



Effect of infrared and microwave treatments alone and in combination on the functional properties of resulting flours from bambara groundnut seeds

Peter Mukwevho, M. Naushad Emmambux^{*}

Department of Consumer and Food Sciences, University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa

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ABSTRACT

The effect of heat treatment (130 °C surface temperature) of pre-conditioned (20 or 53% moisture) bambara groundnut seeds using infrared, microwave energy alone and in combination on functional properties of their flours were determined. Heat treatment caused reduction in water and nitrogen solubility indices of resulting flours. There was a combination effect of moisture conditioning, heat treatment and treatment time. Heat treatment caused starch pre-gelatinisation and denatured protein in resulting flours, which significantly increased with moisture level and treatment time. Endothermic peak by DSC decreased with increased treatment time for all heat treatments in flours from 53% moisture level seeds but not in 20% moisture level seeds. Pasting temperature of bambara groundnut flours significantly increased with heat-treatment times (0,5 and 10 min), but final paste viscosities decreased. Resulting flours from the combined treatment had a more significant reduction in pasting viscosities at both moisture levels than those resulting from infrared or microwave heat treatment. The low viscosity may be explained by unavailability of starch to form a viscous paste due to surrounding protein matrix around the starch granules. Resulting flours had low viscosities, this should be considered when compositing bambara groundnut to wheat for composite bread production.

1. Introduction

Bambara groundnut (*Vigna subterranean*) is an important indigenous African pulse crop and is considered nutrient-dense and drought-tolerant (Collinson et al., 1997; Mazahib et al., 2013). Bambara groundnut seeds are high in carbohydrates (50–60%) (Ajibade & Ijabadeniyi, 2019), proteins (18–24%) (Akande et al., 2009; Nti, 2009; Ogundele et al., 2017) but relatively low in fat (5.3–7.8%) (Ajibade & Ijabadeniyi, 2019). Bambara groundnut seed is also rich in minerals such as phosphorus, potassium, calcium (2.19%), iron and zinc (0.79%) (Yao et al., 2015). The bambara groundnut protein is high in essential amino acids leucine, methionine, and the limiting lysine (Arise et al., 2017; Okpuzor et al., 2010). Bambara groundnut is considered an underutilised indigenous pulse, as both the seed and its products, for example, flour are not widely consumed.

Bambara groundnut seeds are mostly consumed as roasted or cooked in excess water. However, widespread utilisation of bambara groundnut seed is hindered by the development of hard-to-cook phenomenon resulting from storage at high-temperature humidity (Nti, 2009; Plahar et al., 2002). Hard-to-cook (HTC) phenomenon increases the cooking

time to about 2–3 h or more in bambara groundnut seeds (Annan et al., 2002, p. 15). Milling of the bambara groundnut seeds can help to mitigate the hard-to-cook phenomenon challenge and flour properties are not changed due to the hard-to-cook phenomena. Bambara groundnut flour can be used in porridge, soup, and bread (Alozie et al., 2009; Barimalaa et al., 2005). Storage of bambara groundnut flour may lead to undesirable grassy or beany flavour, which can hinder its extensive application (Kudre & Benjakul, 2013). Lipoxygenase initiates the development of beany flavour by catalysing polyunsaturated lipids' oxidation and converts them to pentadienyl, leading to aldehydes and ketones formation (Doi et al., 1980; Kudre & Benjakul, 2013; Solina et al., 2005). Lipoxygenase can be inactivated in isolated bambara groundnut protein by heat treatment in a water bath and solvents, leading to reduced beany flavour (Kudre and Benjakul, 2013, 2014). Microwave (Jiang et al., 2016) and infrared energy (Bo Li et al., 2016) can also inactivate the enzyme, for example, lipoxygenase, that can contribute to beany flavour.

Microwave offer advantages such as selective absorption of heat energy and greater penetration depth, of up to 122 mm at 2.45 GHz (Tiwari & O'Donnell, 2012, pp. 8–220). Infrared advantages are rapid

^{*} Corresponding author. Postal address: Department of Consumer and Food Sciences, University of Pretoria, Private Bag X20, Hatfield, 0028, Pretoria, South Africa.
E-mail address: naushad.emmambux@up.ac.za (M.N. Emmambux).

and even heating with a penetration depth at a range of 0.31–4.76 mm (Eliasson et al., 2015; Skjöldebrand, 2001, pp. 208–228). When applied to moisture conditioned foods, microwave and infrared reduce energy requirements and are therefore more efficient than conventional heating systems, for example, convection oven (Datta & Ni, 2002; Ozturk et al., 2016). Moisture conditioning of seeds is important during microwave heat treatment (Thostenson & Chou, 1999) because water has dielectric properties and thus can absorb microwave energy and generate heat through molecular friction (Román et al., 2015). However, microwave radiation may heat some part of the grains more than others leading to burns in low moisture seeds.

Although heat moisture treatment can cause a reduction in beany flavour (Kudre and Benjakul, 2013, 2014), it can also affect the functional properties of the flour.

Infrared heating is an electromagnetic radiation at infrared range of 1.8–3.4 μm in wavelength that can transfer thermal energy (Zheng et al., 1998). Infrared radiation penetrates the seed coat of moisture conditioned grains. It causes water and seed molecules to vibrate mostly through stretching and bending. Infrared also results in water vapour pressure that may lead to seed rupture. Infrared heat treatment of moisture conditioned seeds lead to changes in resulting flours such as starch pre-gelatinisation, protein denaturation in legumes seed and cause a reduction in pasting viscosities (Arntfield et al., 2001; Cenkowski & Sosulski, 1998; Mwangwela et al., 2007; Ogundele et al., 2017).

It is worth noting that most research work on infrared and microwave processing of pulses was mostly done alone, and research on the use of combine infrared and microwave energy is limited.

The hypothesis is that resulting flours from moisture conditioned and heat-treated bambara groundnut seed using infrared, and microwave energy in combination will impact differently compared to the individual treatment. The functional properties of resultant flours from treated grains will be related to the microstructure and molecular structure. This is due to gelatinised starch and denatured protein, causing changes such as low pasting viscosities in resulting flour. Combining the two heating systems on bambara groundnut seeds may have a combination effect and change functional properties that each heat treatment alone may not provide. The combined microwave and infrared heat treatment benefit from the penetration depth of microwaves heating the interior parts of the food material; along with the surface heating of infrared waves allowing for uniform heating. The objective of this study was to determine the effects of the infrared, microwave, and a combination treatment of 20% moisture conditioned seeds and high moisture (53%) soaked seeds on functional and microstructural properties of resulting flours. Seeds moisture conditioned to 20% moisture will resemble dry cooking of seeds (Yoshida & Takagi, 1997), while seeds soaked to 53% moisture level will resemble moist cooking of seeds.

2. Experimental details

2.1. Materials

Bambara groundnut seeds were obtained from VKB Agriculture (Pvt) Ltd (Mokopane, South Africa). The grains were cleaned to remove any foreign material, broken and shrivelled seeds before use. Cleaned seeds were packaged in an airtight container then stored at 4 °C until use. All treatments were done in triplicate. All reagents were of analytical grade.

