In Vitro Starch Digestion and Physicochemical Properties of Maize Starch and Maize Meal Modified by Heat-Moisture Treatment and Stearic Acid

Isaac Kwabena Asare, Clarity Ropafadzo Mapengo, Mohammad Naushad Emmambux

Department of Food and Consumer Sciences Pretoria University Private Bag X20, Hatfield, Pretoria 0028, South Africa. E-mail: naushad.emmambux@up.ac.za

Abstract

The physicochemical and nutritional properties of heat-moisture treated (HMT) maize starch and maize meal with stearic acid (SA) are studied. The addition of SA followed by HMT produces nongelling starch and maize meal porridge with reduced pasting viscosity. Heat-moisture treatment significantly ($P \leq 0.05$) decreases the starch hydrolysis, increases resistant starch, and lowers estimated glycaemic index of both maize meal and maize starch with SA. These changes are due to a more organized crystalline structure between starch polymers and well as the formation of amylose–lipid complexes as shown by differential scanning calorimeter and X-ray diffraction. There seems to be a synergistic effect between HMT and stearic addition as HMT promotes more starch polymer interaction compared to amylose–lipid formation for stearic acid addition. These results suggest that HMT combined with SA can be used to manufacture starch-based functional ingredients and foods with reduced glycaemic index.

1 Introduction

Maize meal is considered a staple and traditional diet in Southern Africa and other sub-Saharan countries.[1] It is prepared from maize flour through milling and contains about 70–75% of starch.[2] Maize meal can be cooked as soft porridge and as a stiff pap. Soft porridge is a thin gruel which flows but stiff pap is a gel, that does not flow. Most staple foods like maize and cassava are considered to be high glycemic index (GI) foods[3] and have GI of about 74. The high glycaemic index is due to a high percentage of starch assimilation that results in rapid absorption of glucose to increase blood glucose level. There are some concerns over the consumption of high GI foods, as the latter triggers hyper-postprandial glucose responses and has been linked with type 2 diabetes.[4]

Starch can be divided into three portions; rapidly digested starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). Englyst et al.[5] stated that RDS and SDS are starch portions which are hydrolyzed to dextrins by α-amylase within 20 and 120 min after assimilation, respectively. SDS is gradually assimilated all through the small intestine, resulting in a slow and lengthy release of glucose into the bloodstream, associated with low glycemic response. RS is the fraction that is not hydrolyzed after 180 min and continuous to the large intestine where it is fermented by gut microbiota.[5] RS is also subdivided into five categories.[6] RS 1 is physically unavailable starch to be assimilated. RS 2 is composed of native granules with structures making the starch slow to digest.[6] RS 3 is retrograded starch, and RS 4 is chemically improved starch.[7] RS 5 can be considered as amylose–lipid complexes (ALCs).[8] ALCs are said to be dietary fiber and thus have health benefits.[9] ALCs contribute toward controlling postprandial glycemic and insulinemic responses and preventing colon cancer.[10]

Amylose–lipid complexes are formed when a fatty acid and the hydrophobic core of the amylose helix interact.[11] Fatty acids fit into a category of lipids which entails amphiphilic compounds.[12] The ALCs are generally made strong by intermolecular bonding between the amylose glucose residues, water molecules, and ligand.[13] HMT is a procedure that includes treatment of starch at low moisture levels (< 35% moisture w/w) for a specified period (15 min–16 h) and at temperatures (84–120 °C) below the temperature of gelatinization and above the glass transition temperature of the material. HMT induces molecular rearrangement of amylose–amylose interaction (AM–AM) within the amorphous domain, amylose-amylopectin side-chain interaction (AM–AMP) and the complex formed between the amylose helix and the endogenous lipid to form amylose–lipid complexes.[14]

Sievert and Pomeranz[15] reported that HMT reduced enzyme susceptibility of normal and waxy starches at 18% moisture and temperature of 110 °C. Chung et al.[16] worked on RS levels of corn, pea, and lentil starches and conveyed that RS had increased from 4.6%, 10.0%, 9.1.% to 12.3%, 14.5%, 14.7%, respectively, after HMT (120 °C, 1 h, and 30% moisture content). Sang and Seib[17] stated that subjecting Hylon V starch (about 50% amylose) to HMT (45% moisture, 110 °C, 4 h) and phosphorylation (sodium trimetaphosphate/sodium tripolyphosphate) improved RS by 19% and reduced SDS and RDS stages by 12% and 6%, respectively. HMT of starches has been examined as a method of increasing the percentage of SDS and RS due to the altered crystalline nature, making the glycosidic bonds inaccessible for enzyme hydrolysis.[16]

Several researchers have worked on how lipids and HMT affect the properties of starch. Mapengo, Ray, and Emmambux[18] worked on the properties of HMT maize starch after long pasting (120 min holding at 91 °C), while Mapengo and Emmambux[19] reported an increase in RS in heat moisture treated maize meal by infrared with stearic acid. Comparison of the properties of starches with their respective flours after HMT has been done on sorghum starch/flour,[20] rice flour/starch,[21] and wheat starch/flour.[22] However, the physicochemical and digestibility differences between maize starch and maize meal modified by HMT and added lipids have not yet been investigated. The variation in the proximate composition between maize starch and maize meal can impact the functionality of maize starch and maize meal differently. This study investigates the digestibility and functional properties of maize starch and maize meal modified by HMT and stearic acid.

2 Results and Discussion

Pasting of starch and maize meal treated with stearic acid (SA) addition and heat-moisture are shown in Figure 1 and Table 1. Untreated starch had significantly ($P < 0.05$) higher peak, setback, and breakdown viscosities as compared to the heat moisture treated maize starch with stearic acid. The reduced setback viscosity is supported by the nongelling starch paste. Similar findings have been reported by Mapengo et al.[18] for maize starch modified with SA. Richardson et al.[23] highlighted that monoglyceride coated the starch granules to increase its hydrophobicity. This layer reduces the capacity of the granules to imbibe water, thus reducing swelling and producing a reduced peak viscosity. The nongelling starch also suggests that amylose SA complex was formed since amylose intermingles with SA, which hinders the development of junction zones. The latter is an integral part for gel formation.[11]

Table 1. Effects of HMT and SA on the functional and nutritional properties of maize starch/maize meal

	Treatment		Pasted													
	Heat-moisture treatment	**Stearic acid [w/w]**	**FV [mPa s]**	**BV [mPa s**	**SBV [mPa s]**	**Force [N]**	**VP**	**RDS [%]**	**SDS [%]**	**RS [%]**	**EGI**	**Tp_1 [°C]**	**ΔH_1 [J g^{-1}]**	**Tp_2 [°C]**	**ΔH_2 [J g^{-1}]**	**RC [%]**
Starch	None	none	2904±125^{c}	627±14^{c}	690±112^{c}	6.43±0.33^{d}	Gel	77±1.2^{b}	16±1.43^{a}	7±0.23^{a}	91±0.84^{d}	—	—	125±0.4^{b}	0.19±0.1^{a}	5.20±0.28^{a}
	None	1.5	2103±30^{b}	399±139^{b}	198±8^{a}	0.84±0.13^{b}	Paste	47±2.83^{a}	24±1.30^{c}	29±1.81^{b}	74±0.11^{c}	106±2^{a}	1.1±0.1^{a}	122±0.4^{a}	3.86±0.1^{b}	8.35±0.21^{b}
	HMT	none	2075±26^{b}	437±45^{b}	278±13^{b}	4.86±0.36^{c}	Gel	46±1.68^{a}	22±0.81^{bc}	32±2.77^{b}	70±0.35^{b}	106±1^{a}	0.9±0.1^{a}	127±1.3^{b}	6.17±0.2^{c}	7.50±0.28^{b}
	HMT	1.5	1723±43^{a*}	99±6^{a*}	111±11^{a*}	0.48±0.04^{a}	Paste	43±1.11^{a*}	20±0.21^{b}	37±1.11^{c*}	67±0.13^{a*}	106±1^{a}	2.4±0.4^{b*}	121±0.2^{a}	16.85±0.2^{d*}	12.80±0.42^{c*}
Maize meal	None	none	1199±20^{y}	ND	270±2^{z}	1.47±0.09^{y}	Gel	76±1.59^{x}	16±0.87^{w}	8±2.45^{w}	89±0.76^{z}	102±1^{w}	1.3±0.5^{w}	—	—	5.75±0.35^{w}
	None	1.5	1178±97^{y}	ND	193±15^{y}	0.39±0.02^{x}	Paste	45±1.86^{w}	22±2.50^{xy}	33±0.59^{x}	72±0.43^{y}	103±1^{w}	1.9±0.4^{x}	—	—	8.70±0.42^{x}
	HMT	none	843±50^{x}	ND	144±4^{x}	1.57±0.33^{y}	Gel	42±3.1^{w}	25±1.17^{y}	33±1.87^{x}	70±0.01^{x}	105±1^{w}	1.6±0.3^{x}	—	—	6.40±0.28^{w}
	HMT	1.5	863±48^{x}	ND	77±4^{w*}	0.51±0.12^{x}	Paste	44±1.52^{w}	18±0.19^{wx*}	38±1.39^{y*}	67±0.08^{w*}	—	—	111±5.3	37±0.4	11.42±0.35^{y*}

