Emirates Journal of Food and Agriculture

Emirates Journal of Food and Agriculture 36: 1–9
doi: 10.3897/ejfa.2024.119095

RESEARCH PAPER

Characteristics of sorghum flour modified with physical and enzymatic treatments

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Academic editor: Angelo Maria Giuffrè ♦ **Received** 19 January 2024 ♦ **Accepted** 8 May 2024 ♦ **Published** 29 May 2024

Abstract

This study evaluated the physicochemical and thermal properties of modified sorghum flour. The modified sorghum flour was obtained through extrusion -enzymatic treatment using thermostable-α amylase at various feed moisture levels. The results showed that unmodified sorghum flour had the average chemical composition of sample being 87.06% carbohydrate, 9.8% protein, 0.32% fat and 0.64% ash. The unmodified and modified sorghum flour differed in their properties. The swelling power and solubility changed from 16.21 (g/g) to 4.64(g/g) – 28.20 (g/g) and from 4.68% to 4.25%–83.81% respectively. A wide range of pasting properties were noticed, such as peak viscosity (54 cP-3028 cP), breakdown viscosity (59 cP-1029 cP), setback viscosity (-106.50–2351 cP). Peak viscosity was not detected when the modification was performed at high feed moisture (FM 35%) treatment combined with the addition of thermostable-αamylase. Additionally, the onset, peak, and conclusion temperatures of gelatinization, and the enthalpies of modified flours were lower than those of unmodified ones. Morphological changes in the surface of starch granules were noticed as shown by pore formation through SEM observation. This study provided insights into the future direction of extrusion combined with enzymatic treatment for the selective modification of sorghum flour functional properties.

Keywords

α-amylase, extrusion, modified sorghum flour, pasting properties, thermal properties

Introduction

Sorghum was one of the important cereal plants in the world used as a source of food and feed in several countries. This plant grows in semi-arid regions where its kernels have complete nutrition and sap could be used for bioethanol raw material (de Mesa-Stonestreet 2010). The consumption is tolerable, specifically for people with celiac disease and other gluten-intolerant disorders (Ciacci et al. 2007). Over the past few decades, various efforts have been made to improve the quality of non-gluten flour to broaden its applications for baking products. Numerous non-gluten food products had been documented, with some of the more prevalent options originating from sources such as rice (Giuberti et al. 2017), sorghum (Marston et al. 2016), and corn (Bourekoua et al. 2016). Meanwhile, modification methods had been introduced including chemical (Chan et al. 2011), physical (Sun et al. 2014), enzymatic/fermentation (Elkhalifa et al. 2017) and physical modification (Zavareze and Dias 2011). Physical modifications included thermal (pregelatinizing, heat-moisture treatment, annealing, and microwave) and non-thermal (i.e. sonication, milling, hydrostatic treatment, freezing, and thawing) treatments (BeMiller 2018). However, these physical treat-

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ments only caused limited glycosidic bond cleavages of starch components without further chemical or structural modifications (BeMiller 2018). A combination of different modification methods was known to have different effects, although a single modification had a positive impact on changes in flour properties (Elkhalifa et al. 2017).

Extrusion consisted simultaneous operations, such as mixing, conveying, heating, kneading, shearing, and shaping (Vargas-Solórzano et al. 2014). The continuous operation rendered the process conducive to industrialized production, as shown by previous studies (Ye J et al. 2018a, 2018b). Extrusion technology, used for decades, has proven versatile in generating materials with distinct characteristics, particularly within food production. Furthermore, the process served various purposes, functioning as a pretreatment method to eliminate flavors, obviate the need for enzyme bioreactors, ease the presence of undesirable compounds (Xu E et al. 2015; Xu E et al. 2016), pre-gelatinize substances, and modify starches (Masatcioglu et al. 2017). The technology was also applied in the production of protein concentrates, exemplified by its use in the processing of sorghum flour (de Mesa-Stonestreet et al. 2012).

Modification of sorghum flour and starch through extrusion was reported by Vargas-Solórzano et al. (2014) and Jafari et al. (2017). The resulting product characteristics were dependent on the conditions, such as feed moisture, die temperature, barrel temperature, screw speed, torque, and feed rate. Even though the combination of various processing parameters yielded different product properties, feed moisture and screw speed were considered the most influencing factor for pasting properties (Sompong et al. 2011; Chanvrier et al. 2015). This combination of different extrusion processing conditions with enzymes was reported to affect flour properties. Additionally, Xu et al. (2015) reported that thermostable α-amylase highly affected paste viscosities, expansion ratio (ER), bulk density (BD), water absorption/solubility, and final total phenolic content. During the extrusion process, the degree of starch gelatinization and enzyme activity was influenced by moisture content, temperature, pH, enzyme concentration, screw speed, and die nozzle size (Vasanthan et al. 2001). Extrusion with α-amylase addition was also known to release the embedded protein body, namely kafirin in starch granules. The kafirin released was expected to mimic the role of gluten in wheat flour during dough expansion (de Mesa-Stonestreet et al. 2012; Jafari et al. 2017). Therefore, this study aimed to investigate physicochemical changes of sorghum flour modified through extrusion-enzymatic treatment using thermostable α-amylase at different feed moisture levels.

