

Optimized heat-moisture treatment decreases the starch digestibility of polished maize flour from predominant varieties consumed in Uganda

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ABSTRACT

In Uganda, maize flour is the major carbohydrate food source for over 90 % of households. It is largely consumed in polished form characterized by high digestibility, rapidly releasing glucose into the bloodstream, making it unsuitable for metabolic health. This calls for the reduction of maize flour digestibility through increasing its slowly-digestible (SDS) and resistant-starch (RS) contents, typical function of Heat-moisture Treatment (HMT). However, HMT efficacy depends on the botanical starch source and HMT conditions necessitating its optimization. This study investigated optimal HMT conditions to produce polished maize flour with lower starch digestibility by increasing SDS and RS contents for four maize varieties (UH5354, DK777, Longe 10H, and DT Max). Response surface methodology (Box-Bakhen design) was used to set the experimental design with moisture (20–30 %), temperature (80–100 °C), and heating-time (4–8 h) conditions, and corresponding SDS and RS contents, analysed using mega-enzyme procedure. Composite SDS and RS (SDS+RS) were used in the optimization. Quadratic function was the most appropriate model that fitted the data as exhibited by non significant lack of fit ($p > 0.05$) and explained at least 80 % of the variance for each variety considered. Optimum moisture (%), temperature (°C) and heating-time (hours) were 23.17, 80.00, and 4.12 for UH5354, 20.83, 95.43, and 5.42 for DK777, 34.51, 90.46, and 3.01 for Longe 10H and 19.24, 97.02, and 5.88 for DT Max varieties. Relative to the control, these optimal HMT conditions improved the contents of SDS+RS by 8–14 % suggesting their promotion to reduce the high digestibility of polished maize flour among households.

1. Introduction

Starch is the main storage polysaccharide in plants such as cereal grains, roots, and tubers. It is quantitatively the most dominant storage carbohydrate source in the human diet and contributes greatly to the 4 kCal/g energy provided. For its nutritional purposes and based on its hydrolysis by enzymes during digestion, it is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). RDS undergoes rapid digestion within the first 20 min in the small intestine, causing high postprandial glycemic response (Englyst et al., 1992) which increases the risk of non-metabolic diseases. In contrast, SDS is digested slowly (20 and 120 min), whereas RS remains indigestible in small intestine, and is subsequently fermented by gut microorganisms thereby reducing their contribution to postprandial glycemic response (Liu et al., 2020). Several studies have demonstrated SDS and RS role in regulating blood glucose levels, body weight, and

associated metabolic diseases (Miao et al., 2015; Sun et al., 2018; Wang et al., 2019). This makes SDS and RS vital nutritional attributes of carbohydrate quality necessary to reduce the risk of the associated non-communicable diseases.

In Uganda, maize grain is the staple food that provides carbohydrates to about 92 % of households (Wacoo et al., 2018). Therefore, several maize varieties such as longe (5, 5D), UH (5354, 5355, 5051), DK (777 & 9089), Longe 10H, and DT Max are promoted across the country and East African community based on their agronomical performances (Lee, 2020). However, these varieties exhibit variable and relative low levels of SDS and RS ranging from 55.9–59.8 % and 5.14–14.0 %, respectively (Camelo-Méndez et al., 2017; De-Alcántara et al., 2020). The aforementioned statistics suggest that the maize varieties have a high starch digestibility. This makes the glucose from their digestion to be rapidly absorbed into the bloodstream (high glycaemic index) and thus, unsuitable for metabolic health. In addition, polishing of these maize

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grains before milling, being a common practice in Uganda and surrounding countries to improve maize flour organoleptic properties such as sweetness, digestibility, lightness, shelf life, and reduce the cooking time (Kiguli et al., 2019) exacerbates the problem. This is largely because polishing removes the bran which is rich in SDS and RS hence, decreasing the SDS and RS contents of the polished maize flour (Sivakamasundari et al., 2022). Consequently, the reduction in SDS and RS leads to increased starch digestibility and a high glycaemic index, which are associated with health risks such as diabetes type II, obesity, and other non-communicable diseases (NCDs) that are on the rise in the country as per recent National Demographic Health Surveys (UDHS, 2022). Thus, there is a need to improve the SDS and RS contents of polished maize flour most consumed in Uganda and surrounding countries for better nutritional outcomes.

