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Nutritional and functional properties of decorticated and microwave heat moisture treated white sorghum meal with added non-tannin and tannin phenolic extract

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ABSTRACT

Emerging technologies, particularly microwave energy, have proven to be more efficient for heat moisture treatment to enhance starch functionality. In a sorghum food system, interactions between starch-phenolics and protein-phenolics significantly influence the nutritional properties of the food. Microwave heat moisture treatment decreased the starch hydrolysis index of sorghum meals from 69.89 % to 54.33 % in samples without phenolic extracts. The hydrolysis index was further reduced from 59.27 to 35.99 % and 54.74 to 36.18 % in samples containing non-tannin and tannin phenolic extracts. The addition of phenolics led to increased resistant starch content, characterized by higher levels of slowly digestible starch and a lower glycaemic index. The interactions between phenolic compounds, protein, starch, and the α-amylase enzyme contribute to the increased resistant starch content. Specifically, phenolics and proteins form barriers around starch granules that hinder digestion. Furthermore, interactions between phenolics and amylose further decrease digestibility while inhibiting α-amylase activity.

1. Introduction

Sorghum is a rich source of phytochemicals, especially phenolic compounds which include 3-deoxyanthocyanidins, and condensed tannins in some sorghum types (Awika et al., 2005). The three distinct parts of the sorghum grain are the bran layer (pericarp and testa), the endosperm, and the germ. Sorghum can be classified into tannin-free and tannin-containing types based on pigmented testa between the pericarp and endosperm. Phenolic compounds (phenolic acids, flavonoids, and condensed tannins) are found in the pericarp and testa of the sorghum bran. The phenolic content in the bran layer is up to six times higher than the whole grain (Awika et al., 2005). The presence of these phenolic compounds is crucial due to their antioxidant activity and their ability to interact with proteins and digestive enzymes. These interactions can limit protein digestibility and modulate starch digestion, thereby influencing the overall nutritional value of sorghum (Xiong et al., 2019**)**. Condensed tannins are particularly important in this regard due to their higher molecular weight and therefore high ability to bind with proteins and digestive enzymes.

The starch found in the endosperm of sorghum grain is the major source of calories when digested. Among other cereals, sorghum appears to have the lowest starch digestibility, possibly as a result of the protein matrix, which restricts the accessibility of the alpha-amylase enzyme to the starch (Taylor & Duodu, 2023). Sorghum endosperm protein is believed to form new disulphide bridges and was attributed to low protein digestibility (Oria et al., 1995). This protein matrix surrounds the gelatinized starch, implicating its slow starch digestion (Schmidt et al., 2023). On the other hand, starch modification has proven to facilitate the interaction between starch and other macronutrients to enhance the nutritional properties of cereals. Heat moisture treatment (HMT) is one of the physical and thermal modifications of starch, where the functionality of the native starch is improved without destroying the morphology of the granule. Researchers have reported that the HMT of cereals using different thermal processing methods enhances the nutritional and functional properties of starch (Deka & Sit, 2016; Mapengo & Emmambux, 2020).

Our previous study was done to determine the effect of heat moisture treatment by microwave and infrared energy on the functional and nutritional properties of different types of whole sorghum meals (Type I white and red non-tannin, type III red tannin) (Baah et al., 2024). Considering the nutritional properties, the study did not show a significant treatment effect in the *in vitro* starch digestibility but clearly

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showed a significant effect in the *in vitro* protein digestibility between the sorghum types both before and after the heat moisture treatments by microwave and infrared energy. Explanations to these findings were not conclusive as reasons for this outcome could have been an interaction with protein and phenolics, phenolic and starch, and/or starch and protein not forgetting other components such as fibre and fat, which could have played a role as well. To clearly understand the mode of interactions between these compounds, the bran of a white sorghum meal constituting most of the fibre content was removed, and two different phenolic extracts from red non-tannin type sorghum and a red tannin type sorghum were added to the white sorghum meal in this study. The model helped to discern whether the phenolic compounds get infused into the starch endosperm for interaction or to determine if the interaction is with the proteins around the starch.

This research focuses on understanding how tannin and non-tannin phenolic extracts interact with protein and starch components in decorticated white sorghum meals under microwave heat moisture treatment conditions. By examining alterations in starch digestibility, protein digestibility, and other physicochemical properties, this study aims to provide new insights into the complex dynamics between these phenolic compounds and their influence on the functional and nutritional characteristics of sorghum meal. The hypothesis states that, microwave heat moisture treatment of sorghum meal with added non-tannin and tannin phenolic extracts will change the rheological properties, specifically reducing viscosity during pasting and have a lower glycaemic index than untreated sorghum meal without phenolic extracts.

2. Materials and methods

2.1. Materials

Decorticated sorghum grains, type I white non-tannin sorghum (Macia), and two red hybrid cultivars, namely, type I red non-tannin known as GM and type III red tannin known as GH, were obtained locally (African commodity SA, Sunninghill, Johannesburg, South Africa) were used. The whole white sorghum grains were cleaned and decorticated to remove about 30 % of the outer layer of the grain, which is the bran, using the tangential abrasive dehuller device (TADD) (Rural Industries Promotion Company, Kanye, Botswana). The grain was milled using the laboratory hammer mill (Perten Lab mill 3100, PerkinElmer, Waltham) with a 1 mm sieve size. The red hybrid cultivars were also decorticated to obtain the bran from the pericarp of the grain. Analytical grade chemicals of were used and obtained from Merck (Modderfontein, South Africa). Glucose oxidase-peroxidase (GOPOD) assay kit was obtained from Megazyme (Megazyme International, Bray, Ireland).

2.2. Methods

2.2.1. Proximate analysis of decorticated white sorghum meal

The meal samples were analyzed for moisture, ash, and crude fat content according to procedures 925.10, 923.03, and 920.39, respectively, as outlined by the Association of Official Analytical Chemists (AOAC, 2019). Protein content (calculated as nitrogen × 6.25) was determined using the Dumas combustion method. Total starch was then determined using a total starch assay kit (K-TSTA, Megazyme International, Wicklow, Ireland). The starch digestibility was followed according to the published Goñi et al. (1997). The starch digestibility was compared to literature from Chung et al. (2006) and that of protein digestibility was compared with Emmambux and Taylor (2009). Moisture content of the decorticated white sorghum meal was 11.23 %, protein content was 10.53 %, total starch content was 74.86 %, ash content was negligible, and the fat content was negligible.

2.2.2. Phenolic extraction and total phenolic content determination

Phenolics from non-tannin and tannin sorghum bran were extracted as described by Elboughdiri (2018) with modifications. The bran was extracted with 80 % (*v*/v) aqueous ethanol (proportion 1:2 *w*/w) twice at ambient temperature. After centrifugation at 9500 x*g* for 10 min, the supernatant was evaporated in a fume hood overnight to evaporate off the ethanol and then freeze-dried to obtain solid phenolic extracts. The total phenolic content was determined using the Folin-Ciocalteu method by Waterman and Mole (1994) and reported as catechin equivalent.

2.2.3. Heat moisture treatment by microwave energy

White sorghum meal (100 g) was weighed, freeze-dried phenolic extract from non-tannin, and tannin sorghum bran (6 % of the meal weight) was added. The 6 % weight was chosen based on the amount of phenolic extract in 100 g of whole sorghum meal. Samples were mixed evenly using a spatula. The moisture treatment of the different composite sorghum meals was equilibrated at 25 %, as described by Mapengo and Emmambux (2020). Microwave heating was conducted using a microwave reactor Nova 10 (Ertec, Wroclaw, Poland). In a conical flask, sorghum meal (20 g) was placed in the oven with constant stirring. The power was 250 W, with a heating time of 15 mins, the temperature of the vessel was about 110 °C, and a cooling time of 10 min. The heat-moisture treated samples were then rested in the oven at 40 °C for 12 h to obtain a uniform moisture content of about 7 %. The dried sorghum meal samples were stored in an airtight container in the dark at 4 °C for further analysis.