Material and methods

Materials

Sorghum grain of Kawali variety was provided by the Cereal Crops Research Institute, Indonesian Agency for Agricultural Research and Development, Indonesia, and starch-degrading enzyme was obtained through the distributor. Enzyme was Termamyl® SC 4X, Novozyme, Denmark) which had an activity of 240 KNU-S/g. A unit of amylase liberated 1.0 mg of maltose from starch in 3 min, pH 6.9 at 20 °C.

Methods

Sorghum flour preparation and modification

Sorghum flour was prepared by dehulling, followed by dry milling processes and sieving by 200 mesh sieves. Flour was dried in air convection oven for 3–5 h at 50 °C (Memmert GmbH + Co. KG, Germany). Subsequently, the dried sorghum was put into sealed bags and stored in a 20 °C refrigerator before modifications. The modifications were performed according to de Mesa-Stonestreet et al. (2012) and sorghum flour was extruded using a screw extruder (the HAAKE™ Rheomex CTW 100 OS, Thermo Fisher, US) at a temperature of 100 °C (same for each zone), feed rate of 1 kg/hour, screw speed of 100 rpm and die-less. The feed moisture (FM) levels varied at 20%, 30%, and 35% (db). This was controlled by the addition of distilled water with α-amylase (AM) concentrations of 0%, 0.1%, 0.5%, 1% and 1.5%. Thus, the enzyme/substrate ratio at FM 20 was 0. 03 KNU-S/g, 0.16 KNU-S/g, 0. 33 KNU-S/g and 0.49 KNU-S/g, while at FM 30 was 0.07 KNU-S/g, 0.36 KNU-S/g , 0.72 KNU-S/g, 1.08 KNU-S/g, and at FM 35 was 0.10 KNU-S/g, 0.49 KNU-S/g , 0.98 KNU-S/g and 1.47 KNU-S/g, respectively. The extrudates were cooled and dried using a convection oven for 3–5 h at 50 °C, then it was stored overnight at room temperature and sealed in plastic bags. All treatments were repeated twice.

Analytical procedures

Chemical composition

The determination of protein (N × 6.25), fat, water content, and ash was carried out with standard method (AOAC 2006). Protein was also extracted (Wang et al. 2009) from the selected sample and soluble protein was measured (Bradford 1976).

Swelling power (SP) and solubility (%SOL)

Swelling power and solubility were measured as described below. The sample of approximately 800 mg (db) was put into a centrifuge tube with 80 mL distilled water, and heated using a water bath (Thermo Fisher, US) at 95 °C for 30 minutes while occasionally homogenized. The paste was cooled at room temperature and centrifuged using Sorvall™ Legend™ XT/XF Centrifuge (Thermo Fisher, US) at 3000 rpm for 15 minutes. The supernatant was decanted carefully and transferred into an aluminum cup, then it was dried at 105 °C to a constant weight. The weight of the aluminum cup was applied for solubility determination and the residue was weighed for swelling power using the following equations:

$$\%SOL = A / S \times 100$$

$$SP = (B \times 100)/S(100 - \%SOL)$$

Where, %SOL = percent of solubility, SP = swelling power (g/g), A = dried soluble sample (g), B = the weight of sedimental paste (g), S = sample weight (g).

Pasting properties

Pasting properties of flour were analyzed using Rapid Visco Analyzer (RVA-StarchMaster2, Perten Instruments, Sweden), and a temperature-time cycle (heating rate) was applied as follows. The sample was heated at 30 °C for 1 min before heating to 95 °C (~7.5 min) and the value was kept for 5 min. Additionally, it was cooled down to 50 °C in 7.5 min and kept for 2 min before recording the following data pasting temperature (PT), peak viscosity (PV), breakdown viscosity (BD) and setback viscosity (SB). Viscosity was expressed in centipoise (cP).

Thermal properties

Thermal properties were measured by putting the sample (3.0 mg) on the aluminum pan and added distilled water at a ratio of 1:3. This was sealed hermetically and analyzed using Differential Scanning Calorimeter (Lab SYS-DSC 8500 (N5340501), Perkin Elmer, Norwalk, CT) (Sun et al. 2014). The onset temperature (To), peak temperature (Tp), conclusion temperature (Tc) and the gelatinization enthalpy (ΔH) were recorded. ΔH was expressed as J/g.

