STAT 2023 version was used for means comparisons by ANOVA with the Tukey test ($p \leq 0.05$).

Results and discussion

Nutritional composition

The amino acid profile in integral red sorghum flour heat treated (I_TOM), the heat-treated sample with fraction M (OM), and the untreated control fraction M (CM) is displayed in Figure 1.

Among the essential amino acids, leucine exhibited the highest levels across all samples analyzed. Heat treatment of red sorghum grains led to a decrease in leucine content, though the fraction OM still showed higher leucine levels than the integral sorghum flour heat-treated (I_TOM). Additionally, the heat treatment reduced the levels of proline, isoleucine, and histidine. Conversely, the heat-treated fraction OM showed a higher lysine content than the control fraction CM. Similarly, Paucar-Menacho et al. (2018) reported reductions in amino acid content ranging from 17-31% in quinoa grains after puffing, attributing these losses to chemical changes in protein fractions induced by heat treatment, such as glycation, glycoxidation, and oxidation. Moreover, Maillard reactions promoted by heat treatment involve the initial condensation between the carbonyl group of a reducing sugar and amino groups in proteins (Arena et al., 2017; Lohinova and Petrusha, 2023).

Other heat-induced protein changes may include oxidative deamination of N-terminal amino acids, β-elimination of cysteine, and formation of cross-linked lysinoalanine and histidinoalanine derivatives (Arena et al., 2017).

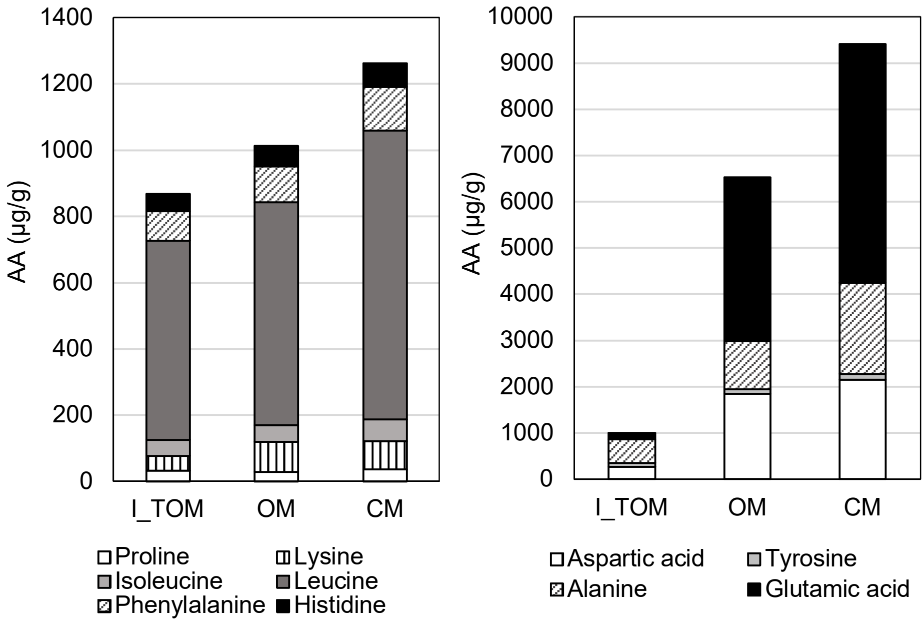

Figure 1. Amino acids (AA) content of sorghum flours

The control fraction of red sorghum (CM) showed a high content of glutamic acid, alanine, and aspartic acid, which diminished after the dry heat treatment (Figure 1). For the treated sample with M fraction (OM), higher levels of non-essential amino acids were observed compared to the values obtained for the integral red sorghum flour heat-treated (I_TOM). It is hypothesized that during heat treatment, condensed tannins bind with proteins to form insoluble complexes (Mohapatra, 2019).

The total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) content in red sorghum grains heat-treated shows significant variations depending on the sample type ($p < 0.05$) (Figure 2).