Mean values ± standard deviation of three independent experiments (n = 3) Means with different superscript alphabets in columns are significantly different at $P \leq 0.05$; HMT = heat-moisture treatment; Stearic acid (SA); *Interaction effect between SA and HMT; FV = final viscosity; BV = breakdown viscosity; SBV = setback viscosity; RDS = rapidly digestible starch; SDS = slowly digestible starch; RS = resistant starch; EGI = estimated glycaemic index; Tp1 = peak temperature for type I ALCs DSC endotherm; Tp2 = peak temperature for type II ALCs DSC endotherm, ΔH1 = enthalpy of melting for type I ALCs; ΔH2 = enthalpy of melting for type II ALCs; RC = relative crystallinity; Gel is when the paste cannot be poured freely after 24 h, but a paste can be poured out freely; ND = not detected; Relative crystallinity estimated excludes pure SA. Different superscript letters within the same column are significantly different ($p \leq 0.05$).

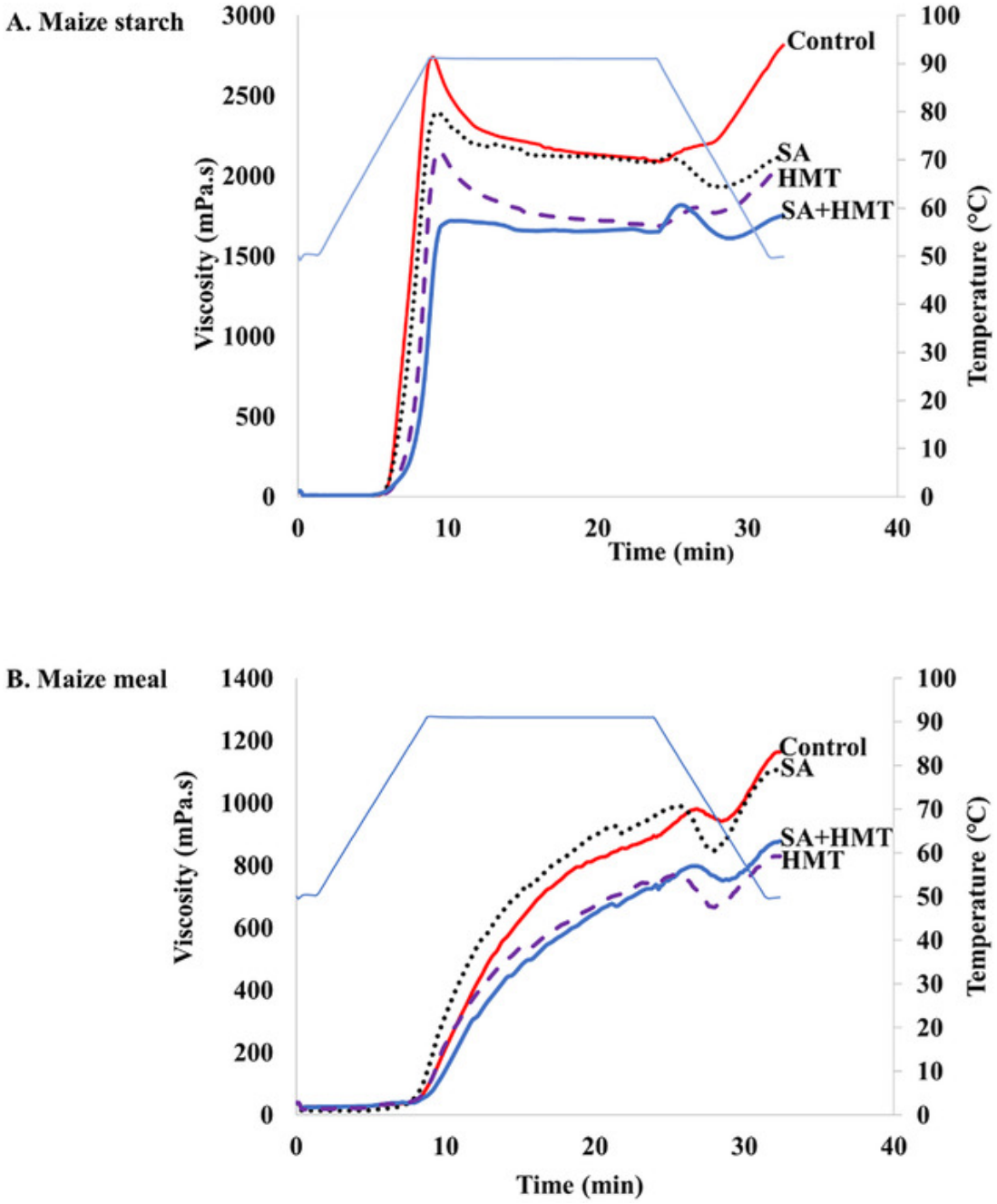

Figure 1. Effects of HMT and SA addition on pasting properties of maize starch A) and maize meal B).

HMT without SA further reduced the peak viscosity and produced a stronger gel than that of raw starch and showed no difference in the breakdown viscosity as compared with SA addition. Adding SA followed by HMT further reduced the peak viscosity, breakdown viscosity, and setback viscosity compared to the raw starch. The reduction in peak viscosity suggests that HMT reduced the amylose leaching and starch granule swelling.[24] HMT alone increased gel hardness, and this is ascribed to the increased cross-linking between the starch polymer chains to form more junction zones.[24] There was an interactive effect between SA and HMT at the peak viscosity and increased in viscosity during cooling.

Untreated maize meal showed no peak and breakdown viscosities, but there was an increased viscosity during holding, final viscosity, and increased in setback viscosity when pasted. Addition of SA produced a nongelling porridge (0.39 N compressive force) compared to gel for control (1.47 N compressive force). HMT of maize meal significantly ($P \leq 0.05$) decreased the viscosity during holding, final viscosity, and setback viscosity as related to the raw maize meal. HMT treated maize meal formed a gel after cooling the paste for 24 h at 25 °C with a compression force of 1.57 N. There was also an interactive effect between the SA and HMT for the final viscosities of treated samples. The combination treatment (HMT with added SA) exhibited a lower viscosity during holding, final viscosity, and setback viscosity compared to the raw maize meal but were similar to HMT treatment alone except for the nongelling property. Although maize meal has ≈80% maize starch, it is clear that pasting maize meal resulted in different pasting profiles to that of maize starch and this can be assigned to the different proximate compositions reported in Section 4.1. The absence of breakdown viscosity in untreated and treated maize meal could be due to the interaction between other maize meal components (high endogenous protein content (≈8%) and fat content (≈1%)) with starch polymers. To further understand the differences between maize starch and maize meal properties, a principal component analysis was done and will be explained in the last section.