2.2. Methods

2.2.1. Pre-conditioning

Bambara groundnut seeds (100 g) with 8.4% initial moisture content were soaked in excess distilled water for 24 h at ambient temperature and covered in foil to reach about 53% moisture level following a method by Ogundele et al., (2018). To achieve the 20% moisture level, a

specific amount of distilled water was calculated following a method described by Arntfield et al. (1997) then sprayed on the grains in polyethylene bags. Seeds were then shaken overnight at ambient temperature to equilibrate to desired moisture content. Seed moisture content was determined after conditioning and the content was 19.6% and 53.3% for the 20% and 53% moisture conditioned seeds, respectively. The pH was 6.5 after pre-conditioning and was determined using a Hanna Instruments HI 2211 pH meter (Hanna Instruments, Woonsocket, RI, USA). Pre-conditioned (20 and 53% moisture levels) seeds were heat-treated as described below Raw untreated control seeds were placed in a polyethylene bag and stored in an airtight container at 4 °C until analysis.

2.2.2. Heat treatment

Infrared (IR), microwave (MW) and combined treatment were conducted using a Microwave/Infrared Hot air combination oven (Dephius Commercial & Industrial Technologies, Pretoria, South Africa). The oven used was fitted with three halogen lamps (0.2–4 μm) for infrared. Bambara seeds were spread in a single layer at a height of 23 cm away from the IR lamps. An infrared thermometer was used to detect the temperature at the surface. Seeds were heat-treated for 0, 5 and 10 min with a power of 1200 W for microwave and temperature set at 130 °C for infrared. Samples were cooled (1 h) at ambient temperature before being dried at 50 °C for 12 h in a hot air-drying oven to reach moisture of 5–11% for all treated samples. The heat-treated seeds were milled into flour to pass through a 500- μm sieve. The resulting flours were stored at 4 °C until the time of use.

2.3. Quality analysis

2.3.1. Proximate analysis

Moisture, crude fat and ash content for the bambara groundnut flour were determined according to AOAC (2000) international method 925.05, 922.06 and 923.02, respectively. The crude protein content of the bambara groundnut flours was determined using the Dumas method. A factor of 5.7 for nitrogen, was used to calculate crude protein in bambara groundnut flour determined using a Dumatherm (DT, Gerhardt Königswinter, Germany). The crude fibre of bambara groundnut flours was determined using the Fibertec FiberCap 2021/2023 system (FOSS Tecator AB, SE-263 21 Hoganas Sweden).

2.3.2. Flour colour measurements

The colour of the flours from untreated and heat-treated bambara groundnut seeds was determined using a Chroma Meter CR-400 (Konica Minolta Sensing Inc, Osaka, Japan). The CIELAB units, L* (lightness), a* (+a*-redness, -a*-greenness) and b* (+b*-yellowness, -b*-blueness) were recorded. The device was calibrated using a Minolta white calibration plate.

2.3.3. Pasting properties

Pasting properties of flours from raw, untreated, and infrared, microwave and combined treated bambara groundnut seeds were determined using a rheometer (Physica MCR101 model, Anton Paar GmbH, Ostfildern, Germany). Bambara groundnut flour (1.6 g dry basis) was dispersed in (w/w) distilled water to make 10% (W/V) suspension. 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.

2.3.4. Water solubility index (WSI) and water absorption capacity (WAC)

Water solubility index (WSI) was also performed, according to Shaman et al. (2017) with modification. Flour samples (2.5 g) were dispersed in 20 mL distilled water at pH 7 and placed in 30 °C water bath, with continuous stirring for 30 min, then allowed to cool and centrifuged at 3000×g for 15 min at 25 °C. The supernatant was

Table 1Effects of heat treatment methods of moisture conditioned (20 and 53%) bambara groundnut seeds on the proximate composition of resulting flours^a.

Moisture content	Heat treatment	Treatment time (min)	Moisture	Protein	Fat	Ash	Crude fibre	Soluble Carbohydrates by difference
Raw seeds 20%		0	8.4 ± 0.05	15.5 ^a ± 0.20	7.0 ^a ± 0.13	3.5 ^d ± 0.06	4.9 ^a ± 0.02	65.8 ^e ± 0.28
		0	9.3 ± 0.72	15.5 ^a ± 0.54	7.0 ^a ± 0.21	3.3 ^b ± 0.02	4.6 ^d ± 0.03	64.8 ^{cd} ± 0.12
	Infrared	5	7.4 ± 0.41	15.5 ^a ± 0.20	7.0 ^a ± 0.09	3.5 ^d ± 0.02	4.8 ^b ± 0.02	65.5 ^e ± 0.29
		10	7.1 ± 0.35	15.4 ^a ± 0.44	7.1 ^b ± 0.09	3.5 ^d ± 0.05	4.8 ^b ± 0.02	66.2 ^{ef} ± 0.78
	Microwave	5	7.3 ± 0.35	15.6 ^a ± 0.28	7.2 ^c ± 0.19	3.5 ^d ± 0.07	4.7 ^c ± 0.02	64.8 ^{cd} ± 0.12
		10	7.0 ± 0.43	15.5 ^a ± 0.45	7.4 ^{de} ± 0.03	3.5 ^d ± 0.02	4.7 ^c ± 0.02	66.8 ^{def} ± 1.04
53%	Combined	5	6.8 ± 0.25	15.7 ^a ± 0.24	7.5 ^{de} ± 0.23	3.5 ^d ± 0.09	4.7 ^c ± 0.03	64.9 ^{cd} ± 0.06
		10	6.1 ± 0.45	15.6 ^a ± 0.41	7.3 ^c ± 0.08	3.5 ^d ± 0.06	4.8 ^b ± 0.01	65.1 ^{de} ± 0.33
		0	10.4 ± 0.97	16.5 ^b ± 0.83	7.3 ^c ± 0.06	3.5 ^d ± 0.02	4.7 ^c ± 0.09	66.3 ^f ± 0.16
	Infrared	5	10.4 ± 0.97	16.7 ^b ± 0.64	7.3 ^{cd} ± 0.05	3.5 ^d ± 0.06	4.8 ^b ± 0.02	63.2 ^{ab} ± 0.45
		10	11.3 ± 0.35	16.9 ^b ± 0.31	7.4 ^{de} ± 0.03	3.3 ^b ± 0.02	4.7 ^c ± 0.04	62.3 ^a ± 0.41
	Microwave	5	9.3 ± 0.70	16.8 ^b ± 0.33	7.9 ^f ± 0.02	3.5 ^d ± 0.02	4.7 ^c ± 0.02	62.5 ^a ± 0.69
10		9.2 ± 0.40	16.1 ^b ± 0.06	7.3 ^c ± 0.04	3.4 ^c ± 0.02	4.7 ^c ± 0.02	63.4 ^{ab} ± 0.14	
Combined	5	8.2 ± 0.47	16.9 ^b ± 0.08	7.4 ^e ± 0.03	3.4 ^c ± 0.02	4.7 ^c ± 0.02	63.3 ^{ab} ± 0.30	
	10	9.3 ± 1.08	16.5 ^b ± 0.24	7.2 ^c ± 0.04	3.2 ^a ± 0.02	4.7 ^c ± 0.05	64.5 ^{cd} ± 0.19	

Mean values (g/100 g, dry basis except moisture) within a column with different letters differ significantly at $P < 0.05$, and the standard deviation (\pm).^a All values other than moisture are presented on a moisture-free basis.

decanted and evaporated in an air-oven at 105 °C for 16 h. WSI was determined as a ratio of dried supernatant's weight to the weight of flour expressed as percent (%). The remaining pellet was considered water absorption capacity and expressed as the weight of pellet (g) obtained per gram of dry ground sample.