The application of dry heat treatment led to a decrease in SFA content and an increase in MUFA and PUFA levels in the heat-treated OM sample compared to the untreated sample (CM). In contrast, for integral red sorghum flour heat-treated (I_TOM), there was a decrease in MUFA and PUFA content, while SFA levels increased. Torbica et al. (2021) also reported a decrease in SFA and the rise of PUFA after heat treatment of sorghum. After dry heat treatment, an increase in n-6 PUFA content was observed, with the highest value recorded for the heat-treated red sorghum flour with M fraction (OM). Integral red sorghum flour heat-treated (I_TOM) showed a significantly lower value compared to OM. Paucar-Menacho et al. (2018) similarly reported a higher omega-6 fatty acid content in quinoa grains after puffing. The omega-3 polyunsaturated fatty acid content displayed significant differences depending on the type of red sorghum flour sample. The highest value was found in the heat-treated M fraction (OM), followed by the untreated sample (CM), and finally, integral red sorghum flour heat-treated (Figure 2). Meanwhile, increasing the intake of omega-3 fatty acids in the diet is essential for human health and well-being (Stabnikova and Paredes-Lopez, 2024). The treatment of red sorghum grains also led to an increase in the MUFA/PUFA ratio in the heat-treated M fraction (OM) compared to the control sample (CM). Integral red sorghum flour heat-treated displayed a lower MUFA/PUFA ratio than OM.