The in vitro starch enzymatic hydrolysis of freeze-dried starch and maize meal pastes are represented in Figure 2 and the derived data in Table 1. White bread was a reference sample (Table 2). After 30 min, untreated starch and maize meal had about 60–70% of starch hydrolyzed. Maximum hydrolysis of about 80% was observed at 60 min for both untreated starch and maize meal, and then it reached a plateau. C_∞ (C infinity) indicates starch digested (until 180 min) expressed as a percentage (Table 2). The raw starch and maize meal after 180 min of digestion had 89% and 94%, respectively, and a corresponding estimated glycaemic index (EGI) of 91 and 90, respectively. The kinetic constant (K) indicates the rate of starch digestion. The low K values suggest a slow rate of digestion, while high K values represent a rapid rate (Table 2). The values from Table 1 and Figure 2 showed that starch in raw starch and maize meal were rapidly digested and with EGI of about 90.

Table 2. Effect of HMT and SA addition on the fractions of starch hydrolyzed and the digestibility kinetics of starch and maize meal

Sample	Treatment		$C\infty$ [%]	K [min^{-1}]	AUC	HI [%]
	HMT [%]	Stearic acid [%]				
Starch	None	None	89 ± 0.50^{b}	0.25 ± 0.02^{c}	$14\,025\pm0.02^{c}$	93 ± 1.53^{d}
	None	1.5%	71 ± 0.21^{c}	0.10 ± 0.01^{b}	$10\,218\pm16.1^{b}$	62 ± 0.20^{c}
	20%	None	57 ± 0.17^{a}	0.05 ± 0.00^{a}	9063 ± 75^{a}	55 ± 0.01^{b}
	20%	1.5%	$54\pm0.01^{a*}$	0.04 ± 0.00^{a}	$8652\pm34.8^{a*}$	$50\pm0.24^{a*}$
Maize meal	None	None	94 ± 1.98^{x}	0.20 ± 0.01^{x}	$14\,032\pm230.7^{y}$	90 ± 1.39^{z}
	None	1.5%	70 ± 1.53^{w}	0.08 ± 0.01^{w}	$10\,448\pm148.7^{w}$	60 ± 0.35^{y}
	20%	None	70 ± 0.77^{w}	0.05 ± 0.01^{w}	$10\,949\pm15.1^{x}$	55 ± 0.01^{x}
	20%	1.5%	$64\pm0.76^{y*}$	0.04 ± 0.00^{w}	$10\,452\pm18.2^{w*}$	$50\pm0.14^{w*}$
Bread			92 ± 2.09^{x}	0.25 ± 0.02^{x}	$15\,096\pm248.6$	100 ± 0.00

Mean values ± standard deviation of three independent experiments (n = 3)

Different alphabets with the same superscript in the same column are significantly different ($p \leq 0.05$)

HMT = heat moisture treatment Stearic acid = SA; EGI = estimated glycemic index (was calculated using the equation (39.71+ 0.549HI) according to Goni et al.,[22].HI = Hydrolysis index expressed as a percentage; K (min−1) = kinetic constant deals with rate of digestion per minute; White wheat bread was the reference sample to calculate EGI; *means interactive effect. Different superscript letters within the same column are significantly different ($p \leq 0.05$).

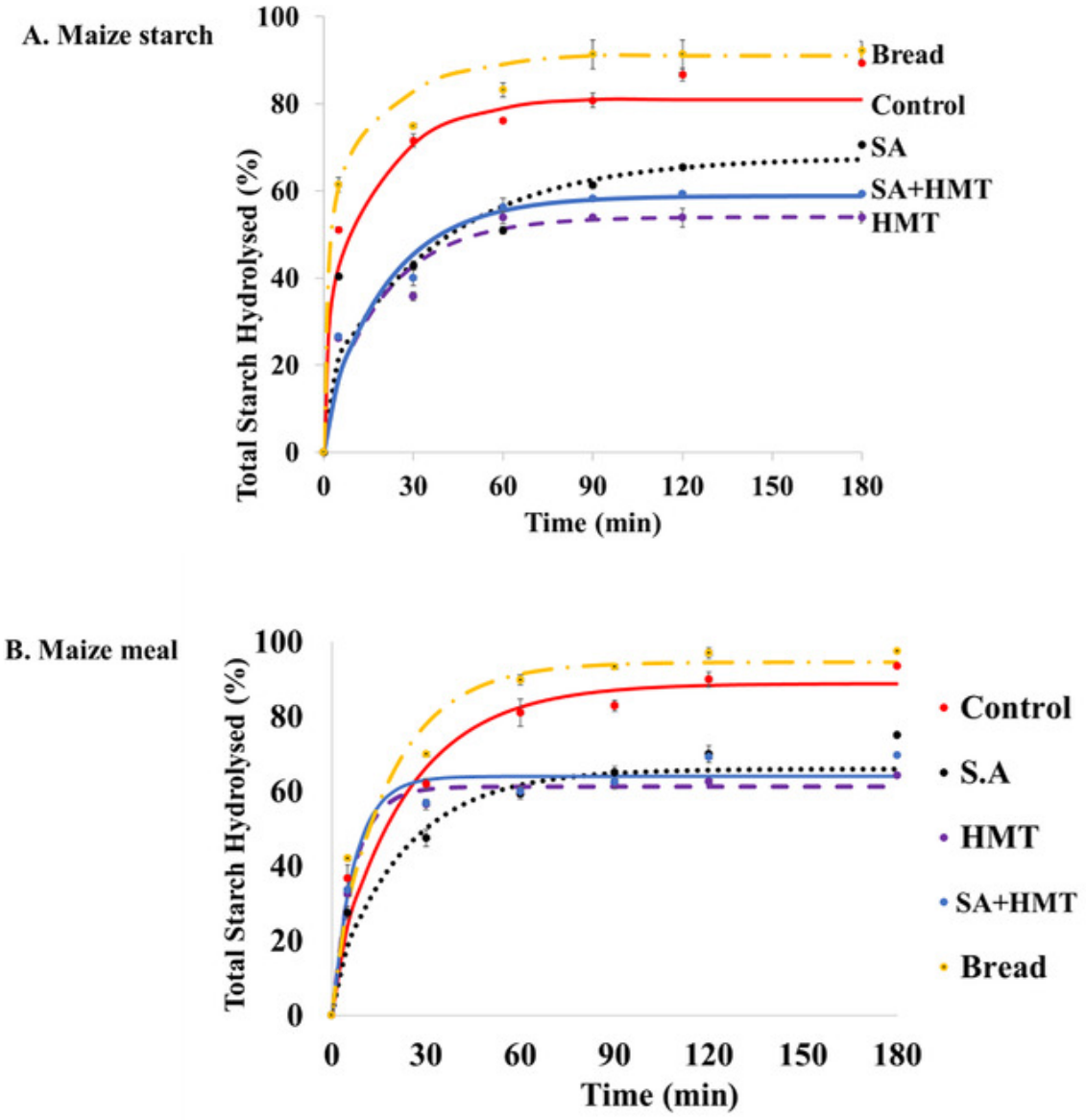

Figure 2. Effect of HMT and SA addition on starch hydrolysis of maize starch A) and maize meal B) after 180 min.