Whereas several starch modification techniques exist and their advantage in increasing SDS and RS of cereals has been demonstrated, physical starch modification, mainly heat moisture treatment (HMT), has shown more promising results over the other methods (Dong et al., 2021; Sandhu et al., 2020; Van Hung et al., 2020). HMT has been shown in several studies to be effective in producing SDS and RS in starch-based foods such as cereals (Asare et al., 2021; Marta et al., 2022). The treated starches obtained from HMT are generally considered safe for use in food (Sandhu et al., 2020). HMT is a natural, more accessible, low-cost physical starch modification method that does not produce any effluents such as salts, or reagents. It involves the treatment of starch granules at low moisture levels (<35 % w/w) for a certain time (15 min–16 h) and at temperatures varying between 80 and 140 °C, below the gelatinization temperature (Wang et al., 2021). In addition, the increase in SDS and RS due to HMT has been shown to decrease their starch digestion rate (Cheng et al., 2019; Gayary & Mahanta, 2020). However, the efficacy of HMT varies according to the botanical source of the starchy food and the conditions applied (Wang et al., 2021), thus necessitate optimizing HMT conditions for a specific starch source typical of response-surface methodology.

Response-surface methodology (RSM) is a useful technique for the investigation of several input variables that influence the performance measures or quality characteristics of the product or process under investigation (Giri & Prasad, 2007). RSM allows not only to explore the optimal process but also to further analyze in depth the extent to which different processes affect a given response factor typical of the starch properties (Liu et al., 2022). RSM is an effective and frequently used tool for optimization and several studies have employed RSM mainly Box-Behnken design (BBD) to optimize HMT conditions resulting in acceptable responses (Dai et al., 2019; Deng et al., 2022; Ye et al., 2016). In particular, BBD reduces the number of additional experiments, takes a shorter time, and eliminates the need for laborious laboratory studies (Yetilmezsoy et al., 2009).

Although, several studies have performed HMT on extracted maize starch (Asare et al., 2021; Liu et al., 2014) and waxy maize starch (Chang et al., 2020; Sui et al., 2015), none have been carried out to reduce the starch digestibility of polished maize flour typical of those consumed in Uganda and several African countries. Whereas the conditions of HMT application for starch modification on polished maize are unknown, they may be variety-dependent (Pieczyk & Domian, 2021). This could be due to the difference in inherent characteristics (amylose, total starch, phenolic compound, proximate composition) that affect the development of resistant starch during HMT (Toutounji et al., 2019; Yang et al., 2023). Therefore, this study aimed to optimize HMT conditions to reduce the starch digestibility of polished maize flour among the predominant maize varieties consumed in Uganda using the response surface methodology.

2. Materials and method

2.1. Materials

In Uganda, while up to eighty maize varieties have been recorded by the National Agricultural Research Organisation (NARO) in 2022, only thirteen are commercialized and grown by the farmers. Although these 13 varieties have different compositions of internal factors affecting starch digestibility (proximate composition, phenolic compounds, amylose, and total starch content), they can be grouped into four significantly different clusters based on those internal factors. These internal factors affect the effectiveness of heat-moisture treatment in decreasing starch digestibility (Qi & Tester, 2016; Sui et al., 2017). Therefore, the most consumed maize variety was selected from each of the four clusters (UH5354, DK777, Longe 10H, and DT Max), and subsequently used in the current study. Seventy-five (75) kilograms of each variety were purchased directly from farmers after the November–December 2023 harvest period. All foreign matter and dirt were removed, grains were sorted, cleaned, and the damaged or broken grains removed. The clean and sorted grains were put in PICS (Purdue Improved Crop Storage) bags, composed of two polypropylene bags, and stored in the laboratory until use within 8–10 weeks.

2.2. Optimization of heat moisture treatment

2.2.1. Experimental design for heat moisture treatment optimization

Response surface methodology was employed to generate the experimental design used to optimize the heat moisture treatment (HMT) conditions with a total of slowly digestible (SDS) and resistant starch (RS) as response factors. The effects of the three HMT factors X_1 (moisture); X_2 (heating length); and X_3 (temperature) on the response Y_1 (SDS + RS) were assessed. The moisture content and temperature play crucial roles during HMT.