2.3.5. Nitrogen solubility index

Nitrogen solubility index for flours from treated and untreated bambara groundnut seeds was determined using the method by [Ogundele et al. \(2017\)](#). Flour samples (1 g) were dispersed in 20 mL of 0.1M NaCl solution and stirred continuously for 1 h at 30 °C at pH 7. The pH was readjusted to pH7 with either NaOH or HCl. The suspension was centrifuged (9154.3 xg, 15 min, and 4 °C) and the supernatant filtered through a Whatman No. 1 filter paper. The residue from the suspension was re-washed twice in 10 mL of 0.1M NaCl solution at pH 7. The nitrogen solubility index was expressed as a percentage of the supernatant's total nitrogen content divided by total nitrogen content in a flour sample on a dry basis.

2.3.6. Thermal properties

The thermal properties of bambara groundnut flour from treated and untreated seeds were analysed with a high-pressure DSC system with STARe (HPDSC827e, Mettler Toledo, Greifensee, Switzerland) according to the method reported by ([Wokadala et al., 2012](#)). Flour samples (10 mg) were mixed with distilled water in the ratio of 3:1 (w/w) and equilibrated for at least 12 h at ambient temperature. An empty aluminium crucible was used as a reference. Scanning was done from 30 °C to 110 °C under high pressure (4 MPa using N₂) at a rate of 10 °C/min. Indium was used as a standard to calibrate DSC ($T_p = 156.6$ °C, 28.45 Jg⁻¹).

2.3.7. Light microscopy (LM)

Light microscopy (LM) of bambara groundnut flour and paste from heat-treated seeds were observed with a Nikon Optiphot Light Microscope (Nikon Corporation, Tokyo, Japan). Unpasted and pasted flours were dispersed in 1 ml of 30% glycerol solution then placed on a glass slide covered with a coverslip, either unstained or stained iodine. Starch birefringence was also observed under polarised light.

2.3.8. Confocal laser scanning microscopy (CLSM)

Pasted bambara groundnut flour was stained for protein using three drops of 0.02% Acid Fuchsin dye in 1% acetic acid. The samples were then incubated for 1 min in an oven at 60 °C then observed using A Zeiss LSM 510 META Confocal Laser Scanning Microscope (Zeiss SMT, Jena, Germany). Plane neoflar100x and Numerical aperture (NA) 1.4 was used

for the blend images. Excitation and emission spectra were 405 nm and 425–475 nm, respectively. The pixel time for both tracks 1 was 12.8 μ s, and the picture size was 512 x 512 pixels.

2.4. Statistical analysis

The statistical design was a 2*3*3 factorial design as two levels of 20% and 53% moisture conditioning, heat treatment of infrared and microwave treatment alone or in combination at different treatment times 0, 5 min and 10 min. Statistical analysis was performed using SPSS 26.0 statistical software for IBM (SPSS, Inc., New York, NY). Multifactor Analysis of Variance (MANOVA) was performed on the data and compared at $P \leq 0.05$ using Fisher's least significant difference (LSD) test following the general linear model (GLM) procedure. The means were separated by using the least significant difference (LSD) test. Independent variables were heat treatment methods (infrared, microwave, and a combination of both), treatment times (0, 5 and 10 min) and moisture levels (20% and 53%). The dependent variables were the measured values.

3. Results and discussion

The proximate of bambara groundnut flour ([Table 1](#)) from untreated seeds showed protein content of 15.5 g/100 g, carbohydrates 65.8 g/100 g, fat content of 7.0 g/100 g, crude fibre content of 4.9 g/100 g, ash content of 3.5 g/100 g and moisture was 8.4 g/100 g. The treated flour had a range of 6.1–11.3 g/100 g for moisture, 15.4–16.9 g/100 g for protein, 7.0–7.9 g/100 g for fat, 3.2–3.5 g/100 g for ash, 4.6–4.9 g/100 g for crude fibre and 62.3–66.8 g/100 g for carbohydrates by subtraction. The variation in final moisture is due to differences in conditioning and soaking along with the differences in heat treatment methods used. In terms of the power, infrared was 500 W per lamp and microwave was 2000 W. The changes in composition among treatments are due to the variations in moisture conditioning (soaking vs conditioning of seeds) and the variations in heat treatments used compounded by the different times that were applied at high temperature and high power (130 °C and 1200 Watts). These differences are similar to those of [Mubaiwa et al. \(2018\)](#) in their study they roasted bambara groundnut seeds. The higher fat content for the treated seeds could be because the lipids were more extractable compared to raw seeds. Pigments and soluble solids, mostly soluble carbohydrates, can leach out of the seed during soaking and this can lead to an increase in the proportion of protein and fat ([Mwangwela, 2006](#); [Siqueira et al., 2013](#))

Table 2

Effects of heat treatment treatments on the colour of resulting flours from moisture conditioned (20 and 53%) bambara groundnut seeds.

Moisture content	Heat treatment	Treatment time (min)	L*	a*	b*	h°	ΔE*ab	
Raw seeds		0	88.6 ^b ± 0.03	1.1 ^k ± 0.02	9.5 ^j ± 0.02	83.3 ⁱ ± 0.01	–	
20%	Infrared	0	88.3 ^c ± 0.3	1.1 ^k ± 0.00	9.0 ^m ± 0.02	83.0 ^{hi} ± 0.01	4.2 ^f ± 0.13	
		5	88.2 ^d ± 0.01	1.5 ^h ± 0.02	10.4 ⁱ ± 0.03	82.0 ^{de} ± 0.02	4.7 ^h ± 0.11	
	Microwave	5	88.0 ^e ± 0.02	1.5 ^h ± 0.01	10.9 ^f ± 0.01	82.2 ^g ± 0.02	4.3 ^f ± 0.05	
		10	87.6 ^g ± 0.08	1.2 ^j ± 0.01	10.6 ^g ± 0.01	82.9 ^g ± 0.02	3.4 ^d ± 0.04	
	Combined	5	85.5 ^m ± 0.01	2.6 ^b ± 0.02	12.9 ^h ± 0.01	76.4 ^b ± 0.02	8.2 ⁱ ± 0.05	
		10	87.3 ⁱ ± 0.01	2.1 ^c ± 0.01	11.6 ± 0.01	79.2 ^{de} ± 0.01	5.4 ⁱ ± 0.12	
	53%	Infrared	0	88.8 ^a ± 0.04	1.1 ^k ± 0.01	9.3 ^j ± 0.02	83.2 ^{hi} ± 0.02	4.2 ^f ± 0.07
			5	88.5 ^b ± 0.02	1.3 ⁱ ± 0.02	9.4 ^k ± 0.02	80.8 ^f ± 0.02	4.6 ^g ± 0.05
Microwave		5	87.5 ^f ± 0.01	1.8 ^f ± 0.04	9.5 ⁱ ± 0.02	78.9 ^e ± 0.01	3.7 ^e ± 0.12	
		10	86.8 ^k ± 0.02	1.5 ^e ± 0.01	10.6 ^d ± 0.01	79.9 ^e ± 0.02	3.2 ^c ± 0.09	
Combined		5	85.5 ^j ± 0.02	1.7 ^e ± 0.02	11.6 ^d ± 0.02	80.3 ^e ± 0.01	3.3 ^c ± 0.14	
		10	86.0 ^h ± 0.01	1.6 ^g ± 0.01	11.2 ^e ± 0.01	81.6 ^f ± 0.02	2.8 ^b ± 0.09	
			10	85.4 ⁱ ± 0.01	1.9 ^d ± 0.01	12.6 ^h ± 0.01	79.8 ^e ± 0.02	2.2 ^a ± 0.10

Each value represents the mean of three determinations, ± S.D. Means followed by different alphabets within a column are significantly different at $P < 0.05$; Seeds of 20% or 53% moisture content were heat-treated at 130 °C; Treatments were performed at 1200 W. RUT-Raw untreated sample. Colour parameters were lightness to darkness (L*), redness to greenness (a*) and yellowness to blueness (b*), hue angle (h°) and total colour difference (ΔE*ab).