Treatment with SA alone exhibited a gradual increase in starch digestion with a decreasing rate for maize starch and maize meal until it plateaued at about 60–90 min (Figure 2A,B). After 180 min, starch and maize meal with added SA had about 71% and 70% starch digestibility (Table 2) and a corresponding EGI of 73 for both. The RDS, SDS, and RS of added SA to starch and maize meal were 47%, 24%, 29%, and 45%, 22%, 33%, respectively. Thus, maize meal showed higher RS compared to maize starch with SA addition. The K value for starch (SA) had 0.1 min^{-1} and maize meal (SA) was also 0.08 min^{-1} suggesting a higher rate of digestibility for maize starch compared to maize meal, but these rates were lower compared to their respective controls (maize starch and maize meal without SA or HMT). Lower starch digestibility suggests that there was a restriction by the enzymes to breakdown the starch polymer. This could be because of the addition of SA which complexes

with the amylose to form amylose–SA complex, restricting alpha-amylase enzyme availability to the alpha 1,4 glycosidic bonds. Cui and Oates[25] reported that the development of amylose–lipid complex decreases the digestibility of starch and modulates lower glycaemic response.

HMT alone of starch and maize meal also showed a plateau after 60 min of hydrolysis in starch and after 30 min of hydrolysis in maize meal. Both HMT starch and maize meal starch digestibility after 180 min were 57% and 70% (Table 2) and a corresponding RDS, SDS, and RS values as 46, 22, 32 and 42, 25, 33, respectively. The *K* value for both samples were 0.05 and 0.04 min^{-1}, suggesting a reduced rate susceptibility to enzymatic digestion, and this corresponds to an EGI of 70 for both samples compared to control. There was a decrease in the RDS values compared to the control, but, SDS and RS displayed a significant ($P \leq 0.05$) increase than the control samples. Chung et al.[16] reported similar results and concluded that HMT induced the starch chains to intermingle and forms double helices to maximize the ample stability of the granule to disruption. This is probably the reason for the rise in the SDS and RS values.

SA addition, in combination with HMT of starch, showed the lowest RDS and EGI values. After 180 min of hydrolysis, the C∞ values for starch and maize meal were 54% and 64% (Table 2) with the corresponding EGI value of about 67 for both. The EGI of ≈67 can correspond to a medium EGI. The RDS, SDS, and RS for both starch and maize meal were 43%, 20%, 37%, and 44%, 18%, 38%, respectively. The *K* value of both starch and maize meal was 0.04 and 0.05 min^{-1}, suggesting a slow rate of hydrolysis. There was significant ($P \leq 0.05$) reduction in the RDS value but increase in the SDS and RS than the control. There was a significant ($P < 0.05$) interactive effect between SA and HMT in terms of RDS, SDS, and RS for starch. In maize meal, the interactive effect between the two treatments occurred for SDS and RS only but not RDS, while in maize starch the interactive effect was observed for RDS and RS. The initial rate of digestibility for maize meal was slower than that of maize starch, as shown by the *k* values. This trend could be explained by the different proximate composition between maize starch and maize meal. The association between maize starch and maize meal with different attributes such as *k* value will be further explained by the principal component analysis (Figure 5) later.

Maize starch and maize meal had melting endotherms represented in Figure 3, and the endotherms' To (onset temperature), Tp (peak temperature), Tc (conclusion temperature), and ΔH (enthalpy of gelatinization) are recorded in Tables 1 and 3. The raw starch and maize meal before pasting had To, Tp, Tc, and ΔH as 64.5, 69.6, 75.7 °C and 8.2 J g^{-1} for starch, 63, 67, 73 °C and 0.9 J g^{-1} for maize meal, respectively (Table 3). Figure 3C,D represents the XRD results of unpasted starch and maize meal. XRD results for raw starch had 2θ degree peaks of about 6°, 10°, 12°, 15°, 17°, and 23° and that of maize meal had 2θ° peaks of about 5.8°, 11.2°, 15°, 17.2° and 23°, respectively. The core diffraction peaks for both raw starch and maize meal before pasting were 15° and 23° and a double peak at 17°. Both starch and maize meal had an A-type proposed crystalline organization.[18]

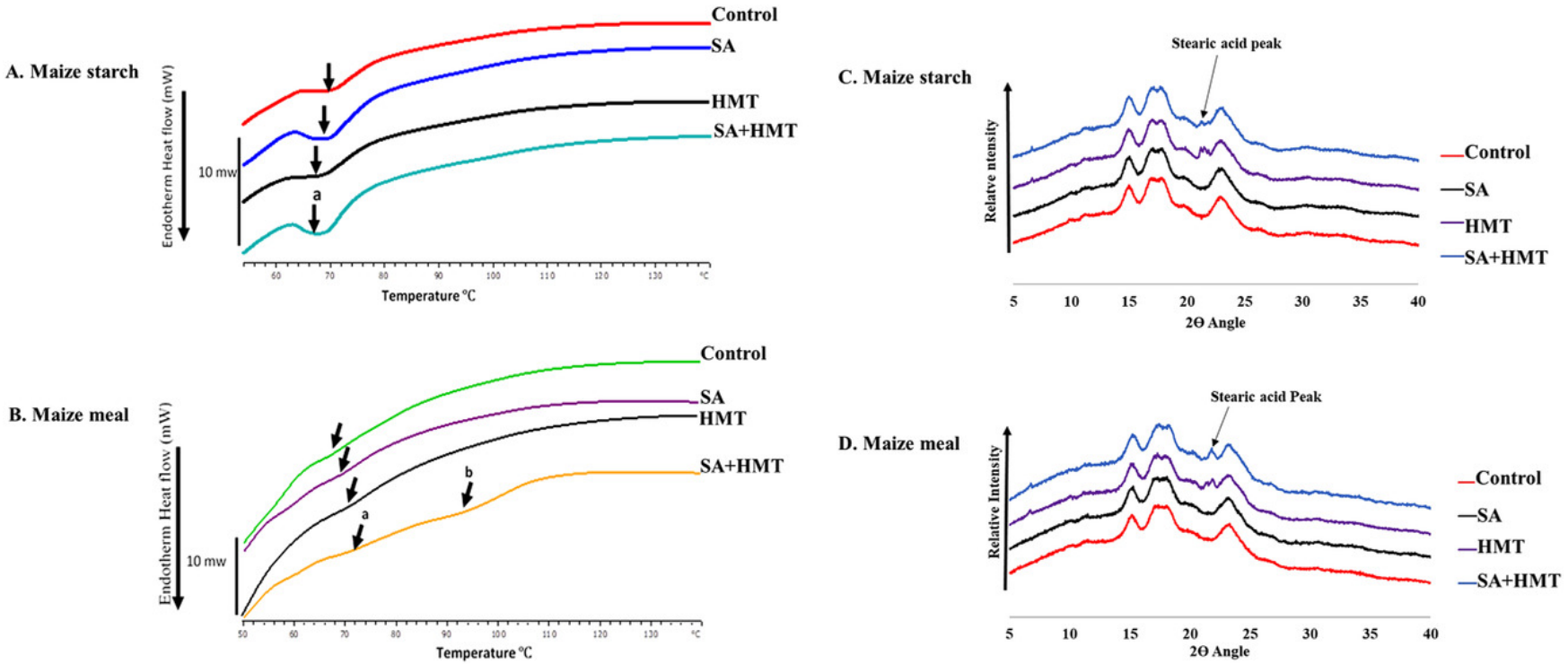

Figure 3. Effects of HMT and SA addition on the thermal properties A,B) and X ray diffractogram C,D) of unpasted maize starch and unpasted maize meal.