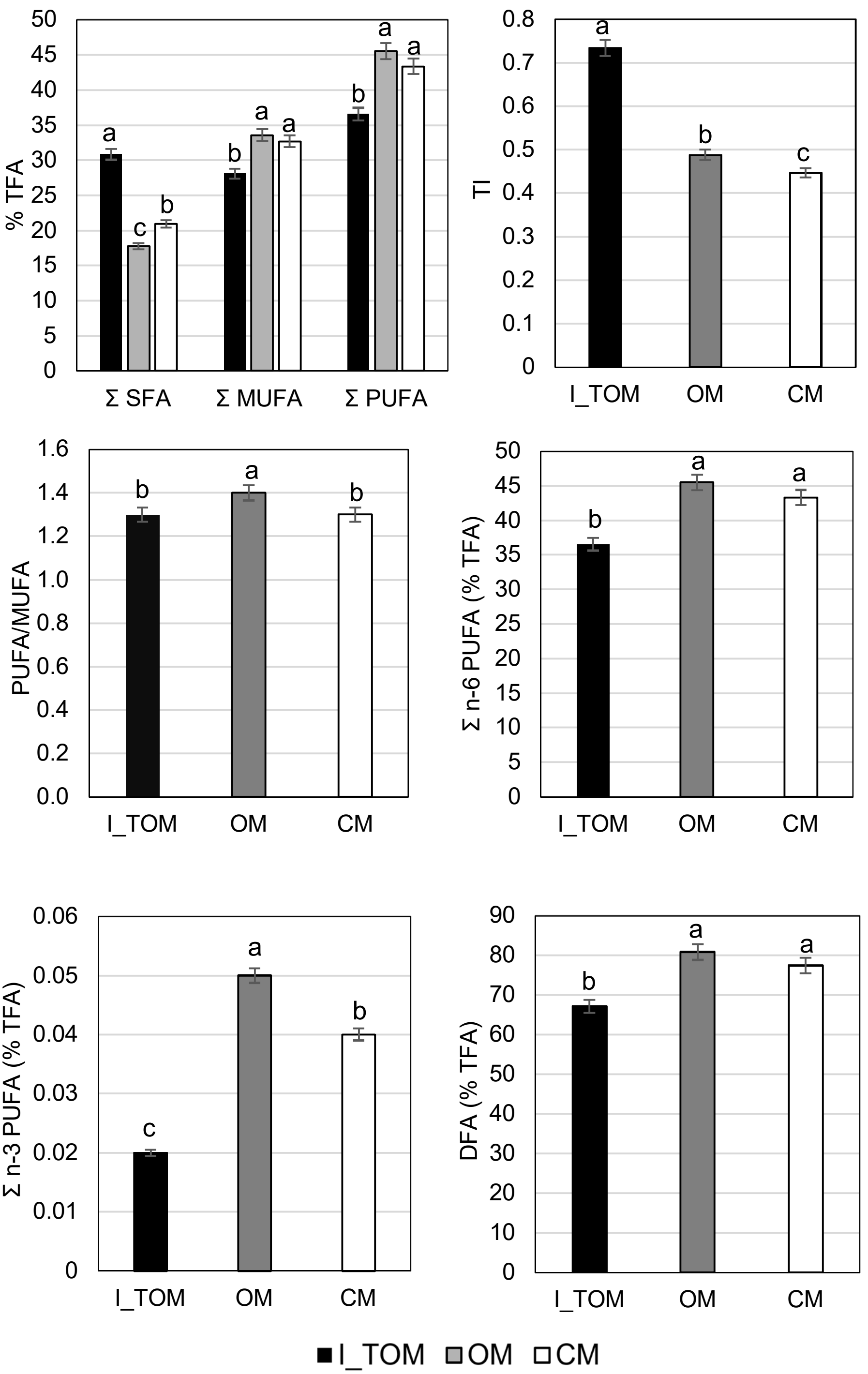

Figure 2. Fatty acids content and thrombogenic index of sorghum flours: Means with different superscripts are significantly different at $p \leq 0.05$

Torbica et al. (2021) reported that hydrothermal treatment reduced the lipid content of barley, rye, triticale, oat, sorghum, and millet flours and modified the profile of fatty acids in flours as a result of complex formation between amylose and protein. Furthermore, the ratio of ω-6 to ω-3 fatty acids raised after heat treatment of barley, rye, triticale, oat, and sorghum (Torbica et al. 2021).

Dry heat treatment and fractionation of red sorghum grains did not lead to significant changes in desirable fatty acids (DFA) content. However, integral red sorghum flour heat-treated (I_TOM) exhibited a significantly lower ($p < 0.05$) DFA value compared to OM (Figure 2). The thrombogenic index (TI) showed significant differences ($p < 0.05$) across the analyzed samples. The heat-treated red sorghum flour, M fraction, (OM) exhibited higher TI values compared to the control fraction (CM). Heat treatment led to an increase in TI in red sorghum flour, but fractionation reduced the thrombogenic index. No data on thrombogenic index variation in sorghum flour were found in the consulted literature. Capouchová et al. (2021) reported that oat samples TI ranged from 0.41 to 0.49, depending on the cultivar.

The mineral composition of the analyzed samples includes calcium and sodium as macroelements, and iron, zinc, and copper as microelements (Table 1).

Table 1

Mineral composition of sorghum flours

Sample	Content (mg/kg)				
	Calcium	Sodium	Iron	Zinc	Copper
I_TOM	18.41±0.46^a	61.82±1.55^b	56.49±1.41^b	20.36±0.51^b	2.62±0.07^c
OM	16.99±0.42^b	63.01±1.58^a	64.18±1.60^a	22.56±0.56^a	4.79±0.12^a
CM	16.22±0.41^b	59.71±1.49^c	38.87±0.97^c	18.47±0.46^c	3.33±0.08^b

Means within the same row with different superscripts are significantly different at $p \leq 0.05$.

No significant differences were identified between the heat-treated sample (OM) and the control sample (CM) of red sorghum flour in terms of calcium content. A higher calcium content was observed in integral red sorghum flour heat-treated (I_TOM) compared to the calcium content in the OM sample. Significant differences ($p < 0.05$) were observed between OM and CM regarding sodium, iron, zinc, and copper content. The molar ratio between phytate and minerals is crucial for determining the potential bioavailability of minerals, with a lower molar ratio indicating greater bioavailability, and vice versa (Wu et al., 2018). Mohapatra et al. (2020) also reported an increase in Na and Ca content after sorghum grains cooking. The reduction of moisture content during dry heat treatment and milling could explain the increase in the mineral content of sorghum flour. This observation was made also by Keya and Sherman (1997) for sorghum grains treated with intense infrared radiation.

The volatile compounds identified in the analyzed red sorghum flours include 2.4-pentadienenitrile, 2.4-hexadienenitrile, and 1,4-pentadiene (Figure 3). In integral red sorghum flour heat-treated, a high percentage of 1,4-pentadiene was identified. In the heat-treated M fraction (OM), 2,4-pentadienenitrile and 2,4-hexadienenitrile were determined. The thermal treatment led to a decrease in the percentage of 2,4-pentadienenitrile and the identification of 2,4-hexadienenitrile in the OM, compared to the control fraction. The reduction of 2,4-pentadienenitrile in the OM fraction was considerable when compared to the percentage identified in the control (CM) and in whole red sorghum flour heat-treated (I_TOM). 1,4-pentadiene aroma compound comes from the ethenolysis of linoleic acid ester present in plants Marvey (2008). It was identified also in other plant sources (Yan et al., 2022).