2.2.4. Pasting properties

The pasting characteristics of the meals were determined using a programmable viscometer with a temperature ramp (RVA 4500, Perten instrument, TCW3 software, Austria). Meal slurry (flour~ 3.5 g, 14 % moisture basis) was brought to a standard weight of 25 g. The slurry was placed in the paddle couple, mixed with a plastic paddle at 960 rpm for 10 s at 50 °C, and changed to 160 rpm throughout the test. The temperature was increased to 95 °C at 12 °C/min, held for 2.5 min, and cooled to 50 °C.

2.2.5. Light microscopy

The micrographs of raw and pasted sorghum meals were observed using light microscopy. About 10 mg (dry basis) of control and HMT sorghum meals were dispersed in 1 ml of distilled water and vortexed. One drop of iodine solution was used to stain the starch granules. Two (2) drops of the suspension were placed on a slide and covered with a coverslip.

The undigested residue of sorghum meals from the *in vitro* starch digestibility analysis was freeze-dried and observed under the light microscope to visualize the undigested starch granules. The freeze-dried samples were dispersed in 1 ml of distilled water and vortexed. One drop of iodine solution was used to stain the starch granules for 5 min, and a drop of the suspension was placed on a slide and covered with a coverslip. Samples were observed using a Nikon Optiphot Transmitted Light Microscope (Tokyo, Japan) with phase-contrast optics.

2.2.6. X-ray diffraction crystallinity

XRD scans were conducted using PANalytical AERIS from Malvern Panalysis (Netherlands) on raw, HMT-treated, and pasted samples as described by Mapengo et al. (2019) with slight modification. The meal samples were equilibrated for 3 days at 95 % estimated relative humidity at room temperature using water. The XRD operating conditions were power settings of 40 kV and 15 mA, X-ray diffraction, fitted with a Cu radiation source. Scanning was done from 5° to 90° (2θ) with an exposure time of about 6 min, step size of 0.022° and a time/step ratio of 5 s. Microsoft Excel was used to plot graphs, and Origin Pro 16 software® was used to calculate the relative crystallinity (RC).

The RC was calculated as; $RC = \frac{\text{Area of crystalline peaks}}{\textit{area of all peaks}} x\,100.$

2.2.7. *Fourier-transform infrared spectroscopy*

FTIR spectroscopy was performed using a Nicolet 6700 FTIR spectrometer (Thermo Electron Cooperation, Madison, USA) on raw, MWHMT-treated, and pasted samples. The Omnic software included in the spectrometer was used to collect the vibrational spectra from 400 to 4000 cm^{-1}. The background was scanned before the sample analysis and was subtracted from the treated and untreated sorghum meals. The sample was placed on the attenuated total reflectance (ATR) crystal to cover the diamond surface. The spectra were obtained at a resolution of 4 cm^{-1} and 132 number of scans. Deconvolution of the amide I group ranging from 1600 to 1700 cm^{-1} was isolated from the whole spectra using Origin Pro software®. Baseline correction was done. The α-helix (1650–1658 cm^{-1}) and β-sheet (1620–1640 cm^{-1}) peaks were identified from the second derivative spectrum. Peak fitting was done using the Gauss curve fit.

2.2.8. *In vitro starch digestibility*

In vitro, starch digestibility of the pasted sorghum meals was done using the method described by (Goñi et al., 1997). A sample of 50 mg was weighed into a conical flask. HCl-KCl buffer (pH 1.5, 10 ml) was added, followed by 0.2 ml of a solution containing 1 g of pepsin (Sigma-Aldrich P7000-100G) in 10 ml of HCl-KCl buffer. Samples were incubated in a shaking water bath at 40 °C for 60 min and made up to 25 ml volume with tri-maleate buffer (pH 6.9). Tris-maleate buffer containing 2.6 IU of pancreatic α-amylase from the porcine pancreas was added (5 ml), and the samples were incubated at 37 °C in a shaking water bath. Aliquots (1 ml) were taken at 20, 60, 90, 120, and 180 min. Aliquots were boiled at 100 °C for 5 min to inactivate the enzyme and refrigerated until the end of incubation time. Sodium acetate buffer (pH 4.75, 3 ml) was added to each aliquot, followed by 60 μl amyloglucosidase (3260 U/ml and incubated at 60 °C for 45 min in a water bath. The volume was adjusted to 10 ml. The released glucose during starch hydrolysis was determined using the GOPOD reagents. The absorbance was read at 510 nm. Each sample was analyzed in triplicate.

The Hydrolysis Index is calculated using the area under the curve (AUC) for starch hydrolysis over time. $HI = \frac{\text{AUC sample}}{\text{AUC reference}}$ X 100. Where reference is white bread.

Starch fractions, rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) were calculated according to Englyst et al. (1992);

$RDS = G20*0.9$
$SDS = (G120 - G20)*0.9$
$RS = (100 - G120)*0.9$

2.2.9. *In vitro protein digestibility*

In vitro protein digestibility was determined using the pepsin method by Hamaker et al. (1986) with modifications. The pasted meal (200 mg) was suspended in 5 ml of citrate buffer (pH 2) and vortexed to ensure complete dispersion. Citrate buffer containing pepsin (28 ml, 131 mg pepsin/100 ml buffer) was added and vortexed. Samples were incubated in a shaking bath at 37 °C for 2 h. Digestion was stopped by adding 2 ml of 2 M sodium hydroxide. Samples were then centrifuged at 9500 *xg* for 10 min. The supernatant was discarded, and the residue was washed twice with 30 ml of distilled water and centrifuged. The residue was dried at 100 °C overnight in an air oven. The protein content of dried residue was determined using the Dumas combustion method. *In vitro* protein digestibility as;

$$IVPD = \frac{\text{the initial total weight of protein} - \text{the residual weight}}{\text{the initial total weight of protein}} x\,100$$

2.2.10. *Statistical analysis*

Data were analyzed using MANOVA with IBM SPSS® statistics. A multivariant general linear model based on a 95 % confidence level was used to compare the differences among the sorghum meals with the different phenolic extracts and heat moisture treatment by microwave energy. The significant difference between means of the values was calculated using Tukey honestly significant difference (HSD) with a $p \leq 0.05$. All experiments were repeated three times.

3. Results and discussions

The total phenolic content of raw sorghum, sorghum with phenolic extract, and the microwave heat moisture treated (MWHMT) samples are shown in Supplementary Table 1 Phenolics were not detected in the raw white sorghum and its MWHMT samples. The addition of non-tannin and tannin phenolic extract to sorghum recorded a total phenolic content of 3.36 mg CE/g and 11.18 mg CE/g, respectively. There was no significant difference in the total phenolic content of respective samples before and after MWHMT.

Fig. 1 and Table 1 show the pasting properties of MWHMT sorghum meals with and without phenolic extract. Phenolic extract addition significantly ($P < 0.05$) reduced the pasting viscosities of the sorghum meals, and the effect was based on the type of phenolic extract. The peak viscosity was lower in sorghum meals with added non-tannin and tannin phenolic extract (2276.50 and 2418.00 mPa.s, respectively) compared to the raw white sorghum meal (2902.00 mPa.s). During cooling, the final viscosities of the raw sorghum and the sorghum meal with non-tannin phenolic extract were higher and were significantly ($P < 0.05$) different to the meal sample with the tannin phenolic extract. The same trend was not observed for the setback viscosity. Setback viscosity increased as the non-tannin phenolic extract was added, but tended to decrease slightly when the tannin phenolic extract was added although not significantly different from the raw sorghum meal. This suggests that the tannin phenolic extract has little to no effect on the retrogradation of the sorghum meal.