Table 3. Effects of HMT and SA addition on the thermal and XRD properties of starch and maize meal before and after pasting

Sample	Treatments		1st endothermic peak (before pasting)				2nd endothermic peak (before pasting)				XRD (before pasting)	1st endotherm (after pasting)	2nd endotherm (after pasting)
Starch	**HMT [%]**	**SA [%]**	**To [°C]**	**Tp [°C]**	**Tc [°C]**	**ΔH [J g^{-1}]**	**To [°C]**	**Tp [°C]**	**Tc [°C]**	**ΔH [J/g^{-1}]**	**Relative Crystallinity [%]**	**ΔH [J g^{-1}]**	**ΔH [J g^{-1})**
	None	None	64.50±0.2^{b}	69.60±0.1^{c}	75.70±0.38^{b}	8.2±1.78^{a}	—	—	—	—	18.3±0.78^{b}	—	0.19±0.1^{a}
	None	1.5%	63.30±0.1^{a}	68.60±0.1^{b}	75.20±0.80^{ab}	19.3±1.39^{c}	—	—	—	—	25.4±1.41^{a}	1.1±0.1^{a}	3.86±0.1^{b}
	HMT	None	63.00±0.4^{a}	68.30±0.3^{ab}	74.60±0.16^{ab}	16.2±0.83^{b}	—	—	—	—	22.7±1.63^{ab}	0.9±0.1^{a}	6.17±0.2^{c}
	HMT	1.5%	63.10±0.1^{a}	68.00±0.1^{a}	73.90±0.79^{a}	22.8±0.90^{d*}	—	—	—	—	27.1±1.27^{a*}	2.4±0.4^{b}	16.85±0.2^{d*}
Maize meal	None	None	63±0.6^{w}	67±1.5^{w}	73±1.2^{w}	0.9±0.1^{w}	—	—	—	—	19.8±1.63^{w}	1.3±0.5^{w}	—
	None	1.5%	63±0.6^{w}	69±1.0^{wx}	76±0.2^{x}	3.2±0.7^{y}	—	—	—	—	25.8±1.27^{xy}	1.9±0.4^{x}	—
	HMT	None	62±0.2^{w}	70±1.1^{x}	75±0.5^{x}	1.5±0.4^{x}	—	—	—	—	22.3±0.78^{wx}	1.6±0.3^{x*}	—
	HMT	1.5%	64±0.6^{w}	70±1.0^{x}	76±0.6^{x}	1.25±0.1^{x}	83±0.1	93±0.5	102±0.7	8.46±0.2	27±1.41^{y*}	—	37±0.4

Mean values± standard deviation of three independent experiments (n = 3); Different superscript letters with the same superscript in the same column are significantly different ($p \leq 0.05$); ***** = Interaction effect.; – = Not Detected; HMT = Heat Moisture Treatment; SA = Stearic acid.

In maize starch with stearic acid, one endothermic peak was observed with Tp of 68.6 °C (Table 3), this may suggest that the gelatinization endotherm merged with pure SA since the melting point of SA is 69 °C and starch is between 64 and 80 °C. The XRD peak at 21° for both starch and maize meal corresponds to uncomplexed SA.[26] There was another peak at 7° and 12.3° for both starch and maize meal suggesting that addition of SA to both starch and maize meal produced V-type complex formation.[18] V-type complexes do not promote retrogradation for gel formation, as explained earlier. The maize starch and maize meal with added SA had crystallinity values significantly ($P \leq 0.05$) increased from 18.3% and 19.8% to 25.4% and 25.8%, respectively.

Starch and maize meal with HMT before pasting showed one endotherm (Figure 3A,B). That endotherm signifies the gelatinization temperature of unpasted starch and maize meal. The gelatinization enthalpy significantly ($P \leq 0.05$) increased for both starch and maize meal to about 16.2 and 1.5 J g^{-1} than that of control. XRD pattern for control was similar to HMT unpasted starch and maize meal but increase to 22.7% and 22.3% for HMT starch and maize meal respectively (Table 3). Increased in relative crystallinity can be ascribed to the development of crystalline helices which are in double form for amylopectin side chain as well as a partial number of complexes formed from the amylose of starch and maize meal reacting with endogenous lipids.[27] This can explain the lower peak viscosity during pasting (Figure 1 and Table 1).

Adding SA followed by HMT starch and maize meal before pasting showed a significant ($P \leq 0.05$) increase in the enthalpy of melting/ gelatinization than the untreated samples (Table 3). Maize meal before pasting had two endotherms with ΔH of about 1.25 and 8.46 J g^{-1} at Tp of 70 and 93 °C, respectively. The second endothermic peak is due to the amylose–lipid complex type I.[28] The amylose in the maize meal interacted with the lipids inside to form a complex known as amylose–lipid complex. The second endotherm noticed by the differential scanning calorimeter (DSC) corresponded with the XRD small peak at 7°. This small peak corresponds with the amylose–lipid complex.[29]

Thermal and XRD properties of starch and maize meal after pasting were also represented in Figure 4A,B and Table 1. Untreated starch after pasting showed one endothermic transition peak with To, Tp, Tc, and ΔH of 119, 125, 132 °C, and 0.19 J g^{-1}. Maize meal also showed one endothermic transition peak with To, Tp, Tc, and ΔH of 97, 102, 110 °C, and 1.3 J g^{-1}, respectively. Both starch and maize meal did not show an endotherm between 65 and 85 °C; suggesting complete starch gelatinization during pasting. Figure 4C,D represents X-ray diffraction of starch and maize meal after pasting. The untreated maize starch and maize meal had 2θ angle peaks at 6.9°, 11.5°, 15.6°, 17.4,° and 21°; their relative crystallinity was 5.2% and 5.75%, respectively.

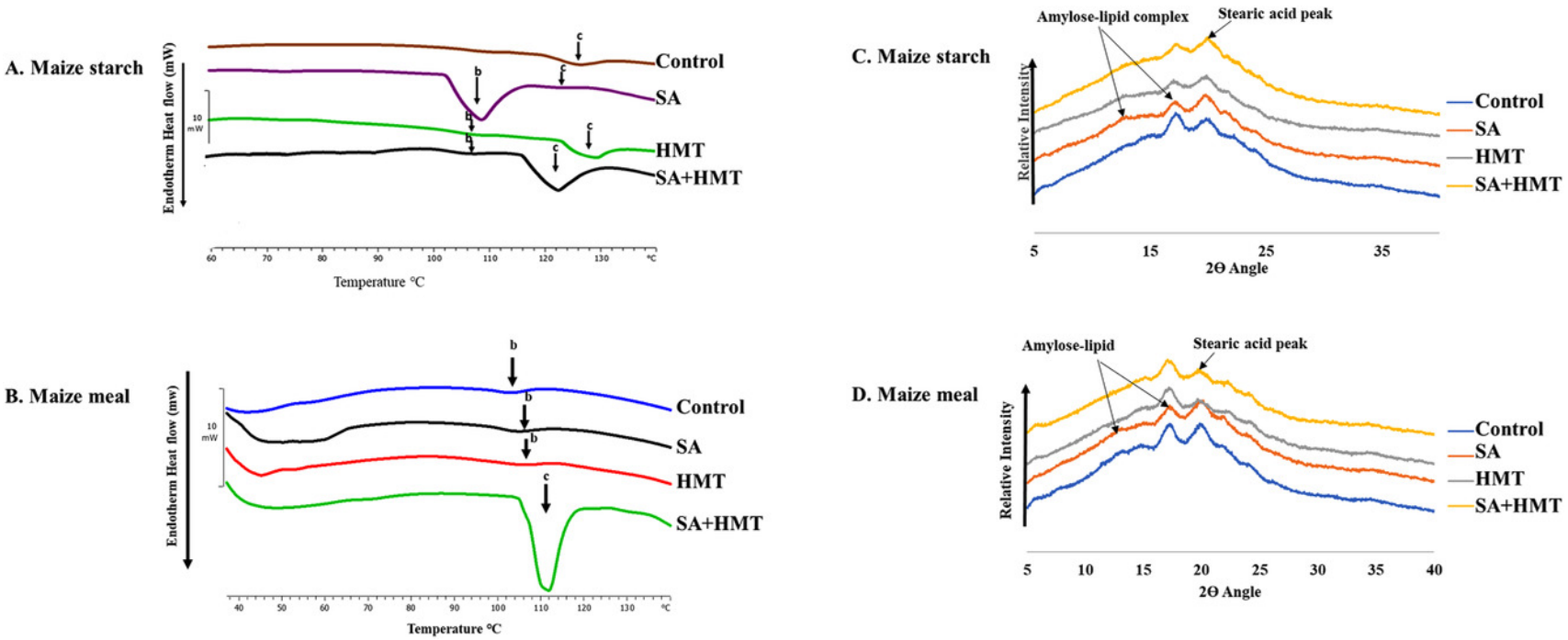

Figure 4. The thermal properties A,B) and X ray diffractogram C,D) of pasted maize starch and unpasted maize meal with SA and HMT.