According to Wang et al. (2016), the increase of moisture content during HMT leads to significant structural disorganization and molecular rearrangements in starch, which can convert RDS into SDS and RS. Although, an increase in moisture content generally promotes the development of SDS and RS in cereals like maize, subjecting maize starch to HMT with moisture content above 30 % tends to decrease SDS and RS content (Chen et al., 2017a; Kumar et al., 2023). On another hand, de la Rosa-Millan et al. (2023) the application of gradual and higher temperatures between 80 and 100, converts RDS into SDS and RS in maize grains due to optimal gelatinization and structural changes. However, when the temperature is above 100 °C, maize starch granules begin excessive gelatinization, swell significantly and lose their granular structure, resulting the SDS and RS (Chen et al., 2017a). Whereas the nutritional content of the maize grains, Ye et al. (2016), found that SDS+RS increases when the maize is subjected to temperatures ranging from 80 to 100 °C, moisture content 20 to 30 %, and heating length 4 to 8 h. In contrast, SDS+RS decreases dramatically when the moisture content, time, and temperature are higher than 30 %, 8 h, and 100 °C, respectively (Ye et al., 2016). Therefore, the ranges of intervals where SDS+RS increases, 20 to 30 % moisture content, 4 to 8 h heating length, and 80 to 100 °C temperature were considered in the present study for setting the experimental design.

A Box-Behnken design, an alternative way to the central composite design most applied to reduce the experimental cost and process (Gündoğdu et al., 2016) was used. The design scheme was comprised of 12 treatments with five replications of the central point to calculate the experimental error for each maize variety. The experimental design was generated using R software version 4.2.2 with rsm packages. For statistical calculations, the process variables were coded at three levels (−1, 0, and +1) representing lower, middle and higher values and the coding was performed using Eq. (1).

$$x_i = (X_i - X_2) / \Delta X_i \quad i = 1, 2, 3, \dots, k \quad (1)$$

where x_1 , is the dimensionless value of an independent variable; X_1 is the real value of an independent variable; X_2 is the real value of an independent variable at the center point; and ΔXi is the step change of the real value of the variable i . The range of independent variables and their levels are presented in Table 1.

2.2.2. Heat moisture treatment process

Heat-moisture treatment (HMT) was performed according to a laboratory protocol applied by Sui et al. (2015). Prior to the HMT application, the initial moisture content of each maize sample was determined gravimetrically using the hot air oven method following the standard AOAC method number 925.09 (Association of Official Analytical Chemists, 1990). Subsequently, the moisture content of maize grains was adjusted by immersing the grains in distilled water to obtain the desired moisture content using the proposed formula of Cordeiro et al. (2018) (Eq. (2)).

$$m_w = \left[\left(\frac{100 - M_i}{100 - M_f} \right) - 1 \right] * m \quad (2)$$

where m_w (g) is the mass of water to be added, M_i (%) is the initial moisture of the sample, M_f (%) is the desired moisture, and m (g) is the maize grain mass used.

The wet maize grains were put in polyethylene zipper bags, sealed, and equilibrated at 4 °C for 24 h in Hisens refrigerator (Model RT488N4ASU, Guangdong, China) before the heat treatment was applied. To avoid the decrease of the moisture content during the heat application, the samples were hermetically sealed in a glass bottle to maintain the moisture content as applied by Zou et al. (2023). The packed samples in glass bottles were heated using an electric hot-air drying oven DHG 9053A (Sui et al., 2015) at the set temperatures and time duration according to the experimental design (Table 1), dried and polished as described under subSection 2.2.3.

2.2.3. Polishing and milling of maize samples

The dried heat-moisture-treated maize samples were polished and milled. Before polishing and milling, the maize grains were dried in an oven with forced air circulation at 50 °C for 24 h (Van Hung et al., 2020). The maize grains treated using HMT were polished using maize huller (Locally fabricated, Gulu, Uganda) following the two common polishing steps typically used in Uganda. Briefly, the whole maize grains were polished from the surface layer to the center. During polishing the germ and bran layer were removed twice from the grain mechanically as typically done in Uganda, resulting into what is locally known as "first

Table 1
Level of factors used in Box-Behnken design for optimization of HMT conditions.