There is limited research on the effect of phenolics on the pasting properties of flour samples available as most research have been on extracted starch. A study by Beta and Corke (2004) showed a significant decrease in peak viscosity of maize starch when ferulic acid was added. A similar study by Li et al. (2018) also showed a significant decrease in peak viscosity of potato starch when complexed with caffeic acid, gallic acid, and ferulic acid. Not overlooking the other components in the sorghum meal, such as protein in this study, the starch could have interacted with the phenolics, reducing peak viscosity. According to Beta and Corke (2004), starch can bind to phenolics. During pasting, which involves the gelatinization of starch in the presence of water and heat, amylose molecules typically contribute to the viscosity of the system by forming a network. Phenolics can form inclusion complexes with amylose in starch. This is an indication that the inclusion complexes formed by the tannin phenolic extract could have stabilized the starch more than the non-tannin phenolic extract for water absorption. Condensed tannin is known to have high molecular weight and complex structure, which could have interfered more with the hydrophobicity nature of the starch chains, thereby resulting in lower setback viscosity compared to the meal with non-tannin phenolic extract (Chen et al., 2022).

MWHMT significantly decreased the peak, final, and setback viscosities of all the sorghum samples. The MWHMT meals with the addition of non-tannin and tannin phenolic extract showed a significant decrease in all the viscosities as compared to the raw sorghum meal. The MWHMT meals were observed to have aggregate formation when viewed under the light microscope, as seen in Supplementary Fig. 1. Even though these aggregates were seen in all MWHMT meals, the sorghum meals with the non-tannin and tannin phenolic extracts showed more aggregates with limited individual starch granules than the raw samples with fewer aggregates. This aggregate formation could be due to an interaction between the starch, protein, and phenolic compounds during the heat treatment. According to Schmidt et al. (2023), proteins can form aggregates during heat treatment.

Furthermore, Wei et al. (2019) also stated that phenolic compounds

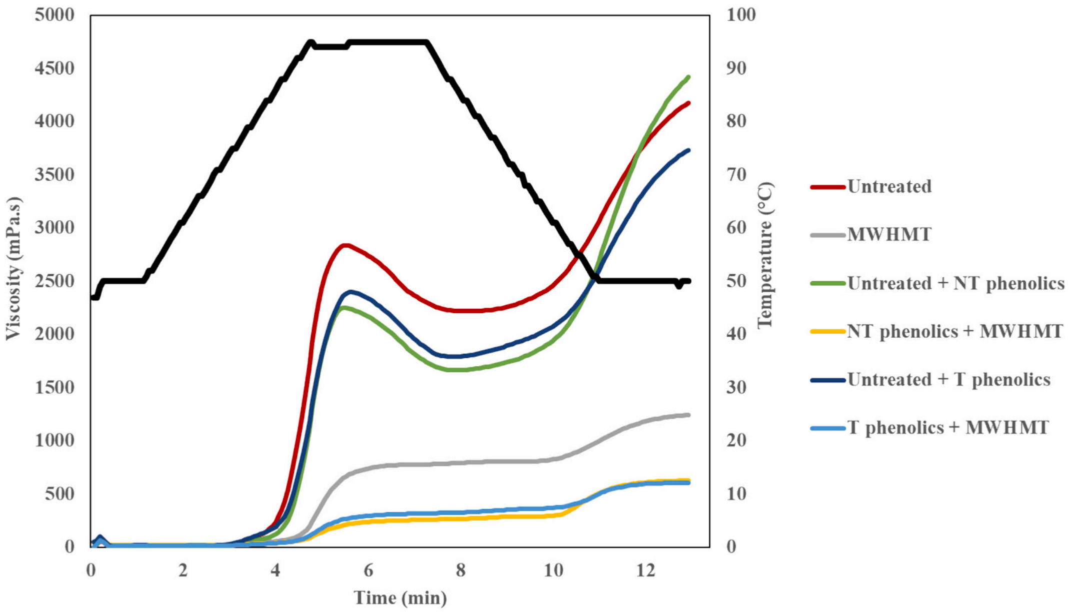

Fig. 1. Effects of non-tannin and tannin phenolic extracts addition and microwave heat moisture treatment (MWHMT) on the pasting properties of white sorghum meals.
NT: Non-tannin, T: Tannin

Table 1
Effects of non-tannin and tannin phenolic extract addition and microwave heat moisture treatment (MWHMT) on the pasting properties of white sorghum meals.

Sample	Treatment	Peak viscosity (mPa.s)	Breakdown (mPa.s)	Final viscosity (mPa.s)	Setback (mPa.s)
Raw sorghum	Untreated	2902.00 ± 11.31^{a}	591.00 ± 11.31^{a}	4333.50 ± 12.02^{a}	2022.50 ± 34.65^{b}
	MWHMT	801.50 ± 31.82^{d}	nd	1273.00 ± 39.60^{c}	503.00 ± 8.49^{c}
Sorghum + non tannin extract	Untreated	2276.50 ± 37.48^{c}	603.00 ± 22.63^{a}	4465.00 ± 66.47^{a}	2791.50 ± 51.62^{a}
	MWHMT	230.50 ± 36.06^{e}	nd	661.00 ± 49.50^{d}	347.00 ± 55.15^{d}
Sorghum + tannin extract	Untreated	2418.00 ± 31.11^{b}	610.50 ± 10.61^{a}	3777.50 ± 62.93^{b}	1970.00 ± 42.23^{b}
	MWHMT	310.00 ± 7.07^{e}	nd	649.50 ± 65.66^{d}	307.00 ± 1.41^{d}

The means within a column with different letters are significantly different ($p < 0.05$).
Nd: not detected.

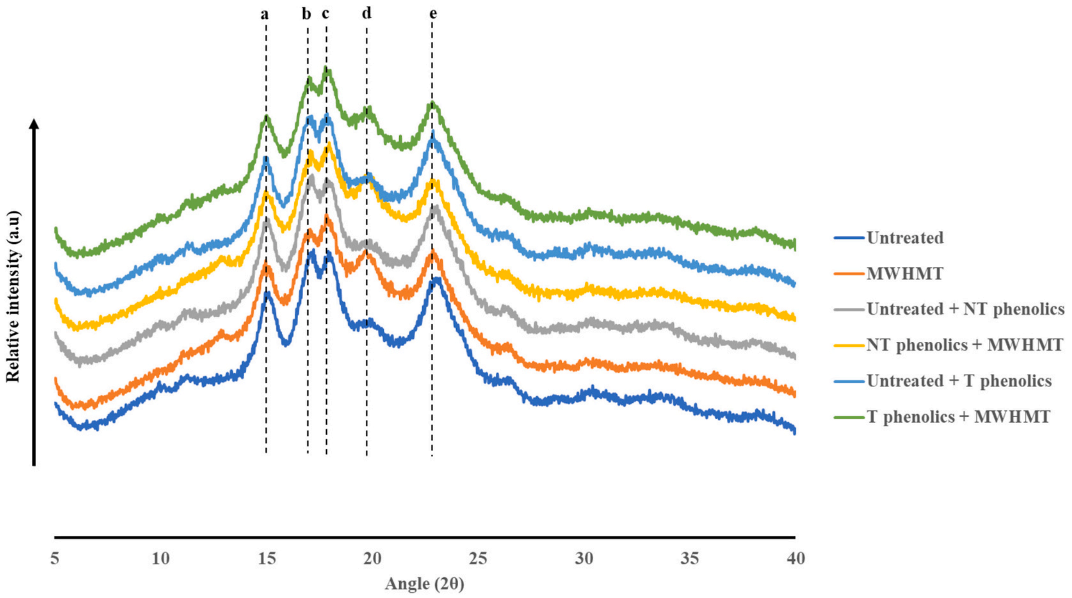

Fig. 2. Effects of non-tannin and tannin phenolic extracts addition and microwave heat moisture treatment (MWHMT) on the X-Ray Diffraction (XRD) of raw sorghum meals.
NT: Non-tannin, T: Tannin, a: peak at $2\theta = 15°$, b: peak at $2\theta = 17°$, c: peak at $2\theta = 18°$, d: peak at $2\theta = 19.9°$, e: peak at $2\theta = 23°$

can form aggregate with other macromolecules. The more complex/higher molecular weight the phenolic compound is, as known for condensed tannin, the more the degree of aggregate formation will be with the macromolecules. This suggests that the aggregate formation with the proteins and phenolic compounds could have limited starch swelling and the release of amylose leading to lower peak viscosity.