Morphological properties

Scanning electron microscope (SEM) (EVO 10 Model, Zeiss, Germany) was used to observe the morphologies of the samples attached to double-sided adhesive tape and coated with gold-palladium before analysis.

Data analysis

All analysis was performed at least in duplicate. Analysis of variance (ANOVA) was performed to compare means and variances with a significant level of 95% ($p < 0.05$). Pearson's correlation coefficients among other parameters were also calculated by using IBM SPSS 24.0 software.

Results

Table 1 summarized the characteristics of native sorghum flour that consisted of average chemical composition, protein content, swelling power and solubility, pasting properties, and thermal properties as well. These characteristics significantly differed from those of modified sorghum flour as presented in Table 2 (protein content, swelling power and solubility), Table 3 (pasting properties). Soluble protein of selected samples was shown in Fig. 1. Concerning the pasting properties, it was found that all the peak viscosity (PV), Break Down (BD) and Set Back (SB) viscosity significantly decreased with the AM treatment.

Table 1. Characteristics of unmodified (native) sorghum flour.

Variable	Value
Total carbohydrate[a]	87.06
Protein (%, db)	9.80
Fat (%, db)	0.32
Water content (%, db)	8.91
Ash (%, db)	0.64
Swelling power (g/g)	16.21
Solubility (%)	4.68
Pasting Temperature (PT, °C) at 20 cP	77.68
Peak Viscosity (PV, cP)	293.08
Breakdown Viscosity (BD, cP)	107.96
Setback Viscosity (SB, cP)	21.08
Onset Temperature (T_o, °C)	79.38
Peak Temperature (T_p, °C)	82.87
Conclusion Temperature (Tc, °C)	88.78
Gelatinization Enthalpy (ΔH, J/g)	6.11

[a] by difference.

Table 2. Protein, Swelling power and solubility of modified sorghum flour.

FM (db)	AM (db)	Protein (%)	Swelling Power (g/g)	Solubility (%)
20%	0%	7.95 ± 0.05	17.60 ± 0.20^c	8.13 ± 1.85^a
	0.1%	7.93 ± 0.14	17.60 ± 2.56^b	29.75 ± 2.42^b
	0.5%	7.80 ± 0.01	20.20 ± 1.36^c	79.89 ± 0.11^c
	1%	8.83 ± 0.06	8.95 ± 1.36^a	80.53 ± 3.23^c
	1.5%	8.98 ± 0.06	10.71 ± 0.19^{ab}	82.86 ± 3.43^c
30%	0%	9.11 ± 0.02	15.78 ± 1.46^a	14.81 ± 0.46^a
	0.1%	8.45 ± 0.08	28.20 ± 0.04^b	21.93 ± 0.06^{ab}
	0.5%	8.43 ± 0.21	13.28 ± 5.16^a	81.28 ± 1.70^c
	1%	7.80 ± 1.04	10.92 ± 1.37^a	72.11 ± 0.21^c
	1.5%	8.19 ± 0.54	27.10 ± 7.92^b	41.77 ± 18.00^b
35%	0%	8.53 ± 0.31	18.97 ± 0.99^d	4.25 ± 0.14^a
	0.1%	8.44 ± 0.57	11.76 ± 0.06^a	83.81 ± 0.52^c
	0.5%	8.41 ± 0.11	15.83 ± 0.77^c	77.96 ± 1.58^c
	1%	8.05 ± 0.08	14.59 ± 1.11^c	79.69 ± 1.44^c
	1.5%	8.90 ± 0.56	4.64 ± 0.50^a	47.19 ± 5.87^b

Data with the same letter in each column of each FM group are not significantly different ($p < 0.05$). FM: feed moisture level; AM: α-amylase concentration. Reported values correspond to the mean ± standard deviation.

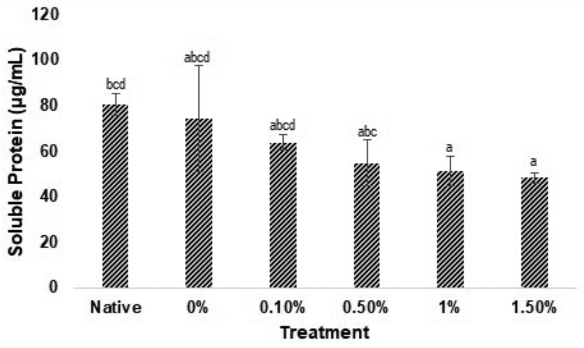

Figure 1. Soluble protein of modified sorghum flour upon treatment of FM 30 with different enzyme addition.

