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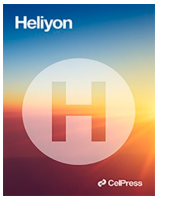

Research article

Influence of protein in low paste viscosities of Bambara groundnut flours from heat-treated Bambara groundnut seeds☆

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ABSTRACT

Heat treatment of Bambara groundnut seeds has been reported to cause low paste viscosities in resulting flours. Structural changes in Bambara groundnut protein, due to heat treatment, causes the protein to encapsulate starch, making it unavailable to paste causing low paste viscosities. In this study, trypsin was used to hydrolyze proteins in the flour and the microstructure analysis confirmed the disappearance of aggregates. Flour microstructure analysis confirmed hydrolysis of protein from previously aggregated status and showed liberated individual starch granules. Following the treatment of flours by trypsin, Confocal laser scanning microscopy did not show a protein signal. Hydrolysing Bambara groundnut proteins significantly increased the flour paste viscosities ($P < 0.05$). The final viscosities for flours from 20 % moisture-conditioned and infrared heat-treated seeds for 5 min were 733.9 mPa s before protein hydrolysis and 2081.71 mPa s after protein hydrolysis. The gelatinization temperature (81 °C) did not show a significant change following protein hydrolysis. Sodium dodecyl-sulphate polyacrylamide gel electrophoresis band intensity increased indicative of disulfide bonding and protein polymerisation when microwave and infrared heat treatment were combined. There were changes in Bambara groundnut protein secondary structures such as an increase of 57 % in β-sheet along with a 60 % reduction in the α-helix as shown by the Fourier-transform infrared spectroscopy. The changes in secondary structure of Bambara groundnut protein were caused by microwave and infrared heating. Heat treatment of Bambara groundnut seeds is partly responsible for the reduction in paste viscosities of their flours.

1. Introduction

Starch and protein are abundant biopolymers in pulses. When pulse seeds are processed using heat treatment and then milled into flour, there is an expected interaction between starch, proteins and other plant material. Application of infrared, microwave and combined heat treatments to moisture-conditioned Bambara groundnut seeds, causes low paste viscosities of their flours. Low paste viscosities have been attributed to protein surrounding starch and restriction of starch pasting during wet heat processing [1]. Heat treatment of legume seeds can lead to structural and functional changes in seeds, which may impact the utilization of their flours. However, due to their low paste viscosities, Bambara groundnut flours from heat-treated seeds may have a specific application in

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complimentary food and beverages as they can increase nutrient density and improve sensory properties such as flavour and texture.

During heat treatment of their seeds, Bambara groundnut protein binds to starch and forms aggregated structures and these aggregates are responsible for the observed low paste viscosities [1,2]. The aggregate formation is likely due to the protein-protein network and protein encapsulating the starch, following protein denaturation. In literature, it has been reported that as heat treatment time or heat intensity increases, the protein network surrounding the starch granule in rice [3] and wheat [4,5] is strengthened due to the disulfide bonding. The denatured protein surrounding the starch may expose the protein's hydrophobic sites and form a barrier around the starch, reducing starch water absorption [2,3]. This is because as proteins denature, they expose buried hydrophobic sites, and as they tightly bind to starch, they form a relatively hydrophobic layer around the starch. Thus, the protein matrix encapsulate the starch granules preventing them from forming a viscous paste [1].

Ding, Cheng [4] used native adlay seed starch to study the changes in their physicochemical properties as affected by lipids and protein. They demonstrated that protein bodies formed a network around the starch which hindered starch gelatinization. Protein was then hydrolysed resulting in an increase in starch viscosity, gelatinization enthalpy, and starch hydrolysis, however there was a reduction in apparent viscosity, and viscoelastic moduli. The authors concluded that endogenous proteins were more crucial than endogenous lipids for the changes in the starch physicochemical properties in rice.

This study determined the influence of protein in low viscosity paste when Bambara groundnut is heat-treated using infrared and microwave heat treatment methods. It has been proposed that low paste viscosities were caused by the encapsulation of starch by protein, rendering starch unavailable to form a viscous paste [1]. The changes in Bambara groundnut protein caused by heat treatment that would lead to protein adherence to starch, causing low paste viscosity of starch are yet to be explored. As far as we know there is no other study that has been done to determine the role of protein from infrared, microwave, and combined heat-treated Bambara groundnut seeds on low paste viscosities of their flours. Knowledge gained in this study can assist in improving Bambara groundnut flour utilization in a various food product.

2. Material and methods

2.1. Materials

The raw material used in this experiment were sourced from VKB Agriculture (Pvt) Ltd (Mokopane, South Africa). The Analysis Association of Analytical Chemists AOAC (2000), official method 925.05 was used to determine the moisture content of the Bambara groundnut flours. The Dumas method was used to assess the crude protein of the flours and 5.7 was used as a factor of nitrogen for the calculation of Bambara groundnut protein content [5], as determined by Dumatherm (DT, Gerhardt Konigswinter, Germany). Porcine trypsin (CAS No: 9002-07-7) was sourced from Sigma-Aldrich (Sigma Chemical Co, Burlington, MA, USA).

2.2. Methods

Bambara groundnut seed moisture conditioning and heat treatment were done as previously described [1]. Briefly, a batch of Bambara groundnut seeds (100 g) was sprayed with a predetermined amount of water to reach 20 % moisture level, while another batch of the seeds were immersed in distilled water at room, for 24 h, to reach a 53 % moisture level. A combination Microwave/Infrared Hot air oven (Dephius Commercial & Industrial Technologies, Pretoria, South Africa) was used to apply microwave and infrared to a single layer of seeds placed at a distance of 23 cm from the oven lamps. The oven temperature was set at 130 °C and the power was set at 1200 W. The temperature on Bambara groundnut seeds surface was measured during heat treatment. Heat-treated seeds were dried at 50 °C in an air oven. The dried Bambara groundnut seeds were milled into flour. Raw untreated seeds were also milled into flour, and all the flours were placed in polythene bags at 4 °C until use.

2.3. Hydrolysis of flours

Untreated and treated whole Bambara groundnut flour (without defatting) (30 g) was dispersed in distilled water at a 1:10 (g/v) ratio and continuously stirred for 1 h using a magnetic stirrer at 40 °C. Porcine trypsin (0.1 %, based on protein content) (Sigma-Aldrich 9002-07-7) was dissolved in Tris-HCl (pH 8) buffer, then added to the slurry and mixed for 15 min. The mixture was incubated in 200 rpm shaking water bath set at the temperature of 40 °C and allowed to run for an hour. After 1 h, the pH was adjusted to 7 and the distilled water was used to wash the slurry (2x). Then, the mixture was centrifuged at 4000×*g* for 20 min at 4 °C. The pellet was lyophilised and then gently ground into flour and the supernatant decanted. The control was prepared following the same procedure as above in pH 8, Tris-HCl buffer, without adding trypsin. The protein content was also determined after treatments.

2.4. Pasting properties

Flours (1.6 g dry basis) of Bambara groundnut samples that were run under buffer and enzyme conditions, for protein hydrolysis, were dispersed in (w/w) distilled water to make 10 % (W/V) suspension. A Physica MCR101 model, Anton Paar GmbH (Ostfildern, Germany) rheometer was used to determine the flour pasting viscosities. The suspension was equilibrated at 50 °C for 1 min, followed by programmed heating to 91 °C at a uniform rate of 5 °C min^{-1} with constant stirring at 160 rpm. The heated slurry was held at 91 °C for 7 min, then cooled to 50 °C at the same rate and held at this temperature for 2 min.

Table 1A
Effects of protein hydrolysis of moisture conditioned (20 and 53 %) and heat-treated Bambara groundnut seeds on the moisture and protein composition of resulting flours run under buffer conditions.

Moisture Conditioning	Heat Treatment Method	Treatment time (min)	Moisture	Protein
		0	2.31 (0.12)	4.43[a] (0.23)
20 %		0	2.18 (0.41)	4.25[c] (0.16)
	Infrared	5	2.46 0.41)	4.12[a] (0.20)
		10	2.38 (0.45)	4.22[a] (0.44)
	Microwave	5	2.34 0.35)	4.15[a] (0.97)
		10	2.00 (0.67)	4.28[a] (0.20)
	Combined	5	1.98 (0.73)	4.31[a] (0.38)
		10	2.13 (0.33)	4.34[a] (0.08)
53 %		0	2.65 (0.31)	4.35[a] (0.27)
	Infrared	5	2.54 (0.61)	4.54[a] (0.63)
		10	2.22 (0.26)	5.10[a] (0.25)
	Microwave	5	2.30 (0.23)	4.76[a] (0.31)
		10	2.62 (0.40)	4.51[a] (0.06)
	Combined	5	2.35 (0.14)	4.86[a] (0.08)
		10	2.12 (0.38)	5.08[a] (0.24)

Mean values within a column with different letters differ significantly at $P < 0.05$, and the standard deviation (±).

Table 1B
Effects of protein hydrolysis of moisture conditioned (20 and 53 %) and heat-treated Bambara groundnut seeds on the moisture and protein composition of resulting flours run with enzyme.

Moisture Conditioning	Heat Treatment Method	Treatment time (min)	Moisture	Protein
		0	2.65 (0.23)	3.22[a] (0.11)
20 %		0	2.05 (0.21)	3.54[a] (0.13)
	Infrared	5	2.21 0.12)	3.52[a] (0.20)
		10	2.11 (0.54)	3.42[a] (0.44)
	Microwave	5	2.05 (0.43)	3.95[a] (0.97)
		10	2.07 (0.15)	3.38[a] (0.20)
	Combined	5	2.22 (0.33)	3.27[a] (0.38)
		10	2.02 (0.25)	3.99[a] (0.08)
53 %		0	2.39 (0.53)	3.09[a] (0.27)
	Infrared	5	2.52 (0.31)	3.54[a] (0.63)
		10	2.37 (0.51)	3.85[a] (0.25)
	Microwave	5	2.54 (0.27)	3.76[a] (0.31)
		10	2.66 (0.30)	3.51[a] (0.06)
	Combined	5	2.24 (0.12)	3.86[a] (0.08)
		10	2.32 (0.28)	3.48[a] (0.24)

Mean values within a column with different letters differ significantly at $P < 0.05$, and the standard deviation (±).