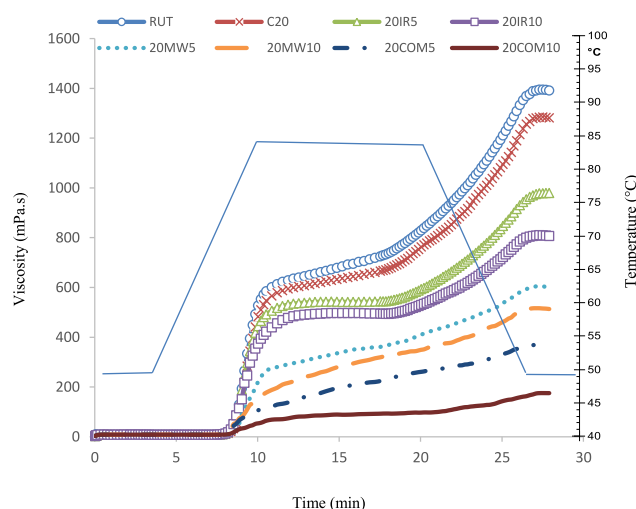


Fig. 1A. Effect of resulting flours from infrared, microwaved processing alone and in combination of bambara groundnut seeds at 20% moisture on viscosity during pasting. RUT-raw seeds, C20- 20% moisture, 20IR5- 5 min infrared, 20IR10-10 min infrared, 20MW5- 5 min microwave, 20MW10- 10 min microwave,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

3.1. Colour

Moisture conditioning of the seeds at 20% decreased the lightness, but high moisture conditioning increased the lightness of the resulting flours (Table 2). This may be due to the leaching of colour compounds during moisture conditioning/soaking of the seeds (Bayram et al., 2004). There was a significant ($P < 0.000$) 3-way interaction of heat treatment*moisture condition*treatment time for all measured colour values. Heat treatment methods caused a reduction in flour lightness value along with an increase in redness and yellowness in recorded colour values across the treatment methods. These changes increased with increasing treatment time. The 20% moisture conditioned seeds resulted in higher a* and b*, h° and ΔE*ab values than those from 53% moisture seeds. Studies conducted on cowpeas, lentils and bambara groundnut seeds ascribed browning of pre-conditioned and heat-treated seeds to Maillard reaction (Mubaiwa et al., 2018; Mwangwela, 2006; Mwangwela et al., 2007). Flours resulting from infrared heat treatment had the lowest change in lightness, yellowness, and redness values at both moisture levels and after 5 and 10 min of treatment time.

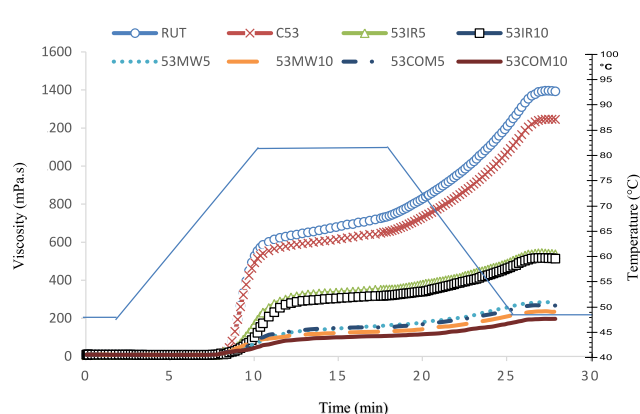


Fig. 1B. Effect of resulting flours from infrared, microwaved processing alone and in combination of bambara groundnut seeds at 53% moisture on viscosity during pasting. RUT-raw seeds, C53- 53% moisture, 53IR5- 5 min infrared, 53IR10-10 min infrared, 53MW5- 5 min microwave, 53MW10- 10 min microwave,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

3.2. Pasting properties of resulting flours

Pasting properties of resulting flours from moisture conditioned infrared, microwave and combined heat treatments are presented in Fig. 1A and B for the 20% and 53% moisture levels, respectively. There was a general reduction in pasting viscosities for the treatment compared to control, with infrared having the least effect followed by microwave and the highest viscosity decrease for combined treatment. Flours from 53% moisture had a more significant reduction for the combined treatment ($P < 0.05$). Other studies have shown similar findings using infrared treated cowpeas (Mwangwela et al., 2007) and bambara groundnut (Ogundele et al., 2017). During heat treatment of bambara groundnut seed, there are microstructural changes within the grain, some of which might reduce viscosities of resulting flours. These are discussed in more detail later.

3.3. Water absorption capacity (WAC) and water solubility index (WSI)

WAC of untreated and resulting flours from heat-treated flours from 20% moisture seeds increased from about 1.8 g/g for untreated to 3.7 g/g for resulting flours from infrared heat-treated seeds (Table 3). The flours resulting from 53% moisture conditioned seeds also increased from 1.9 and increased to 3.1 g/g for 10 min microwaved seeds then

Table 3

Effects of infrared and microwave heat treatment conditioned at 20 and 53% moisture bambara groundnut seeds on functional properties of resulting flours.

Moisture Content	Heat treatment	Treatment time (min)	WAC (g/g)	WSI (%)	NSI (%)	
Raw seeds		0	1.7 ^a ± 0.1	31.8 ^a ± 0.1	155.5 ^c ± 1.2	
20%	Infrared	0	1.8 ^a ± 0.3	31.2 ^b ± 0.3	145.7 ^d ± 3.6	
		5	2.3 ^b ± 0.3	29.5 ^{bcd} ± 0.8	100.6 ^f ± 3.6	
		10	2.6 ^{bc} ± 0.2	23.8 ^{gh} ± 1.4	82.5 ^h ± 3.5	
		10	2.4 ^{bc} ± 0.1	29.4 ^{bcd} ± 0.3	109.1 ^e ± 2.1	
	Microwave	5	2.9 ^{cd} ± 0.2	22.0 ^h ± 1.2	43.2 ^k ± 4.5	
		10	3.7 ^e ± 0.1	15.2 ^j ± 0.7	48.5 ^j ± 1.3	
	Combined	5	3.3 ^d ± 0.2	15.0 ⁱ ± 0.1	32.7 ^h ± 2.3	
		10	3.3 ^d ± 0.2	15.0 ⁱ ± 0.1	32.7 ^h ± 2.3	
	53%	Infrared	0	1.9 ^a ± 0.2	30.9 ^{bd} ± 1.1	172.4 ^a ± 7.3
			5	2.4 ^b ± 0.1	30.1 ^b ± 0.9	159.9 ^b ± 4.0
10			2.5 ^{bc} ± 0.4	26.0 ^{ef} ± 1.3	155.7 ^c ± 4.3	
10			3.0 ^d ± 0.3	27.1 ^{def} ± 0.4	67.1 ⁱ ± 1.0	
Microwave		5	3.1 ^d ± 0.4	24.4 ^g ± 0.4	47.2 ^j ± 3.4	
		10	2.8 ^{cd} ± 0.2	19.5 ⁱ ± 1.0	92.3 ^g ± 8.0	
Combined		5	2.5 ^c ± 0.1	18.1 ⁱ ± 0.2	78.3 ^h ± 1.3	
		10	2.5 ^c ± 0.1	18.1 ⁱ ± 0.2	78.3 ^h ± 1.3	

Each value represents the mean of three determinations, ± S.D. Means followed by different alphabets within a column are significantly different at $P < 0.05$; Seeds of 25% or 53% moisture were heat-treated at 130 °C; Treatments were performed at 1200 W; WAC- water absorption capacity; WSI-water solubility index, NSI- nitrogen solubility index.

decreased to 2.5 g/g in the 10 min combined treatment. WAC increased with increasing heating time for both 20% and 53% moisture seeds, but this trend was reversed for the combined treatment. This may be due to the intensity and severity of the combined treatment on high moisture conditioned seeds. There was higher heat generation due to higher molecular vibrations and rotations as water is a dipolar molecule to

result in higher heat generation. This will lead to higher extent protein denaturation. Denatured protein exposes hydrophobic sites and may have less affinity to water (Naryana & Narasinga Rao, 1982). There was a combination three-way interaction effect of moisture level, heat treatment and treatment time ($P < 0.006$). An increase in moisture level and treatment time resulted in an increased heat treatment effect on WAC.