Inconsistent result of thermal properties was noticed. The properties that correspond to the gelatinization of starch with the enthalpy of gelatinization and its transition temperatures showed variations among samples. The representative DSC thermogram was displayed in Fig. 2A. In addition, two endotherm peak were observed

Table 3. Pasting properties of modified sorghum flour.

FM (db)	AM (%) (db)	PT (°C) at 20 cP	PV (cP)	BD (cP)	SB (cP)
20%	0.00	76.60 ± 0.00	3028.50 ± 45.96	1029.50 ± 20.51	413.50 ± 4.95
	0.10	77.84 ± 0.08	1363.50 ± 50.20	622.00 ± 72.12	78.00 ± 7.07
	0.50	79.05 ± 0.37	54.50 ± 0.71	59.00 ± 1.41	-56.00 ± 1.41
	1.0	ND	ND	ND	ND
	1.5	ND	ND	ND	ND
30%	0	75.83 ± 0.29	1386.50 ± 3.54	450.50 ± 38.89	1018.50 ± 30.41
	0.10	79.58 ± 0.18	569.00 ± 7.07	91.00 ± 2.83	223.00 ± 4.24
	0.50	76.80 ± 0.03	114.00 ± 7.07	114.00 ± 7.07	-106.50 ± 6.36
	1	77.28 ± 0.32	56.00 ± 0.00	61.00 ± 1.41	-57.00 ± 1.41
	1.50	75.97 ± 0.08	1428.50 ± 3.54	529.00 ± 12.73	445.00 ± 12.73
35%	0	75.07 ± 0.14	1622.00 ± 28.28	61.50 ± 38.89	2351.50 ± 55.86
	0.1	ND	ND	ND	ND
	0.5	ND	ND	ND	ND
	1.0	ND	ND	ND	ND
	1.5	ND	ND	ND	ND
P-value	FM	0.142	0.240	0.253	0.181
	AM	0.095	0.585*	0.650**	0.297

Data with the same letter in each column of each FM group are not significantly different ($p < 0.05$). FM: feed moisture level; AM: α-amylase concentration; PT: pasting temperature; PV: pasting viscosity; BD: break down viscosity; SB: set back viscosity. ND: not detected. Reported values correspond to the mean + standard deviation.

on modified sorghum flour upon extrusion at FM 20 or FM 30 with enzyme addition 0.1% (Fig. 2B). Thermal data for other AM treatments was summarized in Table 4. Increasing of gelatinization temperatures and ΔH were observed.

Table 4. Thermal properties of modified sorghum flour at FM 35 with amylase (AM).

AM (%, db)	T_o (°C)	T_p (°C)	T_c (°C)	T_c-T_o (°C)	ΔH (J/g)
0	79.08	82.64	86.17	7.09	2.18
0.1	79.60	83.15	89.19	9.59	5.19
0.5	80.64	84.91	88.60	7.96	3.67
1.0	81.47	85.16	90.47	9.00	3.54
1.5	79.37	83.28	87.82	8.45	8.14

The scanning electron microscopy of native and modified sorghum flour was found to be aggregated and irregularly shaped (Fig. 3). The presence of fissures, pores or holes were detected in the starch granule surface (Fig. 4).

Discussion

Sorghum flour composition

Table 1 showed the properties of unmodified (native) sorghum flour, where it could be observed that the chemical component in terms of protein, ash, and lipid were lower than those reported by Winarti et al. (2023).