2.5. Differential scanning calorimeter (DSC)

The resulting Bambara groundnut flours from samples that were run under buffer and enzyme conditions were freeze-dried, ground into flour then analysed for their thermal properties using the Differential scanning calorimeter (DSC) system (HPDSC827e, Mettler Toledo, Greifensee, Switzerland) which was calibrated using indium ($T_p = 156.6$ °C, 28.45 Jg^{-1}) [6]. A 10 mg Bambara groundnut flour was mixed with 30 mg of distilled water and allowed to equilibrate for 12 h. Samples were scanned at the rate of 10 °C/min between 30 °C to 110 °C under the nitrogen gas with pressure of 4 MPa. Indium was used as a standard to calibrate the DSC prior to analysis.

2.6. Light microscopy

Bambara groundnut flour samples were viewed under light microscope as previously described [1]. Briefly, pasted and unpasted samples made were put in 1 ml of 30 % glycerol which was mixed and put in microscope slide and covered with a cover slip. The sample was either unstained or stained with iodine and then observed under a Nikon Optiphot Light Microscope (Nikon Corporation, Tokyo, Japan) microscope. Polarised light was used to observe starch birefringence.

2.7. Confocal laser scanning microscopy (CLSM)

Protein in Bambara groundnut flour was stained using 0.02 % Acid Fuchsin dye in 1 % acetic acid. Samples were pasted and a few drops of the dye were applied, then the stained samples were put in an oven for an hour, the oven was set to 60 °C. A Zeiss LSM 510 META CLSM (Zeiss SMT, Jena, Germany) with excitation set at 405 nm. The emission spectra was at 425–475 nm [1].

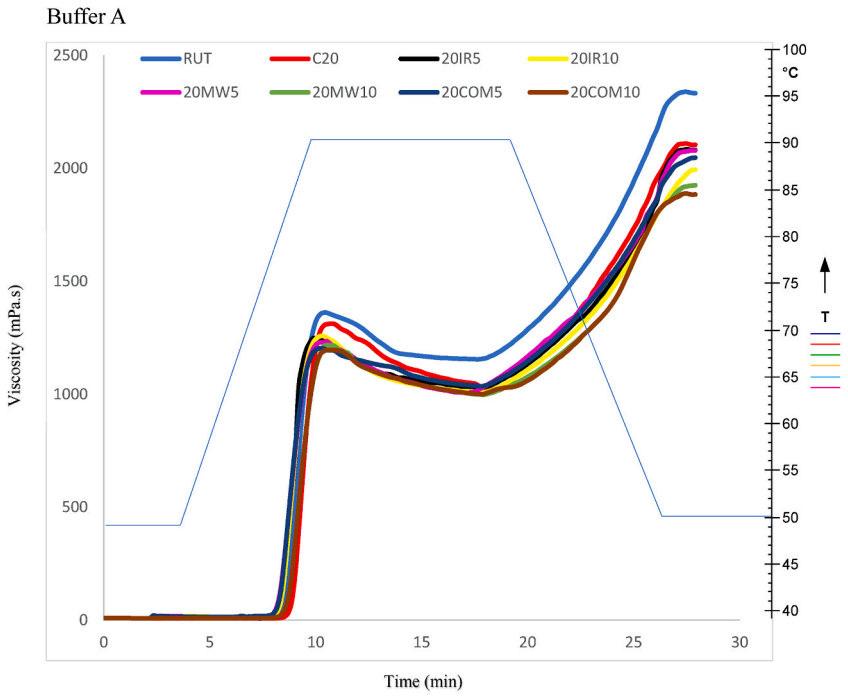

Fig. 1A. Effect of protein hydrolysis of resulting flours from infrared, microwaved processing alone and in combination of Bambara groundnut seeds at 20 % moisture on viscosity during pasting. RUT-raw seeds, C20- 20 % moisture, 20IR5- 5 min infrared, 20IR10-10 min infrared, 20MW5- 5 min microwave, 20MW10 - 10 min microwave, 20COM5- 5 min combined, 20COM10- 10 min combined.

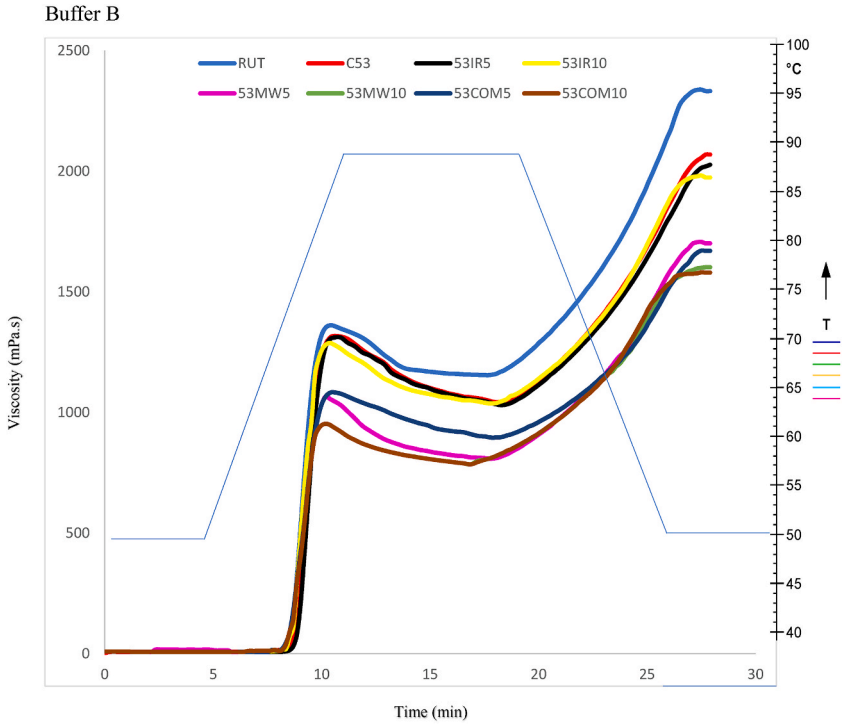

Fig. 1B. Effect of protein hydrolysis of resulting flours from infrared, microwaved processing alone and in combination of Bambara groundnut seeds at 20 % moisture on viscosity during pasting. RUT-raw seeds, C53- 53 % moisture, 53IR5- 5 min infrared, 53IR10-10 min infrared, 53MW5- 5 min microwave, 53MW10- 10 min microwave, 53COM5- 5 min combined, 53COM10- 10 min combined.

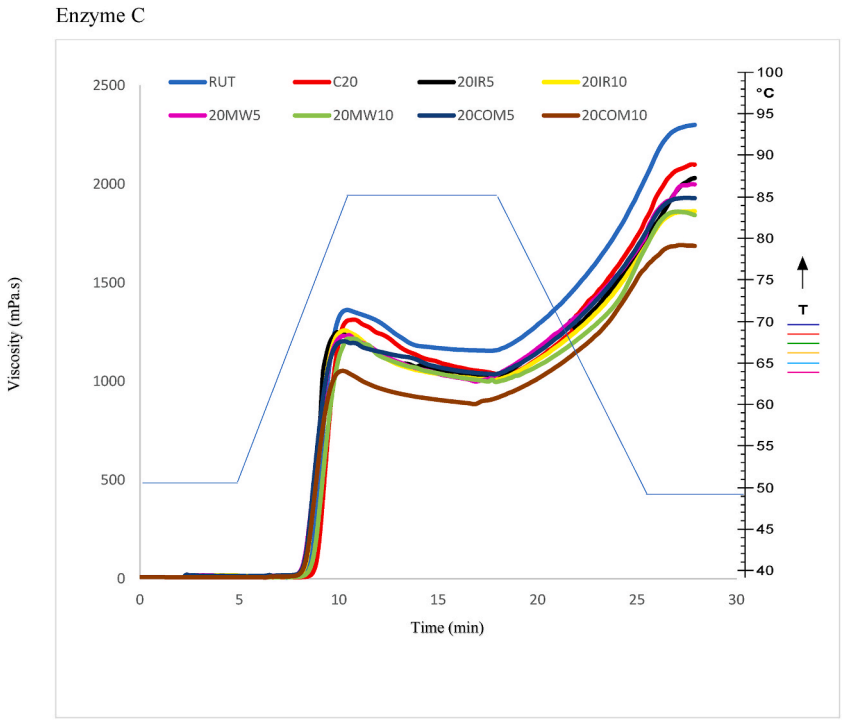

Fig. 1C. Effect of trypsin on protein hydrolysis of resulting flours from infrared, microwaved processing alone and in combination of Bambara groundnut seeds at 20 % moisture on viscosity during pasting. RUT-raw seeds, C20- 20 % moisture, 20IR5- 5 min infrared, 20IR10-10 min infrared, 20MW5- 5 min microwave, 20MW10 - 10 min microwave, 20COM5- 5 min combined, 20COM10- 10 min combined.

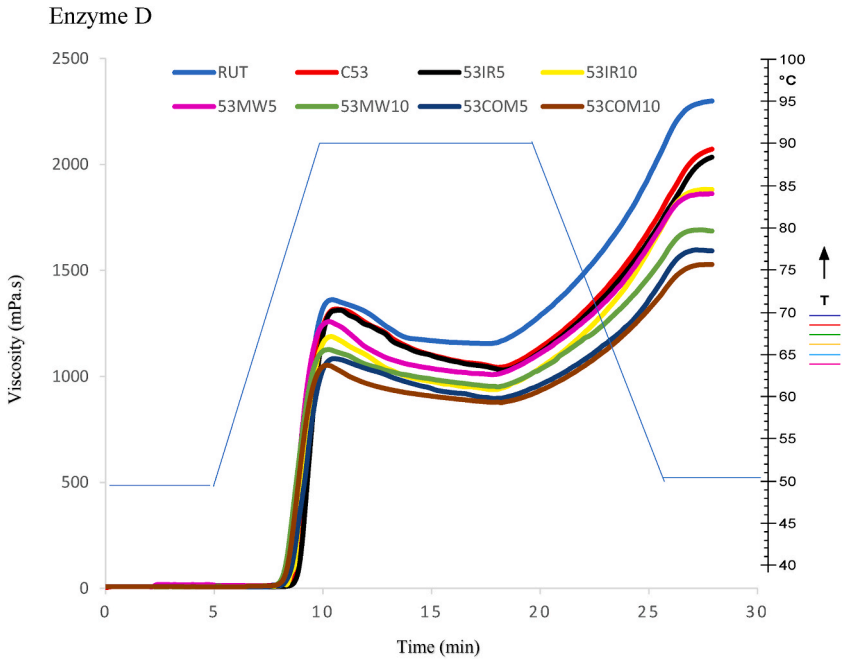

Fig. 1D. Effect of trypsin on protein hydrolysis of resulting flours from infrared, microwaved processing alone and in combination of Bambara groundnut seeds at 20 % and 53 % moisture on viscosity during pasting. RUT-raw seeds, C53- 53 % moisture, 53IR5- 5 min infrared, 53IR10-10 min infrared, 53MW5- 5 min microwave, 53MW10- 10 min microwave, 53COM5- 5 min combined, 53COM10- 10 min combined.