Coded Process Variables			Process variable non-coded		
X_1	X_2	X_3	Moisture content (%)	Heating length (Hours)	Temperature (°C)
0	-1	-1	25	4	80
-1	0	-1	20	6	80
-1	0	-1	30	6	80
0	-1	-1	25	8	80
-1	-1	0	20	4	90
-1	-1	0	30	4	90
0	0	0	25	6	90
0	0	0	25	6	90
0	0	0	25	6	90
0	0	0	25	6	90
0	0	0	25	6	90
0	0	0	25	6	90
-1	-1	0	20	8	90
-1	-1	0	30	8	90
0	-1	-1	25	4	100
-1	0	-1	20	6	100
-1	0	-1	30	6	100
0	-1	-1	25	8	100

class" maize flour. The first class polished maize grains/grits were then grounded in laboratory conditions using the laboratory blender 8010EG (Model HGBTWTG4, Torrington, Connecticut 06,700 USA), and sieved through a 100 μ m sieve. The flour was stored in paper bags at room temperature until laboratory analysis within 15–40 days.

2.2.4. Determination of starch digestibility of HMT-treated maize flour

The starch digestibility of polished maize flour obtained under Section 2.2.3 was determined according to the gastro-intestinal simulated in vitro digestion method of Englyst et al. (1992) using the mega-enzyme procedure (Neogen cooperation, 2023). Briefly, pancreatic α amylase/amyloglucosidase (PAA/AMG) solution was prepared by adding 0.5 g of PAA/AMG powder mixture to 25 mL of sodium maleate buffer 50 mM, pH 6.0. AMG solution was prepared by adding 1 mL of the contents of Amyloglucosidase (3300 U/mL) to 30 mL of sodium acetate buffer pH 4.5. The reagent was prepared immediately before use and stirred on a magnetic stirrer for 5 min and stored on ice until use.

Exactly, 1 g of each sample of polished maize flour was weighed and transferred into 50 mL polypropylene screw-cap tubes and mixed with 1.0 mL of 95 % Ethanol (v/v) and 35 mL of Sodium maleate buffer 50 mM, pH 6.0. The mixture was submersed in a magnetic stirrer water bath (Wagtech OLS 200, Germany) to equilibrate at 37 °C for 5 min at 170 rpm. Exactly, 5 mL of PAA/AMG enzyme solution was added and incubated in Wagtech OLS200 water bath, at 37 °C with agitation at 170 rpm for 180 min. Aliquots (1.0 ml) of the hydrolysate were taken at 0, 20, 120, 180, and 240 min and mixed with 20 ml of 80 % ethanol to inactive the enzyme. Exactly 2.0 mL of each solution was picked and centrifuged at 6500 rpm for 10 min using a Centurion Scientific Benchtop centrifuge (Model 1.C2015, United Kingdom). After centrifuging, the aliquot (0.1 mL) was pipetted in duplicate and mixed with 0.1 mL of AMG solution, incubated at 50 °C for 30 min, and mixed with 3.0 mL of freshly prepared d-Glucose Assay Kit Reagent (GOPOD). Reagent blank solutions were prepared by mixing 0.2 mL of sodium acetate buffer, pH 4.5 with 3.0 mL of GOPOD reagent whereas the d-glucose standards were prepared by mixing 100 μ g of d-glucose with 0.1 mL sodium acetate buffer, 100 mM, pH 4.5 and 3.0 mL of GOPOD reagent. The sample mixed with GOPOD, the reagent blank, and d-glucose standards prepared as described subsequently were incubated at 50 °C for 20 min for color development before reading.

After 240 min of digestion, an aliquot of the sample suspension was mixed with 95 % ethanol to precipitate the resistant starch fraction. The mixture was centrifuged at 4000 rpm for 10 min using a Centurion Scientific Benchtop centrifuge, and the supernatant was decanted. The pellet was then suspended in 50 % aqueous ethanol, vortexed, and centrifuged again at 4000 rpm for 10 min. This wash step was repeated twice to remove any residual digestible starch. This was then followed by adding 2 mL of 1.7 M NaOH to the washed pellet, and the mixture was incubated at 37 °C for 20 min to solubilize the resistant starch. Exactly, 1.0 M sodium acetate buffer (pH 3.8) and AMG solution (3300 U/mL) were added. This mixture was incubated at 50 °C for 30 min to hydrolyze the resistant starch to glucose followed by adding GOPOD reagent. The absorbance of the sample and glucose standards at 510 nm against the reagent blank were measured. The content of RDS (at 20 min), SDS (between 20 and 120 min), and RS (after 240 min) were calculated using the Megazyme formula (K-DSTRS), accessed from the Megazyme website (www.megazyme.com). All experiments were conducted in duplicate. The contents of SDS and RS were added to form a composite factor used during the optimisation process.