Addition of SA to both maize starch and maize meal showed two endothermic peaks in starch (Figure 4A) and one peak in maize meal (Figure 4B) above 90 °C. The ΔH value was significantly ($P \leq 0.05$) higher than the control . Starch with SA alone had two endotherms corresponding to type I and type II ALCs with ΔH values of 1.1 and 3.86 J g^{-1}, respectively. Pasted maize meal with SA had an endothermic peak at about 106 °C with a melting enthalpy of 1.9 J g^{-1}. Endotherms with dissociation temperatures of about (98–105), (106–109),C and (110–120) °C correspond to type I, IIa, and IIb amylose–lipid complexes.[28] Pasted starch and maize meal had diffraction peaks of 2θ degrees at 7.5°, 12.7°, and 19°, which is a significant peak in determining amylose–lipid complexes. V-type X-ray crystallography patterns were observed in pasted starch and maize meal with added SA. This confirms the amylose–lipid complexes detected through DSC in Figure 4A,B. Amylose–lipid complex structures are semicrystalline.[30] This also suggests that the conformational changes were due to amylose–lipid complexes development. This conformational change also showed that the enzyme could not access the alpha 1,4 glycosidic bonds for starch hydrolysis. Pasted starch and maize meal with added SA significantly ($P \leq 0.05$) increased the relative crystallinity from 5.20% and 5.75% to 8.35% and 8.70% than the control.

HMT treated starch after pasting showed two transition endotherms and one endotherm for HMT maize meal (Figure 4B). The melting enthalpies for the two endotherms (type I and type II ALCs) observed in pasted maize starch were 0.9, and 6.17 J g^{-1}, respectively. Maize meal only exhibited type I ALCs endotherm with a melting enthalpy of about 1.6 J g^{-1}. The XRD results showed an increase in the relative crystallinity value from 5.20% and 5.75% to 7.50% and 6.40% for starch and maize meal, respectively. The high relativity of crystallinity is linked with the changes of the amorphous region to a more crystalline nature of starch.[27] The increased relative crystalline nature could explain the decrease in RDS and the increase in RS and SDS values in HMT maize meal.

The thermal properties for HMT starch and maize meal pastes with SA are represented in Figure 4A,B. Pasted starch had two transition endotherms (b & c) with peak temperatures of 106 and 121 °C (Table 3), these correspond to type I and IIb amylose–lipid complexes. Pasted maize meal had one endothermic peak of temperature 115 °C, this agrees with amylose–lipid complex type IIa.[31] The XRD peaks of the combination treatment followed the same pattern as SA addition alone. The relative crystallinity significantly ($P \leq 0.05$) increased from 5.20% and 5.75% to about 12.8% and 11.42% in both maize starch and maize meal, respectively. The V-type crystallite (amylose–lipid complexes) can be associated with lower EGI values reported in Table 1. These changes due to HMT with stearic acid also showed there are molecular changes in starch for restriction of the enzyme hydrolysis of the alpha 1,4 glycosidic bond to increase in RS, SDS, and reduce RDS.

Pasted maize starch and maize meal with SA and HMT showed type II ALCs with a melting enthalpy of 16.85 and 37 J g^{-1}, respectively. The high enthalpy of melting for complexes observed in maize meal than in maize starch could be explained by the possibility of other crystalline structures, such as protein–lipid complexes.[32] This is further explained by the principal component analysis discussed later.

To further understand the functional properties associated with different treatments between maize starch and maize meal, principal component analysis (PCA) was performed. It is first noted that the maize meal and maize starch are from commercial sources and the starch was not extracted from the specific maize meal. Nonetheless, some researchers[32, 33] reported that the pasting properties, Mw and gelatinization properties of normal starches were not

significantly different among native maize starches isolated from different varieties. The first two principal components contributed about 95% of the total variation in maize starch (Figure 5A). The plot in Figure 5A was used to identify possible clusters visually. The different starch samples can be divided into four groups. The starch with stearic acid alone (cluster A) was strongly associated with high peak temperature for type I ALCs. High peak temperature for type II ALCs, summation enthalpy for type I and type II ALCs and resistant starch content was strongly associated with maize starch-containing stearic acid followed by infrared HMT (cluster B). The high *k*-value for starch digestibility, rapidly digestible starch content, high EGI, and gel strength were associated with untreated maize starch (cluster C). A high amount of slowly digestible starch and relative crystallinity was associated with heat-moisture treated starch without stearic acid (cluster D).

The PCA of maize starch above showed many similarities with some slight variations for maize meal. The first two principal components contributed about 87% of the total variation in maize meal. The PC plot in Figure 5B was used to identify possible clusters visually. The different maize meal samples can be divided into four groups. The relative crystallinity, high peak temperature for type II ALCs, and thermal stability of ALCs (increased enthalpy of melting for all ALCs) were associated with maize meal with stearic acid (cluster A). The maize meal with stearic acid and HMT (cluster B) was associated with high content of slowly digestible starch. The high content of rapidly digestible starch and EGI were associated with untreated maize meal (cluster C). Increased final viscosity, gel strength, and k-value (starch digestion kinetic) were associated with heat-moisture treated maize meal (cluster D). The similarity suggests that the properties shown by maize meal in terms of the pasting, starch digestibility, and thermal properties are mainly due to the starch polymers in the maize meal.

The effects (in terms of the trends) of SA alone and HMT alone and their combination on both starch and maize meal were similar, except in the starch digestibility fractions. Heat-moisture treated maize starch with stearic acid was more associated with RS and type II ALCs, while in heat-moisture treated maize meal with stearic acid was mostly associated with SDS. Untreated maize meal was also associated with RS, which could be explained by the high content of protein and fat. The protein and lipids have been reported to favor the formation of ternary complexes (formation of complexes starch–lipid, the formation of complexes protein–lipids, and the disulfide bond-linked protein aggregates).[34] These protein interactions have been reported to restrict starch hydration during pasting and the denatured or hydrolyzed proteins that interact with starch chains during cooking/pasting result in complexes that can by inhibiting enzyme-substrate formation hence slowing down starch digestion.[34] The interactions between maize meal components explain the different pasting profiles observed in Figure 1 as well as the different hydrolysis profiles in Figure 2 between maize starch and maize meal.