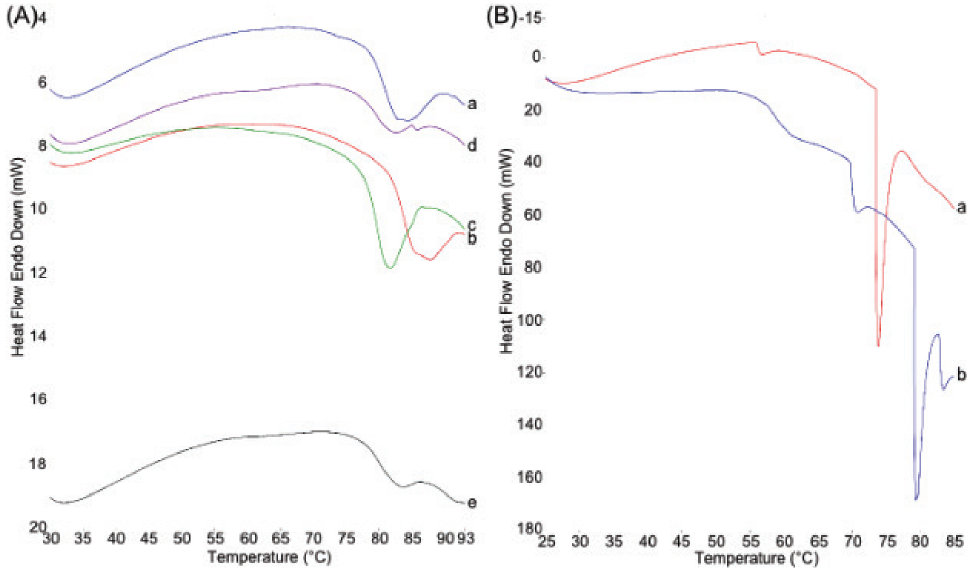

Figure 2. DSC thermograms of modified sorghum flours (**A**) (a: native flour; b: sorghum starch; c: feed moisture of 20% (FM20); d: feed moisture of 30% (FM30); e: feed moisture of 35% (FM35)) and (**B**) (addition of α-amylase of 0.1% with feed moisture of (a) 20% (FM20-AM0.1) (b) 30% (FM30-AM0.1)).

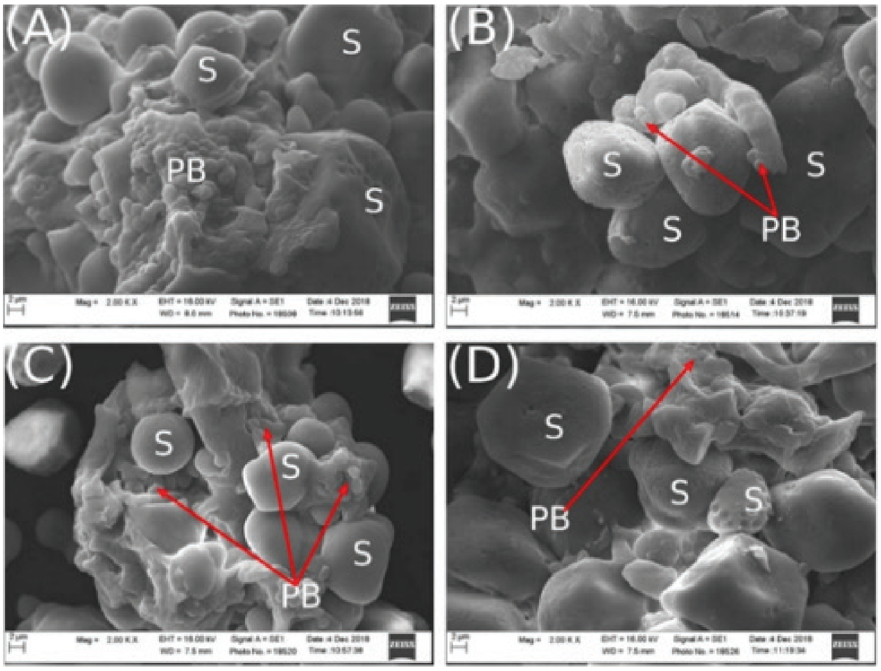

Figure 3. Scanning electron micrograph of native and modified sorghum flours (**A:** native sorghum flour; **B:** FM30; **C:** FM20-AM1; **D:** FM30-AM1). Each micrograph was observed with 2000× magnification. S: Starch granule, PB: protein bodies.

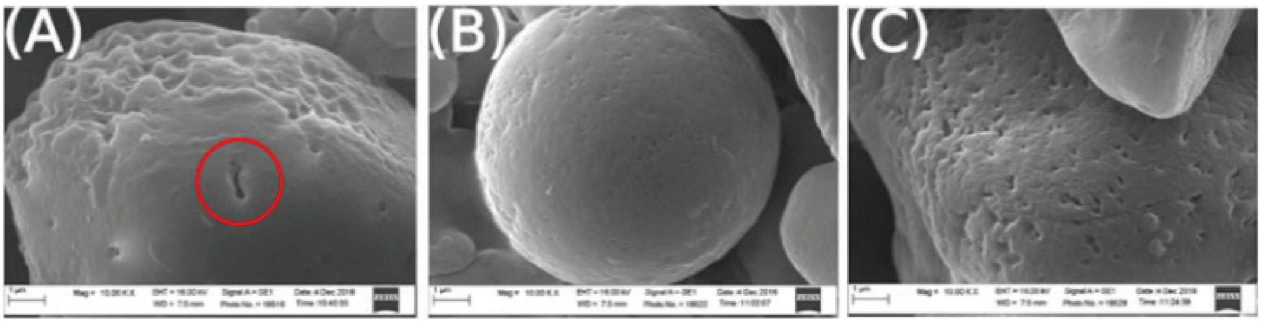

Figure 4. Scanning electron micrograph of the modified sorghum flour pore (**A:** FM30; **B:** FM20-AM1; **C:** FM30-AM1). Each micrograph was observed with 10000× magnification.