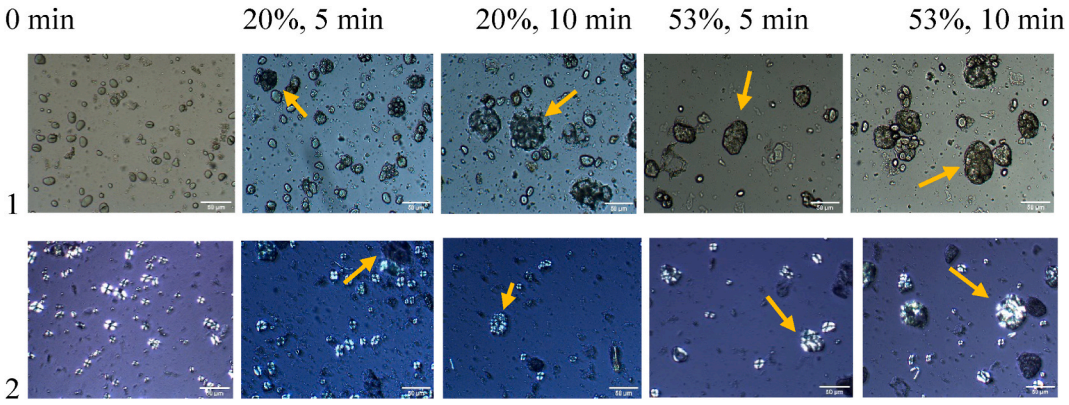

Fig. 2A. Combined heat-treated samples **before** protein hydrolysis. Light micrographs under polarised light and unstained of resulting flours from untreated, combined infrared and microwave heat-treated paste and flour samples of Bambara groundnut seeds moisture conditioned (20 and 53 % moisture). Samples were heat-treated for 0, 5, and 10 min. Before protein hydrolysis micrographs show aggregates. 1-light unstained, 2-polirised light, 3-Iodine stained, 4- pasted unstained, 5-pasted and iodine stained. Bar = 50 μm. Arrows indicating aggregates in combined microwaved and infrared flours and pastes.

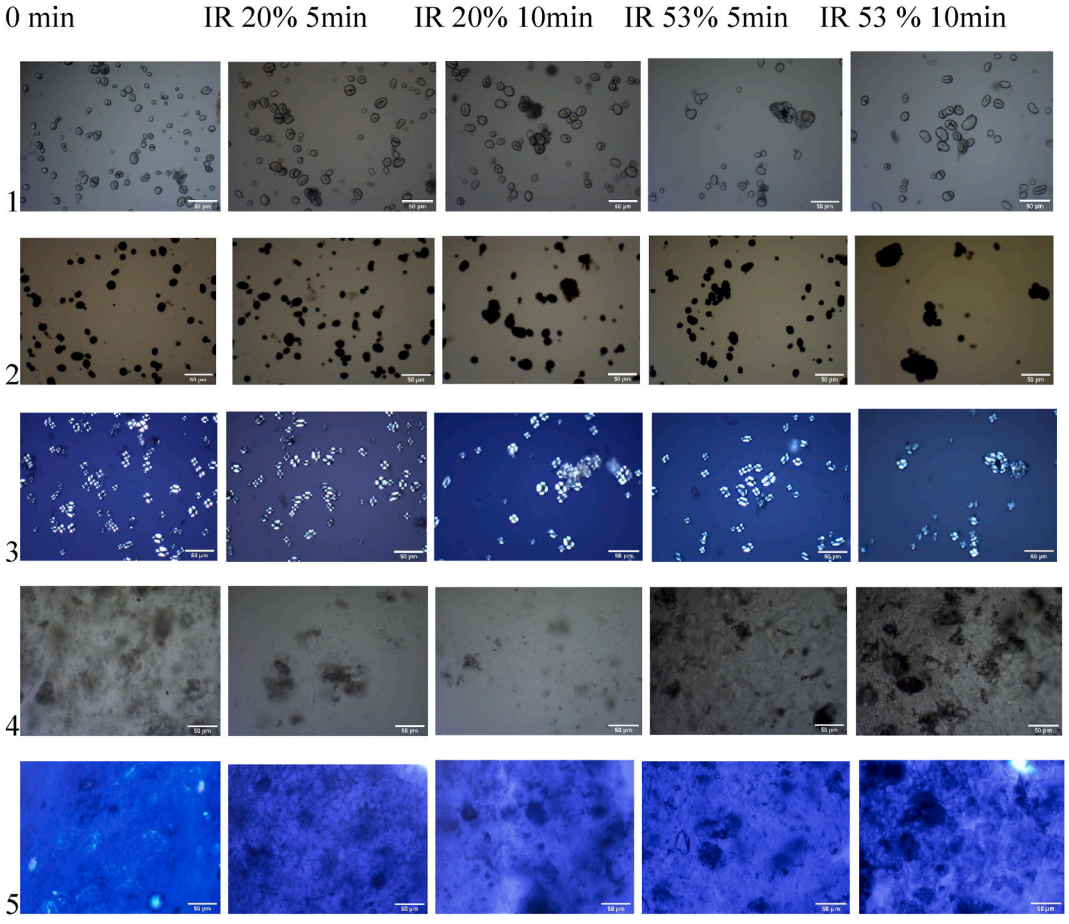

Fig. 2B. Light micrographs under polarised light, iodine stained and unstained of resulting flours from untreated, infrared heat-treated paste and flour samples of Bambara groundnut seeds moisture conditioned (20 and 53 % moisture). Samples were heat-treated for 0, 5, and 10 min. Protein hydrolysis resulted in disappearance of aggregates. 1-light unstained, 2-polirised light, 3-Iodine stained, 4- pasted unstained, 5-pasted and iodine stained. Bar = 50 μm. Arrows indicating aggregates in combined microwaved and infrared flours and pastes.

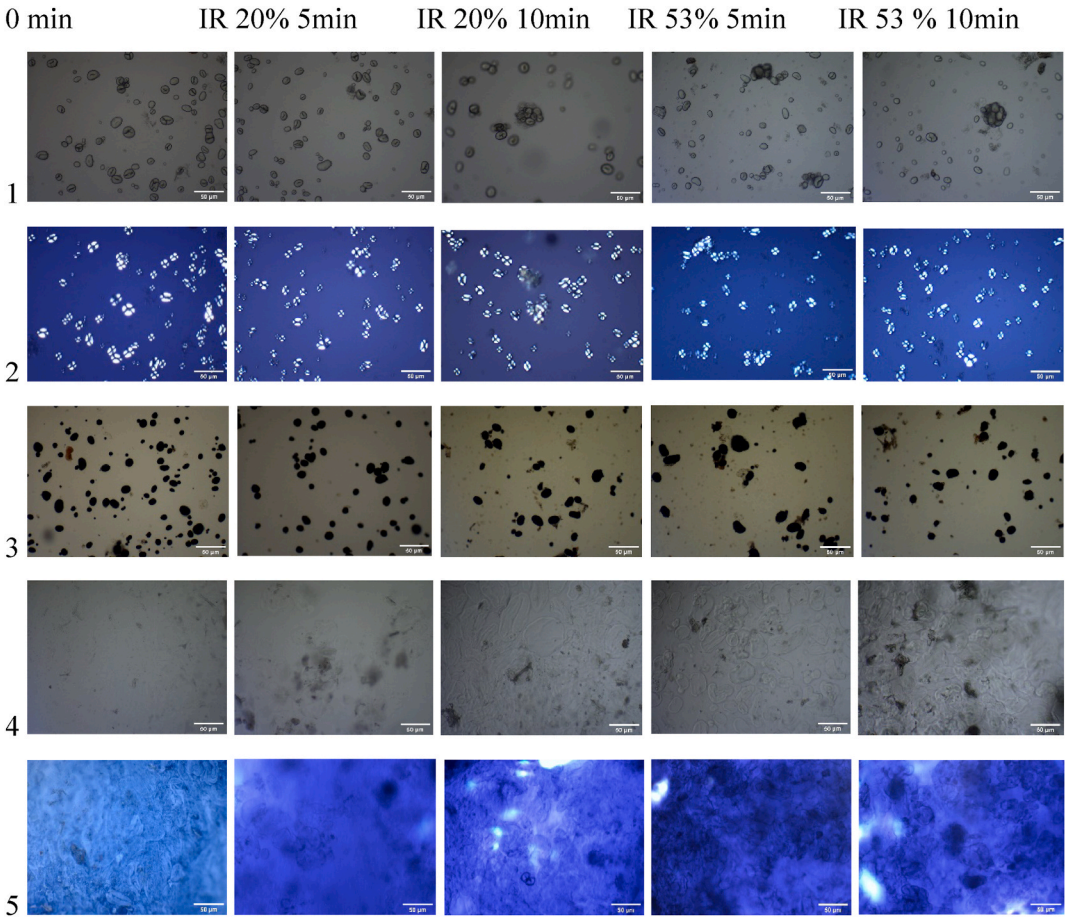

Fig. 2C. Light micrographs under polarised light, iodine stained and unstained of resulting flours from untreated, infrared heat-treated paste and flour samples of Bambara groundnut seeds moisture conditioned (20 and 53 % moisture). Samples were heat-treated for 0, 5, and 10 min. Protein hydrolysis resulted in disappearance of aggregates. 1-light unstained, 2-polirised light, 3-Iodine stained, 4- pasted unstained, 5-pasted and iodine stained. Bar = 50 μm. Arrows indicating aggregates in combined microwaved and infrared flours and pastes.

2.8. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS PAGE)

Bambara groundnut protein from the Bambara groundnut seed samples (non-enzyme hydrolysed) were separated using SDS-PAGE. The Bambara groundnut protein was examined under both the reducing and non-reducing conditions following a procedure by Arise, Nwachukwu [7]. β-mercaptoethanol was used when samples were run under reducing conditions. The Bambara groundnut protein samples (10 μl) were loaded onto the pre-cast TGX gels (Bio-Rad, Richmond, CA, USA), and ran for 30 min. Premixed protein molecular marker was used as molecular markers and the samples were stained by Coomassie Brilliant Blue G-250 after the electrophoresis was run, the gels were washed, and scanned.