Setback viscosity indicates the gelling ability or the retrogradation tendency attained during cooling after pasting. The leached-out amylose realigns through the formation of junction zones between chains, resulting in gel formation (Yuris et al., 2018). The results (Fig. 1 and Table 1) suggest that phenolic extracts likely disrupted the realignment process, resulting in a lower setback viscosity in the sorghum samples containing both non-tannin and tannin phenolic extracts.

Figs. 2 and 3 show the X-ray diffraction pattern of raw sorghum meals, after MWHMT and after pasting, respectively. The raw white sorghum meal showed a typical A-type crystal structure, with a peak at 15°, unresolved doublet at 17° and 18°, 23° and a small peak at 19.9°. The addition of the phenolic extracts did not change the pattern. Furthermore, there was no significant difference in the relative crystallinity of all the samples with and without the addition of phenolics or after MWHMT treatment (Table 2). According to Bhat and Arya (2020), A-type starches are identified with a strong peak reflection at 15°, 23° (2Θ), and a doublet at around 17° and 18°. Some researchers have identified a crystalline pattern with a peak developed at around 19.9° to be a V-type crystal structure (Sarifudin et al., 2019; Zhou & Kong, 2023). This is an indication that the raw and HMT samples exhibited both A and V-type crystalline structures.

After pasting, the low intensity of the peaks was probably due to the gelatinization of the starch present in the meals destroying the semi-crystalline structure, which is evident in Fig. 3. The A-type crystal structure changed into a pattern of V-type crystal structure. Peaks were showing at around 17°, 19.6° and 22°. In literature, the V-type crystal structure could be an interaction between the amylose and lipid-forming amylose-lipid complexes. This study did not show any evidence of a crystalline form of amylose lipid complex formation, as no endotherm was observed in the DSC graph after pasting (Supplementary Fig. 3). The relative crystallinity of pasted sorghum meal with non-tannin phenolic extract was 14.9 %. It increased to 22.98 % after MWHMT treatment. This increase was also observed in the meal samples with tannin phenolic extract from 16.29 % to 20.96 %.

Table 2
Effects of non-tannin and tannin phenolic extract addition and microwave heat moisture treatment (MWHMT) on the percentage relative crystallinity of white sorghum meals.

Sample	Treatment	% relative crystallinity (raw sample)	% relative crystallinity (pasted sample)
Raw sorghum	Untreated	30.69 ± 1.24^{a}	25.07 ± 0.37^{a}
	MWHMT	34.94 ± 0.28^{a}	22.44 ± 4.41^{ab}
Sorghum + non tannin extract	Untreated	31.52 ± 0.38^{a}	14.91 ± 0.96^{b}
	MWHMT	35.06 ± 0.85^{a}	22.98 ± 2.21^{ab}
Sorghum + tannin extract	Untreated	34.84 ± 2.43^{a}	16.29 ± 0.81^{ab}
	MWHMT	35.29 ± 1.93^{a}	20.96 ± 2.32^{ab}

The means within a column with different letters are significantly different ($p < 0.05$).

Figs. 4 and 5 show the deconvoluted spectra in the Amide 1 band from 1600 to 1700 cm^{-1} in MWHMT and pasted samples, respectively. The range for the α-helix peak was 1650–1658 cm$^{-1,}$ and the range for the β-sheet was 1620–1640 cm^{-1}. In the Gaussian deconvolution of the Amide 1 band, small frequency shifts were observed in the MWHMT samples. Table 3 shows the percentage area of the protein secondary structures (α-helix and β-sheet) in the raw and pasted samples. The sorghum meals had a higher percentage area of α-helix compared to β-sheet. The addition of the phenolic extracts decreased the area of the α-helix slightly, but this was statistically insignificant. After MWHMT, there was a slight increase in α-helix in the raw samples from 83.10 to 85.83 %, a slight decrease in the meal sample with non-tannin phenolic extract from 82.96 to 80.98 %, and a significant decrease ($P < 0.05$) in meal samples with tannin phenolic extract from 80.87 % to 69.43 %. The decrease in α-helix upon the addition of the phenolic extracts agrees with a study by Zhao et al. (2020) that showed a change in the conformation of protein as there was a decrease in α-helix upon polyphenol interaction with protein. A contrary trend was observed in the area of the β-sheet. MWHMT reduced the area of the β-sheet in the raw sample. On the other hand, the samples with non-tannin and tannin phenolic extracts showed an increase in β-sheet area, with the sorghum

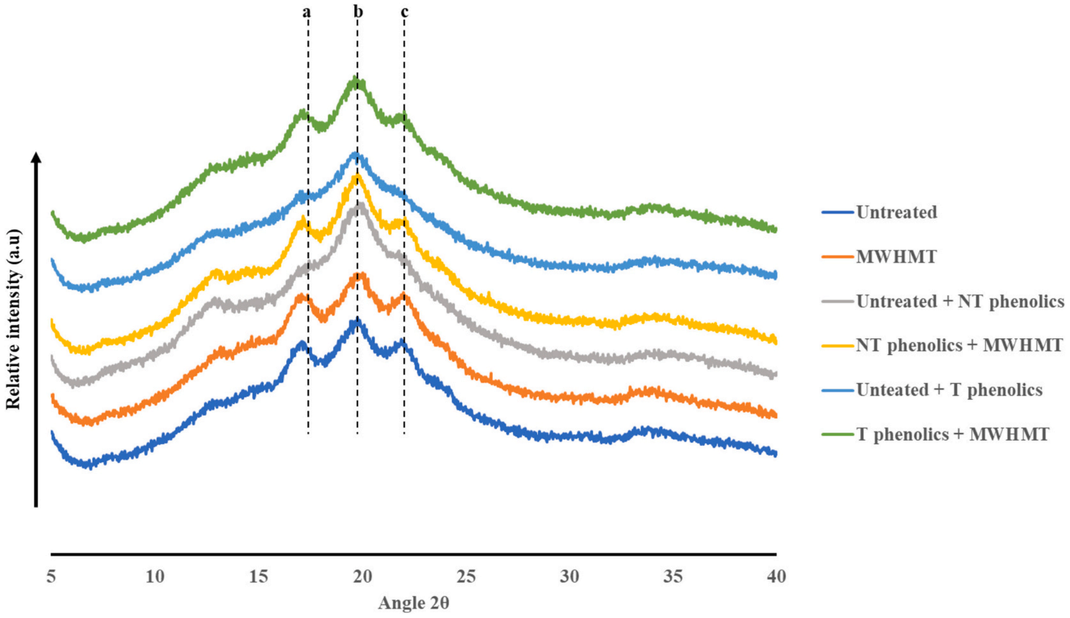

Fig. 3. Effects of non-tannin and tannin phenolic extracts addition and microwave heat moisture treatment (MWHMT) on the X-Ray Diffraction (XRD) of pasted sorghum meals.
NT: Non-tannin, T: Tannin, a: peak at 2θ = 17°, b: peak at 2θ = 19.9°, c: peak at 2θ = 23°.