2.2.5. Statistical models of heat moisture treatment factors

Statistical modelling was performed using R software version 4.2.2. According to Hsu et al. (2015), starch food exhibits distinct digestibility during processing such as HMT due to variations in physicochemical properties (internal factors). As such, optimal processing conditions to reduce starch digestibility should be established on a variety-specific basis. Shapiro test and Leven test were used to assess the normality

and the homogeneity of variance of the data, respectively. The results showed that the data was normally distributed ($p > 0.05$) but violated the assumption of homogeneity of variance ($p < 0.05$). Based on these results, Welch's ANOVA test (not assuming equal variances) was used as applied by Dervisević et al. (2024). These results show that the starch digestibility fractions of polished maize flour in this study varied generally across the maize variety ($p < 0.05$, Supplementary Data 1). Consequently, optimization was conducted on a variety basis in this study. The second-order polynomial regression (Eq. (3)) which is generally able to describe the relationship between the responses and the independent variables was used to check the fitness of the collected data.

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{(i=1)}^2 \sum_{(j=i+1)}^3 \beta_{ij} X_i X_j \quad (3)$$

where Y is the response, the SDS+RS yield (%); β_0 is the constant, β_i is the linear coefficients, β_{ii} represents the quadratic coefficients, β_{ij} is the interaction coefficient, while X_i and X_j are the coding values of the independent variables or levels of each factor.

The linear, interaction, and quadratic relationship effects were expressed using the model. The appropriate model was identified after checking the goodness of fit (lack of fit), the predictive power of the model (R^2 value), and the global significance (model p -value). The best model fitting the data was selected when the lack of fit was not significant, higher, or equal to 0.05 (Fagerland & Hosmer, 2013) and the p -value was significant (≤ 0.05). The acceptable model correlation coefficient (R^2) was set at higher than 0.8, meaning that 80 % of the data are explained by the model, the minimum R^2 value for good model fitting (Khodashenas & Jouki, 2020).

To describe the individual and interactive effects of the factors, three-dimensional (3D) response surface plots were developed. The selected models were used to plot the 3D response surface contour graphs to study the interactive effect of independent variables on the response. The optimum application condition of HMT yielding to the maximum (SDS+RS) development was determined using the selected predictive model. The generated HMT solutions were validated.

2.2.6. Validation of the selected models

Separate validation tests were performed to determine the suitability of the model equations for the prediction of the optimum response variables. The optimized conditions were validated for the maximum SDS+RS contents (%) based on the predictive values obtained using RSM. The composite SDS+RS content was determined under optimized conditions following the procedure described under Section 2.2.4.

2.3. Determination of optimal heat moisture treatment conditions effect

The maize flours were prepared in two batches: control maize flour without HMT and maize flour subjected to HMT-optimised conditions for each variety. The HMT treatment was carried out according to a laboratory protocol applied by Sui et al. (2015) described in Section 2.2.2 except that the optimal HMT conditions found for moisture content, temperature and heating length were applied. After HMT application the maize grains were dried as described under Section 2.2.3. The dried maize grains were polished and ground according to the method described in Section 2.2.3. and starch digestibility fraction analysed. The starch digestibility was analysed according to the procedure described in Section 2.2.4.

2.4. Statistical analysis

The results were statistically tested using a one-sample t -test at the significance level of $p = 0.05$. In order to determine the validity of the model selected, the experimental values were compared with predicted values using a one-sample t -test. The null hypothesis was that there was

no significant difference between the actual and the predicted values (test value). Statistically significant values indicated non-validity whereas statistically insignificant values indicated validity (Giri & Prasad, 2007). The effect of optimal heat moisture treatment conditions was also assessed by comparing control maize flour without HMT to maize flour subjected to optimized HMT conditions using a one-sample t -test. All the analyses were performed using R software version 4.2.2.