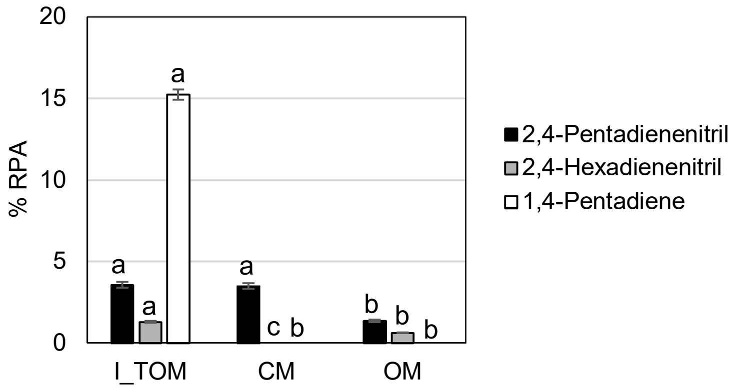

Figure 3. Volatile profile of sorghum flours

Rheological properties

The values obtained for the elastic modulus (G′), which represents the energy stored during deformation, showed that the dough from the heat-treated M fraction (OM) of red sorghum flour exhibited slightly higher elastic energy compared to the dough made from integral red sorghum flour heat-treated (I_TOM) (Figure 4).

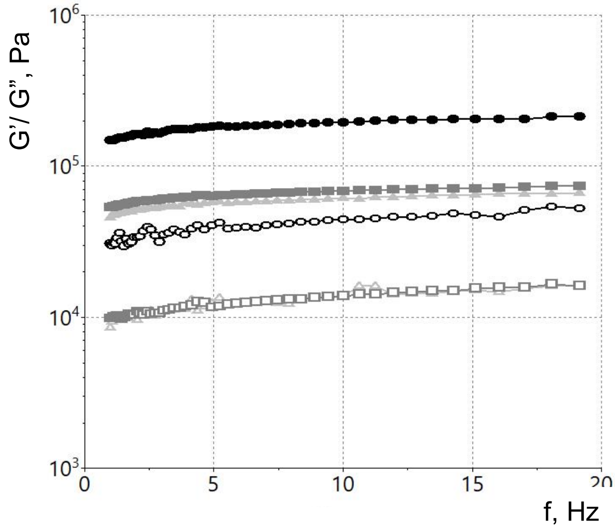

Figure 4. Variations of elastic modulus G′ (filled symbol)
and viscous modulus G″ (empty symbol)
with frequency for I_TOM (-▲-), OM (-■-) and CM (-●-) samples

The viscous modulus (G″) followed a similar trend in the heat-treated M fraction (OM) sample, with the energy lost during deformation being close in value to that of the treated integral red sorghum flour sample. The highest values for both G′ and G″ were observed in the control fraction (CM) dough. These findings indicate that for all samples, G′ values were much higher than G″, suggesting a more elastic than viscous behavior. This behavior is likely due to intermolecular associations among the various components in both treated and untreated red sorghum flour. Similar results were reported by Huang et al. (2022), who found higher G′ values than G″ when examining the effects of thermal treatments and γ-irradiation on millet flour rheology. Measuring the elasticity modulus (G′) and viscosity modulus (G″) provides insight into the rigidity and extensibility of the dough: a high G′ and lower G″ value suggests a rigid dough, while a lower G′ indicates a softer, more extensible dough (Weipert, 1990). Huang et al. (2022) reported higher G′ for sorghum flour after dehulling.

The dough made from the heat-treated M fraction (OM) of red sorghum flour exhibited a behavior similar to that of the dough prepared from integral red sorghum flour heat-treated. The elastic modulus (G′) and viscous modulus (G″) values were higher than those observed in the control fraction (CM) dough (Figure 5).