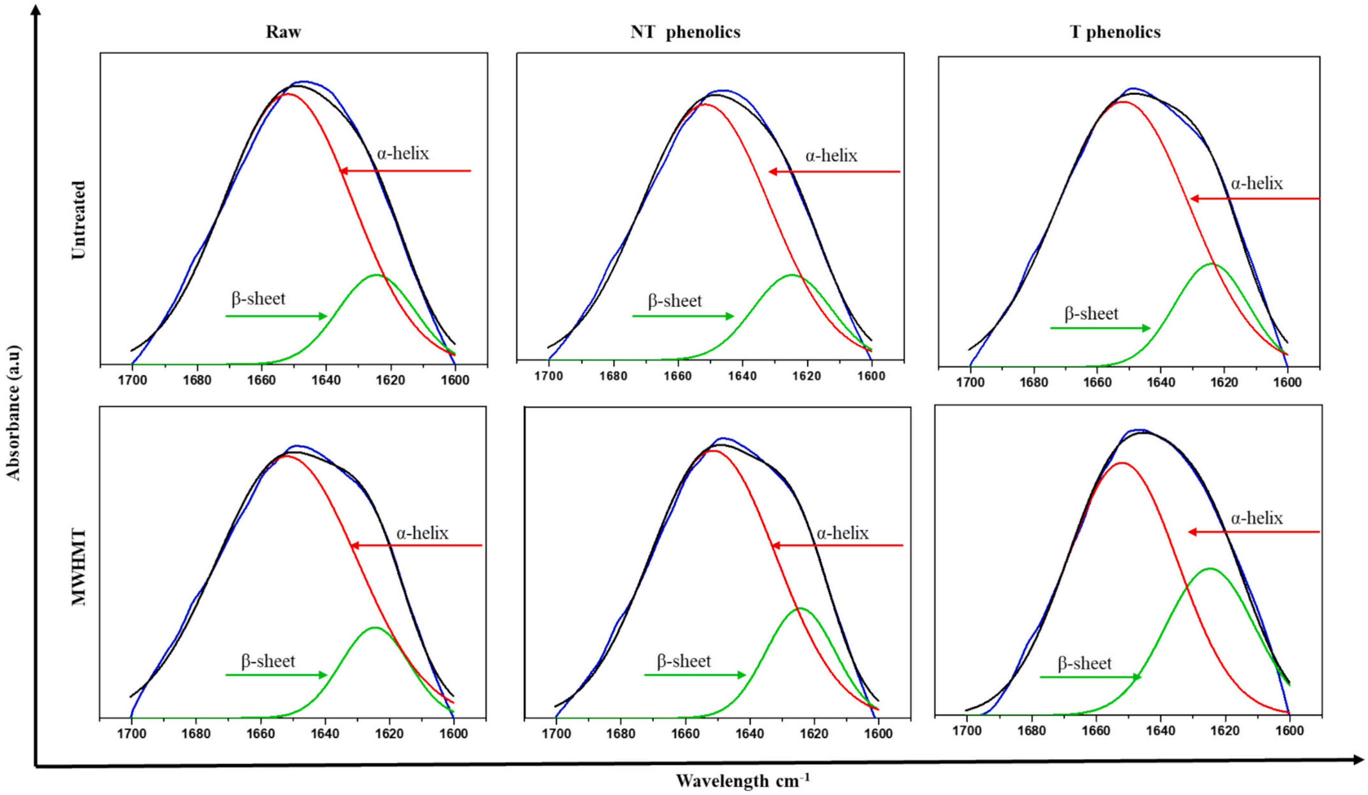

Fig. 4. Effects of non-tannin and tannin phenolic extracts addition and microwave heat moisture treatment (MWHMT) on the Amide 1 band in Fourier transform infrared (FTIR) spectra of raw white sorghum meals.

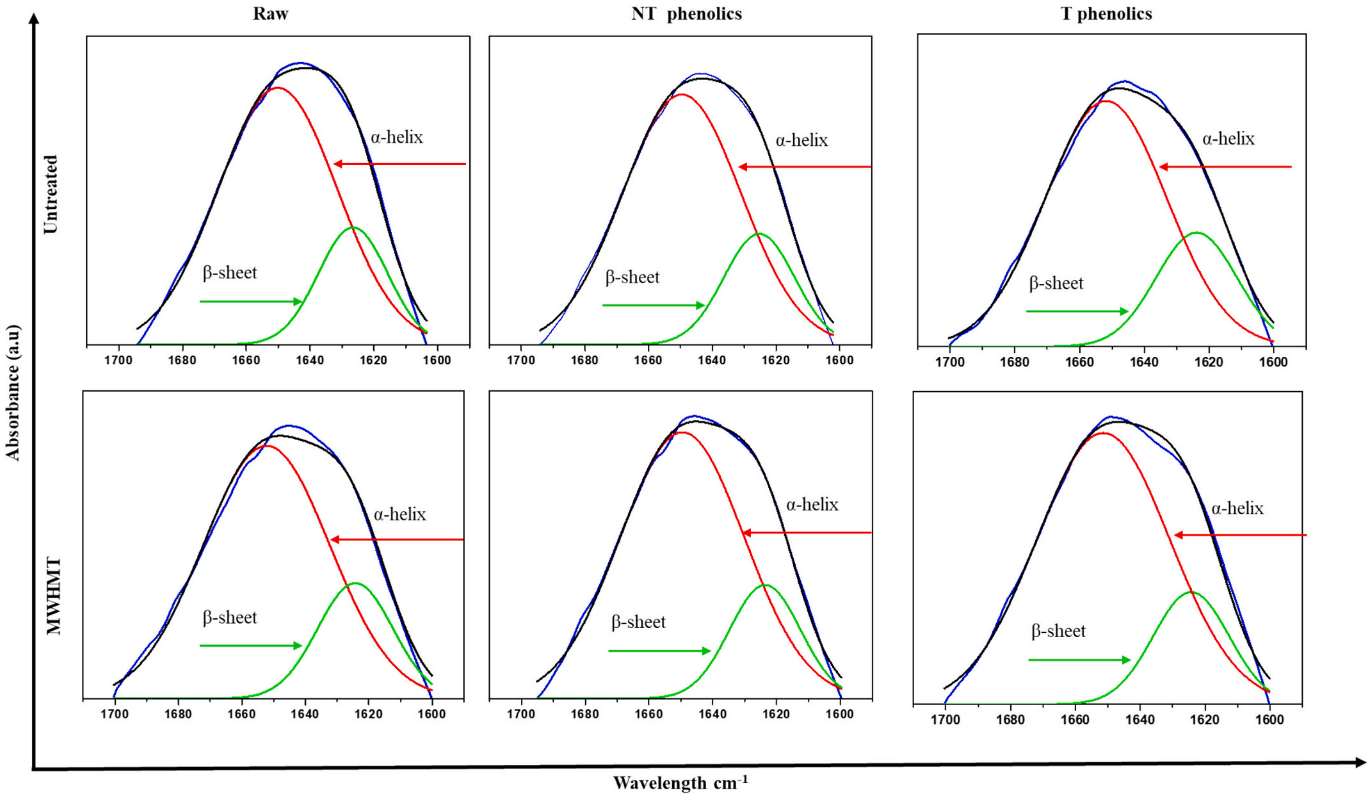

Fig. 5. Effects of non-tannin and tannin phenolic extracts addition and microwave heat moisture treatment (MWHMT) on the Amide 1 band in Fourier transform infrared (FTIR) spectra of pasted white sorghum meals.

Table 3
Effects of non-tannin and tannin phenolic extract addition and and microwave heat moisture treatment (MWHMT) on the percentage protein secondary structure of raw and pasted white sorghum meals.

Sample	Treatment	Area α-helix (%)	Area β-sheet (%)	Area α-helix (%)	Area β-sheet (%)
		Raw samples		Pasted samples	
Raw sorghum	Untreated	83.10 ± 0.78^{ab}	16.90 ± 0.78^{bc}	74.80 ± 5.85^{a}	25.20 ± 5.85^{a}
	MWHMT	85.83 ± 0.44^{a}	14.17 ± 0.44^{c}	77.73 ± 1.25^{a}	22.27 ± 1.25^{a}
Sorghum + non tannin extract	Untreated	82.96 ± 0.27^{ab}	17.04 ± 0.27^{bc}	76.32 ± 3.84^{a}	23.68 ± 3.84^{a}
	MWHMT	80.98 ± 0.85^{b}	19.02 ± 0.85^{b}	78.95 ± 2.02^{a}	21.05 ± 2.02^{a}
Sorghum + tannin extract	Untreated	80.87 ± 1.88^{b}	19.13 ± 1.88^{b}	79.75 ± 4.82^{a}	20.25 ± 4.82^{a}
	MWHMT	69.43 ± 1.61^{c}	30.57 ± 1.61^{a}	77.21 ± 5.23^{a}	22.79 ± 5.23^{a}

The means within a column with different letters are significantly different ($p < 0.05$).

meals containing tannin phenolic extract having a significantly higher β-sheet area.