Flours from the 20% moisture conditioned seeds had WSI ranging from 31.2% for control to a low of 15.0 for the 10 min combined treatment (Table 3). WSI for flours from 53% moisture conditioned seeds ranged from 30.9 for control to a low of 18.1 for the 10 min combined treatment. WSI increased with increased heating time for all heat treatment methods; WSI was lowest in the combined treatment. These findings agreed with Ogundele and Emmambux (2018), who found that infrared heat treatment reduced WSI of flours resulting from whole and dehulled bambara groundnut.

3.4. Nitrogen solubility index (NSI)

There was a significant reduction in NSI of flours resulting from treated seeds compared to those resulting from untreated seeds ($P < 0.05$) (Table 3). For all heat treatment methods (IR, MW and combined treatment), an increase in heat treatment time resulted in a significant reduction in NSI ($P < 0.05$). Ogundele et al. (2017) reported a reduction in NSI of infrared heat-treated bambara groundnut. Shimelis and Rakshit (2005) used common beans to study the effect of microwave heating on protein solubility and reported a decreased protein solubility with increasing heating time. Protein denaturation and aggregation may be responsible for the reduction in NSI (Salazar-Villanea et al., 2016) as heat treatment are increased.

Flours resulting from seeds moisture conditioned to 53% recorded a higher NSI than those from the untreated and 20% moisture content seeds. Ogundele et al. (2017) found NSI of resulting flours from infrared heat-treated bambara groundnut seed increased with increasing heating time on high moisture (53%) conditioned seeds. Mwangwela et al. (2007) reported similar results on low moisture infrared heat-treated cowpea seeds. Seeds conditioned to higher moisture and infrared treated have been reported to have a lower NSI (Arntfield et al., 1997).

3.5. Thermal properties

Flours resulting from IR, MW and combined treatment exhibited a

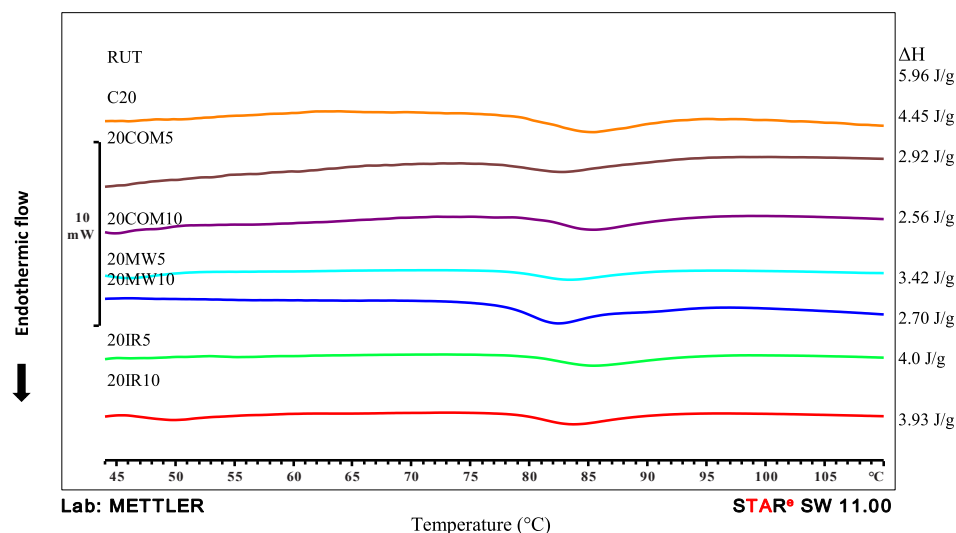


Fig. 2A. Differential scanning calorimeter thermograms of resulting flours from untreated, infrared, microwaved processing alone and in combination of bambara groundnut seeds with 20% moisture level. RUT-raw seeds, C20- 20% moisture, 20IR5- 5 min infrared, 20IR10-10 min infrared, 20MW5- 5 min microwave, 20MW10 - 10 min microwave, 20COM5- 5 min combined, 20COM10- 10 min combined.

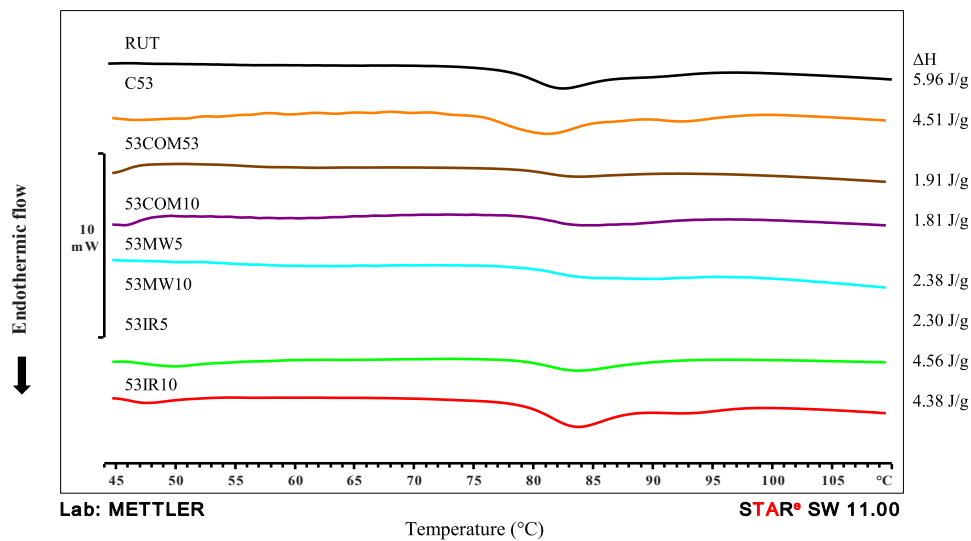


Fig. 2B. Differential scanning calorimeter thermograms of resulting flours from untreated, infrared, microwaved processing alone and in combination of bambara groundnut seeds at 53% moisture level. RUT-raw seeds, C53- 53% moisture, 53IR5- 5 min infrared, 53IR10-10 min infrared, 53MW5- 5 min microwave, 53MW10- 10 min microwave, 53COM5- 5 min combined, 53COM10- 10 min combined.

Table 4
Effects of heat treatment methods of moisture conditioned (20 and 53%) bambara groundnut seeds on thermal temperatures of resulting flour.