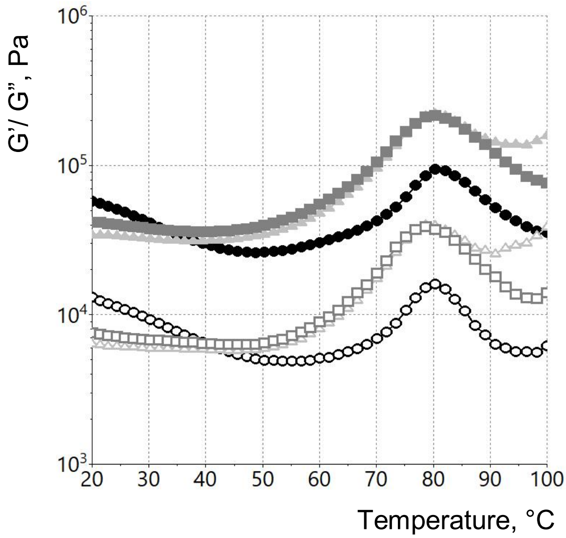

Figure 5. Variations of elastic modulus G′ (filled symbol) and viscous modulus G″ (empty symbol) with temperature for I_TOM (-▲-), OM (-■-) and CM (-●-) samples

The peak gelatinization temperature for the heat-treated M fraction (OM) sample was 78.59 °C, which was lower than that of the control fraction (CM) sample, which has a gelatinization temperature of 80.33 °C. However, there was no significant difference between the OM and the I_TOM sorghum flour sample in terms of peak gelatinization temperature. This suggests that while the heat-treated M fraction shows slight variations in gelatinization properties, it remains largely comparable to the treated integral flour, likely due to similar structural and compositional characteristics achieved through thermal treatment. During heat treatment, the amylose fraction is prone to interact more freely with the amylopectin fractions found in the branched crystalline areas which led to the decrease of mobility of the amylopectin chains and thus generate higher gelatinization temperature (Sun et al., 2014).

Creep and recovery curves of sorghum dough are presented in Figure 6.

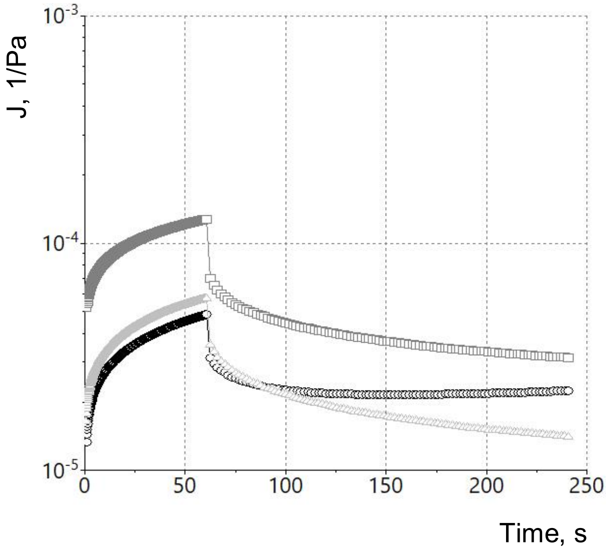

Figure 6. Creep and recovery curves for I_TOM (-■-), OM (-▲-) and CM (-●-) samples

The integral heat-treated sample (I_TOM) of red sorghum flour stood out for its higher maximum compliance in both the creep and recovery phases compared to the control fraction (CM). This suggests that the I_TOM dough has an enhanced ability to deform under stress and recover once the stress is removed, indicating a more elastic and resilient structure compared to the control sample.

The heat-treated M faction (OM) presented higher compliance in the creep phase and lower in the recovery phase compared to the control (CM), indicating a less resilient structure. Creep and recovery behavior is predominantly governed by reorientation of the linkages in the viscoelastic dough (Ahmed, 2014).

Conclusions

Dry heat treatment and fractionation led to significant changes of sorghum flour nutritional properties. Furthermore, the rheological behavior depends on the particle size and treatment applied to sorghum grains. The results obtained showed changes in amino acid profile and an enhancement of n-3, n-6 PUFA and minerals content after application of heat treatment and fractionation. Dough behavior during processing exhibited differences between integral and treated and untreated samples, with the control exhibiting the lowest creep compliances and gelatinization temperatures. These results are helpful for further development of gluten-free backed goods based on sorghum flour.

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