3. Results and discussion

3.1. Model fitting and adequacy checking

The effects of the three independent variables moisture (X_1), length of heating (X_2), and temperature (X_3) on the development of SDS and RS were determined by fitting second-order polynomial models to the experimental data obtained (Supplementary Data 2). The quadratic model was selected as it provided the best fit for the experimental data for each variety of maize. This model generally had the highest values of lack of fit, R -squared, and adjusted R -squared, indicating that it fits the experimental data better compared to other models (Quinino et al., 2012). By implication, this model explains and predicts the response variable better than other models. In addition, the quadratic regression is the only one having an R -squared value greater than 0.8, which is generally considered a good fit (Khodashenas & Jouki, 2020). The quadratic model is statistically significant at the 5 % level for each variety suggesting that the effects modelled by the quadratic function are meaningful and can be used to make predictions.

The lack of fit of the quadratic regression was 0.23, 0.61, 0.09, and 0.63 (>0.05) for the varieties UH5354, DK777, Longe 10H, and DT Max, respectively. This indicates a good fit for the models (Song et al., 2015). The R -squared values were 0.89, 0.91, 0.86, and 0.82 for UH5354, DK777, Longe 10H, and DT Max, respectively which revealed a high accuracy of the models (Polat et al., 2020). This means the quadratic regression could explain up to 89 %, 91 %, 86 %, and 82 % of the experimental data obtained for each variety. The regression coefficients of the linear, quadratic, and interaction terms for each response are presented in Table 2 and their significance was determined using analysis of variance (ANOVA). The model equation for SDS and RS contents (SDS+RS) in terms of coded factors for the quadratic model for each variety are presented in Eqs. (4) to 7 obtained through the ANOVA test.

$$Y_1 = 50.37 + 1.46 X_1 - 0.86 X_2 - 5.89 X_3 + 4.15 X_1 X_2 + 10.53 X_1 X_3 - 6.20 X_2 X_3 + 0.91 X_1^2 + 4.15 X_2^2 - 1.52 X_3^2 \quad (4)$$

$$Y_2 = 47.47 + 0.19 X_1 - 1.06 X_2 + 2.75 X_3 + 1.79 X_1 X_2 - 3.26 X_1 X_3 - 3.85 X_2 X_3 - 0.10 X_1^2 - 7.32 X_2^2 - 5.49 X_3^2 \quad (5)$$

$$Y_3 = 36.71 + 4.87 X_1 - 2.09 X_2 - 0.86 X_3 - 2.72 X_1 X_2 + 1.54 X_1 X_3 + 6.56 X_2 X_3 - 1.75 X_1^2 + 5.74 X_2^2 - 0.95 X_3^2 \quad (6)$$

$$Y_4 = 43.90 - 2.33 X_1 - 0.81 X_2 + 0.51 X_3 - 1.95 X_1 X_2 - 1.55 X_1 X_3 + 0.41 X_2 X_3 - 0.75 X_1^2 + 1.83 X_2^2 + 3.65 X_3^2 \quad (7)$$

With Y_1 , Y_2 , Y_3 , and Y_4 , composite SDS+RS for UH5354, DK777, Longe 10H, and DT Max respectively and X_1 , X_2 , and X_3 coded Moisture content, length of heating, and temperature respectively.

The effect of independent variables (moisture content, heating length, and temperature) on the SDS and RS were strongly predicted with the linear, interaction, and quadratic terms depending on the maize variety (Table 2). For the variety UH5354, the linear term for temperature (X_3) and its interactions with moisture content ($X_1 \times X_3$) and heating length ($X_2 \times X_3$) were statistically significant ($p < 0.05$). In contrast, for the variety DK777, the linear terms for moisture content and temperature,

Table 2
Results of the quadratic polynomial models showing the effect of linear, interaction, and quadratic terms on the slow digestible and resistant starch development across the maize varieties.