Pasting after the MWHMT affected the percentage area of the α-helix and β-sheet protein structure (Table 3). After pasting, there was a decrease in the percentage area of the α-helix and an increase in the β-sheet for samples without phenolic extracts and samples with non-tannin phenolic extract. The contrary was observed for sorghum samples with tannin phenolics. Research on vibrational spectroscopy shows that, upon heating proteins, the occurring α-helix in the secondary structure forms aggregates, leading to an increase in β-sheet and a decrease in α-helix (Bonwell & Wetzel, 2009). This agrees with this study in the former results as the β-sheet increased upon further pasting of the samples. According to Bonwell and Wetzel (2009), proteins during the heat process and upon cooling, the hydrogen bonds reunite to form a stable conformation. In this process, the β-sheet aggregates are affected more than the α-helical secondary structure. The increase in β-sheet structure in sorghum meals after pasting, which is a form of wet cooking, agrees with other researchers (Emmambux & Taylor, 2009; Ezeogu et al., 2008). In the latter results, tannins may have disrupted the hydrogen bonds that stabilize β-sheet structures, leading to a reduction in their content.

The rate of starch hydrolysis of the pasted sorghum samples is shown in Fig. 6. Its corresponding parameters, hydrolysis index (HI), estimated glycaemic index (eGI), and proportions of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) are shown in Table 4. The RDS fraction is a measure of the sudden increase in levels of glucose at the initial 20 min of digestion, SDS fraction undergoes slower digestion and measures the releases of glucose after 120 min of digestion, while RS escapes digestion after 180 min. The relative proportions of RDS, SDS and RS therefore, determine the glycaemic response of a food. The GI is estimated by determining the relative increment in glucose concentration after digestion of a test meal over a set period.

There was a significant ($P < 0.05$) decrease in HI, RDS, and eGI in the pasted sorghum meals with the added non-tannin and tannin phenolic extract compared to the raw meal. After MWHMT, there was a significant decrease in the hydrolysis with the same trend between the samples. This agrees with a study by Li et al. (2021) that recorded a decrease in *in vitro* starch digestibility of sorghum grain after heat moisture treatment using microwave energy. Indicating that microwave heat treatment is effective in decreasing *in vitro* starch digestibility. The sorghum meals with non-tannin and tannin phenolic extracts were higher in slowly digestible starch, and there was a significant ($P < 0.05$) difference as compared to the sorghum meals without the phenolic extracts.

The calculated RS content of raw sorghum meal was about 28.21 %. After the addition of non-tannin phenolic extract, it increased to about 31.97 % and also increased to about 34.65 % with the addition of tannin phenolic extract. There was a significant ($P < 0.05$) increase in RS content after the MWHMT in all sorghum samples. The samples with tannin and non-tannin phenolic extracts recorded the highest resistant starch content at 54.57 % and 55.87 %, respectively, which showed no significant difference between the two types of phenolics. The aggregate formation, as seen in Supplementary Fig. 2, could be associated with the increase in resistant starch content. A study by Mapengo and

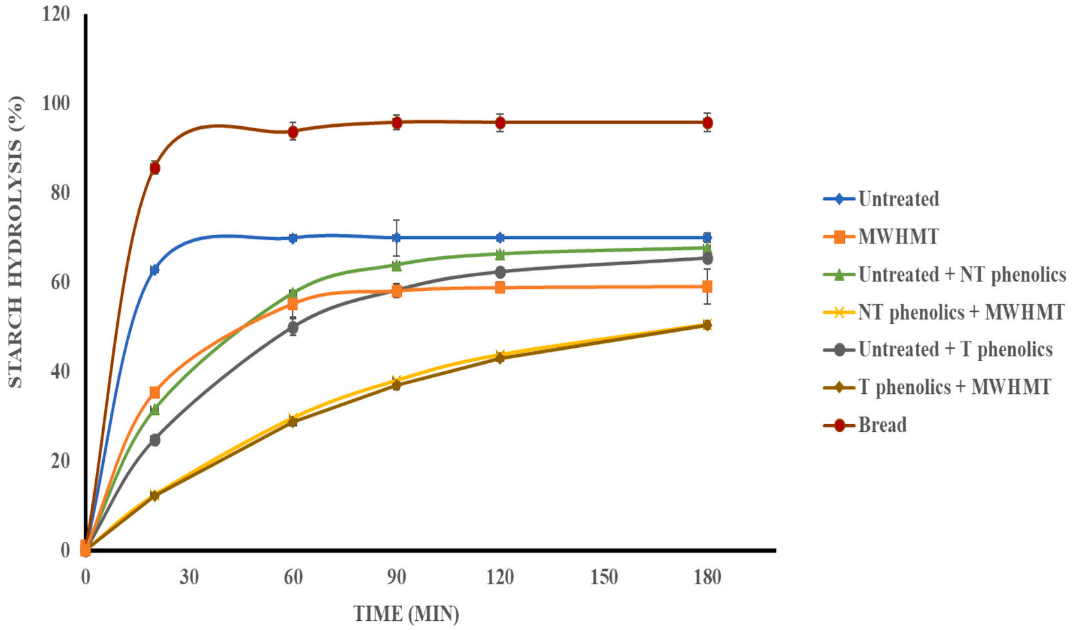

Fig. 6. Effects of non-tannin and tannin phenolic extracts addition and microwave heat moisture treatment (MWHMT) on the *in vitro* starch hydrolysis of white sorghum meals.
NT: Non-tannin, T: Tannin.

Table 4
Effects of non-tannin and tannin phenolic extract addition and microwave heat moisture treatment (MWHMT) on the *in vitro* starch and protein digestibility (%) of white sorghum meals.

Sample	Treatment	HI	eGI	RDS	SDS	RS	IVPD
Raw sorghum	Untreated	69.89 ± 0.90^a	78.08 ± 0.49^a	61.92 ± 0.89^a	9.88 ± 0.91^e	28.21 ± 0.75^e	54.77 ± 1.12^a
	MWHMT	54.33 ± 0.45^c	69.54 ± 0.25^c	40.42 ± 2.86^b	15.40 ± 3.31^d	44.18 ± 0.45^b	48.18 ± 2.84^b
Sorghum + non-tannin extract	Untreated	59.27 ± 0.26^b	72.25 ± 0.14^b	30.94 ± 0.52^c	37.09 ± 0.52^a	31.97 ± 1.72^d	54.07 ± 0.63^a
	MWHMT	35.99 ± 0.14^d	59.47 ± 0.08^d	26.28 ± 1.04^d	19.14 ± 1.47^c	54.57 ± 0.75^a	37.71 ± 1.18^c
Sorghum + tannin extract	Untreated	54.74 ± 0.34^c	69.76 ± 0.19^c	26.33 ± 0.74^d	39.01 ± 0.45^a	34.65 ± 0.61^c	18.14 ± 1.80^d
	MWHMT	36.18 ± 0.62^d	56.57 ± 0.34^d	12.97 ± 0.88^e	31.16 ± 1.19^b	55.87 ± 0.61^a	16.31 ± 0.11^d

The means within a column with different letters are significantly different ($P < 0.05$).
HI: Hydrolysis index, eGI: estimated glycaemic index, RDS: Rapidly digestible starch, SDS: slowly digestible starch, RS: Resistant starch, IVPD: *In vitro* protein digestibility.