2.9. Fourier transform infrared (FTIR) spectroscopy

Bambara groundnut flour samples were characterised by FTIR using A PerkinElmer Spectrum 100 spectrometer (USA). The spectra were obtained in 550–4000 cm^{-1} wavelength. An average of 32 scans were added spectra and the resolution set at 16 cm^{-1}. The spectra of the secondary structure of the Bambara groundnut protein were analysed using the OriginLab® Software (version 8.5). The spectra along with the second derivatives were used to analyse the structural changes resulting from different heat treatments of Bambara groundnut flours in the Amide I region. Baseline subtraction was done before deconvolution and determination. The second derivative data was analysed to detect the band frequencies, which were used to assign major bands in the amide I region. Gaussian peak analyser was used, and the curve fitting was conducted with iterations until the best fit was achieved. The area under the curve was used for band quantification, and these were reported as percentages.

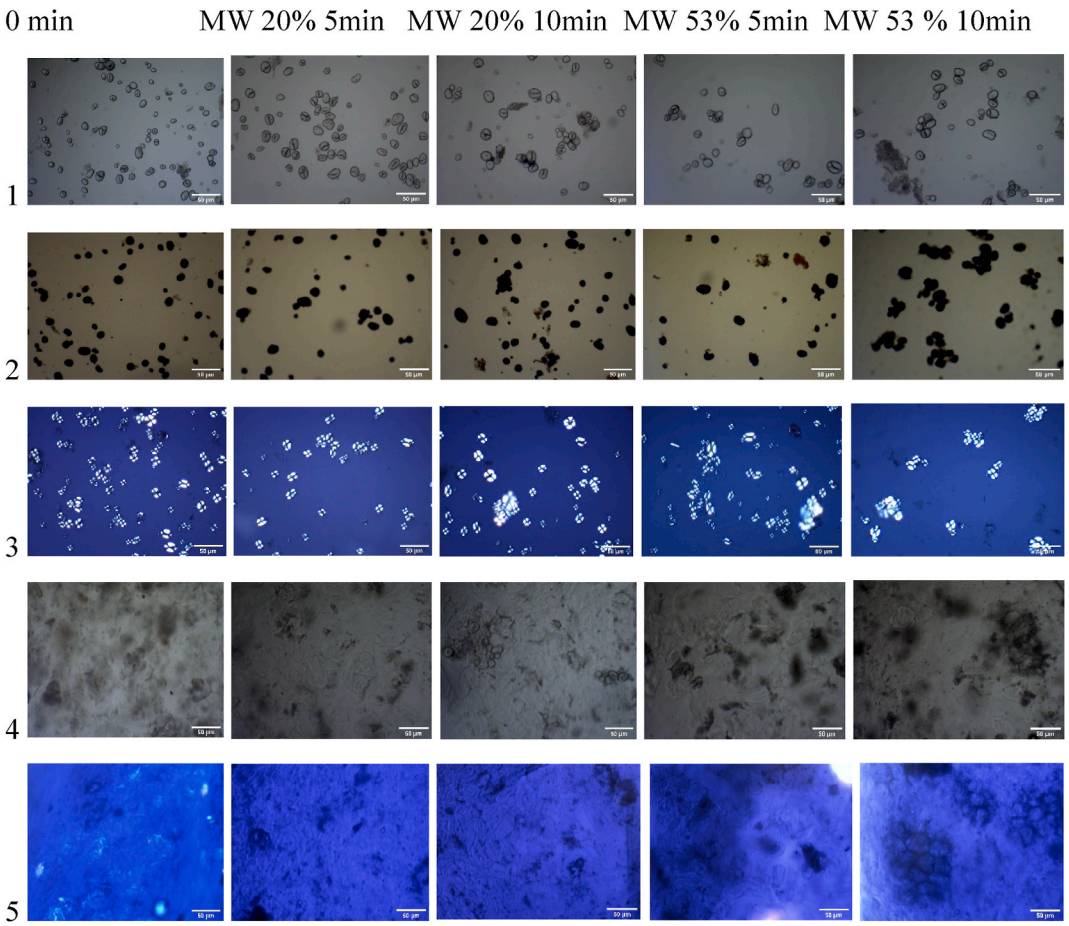

Fig. 2D. Light micrographs under polarised light, iodine stained and unstained of resulting flours from untreated, microwave heat-treated paste and flour samples of Bambara groundnut seeds moisture conditioned (20 and 53 % moisture). Samples were heat-treated for 0, 5, and 10 min. Protein hydrolysis resulted in disappearance of aggregates. 1-light unstained, 2-polirised light, 3-Iodine stained, 4- pasted unstained, 5-pasted and iodine stained. Bar = 50 μm. Arrows indicating aggregates in combined microwaved and infrared flours and pastes.

2.10. Statistical analysis

Multifactor Analysis of Variance was performed on the data of buffer and enzyme hydrolysed flour using SPSS 28.0 statistical software form IBM (SPSS, Inc., New York, NY) and compared at ($P \leq 0.05$) using Fisher's least significant difference (LSD) test. The response variables were the measured values. Independent variables were the 20 % and 53 % seed moisture levels, the three heat treatment types and the heating times of 0, 5 and 10 min.

3. Results and Discussion

3.1. Moisture and protein analysis

As expected, the buffer and enzyme incubation reduced the protein content of Bambara groundnut flours (Table 1A and B). The protein content for buffer and enzyme-treated samples were 4.18–5.10 and 3.09-3,86, respectively. This is consistent with other studies [8,9]. It also looks like the buffer can also denature some protein. Tris-HCL protein extraction buffer has been successfully used to extract protein from plant tissue [10,11].

3.2. Pasting Profile

Fig. 1 show pasting properties of Bambara groundnut flours treated under buffer conditions while Fig. 1C and D shows pasting properties of flours that were hydrolysed by trypsin. The enzyme hydrolysed flours viscosities than the buffer flour samples. There was no significant difference in both the pasting and peak temperatures of the samples ran under buffer and the trypsin hydrolysed samples ($P > 0.05$). The high starch content in these flours may have contributed to the higher pasting viscosity than the resulting flours before

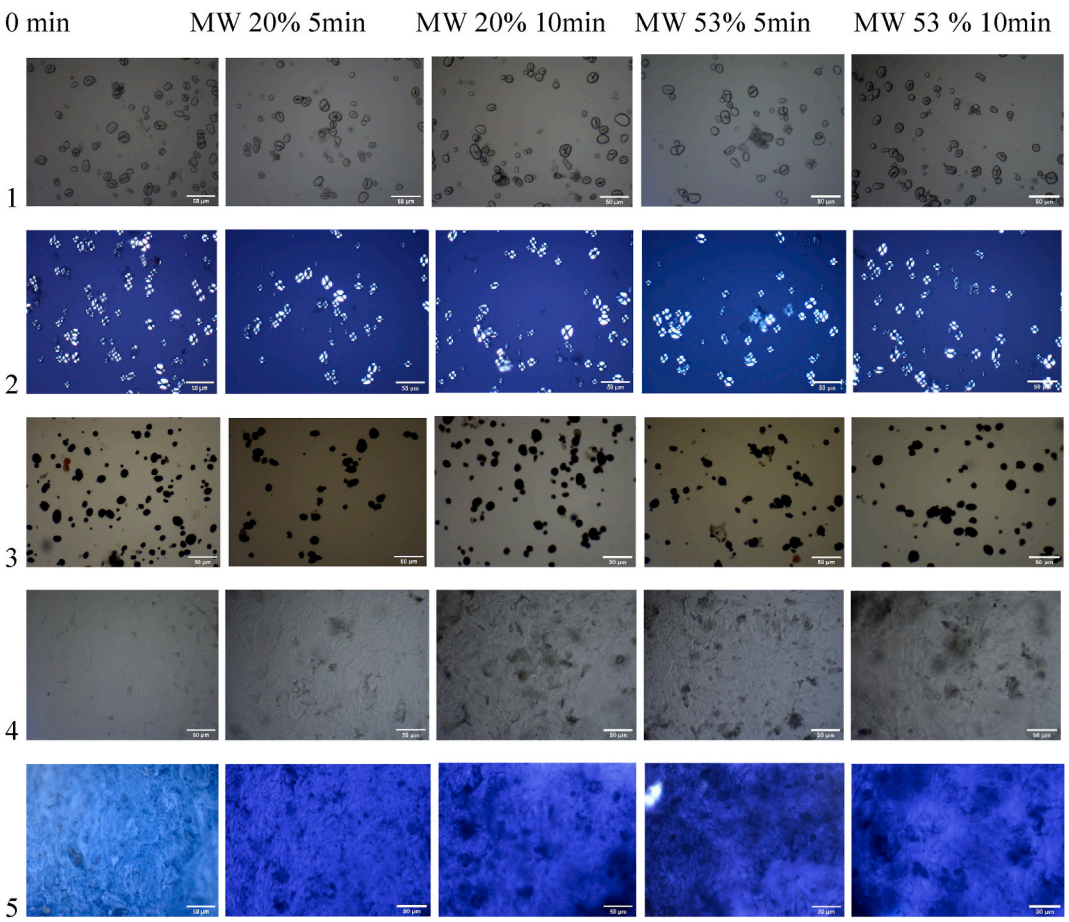

Fig. 2E. Light micrographs under polarised light, iodine stained and unstained of resulting flours from untreated, microwave heat-treated paste and flour samples of Bambara groundnut seeds moisture conditioned (20 and 53 % moisture). Samples were heat-treated for 0, 5, and 10 min. Protein hydrolysis resulted in disappearance of aggregates. 1-light unstained, 2-polirised light, 3-Iodine stained, 4- pasted unstained, 5-pasted and iodine stained. Bar = 50 μm. Arrows indicating aggregates in combined microwaved and infrared flours and pastes.

protein hydrolysis. As an example, the peak (442.1) hold (373.0) and final viscosities (733.9) for 20 % infrared heat treated for 5 min [1] compared to the peak (1202.12) hold (1107.75) and final viscosities (2081.71) for the same samples after protein hydrolysis with trypsin (Table S1B). The hydrolysis of protein that had adhered to the starch is the most important factor that led to higher paste viscosities. Tao, Lu [12] found that removing a surface protein from wheat starches significantly increased starch pasting viscosity. Starch granules could swell to reach maximum capacity when protease further hydrolysed the protein [13]. The higher peak pasting viscosities of flours may be related to the starch that is now freed from aggregated proteins. These starch granules can increase their swelling capacity and have higher peak viscosity. Trypsin-digested samples had an even higher paste, peak, breakdown viscosities indicative of further protein hydrolysis, leading to an even more swelling and high paste viscosity.