Model Statistics	UH5354			DK777			Longe 10H			DT Max		
	Quadratic			Quadratic			Quadratic			Quadratic		
	Coef	F value	P value	Coef	F value	P value	Coef	F value	P value	Coef	F value	P value
Model		6.690	0.010**		8.170	0.006***		4.910	0.024**		3.670	0.050**
X ₂	1.460	0.981	0.355	0.187	7.970	0.026**	4.870	14.170	0.007***	−2.330	9.500	0.018**
X ₃	−0.859	0.338	0.579	−1.060	0.037	0.854	2.090	2.620	0.150	−0.813	1.160	0.317
X ₁	−5.900	15.940	0.005***	2.750	8.170	0.006***	−0.862	0.444	0.527	0.511	0.459	0.520
X ₁ × X ₂	4.150	3.940	0.087*	1.790	7.830	0.027**	−2.720	2.220	0.180	−1.960	3.350	0.110
X ₁ × X ₃	10.530	25.380	0.002***	−3.270	1.180	0.313	1.550	0.713	0.426	−1.560	2.130	0.188
X ₂ × X ₃	6.200	8.800	0.021**	−3.860	5.620	0.050**	6.560	12.840	0.009***	0.412	0.149	0.711
X ₁ ²	0.915	0.202	0.667	−0.103	16.710	0.005***	−1.750	0.959	0.360	−0.754	0.525	0.492
X ₂ ²	4.150	4.150	0.081*	−7.320	0.006	0.941	5.740	10.340	0.015**	1.830	3.080	0.123
X ₃ ²	−1.520	0.555	0.481	−5.490	1.680	0.236	−0.955	0.287	0.609	3.650	12.330	0.010**
Lack of Fit		2.180	0.233		0.676	0.611		4.560	0.088		0.254	0.856
R ²	0.896			0.913			0.863			0.825		
Adjusted R ²	0.762			0.801			0.687			0.600		
Predicted R ²	−0.096			0.442			−0.742			0.323		
Adeq Precision	10.634			8.796			8.033			6.912		

*, **, ***: denote statistical significance at 10 %, 5 % and 1 % level, respectively X₁: Moisture content, X₂: Heating length and X₃: Temperature.

as well as the interactions between moisture and heating length, and moisture and temperature, along with the quadratic term of moisture content (X₁), were significant ($p < 0.05$) (Table 2). These show that the development of the SDS and RS are strongly modelled with the linear and interaction terms for the UH5354 variety whereas with the quadratic and interaction terms for the DK777 variety. For the variety Longe 10H, all three terms modelled strongly the development of SDS and RS whereas only the linear and quadratic terms modelled strongly the development of SDS and RS for DT Max variety (Table 2). These results indicate that the suggested models are describing appropriately the development of SDS and RS for each of the four maize varieties. The

observed and predictive data in terms of coded factors for the quadratic model are presented in Supplementary Data 3.

3.2. Effect of independent variables and optimal HMT conditions for the maize varieties

3.2.1. Interactive effect of independent variables on the response

Prior to the optimal heat moisture condition determination, the effect of the independent variable on the composite slowly digestible and resistant starch was analysed. The quadratic regression enabled the prediction of the effects of the three independent variables on the