Chemical component of sorghum flour was affected by several factors such as genetics, environmental factors and milling system. The total protein content of the modified sorghum flour was slightly changed due to the addition of amylase enzyme. Extrusion processing had the potential to denature and degrade proteins and thus change the protein properties. Kafirin, the major sorghum storage protein was alcohol-soluble protein. This study revealed that the protein solubility of modified sorghum flour decreased especially when the modification was carried out at FM 35 with enzyme as shown in Fig. 1. A decrease in the soluble protein fraction was also reported by de Mesa-stonestreet et al. (2012). This could be caused by the large number of hydrophobic amino acid residues in sorghum protein (Belton et al. 2006), thus allowing interaction and formation of protein aggregates and disulfide bridges to be re-formed which reduced the protein solubility. Protein content played an important role in human nutrition, especially sorghum proteins, since sorghum flour is safe for celiac patients and allows palatable.

Swelling power (SP) and Solubility (%SOL)

Differences in SP values related to the addition of α-amylase enzyme. Here, sorghum flour showed a diversity in the SP values, ranging from 4.64 g/g to 28.20 g/g (Table 2). Enzyme activity might have similar effects to acid hydrolysis on starch after 20 and 35 days as reported by (Wang and Copeland 2012). The insoluble starch showed a fraction of water binding, reaching up to 75 and 80 molecules per glucose. The binding capacity was subjected to augmentation post-hydrolysis, attributed to the development of amylose chains, which were partially degraded to attain optimal lengths. Additionally, reports showed that subjecting starch to α-amylase leads to an augmentation in its swelling capacity (Dura et al. 2014; Zhang et al. 2016). However, different results were stated by (Jafari et al. 2017) where swelling power of sorghum flour decreased after extrusion treatment without the addition of enzyme. A higher feed moisture would obtain a decrease in swelling power-modified sorghum flour and each FM group had different results based on observations. The group combined with individual enzymatic treatments showed an optimal swelling power. For FM20, FM30, and FM35 swelling power, the optimal values were determined at α-amylase additions of 0.5%, 0.1%, and 0%, respectively.

The extrusion-enzymatic modification was also found to affect solubility of carbohydrate fraction in water. The highest and lowest solubility were found at FM35-AM0.1 and FM35-AM0 modifications with an increase and decrease of almost 18-fold and 9.19%. The results were in line with (Guha et al. 1997), where extrusion without die increased solubility in water. Meanwhile, extrusion with die as reported by (Hagenimana et al. 2006; Jafari et al. 2017) led to an increase in carbohydrate solubility index from extrudate flour. The increase of solubility was expected through α-amylase activity due to high FM. Enhancement of enzyme activity would yield in higher degree of hydrolysis which resulted in water-soluble carbohydrate derivatives (Likimani et al. 1991). In each FM group, for FM20, FM30, and FM35, optimum solubility was found at α-amylase addition of 1.5%, 0.5% and 0.1%, respectively. The correlation coefficients between FM to swelling power and solubility of modified sorghum flour were calculated. There was a non-signification correlation between FM on swelling power and solubility ($r = 0.376$ and $r = 0.476$ at $p < 0.05$). The different α-amylase activity due to different concentrations added and FM was substantially contributed to variations of swelling power and solubility.

Pasting properties

Pasting properties determine the suitability of ingredients for several foods. Table 3 summarized the RVA parameters of samples, which resulted in a wide range. In general, the gelatinization temperature of native sorghum starch ranged from 70 to 73 °C (Chandrashekar and Kirleis 1988). According to Sitanggang et al. (2018) for Numbu and Genjah sorghum varieties, starch gelatinization temperatures were 75.97 and 77.30 °C, respectively. In this study, the gelatinization temperature of native sorghum was approximately 77 °C. Modified flour had temperatures that varied from 75.07 to 79.58 °C and the transition could occur due to different water uptakes between sorghum starch and flour. At a temperature of <70 °C, the water uptake of flour was greater than starch but at >70 °C, more water could be held (Chandrashekar and Kirleis 1988). This phenomenon was influenced by the presence of non-starch components such as proteins (Chandrashekar and Kirleis 1988) and lipid (Ye J et al. 2018a, 2018b), influencing the gelatinization temperature of flour. Gelatinization temperatures of flour FM20, FM30, and FM35, decreased as compared to native flour by a factor of 1.41%, 2.44%, and 3.48%. The decrease in PT of corn flour on extrusion at low moisture content (22–27% db) was also reported (Zhang et al. 2016). According to Jafari et al. (2017), the extrusion increased the intensity of shear forces which could damage starch crystal structure, causing the formation of an amorphous structure of starch.