Protein hydrolysis influenced the pasting parameters because the denatured protein from heat-treated samples was hydrolysed and not available to cover the starch granules and prevent starch swelling and amylose leaching out of the granule to make a viscous paste [14,15]. Yang, Jiao [9] found that removing non-starch flour components increased starch paste viscosity. This was attributed to increased starch swelling power, and the components (protein, fat and β-glucan) that may have hindered starch swelling were removed. Hydrolysis of protein adhered to starch may improve the association of water with starch granule water [12] and, therefore, promote the development of a viscous paste. Yang, Jiao [9] also found that the hydrolysis of protein had a compounded effect on paste viscosity than the hydrolysis of fat or β-glucan alone. This may indicate that protein plays a major role in reducing flour paste viscosity. It is also possible that the increase in starch concentration may play a role in increasing paste viscosity. Yang, Jiao [9] found that the hydrolysis of non-starch material from flour increased peak viscosity and they postulated that this may be attributed to an increase in starch concentration. It is also noted that as the paste viscosity increased with hydrolysis of protein, it is unlikely that the starch was depolymerised by infrared and microwave treatment.

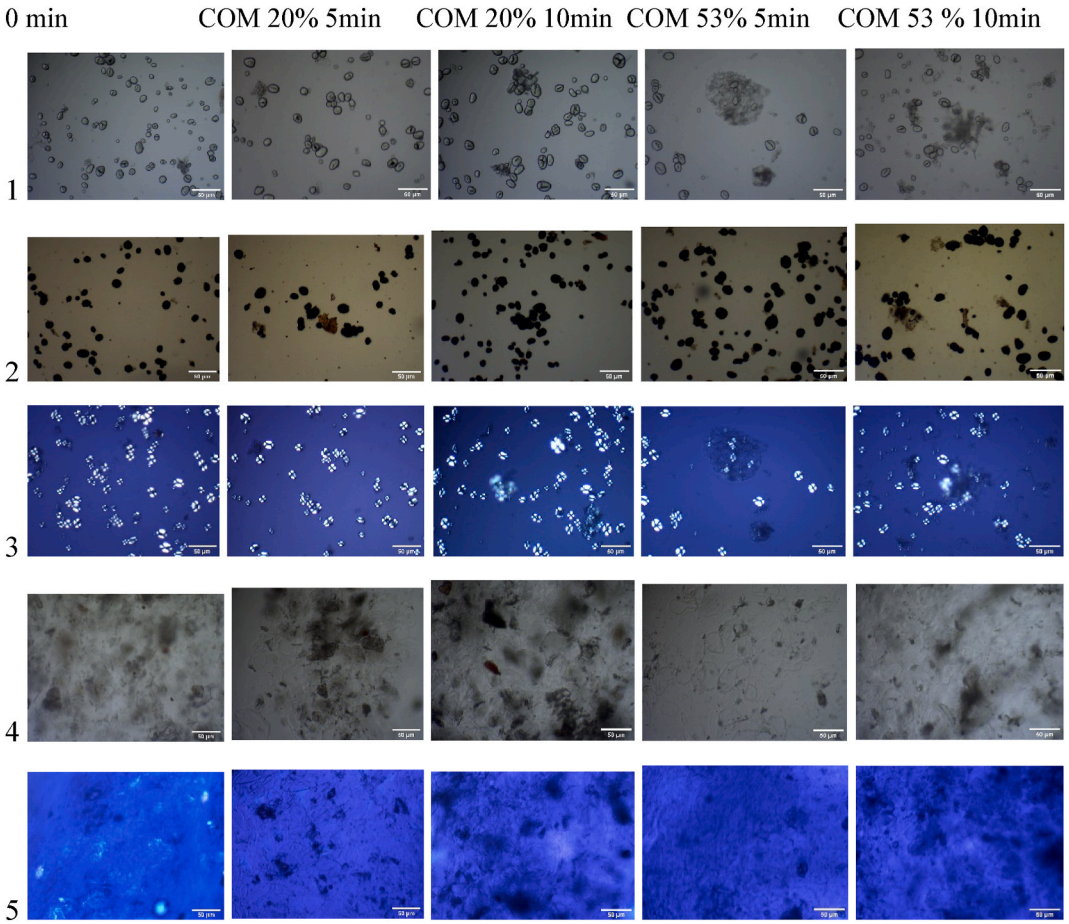

Fig. 2F. Light micrographs under polarised light, iodine stained and unstained of resulting flours from untreated, combined infrared and microwave heat-treated paste and flour samples of Bambara groundnut seeds moisture conditioned (20 and 53 % moisture). Samples were heat-treated for 0, 5, and 10 min. Protein hydrolysis resulted in disappearance of aggregates. 1-light unstained, 2-polirised light, 3-Iodine stained, 4- pasted unstained, 5-pasted and iodine stained. Bar = 50 μm. Arrows indicating aggregates in combined microwaved and infrared flours and pastes.

3.3. Microscopy

The light microscopy micrographs were studied under polarised light, iodine-stained and unstained flours and paste (stained and unstained) from the buffer and enzyme-treated samples. The aggregates observed in the flour samples from heat-treated seeds before protein hydrolysis (Fig. 2) (combined heat-treated, Fig. S3 A and C) were hydrolysed. Starch granules of different sizes were visible, unlike in the previous study [1], Fig. 2A and B, where mostly large Bambara groundnut starch granules were observed (Fig. S3 A and C). Bambara groundnut starches of different sizes were distinguished in IR, MW and combined samples for the buffer (Fig. 2) and enzyme (Fig. 2C–E, G) samples. The few remaining starch-protein extracts were barely visible in the combined heat-treated and microwave-treated samples at 10 min. Besides these, there were no distinguishable differences between the heat-treated samples and the control (non-treated). The protein hydrolysis processes successfully hydrolysed the protein-starch aggregates in all samples, freeing the previously trapped starch. Under polarised light, starch samples showed birefringence with Maltese crosses perceptible. Micrographs of samples that were heat-treated for 5 min had more Maltese crosses than those samples that were heat treated for a longer time (10min). Removing proteins bound to starch in starch-protein aggregates led to free starch molecules and allowed water to gain more access to the starch, increasing viscosity.

Similarly, in CLSM (Fig. S3), micrographs obtained before protein hydrolysis showed starch appearing as black spheres encapsulated by a red protein matrix (Fig. S3 A and C), While the starch appeared green in micrographs of flours emanating from heat-treated Bambara groundnut seeds taken before protein hydrolysis (Fig. S3 B). Fig. S3 C shows starch aggregates appearing green after staining for starch with FITC. Fig. S3 D shows micrographs after protein hydrolysis showing starch evenly distributed in both control and heat-treated samples, indicating the successful hydrolysis of most protein except for some remaining, as shown by the protein content in Table 1A and B. The microstructures of the buffer and enzyme-digested samples are depicted in Fig. S3 A and B. The micrographs show a three-dimensional protein, starch, and other materials network. When the protein was hydrolysed, the red colour disappeared, and the green colour dominated the microstructure. In the samples that underwent heat treatment for 10 min, residual

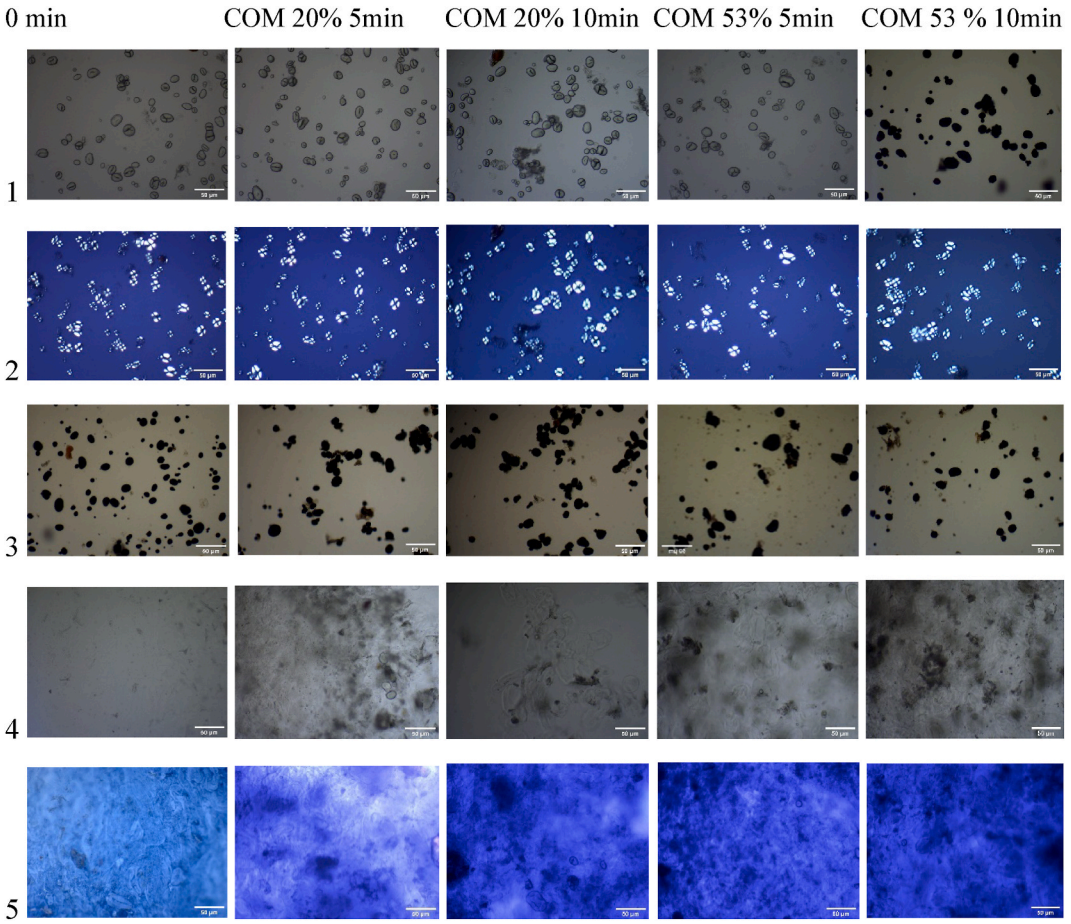

Fig. 2G. Light micrographs under polarised light, iodine stained and unstained of resulting flours from untreated, combined infrared and microwave heat-treated paste and flour samples of Bambara groundnut seeds moisture conditioned (20 and 53 % moisture). Samples were heat-treated for 0, 5, and 10 min. Protein hydrolysis using trypsin resulted in disappearance of aggregates. 1-light unstained, 2-polirised light, 3-Iodine stained, 4-pasted unstained, 5-pasted and iodine stained. Bar = 50 μm. Arrows indicating aggregates in combined microwaved and infrared flours and pastes.

Table 2A
Effects of heat treatment methods of moisture conditioned (20 and 53 %) Bambara groundnut seeds under buffer conditions on thermal profiles of resulting flours.