Moisture content	Heat treatment	Treatment time (min)	Onset, T _o (°C)	Peak, T _p (°C)	Endset, T _e (°C)		
20%	Raw seeds	0	72.7 ^a ± 0.1	80.0 ^a ± 0.3	87.1 ^b ± 0.2		
		Infrared	5	76.1 ^c ± 0.3	81.9 ^b ± 0.2	88.9 ^c ± 0.2	
			10	77.7 ^{def} ± 0.7	83.4 ^c ± 0.4	90.4 ^c ± 0.4	
	Microwave	5	77.8 ^{def} ± 1.0	84.5 ^{cd} ± 0.5	92.1 ^{ef} ± 0.6		
		10	77.4 ^{de} ± 0.6	83.1 ^c ± 0.3	90.9 ^{cd} ± 0.7		
			77.2 ^d ± 0.5	82.7 ^{bcd} ± 1.1	89.3 ^d ± 0.2		
		Combined	5	79.1 ^{def} ± 0.9	85.2 ^c ± 0.1	92.7 ^e ± 0.2	
	10		79.9 ^e ± 0.3	85.3 ^d ± 1.2	93.2 ^f ± 0.3		
	53%	Raw seeds	0	75.2 ^b ± 0.2	79.9 ^a ± 0.3	85.7 ^a ± 0.1	
			Infrared	5	77.4 ^d ± 0.3	83.5 ^c ± 0.4	92.3 ^e ± 0.2
				10	79.0 ^{de} ± 0.4	84.1 ^{cd} ± 0.2	94.0 ^{ef} ± 0.6
		Microwave	5	77.2 ^d ± 0.2	82.3 ^c ± 0.3	87.8 ^b ± 0.3	
10			78.4 ^d ± 0.3	84.3 ^{cd} ± 0.5	93.0 ^e ± 0.2		
			81.2 ^f ± 0.1	86.4 ^e ± 0.2	94.0 ^f ± 0.6		
Combined			5	81.8 ^f ± 0.4	87.8 ^f ± 0.3	94.5 ^{fg} ± 0.5	
		10	81.8 ^f ± 0.4	87.8 ^f ± 0.3	94.5 ^{fg} ± 0.5		

T_o is gelatinisation onset temperature, T_p is gelatinisation peak temperature, and T_e is end of gelatinisation temperature. Each value represents the mean of three determinations, ± S.D. Means followed by different alphabets within a column are significantly different at P < 0.05.

single endothermic peak (about 72.2–94.5 °C) (Fig. 2A and B). There was a three-way interaction effect of moisture conditioner (20 and 53% moisture level), heat treatment (130 °C, 1200 Watts) and treatment time (5 and 10 min) on endothermic peak and enthalpy of the endothermic peak. The endothermic peak energy of resulting flours decreased

significantly with increasing heat treatment time from 0, 5 and 10 min (P > 0.050) at both moisture levels. The decrease in endothermic peak has already been reported by Mwangwela et al. (2007) for flours from infrared treated cowpea seeds and Ogundele et al. (2017) for flours from infrared treated bambara groundnut. This may mean that the starch in the treated samples were partly gelatinised.

There was a higher decrease in endothermic enthalpy for microwave than the infrared treated sample, with combined treatment having the highest reduction compared to control. Infrared heating and microwave heating use different ways to heat food material. During infrared heating of food material, electromagnetic radiation in the infrared region generates heat within the food using infrared waves (0.78–1000 μm) through molecular vibration. Infrared waves have low penetration depth, and not all the infrared radiation is absorbed as some are reflected and scattered.

On the other hand, microwaves have a higher penetration depth compared to infrared energy. Microwave cause dipolar rotation and ionic conduction of water and other polar biomolecules within the food. Microwave heating uses an energy conversion mechanism where electromagnetic energy is transferred to thermal energy within the food (Thostenson & Chou, 1999). Thus, the different heating mechanisms, penetration depth and heat intensities for infrared and microwave may be responsible for the different effects in terms of reduction in the endothermic peak area relate to the energy during the thermal transition of bambara groundnut flours.

Even though there was a significant decrease in endothermic peak energy of the flours from treated grains, there was an increase in the endothermic temperatures from infrared, microwave, to combined treatments. The combined treatment had the highest endothermic temperatures for the 10 min and 53% moisture treatment (Table 4). This may suggest that some crystalline structures that melt at higher temperatures could be formed. Starch molecules are known to reassociate and can form higher-order structures during heat treatment. Mapengo and Emmambux (2020) suggested that new bonds may be formed because of infrared heat treatment.

3.6. Light and confocal laser scanning microscopy

Fig. 3A–C shows light microscopy micrographs of resulting flours (1–3) and paste (4–5) from IR, MW, and combined treatment. Under polarised light, Maltese crosses were visible in untreated samples and

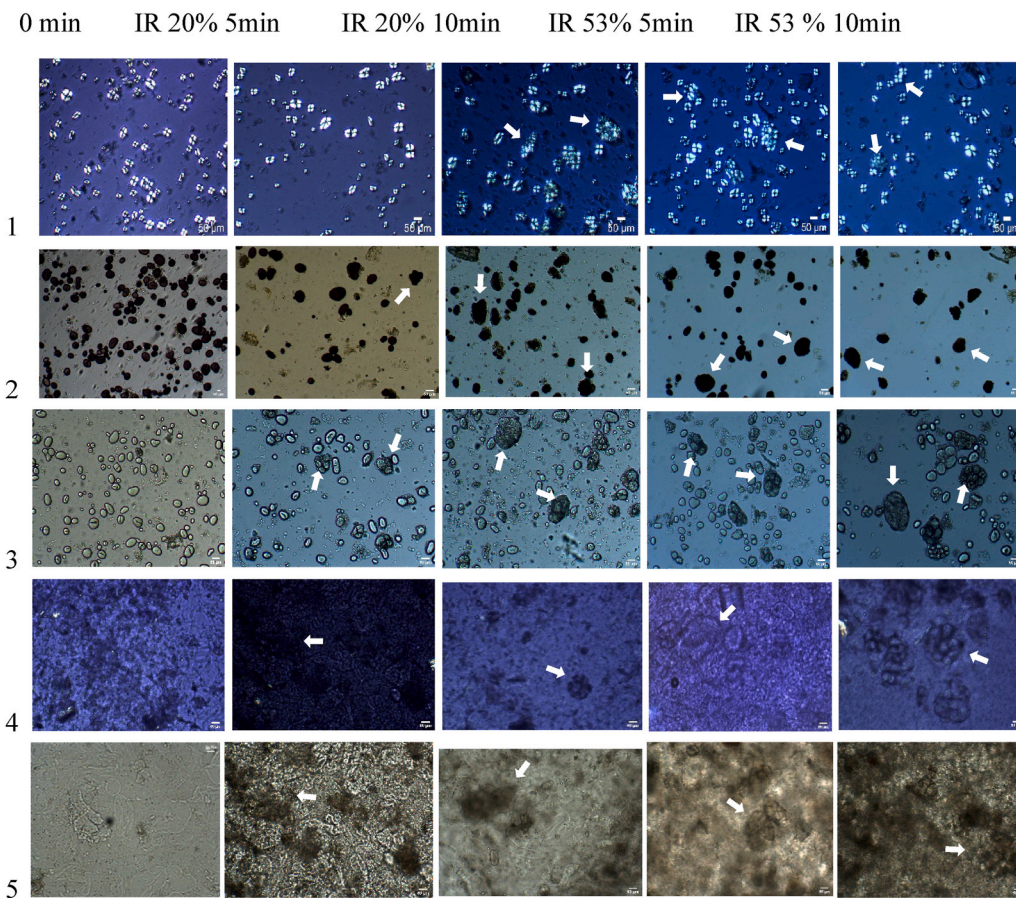


Fig. 3A. Light micrographs under polarised light, iodine stained and unstained of resulting flours from untreated, Infrared heat-treated paste and flour samples of bambara groundnut seeds moisture conditioned (20 and 53% moisture). Samples were heat-treated for 0, 5, and 10 min. Increase in heat treatment time increased the aggregates. 1-polarised light, 2-Iodine stained, 3- light unstained, 4-pasted and iodine stained, 5- pasted unstained. Bar = 50 µm. Arrows indicating aggregates in infrared flours and pastes.