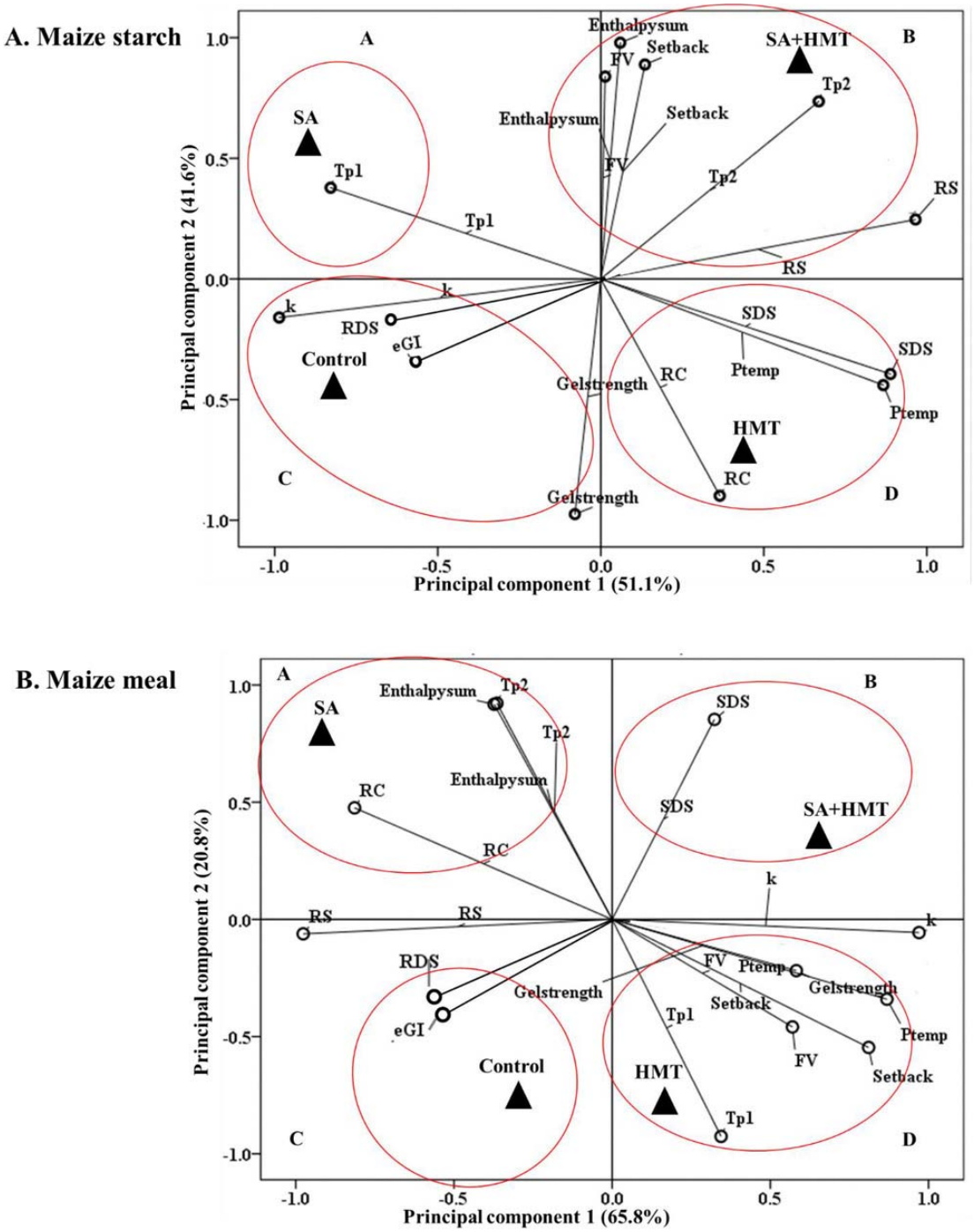

Figure 5. Principal component analysis: A) score plot describing overall variation in the first and second components in maize starch samples; and B) score plot describing overall variation in the first and second components in maize meal pasted samples. (Ptemp = pasting temperature; Setback = setback viscosity; FV = final viscosity; RC = relative crystallinity for pasted samples; RDS = rapidly digestible starch; SDS = slowly digestible starch; RS = resistant starch; Gelstrength = force required to penetrate gel after 24 h; Tp1 = DSC peak temperature of Type I ALCs; Tp2 = DSC peak temperature of Type II ALCs; Enthalpysum = enthalpy of melting type I and Type II ALCs.

3 Conclusions

SA and HMT as a combination treatment has the potential to yield a medium EGI starch and maize meal. The HMT most likely to encourage the rearrangement of starch chains and the development of more ordered double-helical amylopectin side-chain clusters within the starch granules. At the same time, the presence of SA facilitates amylose–lipid complex formation. These changes make the alpha 1–4 glycosidic bonds not as much accessible for enzymatic hydrolysis thereby making starch and maize meal less susceptible to enzyme digestion. SA and HMT of starch and maize meal produce a soft nongelling porridge. The main difference between the functional properties of maize meal and maize starch is due to the high content of nonstarch components (protein and fat) present in the maize meal. This significantly affected the digestibility of both samples to a large extent.

4 Experimental Section

Materials

Superfine maize meal was obtained from Rainbow Chicken Limited (RCL) (Pretoria, South Africa). It contained about 15.3% moisture, 8.2% protein, 1.1% fat content, 0.65% Ash, and a total starch of ≈81.3%. Commercial normal starch, Amyral with 12.9% moisture, 0.64% protein, 0.31% fat content, 0.08% Ash, and total starch content of 95% was sourced from Tongaat Hulett Starch (Edenvale, South Africa). Stearic acid (SA) with CAS number of 57-11-4 was acquired from Sigma-Aldrich Company (St. Louis, MO). The reagents used for this work were analytical standard.

Methods—Adding SA into Starch and Maize Meal

SA (1.5% w/w) was incorporated into samples using the method of Mapengo et al.[18] Pure ethanol was used as a solvent to dissolve SA, and then the powder samples were added. The blend was placed in the water bath and shaken at 50 °C for 30 min at 120 rpm. The samples were placed in an oven for solvent evaporation (at 40 °C).

HMT

The samples with and without SA were standardized to ≈20% moisture which is the desired moisture for HMT. The starch mixture was heated at 110 °C for 16 h in an oven.[18]

Analyses—Starch and Maize Meal Pasting Properties

Samples were pasted, according to Wokadala et al.[28] with modification using a cup and a stirrer (ST24-2D/2V/2V-30) in a Rheometer (Physica MCR 101, Anton Paar, Germany). Starch slurries (10% w/w) were pasted by stirring primarily at 960 rpm at 50 °C for 10 s, heating to 91 °C at 7.5 min at 160 rpm and holding at 91 °C for 15 min at 160 rpm. The paste was allowed to cool from 91 to 50 °C at 7.5 min, then allowed to stand for 1 min at 50 °C. After which the pastes were freeze-dried, the samples were milled into a fine powder using mortar and pestle. Samples were kept in an airtight container at 5 °C until analysis.

Pasted Starch Texture

The texture of the pasted starch was analyzed with a modified procedure reported by Mapengo et al.[18] The produced paste was emptied into small cylinder-shaped vessel of diameter 39.5 mm and a depth of 11.5 mm. The depth of each dish was increased by ≈5 mm by paper tape around its rim. The gels were kept at 25 °C overnight in covered containers. After the paper tape was removed, excess gel above the rim was removed by cutting the surface to be smooth using a wire cheese cutter. A cylindrical probe of 20 mm diameter was used to analyze the gel firmness using texture analyzer EZ-L, Shimadzu (Tokyo, Japan). The probe plunged into the gels (5 mm deep) at 2 mm s^{-1} speed. The maximum force and least force was taken as the firmness and adhesiveness.

In Vitro Starch Digestibility and Estimated Glycemic Index

The modification was done based on Goňi et al.[35] procedure. The cooked and freeze-dried starch and maize meal (≈50 mg) was weighed for analysis. HCl–KCl buffer (pH 1.5) and a solution of 0.2 mL comprising 1 mg pepsin (Sigma-Aldrich P7000-100 G) were added to the samples. Samples were then allowed to stay for 60 min at 40 °C with constant shaking. The rest of the reagents and enzyme solutions were added as per the steps/sequence outlined by Oladiran and Emmambux.[36] Concentrated glucose was estimated using the glucose oxidase–peroxidase kit, and the degree of in vitro starch digestion was calculated as the percentage of the total starch assimilated at time intermediaries ranging from 0 to 180 min.