Moisture Content	Heat Treatment Method	Treatment time(min)	Onset, T_o (°C)	Peak, T_p (°C)	Endset, Tp (°C)	Enthalpy, ΔH J/g
RUT		0	77.36^{a} (0.01)	81.67^{a} (0.05)	86.91^{c} (0.09)	8.49^{a} (0.02)
20 %		0	77.89^{a} (0.01)	81.32^{a} (0.01)	86.65^{c} (0.01)	8.14^{a} (0.09)
	Infrared	5	77.38^{a} (0.02)	81.17^{a} (0.05)	84.53^{a} (0.09)	3.62^{c} (0.01)
		10	77.85^{a} (0.02)	81.67^{a} (0.01)	84.81^{a} (0.01)	3.39^{c} (0.15)
	Microwave	5	76.83^{a} (0.03)	80.07^{a} (0.04)	84.49^{a} (0.09)	4.29^{c} (0.09)
		10	77.73^{a} (0.20)	80.21^{a} (0.05)	84.33^{a} (0.20)	4.12^{c} (0.21)
	Combined	5	76.20^{a} (0.05)	80.32^{a} (0.09)	84.43^{a} (0.01)	4.59^{b} (0.09)
		10	77.28^{a} (0.01)	81.18^{a} (0.01)	84.74^{a} (0.15)	4.03^{c} (0.20)
53 %		0	77.87^{a} (0.14)	81.67^{a} (0.01)	86.28^{c} (0.05)	8.04^{a} (0.30)
	Infrared	5	77.29^{a} (0.20)	81.32^{a} (0.02)	84.65^{b} (0.01)	4.88^{c} (0.05)
		10	77.11^{a} (0.15)	81.32^{a} (0.02)	83.71^{b} (0.01)	4.51^{c} (0.09)
	Microwave	5	77.12^{a} (0.01)	81.02^{a} (0.03)	83.68^{b} (0.04)	5.71^{b} (0.20)
		10	76.88^{a} (0.09)	81.03^{a} (0.03)	83.81^{b} (0.02)	5.23^{b} (0.01)
	Combined	5	76.56^{a} (0.01)	80.52^{a} (0.05)	83.51^{b} (0.02)	4.78^{a} (0.05)
		10	77.31^{a} (0.02)	81.69^{a} (0.01)	83.43^{b} (0.03)	4.54^{ab} (0.02)

A Each value represents the mean of three determinations, SD in parenthesis. Means followed by different alphabets within column are significantly different at $p < 0.05$; Bambara groundnut seeds of 20 % or 53 % moisture content were heat-treated at 130 °C; Treatments were performed at 1200W. RUT-Raw untreated sample. T_o is gelatinization onset temperature, T_p is gelatinization peak temperature, and T_e is end of gelatinization temperature.

Table 2B
Effects of heat treatment methods of moisture conditioned (20 and 53 %) Bambara groundnut seeds on thermal profiles of resulting flours treated with an enzyme. A*

Moisture Content	Heat Treatment Method	Treatment time(min)	Onset, T_o (°C)	Peak, T_p (°C)	Endset, Tp (°C)	Enthalpy, ΔH J/g
RUT		0	77.94^{a} (0.02)	81.82^{a} (0.02)	87.22^{b} (0.01)	7.59^{a} (0.01)
20 %		0	77.31^{a} (0.01)	81.16^{a} (0.01)	87.30^{b} (0.02)	7.69^{a} (0.02)
	Infrared	5	77.31^{a} (0.01)	81.07^{a} (0.06)	86.51^{a} (0.01)	3.85^{c} (0.05)
		10	76.05^{a} (0.01)	81.62^{a} (0.19)	86.56^{a} (0.09)	3.48^{c} (0.02)
	Microwave	5	76.79^{a} (0.02)	81.01^{a} (0.01)	86.71^{a} (0.03)	4.06^{c} (0.01)
		10	76.31^{a} (0.09)	81.68^{a} (0.01)	88.19^{b} (0.02)	3.46^{c} (0.01)
	Combined	5	76.58^{a} (0.01)	81.11^{a} (0.18)	86.51^{a} (0.07)	5.55^{b} (0.01)
		10	76.31^{a} (0.09)	81.03^{a} (0.01)	85.31^{a} (0.05)	4.96^{b} (0.05)
53 %		0	77.31^{a} (0.05)	81.16^{a} (0.02)	87.31^{a} (0.02)	7.69^{a} (0.02)
	Infrared	5	76.53^{a} (0.04)	80.17^{a} (0.03)	86.23^{a} (0.01)	4.81^{c} (0.01)
		10	76.62^{a} (0.01)	81.32^{a} (0.05)	86.91^{b} (0.01)	4.75^{c} (0.02)
	Microwave	5	76.22^{a} (0.02)	81.52^{a} (0.03)	86.28^{a} (0.07)	5.91^{b} (0.04)
		10	76.19^{a} (0.03)	81.87^{a} (0.03)	86.20^{a} (0.56)	5.15^{b} (0.03)
	Combined	5	76.58^{a} (0.02)	80.23^{a} (0.26)	85.47^{a} (0.02)	4.16^{c} (0.03)
		10	76.05^{a} (0.20)	80.51^{a} (0.03)	85.93^{a} (0.01)	4.04^{c} (0.02)

A Each value represents the mean of three determinations, SD in parenthesis. Means followed by different alphabets within column are significantly different at $p < 0.05$; Seeds of 25 % or 53 % moisture content were heat treated at 130 °C; Treatments were performed at 1200W. RUT-Raw untreated sample. T_o is gelatinization onset temperature, T_p is gelatinization peak temperature, and T_e is end of gelatinization temperature.

proteins seemed to still surround or be on the starch granule [8]. Starch amylose and amylopectin can form a parallel layer on the protein with hydrogen bond formation [9].

3.4. Differential scanning calorimetry (DSC)

Bambara groundnut flours that resulted from buffer and enzyme protein hydrolysis conditions showed no significant differences in T_o and T_p endothermic temperatures ($P > 0.05$) (Table 2A and B, Fig S1 A-D). The untreated samples (RUT, C20 and C53) had the highest endothermic conclusion temperatures (T_e) and enthalpies (ΔH) for the 20 and 53 % moisture treatment when compared to heat-treated samples. The endothermic peak of the flour is most likely due to starch gelatinization, as discussed in our previous study [1]. As expected, the enzyme pre-treatment of flours showed an increased endothermic enthalpy (ΔH) due to increased starch content. These results are consistent with those of Yu-Sheng and Hai-Hua [16], who found an increase in gelatinization enthalpy of wheat flours after defatting or protein hydrolysis. Protein restricts starch swelling and competes for water with starch, and less water is available for starch gelatinization. A decrease in gelatinization enthalpy of samples that were heat-treated using combined heat treatment for 10 min may be related to the severity of heat treatment that have partially pre-gelatinised some of the starch granules. This is also evident in the microscopy micrographs that shows loss of birefringence.

3.5. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE)

In this study, SDS-PAGE of Bambara groundnut flours showed three major bands of molecular mass 118 kDa, 63 and 50 kDa, identified as legumin, α-vicilin and β-vicilin [17]. Bambara groundnut proteins are globulins of vicilin, legumin, and albumins. Prolamin and glutelin making up a small fraction. Vicilin is composed of three subunits (α, β, and γ), while legumin is a hexametric protein of α, β subunits that has cysteine residues that can form disulfide bonds [17–19] and is bonded together by hydrophobic interactions in legumes [20,21]. Native SPS-PAGE of Bambara groundnut proteins show legumin bands at 385 kDa and vicilin at 170 kDa [17]. Bambara groundnut vicilin protein bands (53 kDa, 65 kDa and 118 kDa) have been identified using SDS-PAGE [7,17,22].

The band intensity was low when samples were run under nonreducing conditions (Fig. 3A and B and C) and (Figure S4A, B and C). The intensity of the bands (legumin) increased from Infrared to microwave, and the highest intensity was among the combined heat-treated flours. The formation of high molecular weight protein polymers in Bambara groundnut seeds can be attributed to the high heat intensity of the combined heat treatment. The highly polymerized Bambara groundnut protein is caused by increased disulfide bonding of Bambara groundnut protein due to heat treatment. Peng, Kong [23] found that disulfide bonds increased in heated pea protein. Due to oxidation reactions, disulfide bonds are formed when the cysteine amino acids are joined at the intra or intermolecular bonds. Bambara groundnut protein unfolds as it denatures in response to heat treatment, exposing sites that allow for binding with free thiol groups and disulfide bonding formation. The changes in the bond formation of Bambara groundnut protein, forming polymeric structures joined by hydrogen bonds, ionic interactions, hydrophobic interactions, hydrogen bonding and possibly dityrosine cross-links were caused by heat treatment of Bambara groundnut seeds. Kudre and Benjakul [18] reported that heat treatment of Bambara groundnut protein isolates formed protein aggregates via disulfide bonds. Hydrophobic interactions may also be partly responsible for Bambara groundnut aggregation.