The modified sorghum flour had different pasting profiles from native counterparts reported by (Hagenimana et al. 2006; Sarawong et al. 2014). Pasting viscosity (PV) of modified flour decreased for each FM group. According to Sarawong et al. (2014), the viscosity of green banana flour decreased with a low FM. This was presumably caused by the near-complete starch gelatinization due to various levels of depolymerization and molecular attachment (Hagenimana et al. 2006). There was a relatively strong correlation of PV reduction with enzyme during modification (AM). Pearson's correlation coefficient (r) between AM to PV was 58,5 with $p < 0.05$.

After a holding period at a high temperature (95 °C), swollen granules started to rupture, and the dissolution of the polymer continued with a shear force maintained during the mixing. A decrease in viscosity was recorded as BD (Adedokun et al. 2010) and native flour had the highest BD compared to all modified flours. Similar results were also reported by Sarawong et al. (2014) where the decrease showed the magnitude of starch granules disintegration during the extrusion process. In this study, the decrease of BD showed a relatively strong correlation with enzyme ($r = 0.650$, $p < 0.05$). Generally, amylose and amylopectin polymers were reassociated (retrogradated) and the viscosity increased as shown by the final result known as setback (SB) (Adedokun et al. 2010). In this study, two modified flour showed a very significant increase in SB, such as FM30 (four-fold) and FM35 (nine-fold). Based on the analysis, there was no strong correlation between FM and the addition of enzyme ($r = 0.181$, $p < 0.05$). However, there was a strong correlation (SB-PT with $r = 533$, $p < 0.05$; SB-PV with $r = 0.633$, $p < 0.01$, and SB-BD with $r = 0.682$, $p < 0.01$.

Pasting properties were important for controlling viscosity behaviour during processing and storage of several food system. Modified sorghum flour resulted from treatment FM 20 or FM 30 with enzyme addition could be utilized to control viscosity, e.g in sauce, soup-based product formulation. In the other hand, modified sorghum flour processed at FM 35 with enzyme addition was suitable for carrier or filler due to very low viscosity value.

Thermal properties

The endothermic peak of gelatinization appeared at about 83 °C. There were differences obtained from RVA analysis in the previous section, where the temperature of modified gelatinization of sorghum flour ranged from 75.05–79.59 °C. The differences obtained from measurements using DSC and RVA were reported (Ye L et al. 2016), and this phenomenon was due to the interaction between starch and protein/fat, causing a different change between the heat flux on DSC and viscosity in RVA. The presence of gluten during the mixing of the wheat starch fraction was able to shift the gelatinization temperature monitored using DSC. This gelatinization temperature would increase along with the amount of gluten added (Jekle et al. 2016).

Without enzyme addition, the enthalpy (ΔH) value changed significantly from 9.81 J/g (FM 20) to 2.60 J/g (FM 30) or 2.18J/g (FM 35). The native sorghum flour showed comparable ΔH value with those of modified sorghum flour derived from FM 20. This trend was like the trend as reported by (Maaruf et al. 2001). In the thermogram of FM20-AM0.1 and FM30-AM0.1 (Fig. 1B), two endothermic peaks reported were considered as the glass transition (Tg) and gelatinization temperatures. The increase in Tg can be caused by the stress experienced by a material due to processing, and handling specifically when heated beyond the initial glass transition temperature. The appearance of Tg peak in the thermogram was presumably because most of starches were not perfectly gelatinized during the process. The glass transition was related to the rearrangement of the amorphous solid matrix which included bonds breaking and formation on a molecule (Falade and Christopher 2015). In this study, the first peak (Tg) was considered due to the disorganization of starch crystals, and in the second peak with the presence of water and application of heat, the remaining crystals were melted. The second peak protruded intensely with higher FM (Donovan 1979) and the phenomenon was relatively obvious when compared to the modified flour FM20-AM0.1 and FM30-AM0.1. The first peak in FM30-AM0.1 protruded more intensely when compared to the peak of FM20-AM0.1. Additionally, the peak difference in endotherms could be explained by the theory of terminal extent of gelatinization (TEG). TEG was interpreted as the upper limit until the occurrence of gelatinization depended on the temperature and water content of the material (Fukuoka et al. 2002). These temperatures ranged from 60–100 °C and the appearance of Tg at 55.95–59.26 °C (FM20-AM0.1) and 69.8–75.13 °C (FM30-AM0.1) corresponded to the endothermic peaks of Tg at 55–75 °C and 35.2–50.2 °C with wheat (Fukuoka et al. 2002) and rice flours (Falade and Christopher 2015). In this study, the range of temperatures for the appearance of Tg peaks was varied. This was not clear about the influence of enzyme addition in modified flour FM20-AM0.1 and FM30-AM0.1 on Tg peaks. However, enzyme activity during hydrolysis depended on the amount of water in the environment. The enzyme/substrate (E/S) ratio in modified flour treated with 0.1% enzyme at FM20 and at FM 30 were 0.03 KNU-S/g and 0.07 KNU-S/g respectively. Enzymatic hydrolysis increased the gelatinization temperature and ΔH value of modified sorghum flour upon treatment at FM 35. It indicated that the proportions of amorphous regions of granules could be reduced because of the enzymatic treatment, leading to higher temperatures to disrupt the remaining crystalline regions, which were thermally stable. It could be applied as a strategy to increase the functional ingredient of resistant starch content in food product. Baptista et al. (2024) reported the health impact of resistant starch and their processing challenges. From this study, a similar findings were noticed. Hence, it may be helpful for food industry to develop novel fuctional food ingredients.