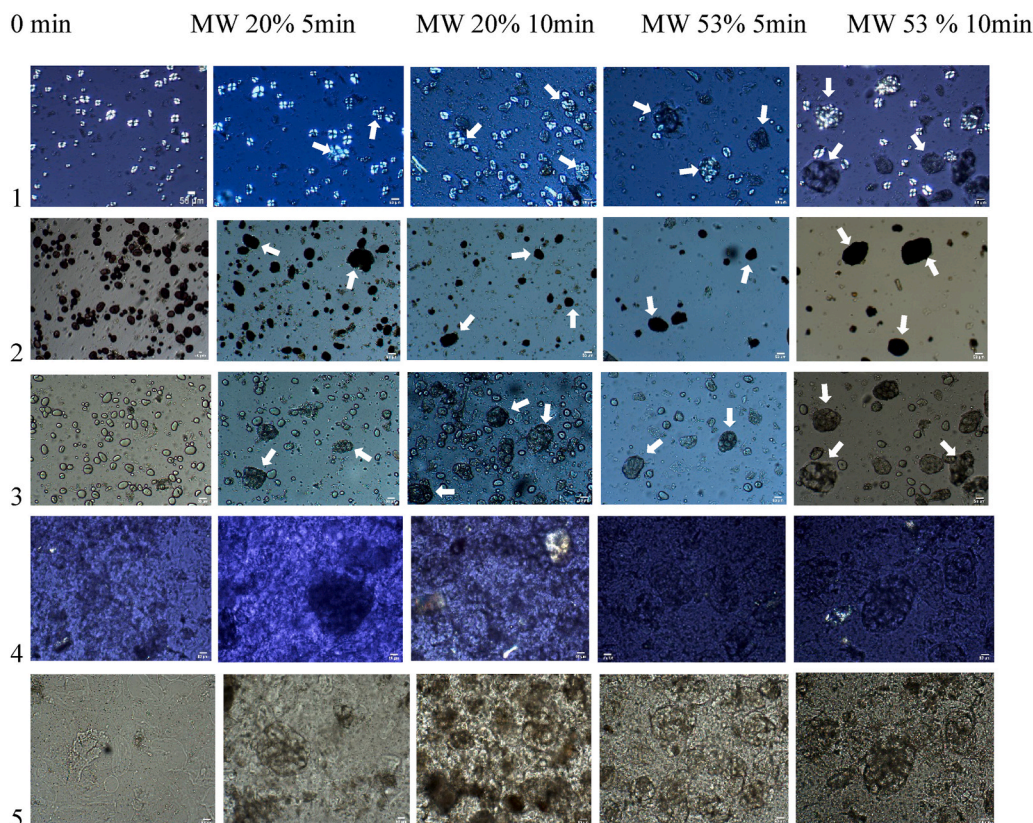


Fig. 3B. Light micrographs under polarised light, iodine stained and unstained of resulting flours from untreated, microwave heat-treated paste and flour samples of bambara groundnut seeds moisture conditioned (20 and 53% moisture). Samples were heat-treated for 0, 5, and 10 min. Increase in heat treatment time increased the aggregates. 1-polarised light, 2-Iodine stained, 3- light unstained, 4-pasted and iodine stained, 5- pasted unstained. Bar = 50 µm. Arrows indicating aggregates in microwave flours and pastes.

0 min IR/MW 20% 5min IR/MW 20% 10min IR/MW 53% 5min IR/MW 53 % 10min

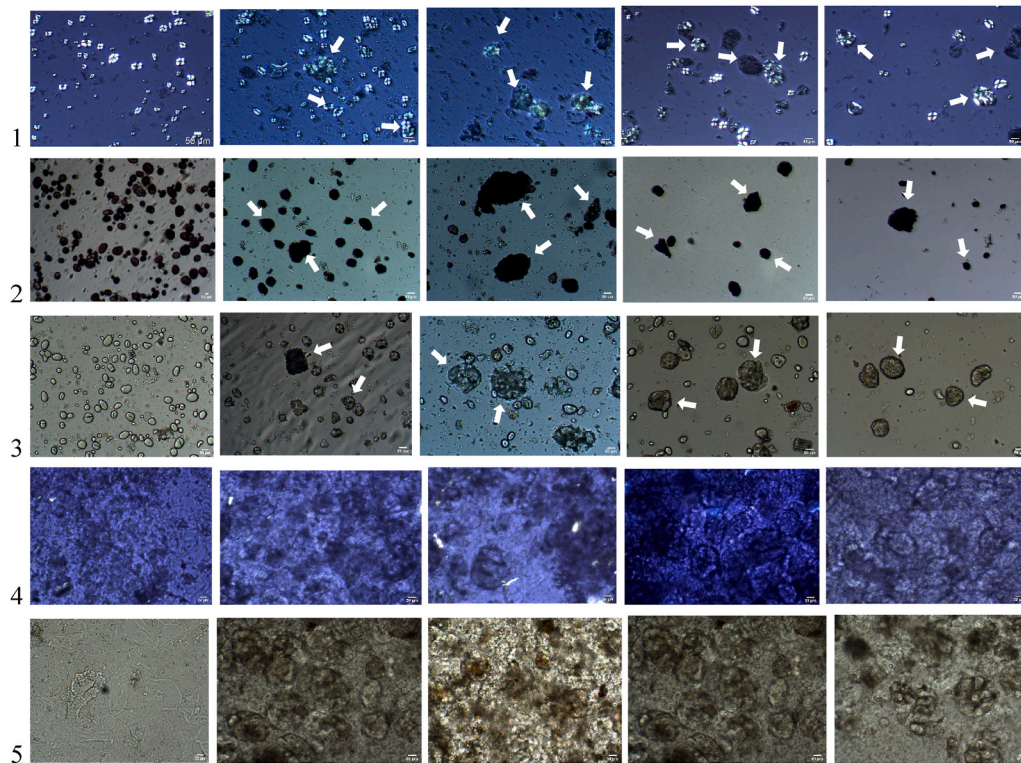


Fig. 3C. Light micrographs under polarised light, iodine stained and unstained of resulting flours from untreated, combined infrared and microwave heat-treated paste and flour samples of bambara groundnut seeds moisture conditioned (20 and 53% moisture). Samples were heat-treated for 0, 5, and 10 min.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