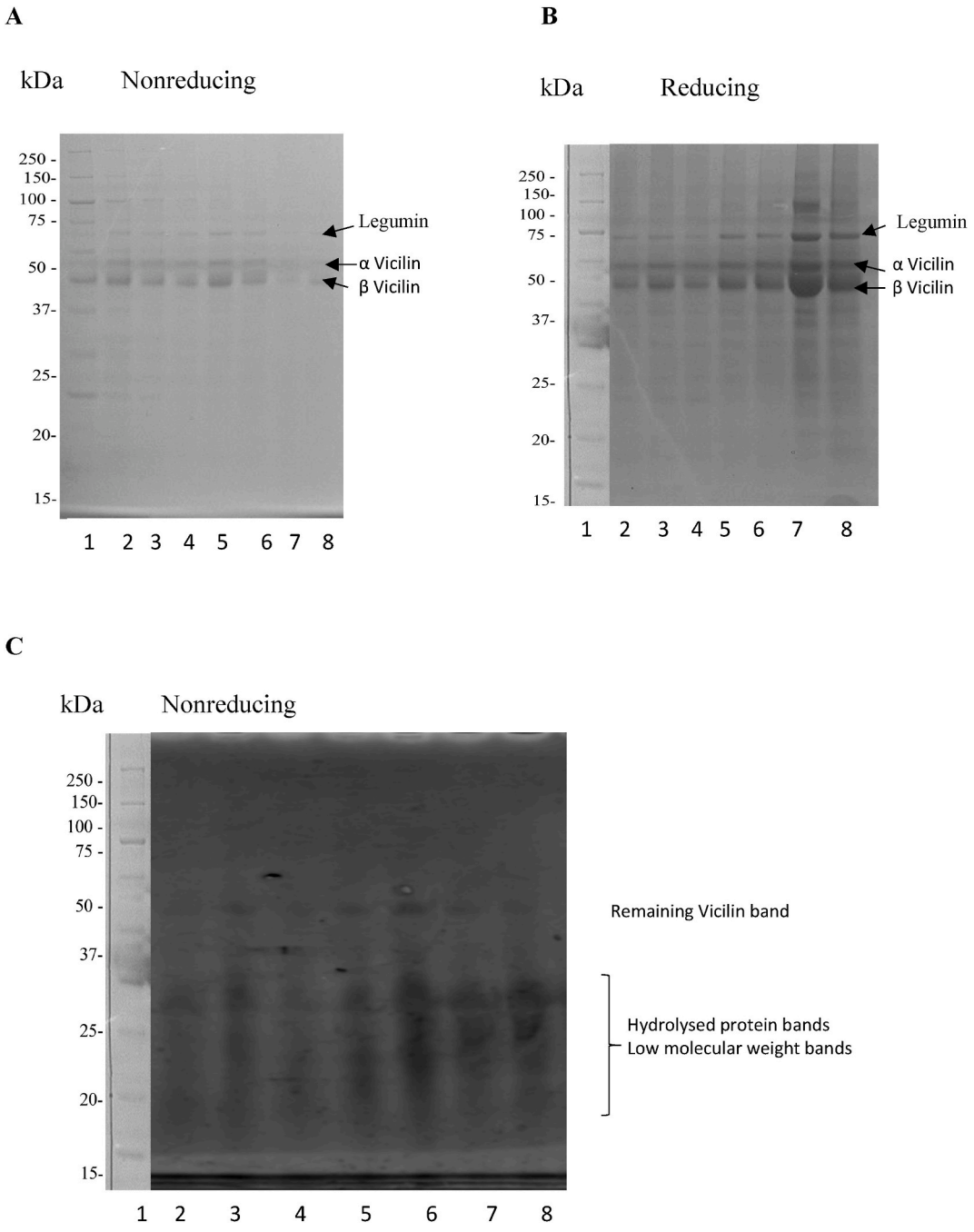

Fig. 3. A and B: SDS-PAGE under reducing and non-reducing condition of Bambara groundnut seeds of 20 % or 53 % moisture content were heat treated at 130 °C; Treatments were performed at 1200W.A and B:1- Standard proteins, 2- raw seeds, 3–20 % moisture 10 min infrared, 4–53% moisture 10 min infrared, 5–20 % moisture 10 microwave, 6–53 % moisture 10 min microwave, 7–20 % moisture,10 min combined, 8–53 % moisture 10 min combined. Fig. 3C depicts SDS-PAGE under non reducing conditions of 20 % and 53 % Bambara groundnut seed flours after protein hydrolysis with trypsin.

3.6. Fourier transform infrared (FTIR) spectroscopy

The Bambara groundnut flours FTIR spectra was analysed in the Amide 1 and II regions, which correspond to the 1600–1700 cm $^{-1}$ and 1500–1600 cm $^{-1}$ wavenumber respectively and can be found in Fig. S2 A and B. The C=O stretching vibrations can reveal changes in Amide I band and are used for secondary structure spectra analysis [24]. The amide II band is associated with N-H bend together with C-N stretching vibrations [25]. There was an increase in intensity with moisture conditioning at 20 and 53 % moisture levels

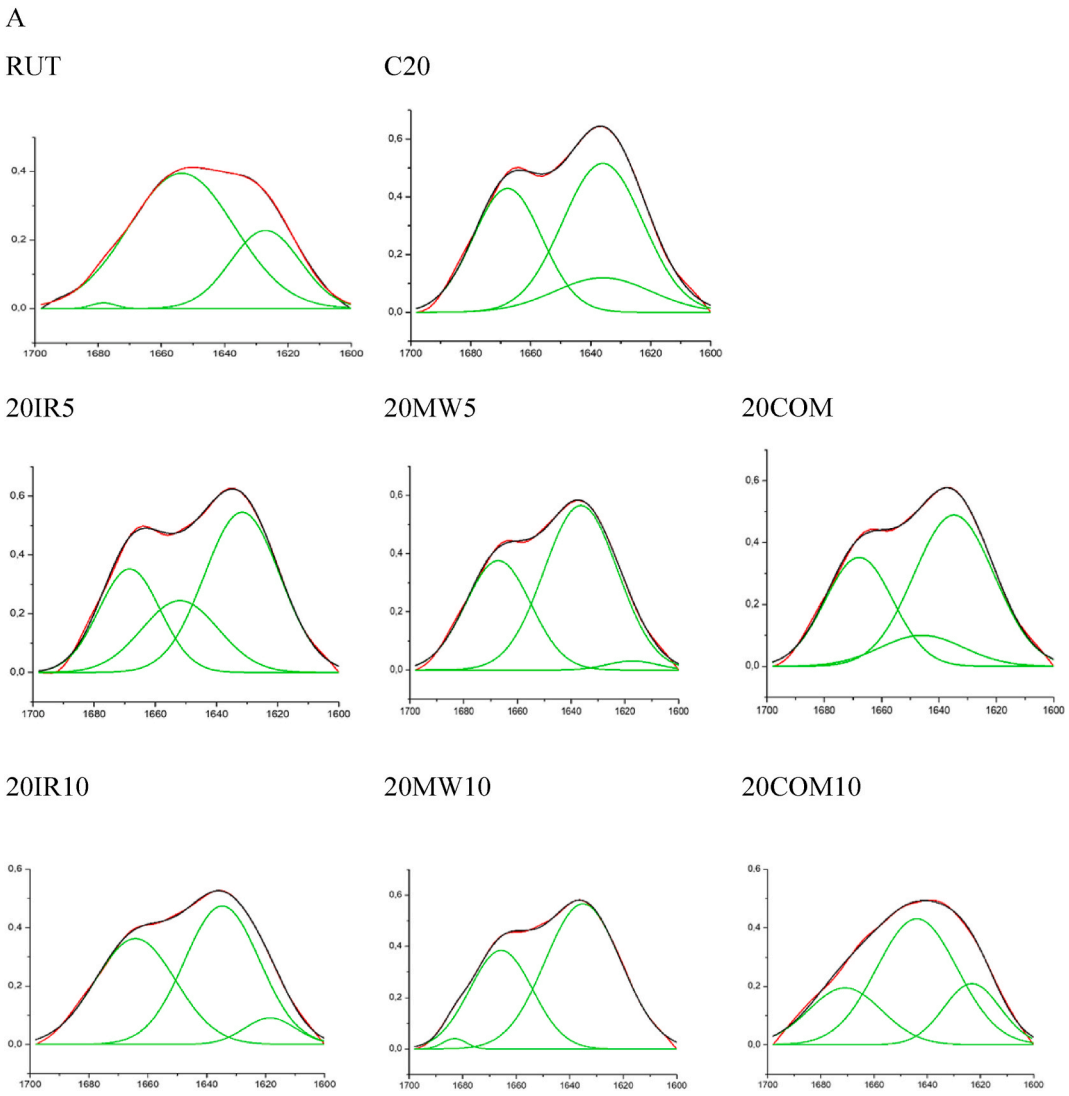

Fig. 4A. Effect of heat treatment using infrared, microwave and a combined treatment on Deconvoluted peaks of Amide I region of Bambara protein. RUT-raw seeds, C20- 20 % moisture, 20IR5- 5 min infrared, 20IR10-10 min infrared, 20MW5- 5 min microwave, 20MW10 - 10 min microwave, 20COM5- 5 min combined, 20COM10- 10 min combined. B.

compared with the untreated control, and these were slightly reduced with the application of heat treatment. The Bambara groundnut spectra were deconvoluted to further elucidate the changes caused by microwave and infrared heat treatment (Table S1 and Fig. 4A and B). The control showed a high level of α-helix of the native state of the untreated protein. Surprisingly, there was a change in the protein bands resulting from moisture conditioning. This may have been due to the heat during the drying of the seeds at 50 °C leading to changes in hydrogen bonding affecting the α-helix of the protein [26–28].

The application of infrared, microwave and a combined heat treatment leads to structural changes in Bambara groundnut protein as evidenced by alterations in amide I and amide II bands. For the flours from both infrared and microwave heat-treated seeds there a reduction in α -helix and an increase β-sheet. It was noted that the percentage of α -helix structure significantly reduced, and the β-sheet structure significantly increased along with an increase in heating time from 5 min to 10 min. The β-sheets were highest in the combined heat-treated seeds that were moisture conditioned to 53 % moisture level. There was a gradual reduction in the number of random coils as the heat treatment time was increased. The increase in β-sheets has been associated with the denaturation and rearrangement of protein and formation of aggregates of Bambara groundnut protein [29] and wheat protein [30]. A study by Wang, Luo [30] reported an increase in β-sheet in wheat protein as caused by heat treatment. Han, Wu [31] found similar results in rabbit myosin in response to heat treatment. Andlinger, Schrempel [32] studied the aggregation behaviour of potato protein (patatin) caused by heat treatment. They concluded that hydrophobic interactions were a major force behind aggregation at higher temperatures than disulfide bonding formation. In the current study, the observed changes in Bambara groundnut protein secondary structures, that lead to aggregation formation via disulfide bonding and hydrophobic interactions were caused by the heat treatment of Bambara groundnut