Emmambux (2020) reported the development of aggregate formation in heat moisture-treated maize meals. This may result from denatured proteins forming a hydrophobic layer around the starch (Puncha-Arnon & Uttapap, 2013).

It is important to note that even though aggregate formation was seen in samples without the phenolic extracts, the size of the aggregates increased in the sorghum meals with the non-tannin and tannin phenolic extracts. This could suggest that the phenolic extracts promoted more interactions for aggregate formation. The denatured protein matrix could have formed complexes with the phenolic compounds, limiting the starch expansion and amylase access and, hence, the higher resistant starch content. To better understand whether the aggregate formation was related to resistant starch content, the residue of the undigested samples was stained with iodine and observed under the bright light microscope, as shown in Supplementary Fig. 4. The visual difference between the sorghum samples without the phenolic extracts and the sorghum samples with phenolic extract was the intensity iodine stain of the aggregates. More aggregates were visible after digestion in the samples with added phenolic extract. It can be said that a compact molecular structure was developed after addition of the phenolic extract and MWHMT, which limited the accessibility of the digestive enzyme, thereby creating a possible resistant starch type 1 (RS1) causing the matrix effect. The latter can be due to indigestible as discussed later. In addition, RS5 as the V-type complexes can be formed mostly developed as a result of starch-protein, starch-polyphenols, and starch-other polysaccharide interactions (Gutiérrez & Tovar, 2021). In this study, the possible complex formation could be starch-protein-phenolics interactions.

Foods containing RS often have a low glycaemic index because the small intestine does not break down RS (Haub et al., 2010). This was evident in this study as the RS content present affected the estimated glycaemic index. The estimated glycaemic index was higher in raw samples with lower resistant starch content. MWHMT treatment and the addition of phenolic extracts resulted in the creation of more RS content, lowering the release of glucose and, further, lowering eGI.

The decrease in starch hydrolysis can also be correlated with the higher relative crystallinity as observed in the MWMHT pasted samples of the XRD. This trend agrees with a study by You et al. (2014), who found that the increase in relative crystallinity was attributed to starch hydrolysis, resulting in lower RDS content, higher RS content, and lower eGI. The pasted sorghum meals with non-tannin and tannin phenolic extracts increased in relative crystallinity and had the lowest rate of starch hydrolysis. It has been suggested that the crystallites place a physical restriction on enzyme accessibility. Consequently, as the crystalline regions become more rigid and extensive, the susceptibility of the amylase enzyme decreases, resulting in reduced starch hydrolysis (Chung et al., 2006).

The *in vitro* protein digestibility of the sorghum meals with and without the addition of phenolic extracts and MWHMT is presented in Table 4. For raw sorghum meal and sorghum meal with non-tannin phenolic extracts, the addition of the non-tannin phenolic extract did not show any significant difference in protein digestibility. MWHMT led to a significant decrease in the protein digestibility in both samples. According to Ezeogu et al. (2005), heat treatment can lead to a decrease in *in vitro* digestibility by the formation of enzymatically- resistant protein polymers formed by disulphide crossing by the β and γ- kafirin. The increase in the β-sheet after pasting of the samples could also explain the lower *in vitro* protein digestibility. As stated earlier, some research has confirmed the formation of β-sheet of sorghum meal protein after wet cooking. A study by Emmambux and Taylor (2009) on sorghum meal and its prolamin protein showed the formation of β-sheet structure from the FTIR results and a decrease in protein digestibility after wet cooking. They suggested that the formation of β-sheet structures could have caused the reduction in the susceptibility to proteolysis by the pepsin enzyme leading to the reduction in protein digestibility. According to Yang et al. (2016), an increase in β-sheet results in a regular and tight structure of protein aggregates leading to lower protein digestibility.

A significant difference ($P < 0.05$) was observed when the tannin phenolic extract was added. *In vitro* protein digestibility decreased from 54.77 % to 18.14 % when tannin phenolic extract was added and pasted before MWHMT. However, MWHMT did not have any significant change in the protein digestibility. In literature, the understanding of the poor protein digestibility of sorghum is mostly related to the polymerization of the protein kafirin as a result of disulphide bonding of kafirin monomers during cooking. With tannin addition, the digestibility was further reduced, possibly due to the complex formation between tannin and the protein. According to Emmambux and Taylor (2003), condensed tannins can form complexes with sorghum protein (kafirin). These authors showed that sorghum condensed tannins can bind irreversibly to kafirin and suggested that it can play a role in decreasing of protein digestibility in high tannin sorghum. The low protein digestibility, especially those with the added phenolics, may also suggest that the protein will still be present after protease digestion and, thus, can have a matrix effect to produce RS1.

4. Conclusions

This study highlights the effectiveness of MWHMT in reducing starch digestibility by modifying the starch molecular configurations. The incorporation of non-tannin and tannin phenolic extracts was shown to significantly lower starch hydrolysis, demonstrating the complex interplay between phenolic compounds, proteins, starch, and the α-amylase enzyme within the sorghum food system. The formation of protective barriers around starch granules by phenolics and protein interaction plays a crucial role in impeding digestion. Also phenolic and starch interactions further contributed to the decrease in digestibility and inhibition of α-amylase activity. These findings emphasise the vital role that phenolic compounds play in modulating starch digestibility, offering valuable insights for the development of functional foods aimed at improving health outcomes through enhanced resistant starch content. Future research could explore the practical applications of these findings in food formulation and dietary strategies to optimize starch utilization and promote digestive health.

CRediT authorship contribution statement

Rose Otema Baah: Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Kwaku Gyebi Duodu:** Writing – review & editing, Validation, Supervision, Conceptualization. **Joanna Harasym:** Writing – review & editing, Visualization, Validation, Supervision, Resources. **Mohammad N. Emmambux:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that there are no conflicts of interest with this article submitted for publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.143261.

Data availability

Data will be made available on request.

References

AOAC. (2019). *Official methods of analysis of AOAC international Official methods: 925.10 (moisture), 923.03 (ash), 920.39 (fat) (21th ed.) (2019). Gaithersburg, MD, USA*.

Awika, J. M., Mcdonough, C. M., & Rooney, L. W. (2005). Decorticating sorghum to concentrate healthy phytochemicals. *Journal of Agricultural and Food Chemistry, 53* (16), 6230–6234. https://doi.org/10.1021/jf0510384

Baah, R. O., Duodu, K. G., Taylor, J. R. N., & Emmambux, M. N. (2024). Functional and nutritional properties of infrared and microwave heat moisture-treated sorghum meals. *Cereal Chemistry., 101*(5), 1071–1083. https://doi.org/10.1002/cche.10808

Beta, T., & Corke, H. (2004). Effect of ferulic acid and catechin on sorghum and maize starch pasting properties. *Cereal Chemistry, 81*(3), 418–422. https://doi.org/10.1094/CCHEM.2004.81.3.418

Bhat, M. S., & Arya, S. S. (2020). Physico-functional, pasting and structural properties of gorgon nut (Euryale ferox) flour as affected by heat-moisture and acid treatment. *Journal of Food Measurement and Characterization, 14*(3), 1656–1664. https://doi.org/10.1007/s11694-020-00413-4

Bonwell, E. S., & Wetzel, D. L. (2009). Innovative FTIR imaging of protein film secondary structure before and after heat treatment. *Journal of Agricultural and Food Chemistry, 57*(21), 10067–10072. https://doi.org/10.1021/jf902225p

Chen, N., Gao, H. X., He, Q., Yu, Z. L., & Zeng, W. C. (2022). Influence of structure complexity of phenolic compounds on their binding with maize starch. *Food Structure, 33*, Article 100286. https://doi.org/10.1016/j.foostr.2022.100286