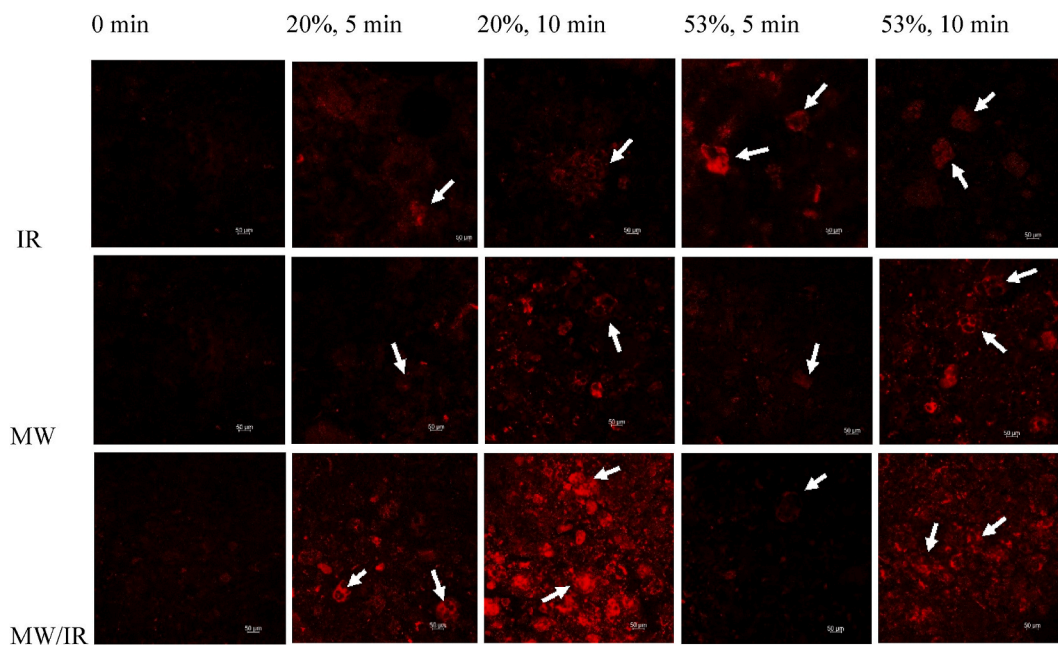


Fig. 4. Confocal laser scanning microscopy micrographs of resulting flours from untreated, infrared, microwave, and combine microwave/infrared heat-treated paste of bambara groundnut seeds moisture conditioned (20 and 53% moisture). Samples were heat-treated for 0, 5, and 10 min. An increase in heat treatment time increased the aggregates and aggregates. Bar = 50 µm. Pastes stained for protein with 0.02% Acid Fuchsin dye in 1% acetic acid dye. Arrows indicate starch-protein aggregates, black spots inside the red stain indicate the starch, and the red indicates the protein matrix. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

evidenced birefringence due to crystalline starch structure. Maltese crosses gradually disappeared with increased treatment time for all samples, suggesting progressive starch gelatinisation. The highest effects in terms of the disappearance of birefringence were observed in

combined treated samples. This confirms that the decrease in endothermic enthalpy observed by DSC is partly due to progressive starch gelatinisation. [Ogundele et al. \(2017\)](#) reported that an increase in infrared heating time resulted in a reduction in starch birefringence.

Similar results were reported by Li et al. (2019) for MW heat-treated millet starch. Pastes from untreated samples seem to be continuous with no visible starch granules (Fig. 3A–C/4 and 5). The micrographs from heat-treated samples (0, 5, 10 min) showed an increasing aggregation from 0 to 10 min of heat-treated samples (Fig. 3A–C). The combined treatment had more pronounced effects, in terms of the aggregation, followed MW then IR.

CLSM micrographs (Fig. 4) recorded the nature of pasted aggregates viewed under light microscopy. The obtained micrographs indicated a protein matrix appearing red, surrounding black spherical starch granules (non-stained) in heat-treated samples. Control samples (0 min) had a continuous pasted starch appearing black with protein matrix appearing red and without distinct clustering or aggregations. Samples from 20% moisture conditioning had well-distributed aggregates than those from 53% soaked seeds. A higher number of aggregates were observed in combined treatment samples at 53% moisture level.

High moisture has been associated with protein denaturation during heat treatment to form aggregates (Ogunde et al., 2017). Proteins may be forming a matting network around starch and causing starch aggregates. Combination of IR waves causing vibrations and MW waves, causing rotational movements within the seed may have increased the level of protein denaturation and accelerated development of these aggregates. Microwave, infrared and combined heat treatment of pre-soaked 20% and 53% bambara groundnut seeds led to protein denaturation in resulting flours. A reduction in nitrogen solubility index (Table 3) seems to support this. As protein unfolded, they expose hydrophobic sites. Heating increased aggregation formation of flours that have partially gelatinised starch embedded in denatured protein matrices (Ogunde & Emmambux, 2018).

The combined treatment had a more pronounced effect on protein denaturation than individual treatment alone. The role in terms of how protein causes aggregation still need to be determined. It looks like protein crosslinking via disulphide bonds may be responsible for the protein's role in forming aggregates. Heat treatment leads to protein denaturation and crosslinking of sulphhydryl groups. Denatured protein expose their hydrophilic sites leading to more association with water. As heating time increased, there was a reduction in WSI and NSI for all heat treatment methods. Mwangwela et al. (2007) and Ogunde and Emmambux (2018) related the reduction in WSI to decreased protein solubility in cowpea and bambara groundnut, respectively. However, this explanation needs further investigation.

Reduction in pasting viscosities is due to denatured protein restricting expansion and causing starch-protein aggregates. The latter is denatured protein with the exposed hydrophobic sites that coat starch granules. It looks like the denatured protein exposes hydrophobic sites restricting starch access to water for pasting, thus a reduction in viscosities caused by heat treatment. It is possible that there may be amylase activity during soaking, Balasubramanian and Sadasivam (1989) found high amylase activity after soaking amaranth grains overnight. Amylase activity may lead to reduction in paste viscosities because amylase hydrolyses starch to shorter chain dextrin. Heat treatment increases WAC while reducing WSI in resulting flours as proteins dissociate and change conformation, exposing polar binding sites as they denature, leading to more association with water molecules (Naryana & Narasinga Rao, 1982). An increase in flour WAC could be advantageous in bread processing as flours with high water absorbance have improved characteristics (Wolf, 1970).

The combined heat treatment resulted in the greatest reduction in WSI, NSI, endothermic enthalpy, pasting viscosities, highest WACs, and most pronounced aggregate formation. This is due to combination treatment causing more starch gelatinisation and increased protein denaturation during heat treatment which led to more aggregate formation. There was improved heating on the bambara groundnut seeds resulting from the combined microwave and infrared heat treatment, leading to a bigger effect on resulting flours' functional properties. The combination effect was due to a combination of microwave energy

causing water and charged biomolecules to rotate, together with infrared causing molecular vibrations leading to a compounded thermal effect on the seed. Combined treatment has a more uniform temperature distribution, and a rapid rise in surface temperature contributed from infrared waves and is distributed to the interior of the food through microwaves (Datta & Rakesh, 2013).

The moisture levels were chosen to resemble dry heating for 20% moisture seeds and cooking for the 53% moisture seeds. To explore the effects of moisture conditioning and soaking of bambara seeds on the resulting flours. Moisture conditioning may lead to improved rehydration capacities and colour changes of the final product. Different moisture conditioning/soaking have been applied to different legume seeds such as peas (10%) by Andrejko et al. (2008), lentils (33%) by Arntfield et al. (2001), cowpea (41%) by Mwangwela et al. (2007) (53%) bambara groundnut by Ogunde et al. (2017), and mung beans (55%) by Padmashree et al. (2016). The two moisture levels were chosen to give the seeds high moisture to allow for high molecular changes in biomolecules.

4. Conclusions

There is a combination effect in terms of the properties of the resulting flours from heat treatment of conditioned bambara groundnut seeds using infrared and microwave energy. The combination effect can be due to molecular vibration from infrared energy and molecular rotations from microwave energy of water and other biomolecules in moisture conditioned seeds. Starch pre-gelatinisation and protein denaturation in resulting flours play an important role in changing the properties. It looks like denatured protein can form an adherent network to starch granules which leads to reduction in viscosity of the flours. The low viscosities of resultant flours from heat-treated bambara groundnut seeds can be advantageous when compositing with wheat flour for composite bread production.

Conflict of interest and authorship conformation form

Please check the following as appropriate:

- o All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
- o This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
- o The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript
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Declaration of competing interest

The authors declare no conflict of interest.

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