The starch hydrolysis kinetics were estimated based on the equation by Goňi et al.[35]

$$C = C_{\infty}\left(1 - e^{-kt}\right) \tag{1}$$

where C is the concentration at time t, C_{∞} is the starch hydrolyses percentage after 180 min, k is kinetic constant (min^{-1}) and t is time (min). The parameters k and $C\infty$ were calculated for each treatment founded on the data obtained from the in vitro hydrolysis technique. Jaisut et al.[37] proposed an equation to compute the area under the curve (AUC)

$$\mathrm{AUC} = C_{\infty}\left(t_{\mathrm{f}} - t_0\right) - \left(C_{\infty}/k\right)\left(1 - \exp\left(-k\left(t_{\mathrm{f}} - t_0\right)\right)\right) \tag{2}$$

t_f is the final time (180 min), $t0$ is the initial time (time 0)

$$\text{The hydrolysis index (HI)} = \frac{\text{AUC of the sample}}{\text{AUC of the reference sample}} \times 100 \tag{3}$$

The estimated glycaemic index (EGI) = 39.71+ 0.549 HI (Goňi et al.[35])

RDS, SDS, and RS fractions were estimated using the enzymatic hydrolysis technique.[35] RDS is calculated as a fraction of starch assimilated at 30 min, and SDS was calculated as the fraction of starch digested at 120 min. RS was the entirety of starch undigested that was deducted from total starch.

$$\text{Equations are as follows: } \mathrm{RDS} = G_{30} \times 0.9; \mathrm{SDS} = \left(G_{120} - G_{30}\right) \times 0.9; \mathrm{RS} = \text{Glucose} \times 0.9 \tag{4}$$

Thermal Properties

Pasted and unpasted starch and maize meal were analyzed using a DSC system (HP DSC827e, Mettler Toledo, Greifensee, Switzerland) Wokadala et al.[28] An aluminum pan was used to weigh in the starch powder (10 mg, db) and distilled water was added in a proportion of 1:3 w/w starch-to-water (to make a homogeneous suspension). Pans were then sealed and were allowed to stay at room temperature for at most 24 h. Scanning was done at a high pressure (using N_2 at 4 Mpa) and a temperature of 25 to 140 °C at the rate of 10 °C min^{-1}. Indium with Tp = 156.6 °C and ΔH of 28.45 J g^{-1} was the standard and an empty a pan was used as a reference.

X-Ray Diffractions for Starch Crystallinity

X'Pert PANalytical diffractometer (Eindhoven, Netherlands) was used for the wide-angle X-ray diffraction scattering on cooked and uncooked starch and maize meal. The cooked were freeze-dried and were allowed to stay for 5 days, with an estimated relative humidity of 95% at 25 °C using glycerol.[28] The conditions for the XRD to operate well were 45 kV, 40 mA, Cu Kα (0.154 nm). The overall level of crystallinity was calculated as the percentage of the incorporated territory peaks of crystallinity to the absolute coordinated area on a straight baseline.[28]

Data Analysis

A factorial design (2*2) was used (SA which had 2 levels, i.e., 0% and 1.5% w/w and heat-moisture treated with levels 20% w/w moisture and the control (no HMT). The work was done in three independent experiments. MANOVA was used to determine the significant differences between SA addition and HMT sample. The data for starch and maize meal were analyzed separately. Fischer's LSD Test at $P \leq 0.05$ was used to compare the means.

Acknowledgements

Acknowledgement goes to DSI/NRF Centre of Excellence in Food Security project ID 91490.

Conflict of Interest

The authors declare no conflict of interest.

References

1. B. Van der Merwe, C. Erasmus, J. R. N. Taylor, *J. Food Chem.* 2001, 72, 347.
2. E. K. Arendt, E. Zannini, Cereal Grains for the Food and Beverage Industries, Woodhead Publishing limited, United Kingdom 2013.
3. C. A. Kouamé, N. K. Kouassi, *Food Nutr. Sci.* 2014, 5, 308.
4. M. Benmoussa, B. Suhendra, A. Aboubacar, B. R. Hamaker, *Starch/Stärke* 2004, 58, 92.
5. K. N. Englyst, S. Vinoy, H. N. Englyst, V. Lang, *Br. J. Nutr.* 2003, 89, 329.
6. H. Jiang, J. Jane, in Resistant Starch Sources, Applications and Health Benefits (Eds: Y. C. Shi, C. C. Maningat) John Wiley & Sons, Ltd., Chichester 2013, Ch. 2.

7. G. O. Rombo, J. R. N. Taylor, A. Minnaar, *J. Sci. Food Agric.* 2004, 84, 350.
8. J. Hasjim, Y. Ai, J Jane, in Resistant Starch Sources, Applications and Health Benefits (Eds: Y. C. Shi, C. C. Maningat) John Wiley & Sons, Ltd., Chichester 2013, Ch. 4.
9. Codex Alimentarius, Guidelines on Nutrition Labelling CAC/GL 2–1985 as Last Amended 2010, Joint FAO/WHO Food Standards Programme, Secretariat of the Codex Alimentarius Commission, FAO, Rome 2010.
10. A. P. Nugent, *Nutr. Bull.* 2005, 30, 27.
11. G. Gelders, T. Vanderstukken, H. Goesaert, J. Delcour, *Carbohydr. Polym.* 2004, 56, 447.
12. M. K. Campbell, S. O. Farrell, Lipids and Proteins are Associated in Biological Membranes Biochemistry, 4th ed., Thomson Learning, Pacific Grove, CA 2003.
13. G. G. Gelders, H. Goesaert, J. A. Delcour, *J. Agric. Food Chem.* 2006, 54, 1493.
14. K. O. Adebowale, O. S. Lawal, *Food Hydrocolloid* 2003, 17, 265.
15. D. Sievert, Y. Pomeranz, *Cereal Chem.* 1989, 66, 342.
16. H. J. Chung, R. Hoover, Q. Liu, *Int. J. Biol. Macromol.* 2009, 44, 203.
17. Y. Sang, P. A. Seib, *Carbohydr. Polym.* 2006, 63, 167.
18. C. R. Mapengo, S. S. Ray, M. N. Emmambux, *Food Chem.* 2019, 289, 396.
19. C. R. Mapengo, M. Naushad Emmambux, *Food Chem.* 2020, 325, 126846.
20. Q. Sun, Z. Han, L. Wang, L. Xiong, *Food Chem.* 2014, 145, 756.
21. S. Puncha-arnon, D. Uttapap, *Carbohydr. Polym.* 2013, 91, 85.
22. X. Chen, X. He, X. Fu, Q. Huang, *J. Cereal Sci.* 2015, 63, 109.
23. G. Richardson, M. Langton, A. Bark, A.-M. Hermansson, *Starch/Stärke* 2003, 55, 150.
24. H. Liu, H. Corke, L. Ramsden, *Starch/Stärke* 2000, 52, 353.
25. R. Cui, C. G. Oates, *Food Chem.* 1999, 65, 417.
26. G. G. Gelders, H. Goesaert, J. A. Delcour, *Biomacromolecules* 2005, 6, 2622.
27. R. Hoover, *Food Rev. Intl.* 2000, 16, 369.
28. O. C. Wokadala, S. S. Ray, M. N. Emmambux, *Carbohydr. Polym.* 2012, 90, 616.
29. 29M. Godet, V. Tran, M. Delage, A. Buleon, *Int. J. Biol. Macromol.* 1993, 15, 11.
30. 30E. O. Arijaje, Y. J. Wang, *Starch/Stärke* 2016, 68, 700.
31. 31C. G. Biliaderis, G. Galloway, *Carbohydr. Res.* 1989, 189, 31.
32. 32A. Aparicio-Saguilán, G. Méndez-Montealvo, J. Solorza-Feria, L. A. Bello-Pérez, *J. Sci. Food Agric.* 2006, 86, 1078.
33. 33M. S. Milašinović-Šeremešić, M. M. Radosavljević, L. P. Dokić, *Acta Period. Tecnol.* 2012, 61.
34. G. Zhang, B. Hamaker, *J. Agric. Food Chem.* 2003, 51, 2797.
35. I. Goñi, A. Garcia-Alonso, F. Saura-Calixto, *Nutr. Res.* 1997, 17, 427.
36. D. A. Oladiran, N. M. Emmambux, *Starch/Stärke* 2017, 69, 7.
37. D. Jaisut, S. Prachayawarakorn, W. Varanyanond, P. Tungtrakul, S. Soponronnarit, *J. Food Eng.* 2008, 86, 251.