Chung, H. J., Lim, H. S., & Lim, S. T. (2006). Effect of partial gelatinization and retrogradation on the enzymatic digestion of waxy rice starch. *Journal of Cereal Science, 43*(3), 353–359. https://doi.org/10.1016/j.jcs.2005.12.001

Deka, D., & Sit, N. (2016). Dual modification of taro starch by microwave and other heat moisture treatments. *International Journal of Biological Macromolecules, 92*, 416–422. https://doi.org/10.1016/j.ijbiomac.2016.07.04

Elboughdiri, N. (2018). Effect of time, solvent-solid ratio, ethanol concentration and temperature on extraction yield of phenolic compounds from olive leaves. *Engineering Technology and Applied Science Research, 8*(2), 2805–2808. https://doi.org/10.13140/RG.2.2.26002.81601

Emmambux, M. N., & Taylor, J. R. (2009). Properties of heat-treated sorghum and maize meal and their prolamin proteins. *Journal of Agricultural and Food Chemistry, 57*(3), 1045–1050. https://doi.org/10.1021/jf802672e

Emmambux, N. M., & Taylor, J. R. (2003). Sorghum kafirin interaction with various phenolic compounds. *Journal of the Science of Food and Agriculture, 83*(5), 402–407. https://doi.org/10.1002/jsfa.1379

Englyst, H. N., Kingman, S. M., & Cummings, J. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition, 46*, S33–S50 (PMID: 1330528).

Ezeogu, L., Duodu, K., & Taylor, J. (2005). Effects of endosperm texture and cooking conditions on the *in vitro* starch digestibility of sorghum and maize flours. *Journal of Cereal Science, 42*(1), 33–44. https://doi.org/10.1016/j.jcs.2005.02.002

Ezeogu, L. I., Duodu, K. G., Emmambux, M. N., & Taylor, J. R. (2008). Influence of cooking conditions on the protein matrix of sorghum and maize endosperm flours. *Cereal Chemistry, 85*(3), 397–402. https://doi.org/10.1094/CCHEM-85-3-0397

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*(3), 427–437. https://doi.org/10.1016/S0271-5317(97)00010-9

Gutiérrez, T. J., & Tovar, J. (2021). Update of the concept of type 5 resistant starch (RS5): Self-assembled starch V-type complexes. *Trends in Food Science & Technology, 109*, 711–724. https://doi.org/10.1016/j.tifs.2021.01.078

Hamaker, B. R., Kirleis, A. W., Mertz, E. T., & Axtell, J. D. (1986). Effect of cooking on the protein profiles and *in vitro* digestibility of sorghum and maize. *Journal of Agricultural and Food Chemistry, 34*(4), 647–649. https://doi.org/10.1021/jf00070a014

Haub, M. D., Hubach, K. L., Al-Tamimi, E. K., Ornelas, S., & Seib, P. A. (2010). Different types of resistant starch elicit different glucose reponses in humans. *Journal of Nutrition and Metabolism, 2010*(1), Article 230501. https://doi.org/10.1155/2010/230501

Li, M., Pernell, C., & Ferruzzi, M. G. (2018). Complexation with phenolic acids affect rheological properties and digestibility of potato starch and maize amylopectin. *Food Hydrocolloids, 77*, 843–852. https://doi.org/10.1016/j.foodhyd.2017.11.028

Li, R., Dai, L., Peng, H., Jiang, P., Liu, N., Zhang, D., Wang, C., & Li, Z. (2021). Effects of microwave treatment on sorghum grains: Effects on the physicochemical properties and in vitro digestibility of starch. *Journal of Food Process Engineering, 44*(10), Article e13804. https://doi.org/10.1111/jfpe.13804

Mapengo, C. R., & Emmambux, M. N. (2020). Functional properties of heat-moisture treated maize meal with added stearic acid by infrared energy. *Food Chemistry, 325*, Article 126846. https://doi.org/10.1016/j.foodchem.2020.126846

Mapengo, C. R., Ray, S. S., & Emmambux, M. N. (2019). Pasting properties of hydrothermally treated maize starch with added stearic acid. *Food Chemistry, 289*, 396–403. https://doi.org/10.1016/j.foodchem.2019.02.130

Oria, M.P., Hamaker, B.R. & Shull, J.M., (1995). Resistance of sorghum. alpha.-,. beta.-, and. gamma.-Kafirins to Pepsin Digestion. Journal of Agricultural and Food Chemistry, 43(8), pp.2148–2153. doi:https://doi.org/10.1021/jf00056a036.

Puncha-Arnon, S., & Uttapap, D. (2013). Rice starch vs. rice flour: Differences in their properties when modified by heat moisture treatment. *Carbohydrate Polymers, 91*(1), 85–91. https://doi.org/10.1016/j.carbpol.2012.08.006

Sarifudin, A., Soontaranon, S., Rugmai, S., & Tongta, S. (2019). Structural transformations at different organizational levels of ethanol-treated starch during heating. *International Journal of Biological Macromolecules, 132*, 1131–1139. https://doi.org/10.1016/j.ijbiomac.2019.04.025

Schmidt, L. C., Ozturk, O. K., Young, J., Bugusu, B., Li, M., Claddis, D., Mohamedshah, Z., Ferruzzi, M., & Hamaker, B. R. (2023). Formation of cereal protein disulfide-linked stable matrices by apigeninidin, a 3-deoxyanthocyanidin. *Food Chemistry, 404*, Article 134611. https://doi.org/10.1016/j.foodchem.2022.134611

Taylor, J. R., & Duodu, K. G. (2023). Resistant-type starch in sorghum foods. Factors involved and health implications. *Starch-Stärke, 75*(9–10), 2100296. https://doi.org/10.1002/star.202100296

Waterman, P.G. & Mole, S. (1994). Analysis of phenolic plant metabolites. In Blackwell scientific (pp. 210-230).

Wei, X., Li, J., & Li, B. (2019). Multiple steps and critical behaviors of the binding of tannic acid to wheat starch: Effect of the concentration of wheat starch and the mass ratio of tannic acid to wheat starch. *Food Hydrocolloids, 94*, 174–182. https://doi.org/10.1016/j.foodhyd.2019.03.019

Xiong, Y., Zhang, P., Warner, R. D., & Fang, Z. (2019). Sorghum grain: From genotype, nutrition, and phenolic profile to its health benefits and food applications. *Comprehensive Reviews in Food Science and Food Safety, 18*(6), 2025–2046. https://doi.org/10.1111/1541-4337.12506

Yang, Y., Wang, Z., Wang, R., Sui, X., Qi, B., Han, F., Li, Y., & Jiang, L. (2016). Secondary structure and subunit composition of soy protein *in vitro* digested by pepsin and its relation with digestibility. *BioMed Research International, 1*, 5498639. https://doi.org/10.1155/2016/5498639

You, S.-Y., Lim, S.-T., Lee, J. H., & Chung, H.-J. (2014). Impact of molecular and crystalline structures on *in vitro* digestibility of waxy rice starches. *Carbohydrate Polymers, 112*, 729–735. https://doi.org/10.1016/j.carbpol.2014.06.065

Yuris, A., Matia-Merino, L., Hardacre, A. K., Hindmarsh, J., & Goh, K. K. T. (2018). Molecular interactions in composite wheat starch-mesona chinensis polysaccharide gels: Rheological, textural, microstructural and retrogradation properties. *Food Hydrocolloids, 79*, 1–12. https://doi.org/10.1016/j.foodhyd.2017.12.007

Zhao, Q., Yu, X., Zhou, C., Yagoub, A. E. A., & Ma, H. (2020). Effects of collagen and casein with phenolic compounds interactions on protein *in vitro* digestion and antioxidation. *Lwt, 124*, Article 109192. https://doi.org/10.1016/j.lwt.2020.109192

Zhou, J., & Kong, L. (2023). Complexation with pre-formed "empty" V-type starch for encapsulation of aroma compounds. *Food Science and Human Wellness, 12*(2), 488–494. https://doi.org/10.1016/j.fshw.2022.07.050