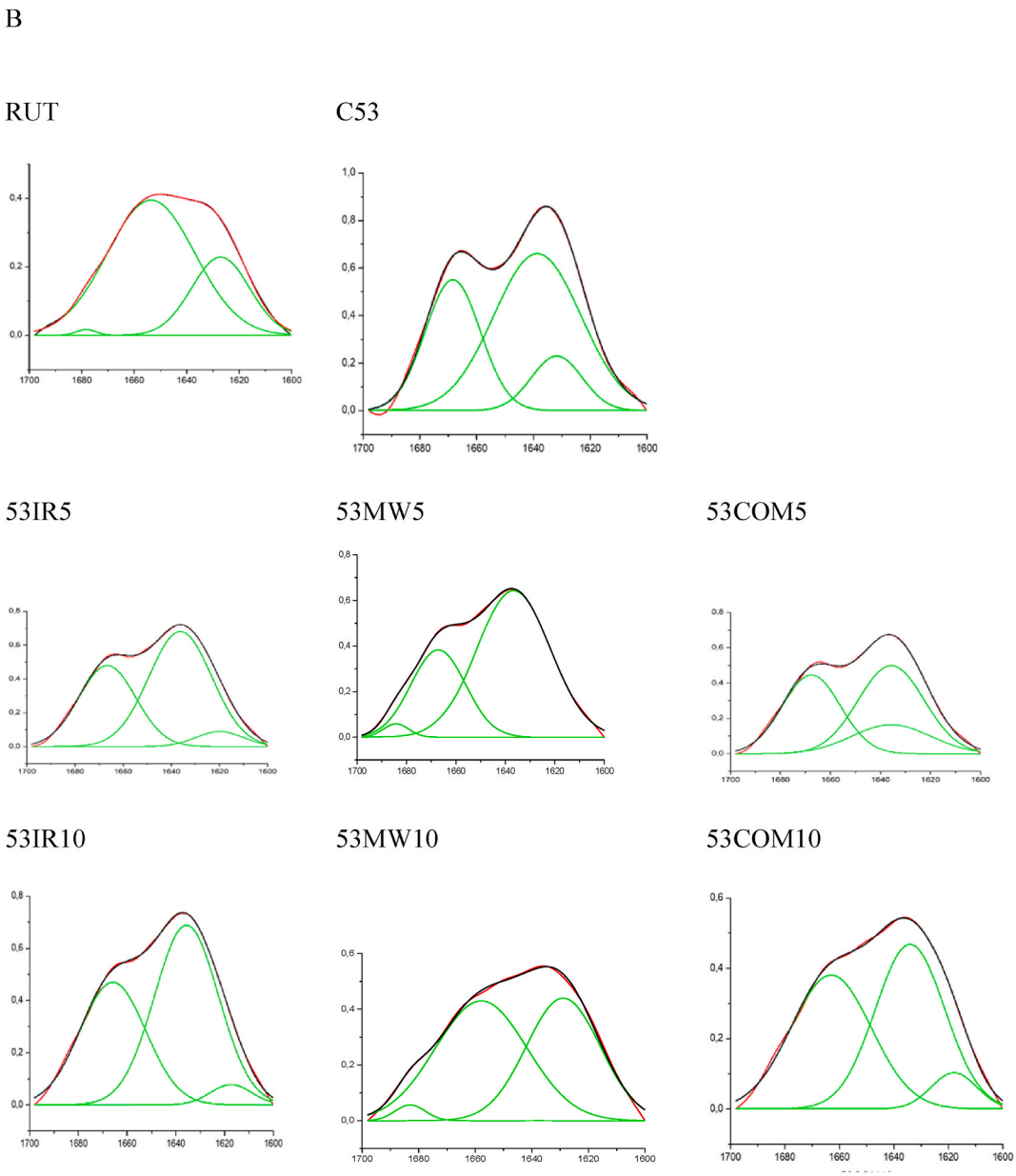

Fig. 4B. Effect of heat treatment using infrared, microwave and a combined treatment on Deconvoluted peaks of Amide I region of Bambara protein.
A. RUT-raw seeds, C53- 53 % moisture, 53IR5- 5 min infrared, 53IR10-10 min infrared, 53MW5- 5 min microwave, 53MW10- 10 min microwave, 53COM5- 5 min combined, 53COM10- 10 min combined.

seed.

4. Conclusions

This study demonstrates that microwave and infrared heat treatment of Bambara groundnut seeds results in structural changes in Bambara groundnut protein. Heat treatment caused the protein to denature, exposing hydrophobic sites and adhering to starch, forming a hydrophobic barrier. CLSM micrographs revealed aggregates of protein and starch caused by heat treatment. Disulfide bonds strengthen the protein barrier and further reduce water access to starch, causing low paste viscosities. In response to heat treatment, secondary structure protein conformational changes by FTIR as evidenced by a decrease in α-helix along with an increase in β-sheet sheet. It seems like the conformational changes in Bambara groundnut protein probably caused the formation of disulfide bonding in response to heat treatment, causing a reinforcement of the matrix around the starch, forming aggregated structures. After protein

hydrolysis, there was no protein signal detected by CLSM, and the starch was well-distributed and not aggregated. Hydrolysis of protein from heat-treated flours led to an increase in paste viscosities, which indicates the successful hydrolysis of the protein barrier around the starch, which allowed starch to imbibe more water and form a viscous paste.

CRediT authorship contribution statement

Peter Mukwevho: Writing – review & editing, Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Naushad M. Emmambux:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Statement of data availability

To be made accessible on a reasonable request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Peter Mukwevho reports was provided by National Research Foundation of South Africa. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40093.

References

[1] P. Mukwevho, M.N. Emmambux, Effect of infrared and microwave treatments alone and in combination on the functional properties of resulting flours from Bambara groundnut seeds, LWT 153 (2022).
[2] A. Mwangwela, R. Waniska, A. Minnaar, Effect of micronisation temperature (130 and 170°C) on functional properties of cowpea flour, Food Chem. 104 (2007) 650–657.
[3] O.M. Ogundele, M.N. Emmambux, *Effect of infrared heating of pre-soaked whole and dehulled Bambara groundnut (***Vigna subterranea***) seeds on their cooking characteristics and microstructure*, LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 97 (2018) 581–587.
[4] Y. Ding, et al., Effects of endogenous proteins and lipids on structural, thermal, rheological, and pasting properties and digestibility of adlay seed (Coix lacryma-jobi L.) starch, Food Hydrocolloids (2021) 111.
[5] J. Mubaiwa, et al., Bambara groundnut (Vigna subterranea (L.) Verdc.) flour: a functional ingredient to favour the use of an unexploited sustainable protein source, PLoS One 13 (10) (2018) e0205776.
[6] O.C. Wokadala, S.S. Ray, M.N. Emmambux, Occurrence of amylose-lipid complexes in teff and maize starch biphasic pastes, Carbohydrate Polymers 90 (1) (2012) 616–622.
[7] A.K. Arise, et al., Structure, composition and functional properties of storage proteins extracted from Bambara groundnut (Vigna subterranea) landraces, International Journal of Food Science & Technology 52 (5) (2017) 1211–1220.
[8] J. Ye, et al., Effect of endogenous proteins and lipids on starch digestibility in rice flour, Food Res. Int. 106 (2018) 404–409.
[9] Y. Yang, et al., Effect of removal of endogenous non-starch components on the structural, physicochemical properties, and in vitro digestibility of highland barley starch, Food Hydrocolloids 117 (2021).
[10] C. Barbara, et al., Proteomics and Bryophytes: a comparison between different methods of protein extraction to study protein synthesis in the aquatic moss Leptodictyum riparium(Hedw.), Caryologia 60 (1–2) (2007) 102–105.
[11] S. Yadav, et al., Comparison and optimization of protein extraction and two-dimensional gel electrophoresis protocols for liverworts, BMC Res. Notes 13 (2020), https://doi.org/10.1186/s13104-020-4929-1.
[12] H. Tao, et al., Removing surface proteins promote the retrogradation of wheat starch, Food Hydrocolloids (2021) 113.
[13] M.I. Saleh, J.-F. Meullenet, Effect of moisture content at Harvest and degree of milling (based on surface lipid content) on the texture properties of cooked long-grain rice, Cereal Chem. 84 (2) (2007) 119–124.
[14] M.A. Fitzgerald, et al., Viscosity of rice flour: a rheological and biological study, J. Agric. Food Chem. 51 (8) (2003) 2295–2299.
[15] A. Becker, S.E. Hill, J.R. Mitchell, Relevance of amylose-lipid complexes to the behaviour of thermally processed starches, Starch - Starke 53 (3–4) (2001) 121–130.
[16] W. Yu-Sheng, C. Hai-Hua, Effect of defatting or protein-removing treatment of wheat flour on its pasting, thermal and rheological properties, Curr. Top. Nutraceutical Res. 17 (1) (2018) 99–104.
[17] C.F. Diedericks, et al., Extraction, gelation and microstructure of Bambara groundnut vicilins, Food Hydrocolloids 97 (2019).
[18] T.G. Kudre, S. Benjakul, *Effects of binary organic solvents and heating on lipid removal and the reduction of beany odour in Bambara groundnut (***Vigna subterranean***) flour*, Food Chem. 141 (2) (2013) 1390–1397.

[19] J. Yang, et al., Foaming and emulsifying properties of extensively and mildly extracted Bambara groundnut proteins : a comparison of legumin. Vicilin and Albumin Protein, 2022.
[20] A.C.Y. Lam, et al., Physicochemical and functional properties of protein isolates obtained from several pea cultivars, Cereal Chem. 94 (1) (2017) 89–97.
[21] B. Guldiken, et al., Effect of variety and environment on the physicochemical, functional, and nutritional properties of navy bean flours, European Food Research and Technology 247 (7) (2021) 1745–1756.
[22] N.M. Busu, E.O. Amonsou, *Fractionation pH of Bambara groundnut (***Vigna subterranea***) protein impacts the degree of complexation with gum Arabic*, Food Hydrocolloids 87 (2019) 653–660.
[23] W. Peng, et al., Effects of heat treatment on the emulsifying properties of pea proteins, Food Hydrocolloids 52 (2016) 301–310.
[24] H. Susi, D.M. Byler, Fourier transform infrared study of proteins with parallel beta-chains, Arch. Biochem. Biophys. 258 (2) (1987) 465–469.
[25] L.P. Deflores, et al., Amide I'-II' 2D IR spectroscopy provides enhanced protein secondary structural sensitivity, J. Am. Chem. Soc. 131 (9) (2009) 3385–3391.
[26] J.D. Schofield, et al., The effect of heat on wheat gluten and the involvement of sulphydryl-disulphide interchange reactions, J. Cereal. Sci. 1 (4) (1983) 241–253.
[27] R.Y. Yada, Proteins in Food Processing, CRC Press, Woodhead Pub.: Boca Raton Cambridge, Eng, 2004.
[28] C. Pavalee, et al., Gluten conformation at different temperatures and additive treatments, Foods 11 (2022) 430, https://doi.org/10.3390/foods11030430.
[29] F.M. Ruzengwe, E.O. Amonsou, T. Kudanga, Transglutaminase-mediated crosslinking of Bambara groundnut protein hydrogels: implications on rheological, textural and microstructural properties, Food Res. Int. 137 (2020).
[30] K.-Q. Wang, et al., Changes in chemical interactions and protein conformation during heat-induced wheat gluten gel formation, Food Chem. 214 (2017) 393–399.
[31] Han, et al., The changes and relationship of structure and functional properties of rabbit myosin during heat-induced gelation, CyTA - J. Food 13 (1) (2015) 63–68.
[32] D.J. Andlinger, et al., Heat-induced aggregation kinetics of potato protein - investigated by chromatography, calorimetry, and light scattering, Food Chem. 389 (2022).