Modified sorghum flour microstructure

The surface of native starch granules was relatively smooth. Additionally, the absence of pores or holes on the surface of the control starch granules showed the absence of endogenous enzymes (Dura et al. 2014). Porous structures only occur on the surface of starch caused by enzyme activity (Chen Y et al. 2011). Crater-like shapes were possible on the surface of starch granules which were the result of protein body suppression of soft endosperm structures during the growth process. These crater-like shapes were indicated as small cracks through observation under SEM with greater magnification (Huber and BeMiller 2000).

The modification of flour by extrusion and the addition of thermostable α-amylase enzyme caused various changes to the morphologies of modified flours. In this study, starch granules did not explode and could be found easily because of die-less extrusion. In extruded flour without the addition of enzyme (Fig. 3A), the damage was formed but not evenly distributed on the surface of starch granules. Damages occurred mostly at the edge of starch granules which were induced by shear forces during the extrusion. Considering the inherent presence of shapes and cracks, occurring and further increased by the mechanical forces applied during the extrusion process, the development of coarser surfaces could be anticipated. According to Xu E et al. (2015), the particles of starch were loosely dispersed by the presence of high-speed grinding during the extrusion. Therefore, friction occurs between the walls and causes damage to the surface. According to Rocha et al. (2012), α-amylase enzyme activity could cause surface damage. In contrast to the modification using extrusion, the addition of the α-amylase enzyme did not cause damage to the edge of starch granules but to the pore formation with various sizes (Fig. 3B–C). The depth and distribution of pores also depend on the botanical sources of starch granules and the types of enzymes used (Benavent-Gil and Rosell 2017a). The increase in FM combined with enzyme yielded considerably different effects on the surface of starch granules. In FM20, small pore was formed which were almost evenly distributed in the surface of starch granules, as shown in Fig. 3B. In FM30, the pore formed was larger and flatter on the entire starch surfaces (Fig. 3C). The difference in FM seemed to also influence enzyme activity on the hydrolysis of starch. The results were similar to the investigation conducted by (Zhang et al. 2012). Enzymatic hydrolysis was not well executed when the ratio of water and starch mass was lower, resulting in fewer pores (Zhang et al. 2012). The difference in pore sizes and shapes between FM20-AM1 and FM30-AM1 also showed that the action of enzymes was pronounced on the surface and in starch granules (Benavent-Gil and Rosell 2017b). The results were also in line with (Huber and BeMiller 2000), which conducted observations using coloring agents to determine the mechanism of water entry into sorghum and corn starch granules. Rapid penetration of the dye into the matrix proceeded from the inside out when rehydration occurred. Therefore, FM is directly proportional to enzyme activity and the pore sizes, which cause changes in functional properties. Porous starch granule was desirable and promising for use in functional food development, e.g in incorporation and protection of bio-active compound. Piloni et al. (2022) reported that better adsorptive capacity toward chia oil was provided by porous starch compared to those of native starch.

Conclusions

The extrusion-enzymatic treatment on sorghum flour had a significant effect on swelling power, solubility, pasting and thermal properties, and morphology of modified sorghum flour. The modified sorghum flour exhibited a considerable variability which could potentially be suitable for application in various commercial interest. Based on the pasting properties, it was advisable that modified sorghum flour resulted from FM30 with enzyme addition could be utilized as visco controller in , e.g in sauce, soup-based product formulation. On the other hand, modified sorghum flour from FM 35 with enzyme was suited for filler or carrier. It was also found that the the proportions of amorphous regions of granules could be reduced as a result of the enzymatic treatments leading to higher temperatures to disrupt the remaining crystalline regions, which were thermally stable. Porous starch granule was desirable and promising for use in functional food development.

Conflict of interest

The authors declared no potential conflict of interest.

Acknowledgments

The authors were grateful to the Centre of Agricultural Postharvest Research and Development (Bogor, Indonesia) for the technical assistance provided.

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