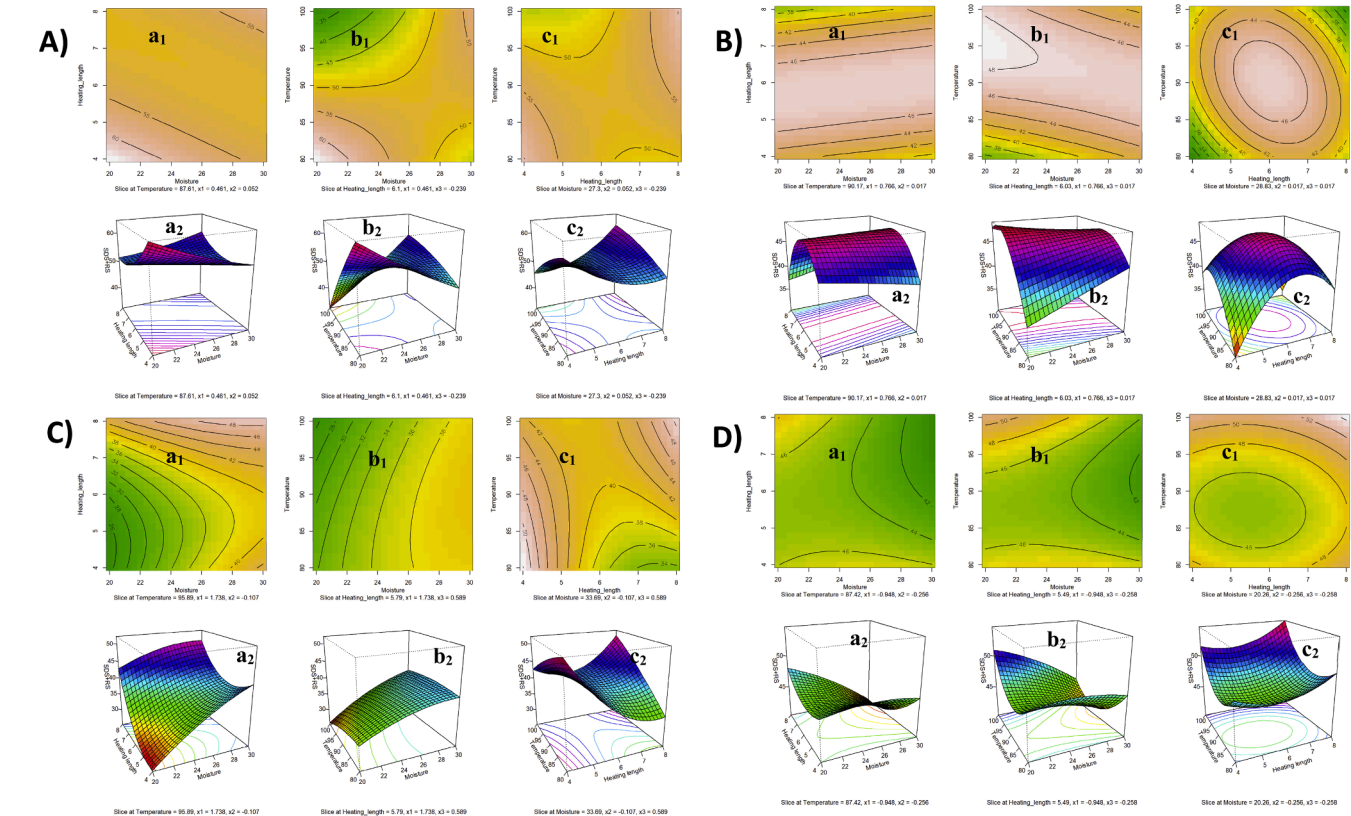


Fig. 1. Response surface plots and contour plots showing the effect of moisture content, length of heating, and temperature on the development of composite slow digestible and resistant starch content of UH5354 (A), DK777 (B), Longe 10H (C) and DT Max (D) maize varieties.

development of SDS and RS content of flour for each maize variety. The relationship between the independent and dependent variables is depicted through two-dimensional contour plots (Fig. 1(A–D): a_1 , b_1 , c_1) and three-dimensional response surface plots (Fig. 1(A–D): a_2 , b_2 , c_2). In these plots, the dependent variable was obtained with two continuous independent variables (Heating length and Moisture), whereas the third independent variable was fixed (Temperature) in the case of Fig 1(A– a_1) for example. The circular contour plot indicated that the interactions between the corresponding variables were negligible, whereas the elliptical contour plot indicated perfect interactions (Guo et al., 2010).

As shown in Fig. 1, the development of resistant starch fractions varied significantly when the moisture content and temperature of the HMT varied (Fig 1A: b_1). The elliptical contour plots revealed a significant interaction between the three independent variables. This interaction effect is more pronounced for the variations in moisture and temperature (Fig. 1A: b_2) but less pronounced for the heating variation (Fig. 1A: b_1). This indicates that the variation in moisture content and temperature influenced SDS and RS formation in these maize varieties. When maize starch is exposed to high moisture content and temperatures during HMT, the starch granules absorb water, swell, and undergo partial gelatinization. This process disrupts the native crystalline structure of starch, allowing for molecular rearrangement. However, because HMT occurs under limited water availability (compared to full gelatinization in excess water), complete granule breakdown is avoided, causing the formation of SDS and RS (Liu et al., 2016; Wang et al., 2016). For instance, Ye et al. (2016) and Zou et al. (2023) reported that increasing both moisture content and temperatures increases SDS and RS contents in maize starch which aligns with the findings of the present study.

The increase of moisture content during HMT promotes molecular rearrangement, transforms the crystalline structure of starch, often from B-type to A-type, decreases relative crystallinity, and increases SDS and RS content (Pieczyk & Domian, 2021; Sun et al., 2024; Xiao et al., 2017). For instance, increasing moisture levels during HMT between 25 and 35 % has been shown to lead to the increased of SDS and RS content of maize starch (Wang et al., 2016) waxy maize and native maize starch (Chen et al., 2017b) and native maize flour (Ye et al., 2016). On the other hand, increase HMT temperature has been shown to enhance SDS and RS content due to structural changes that facilitate starch retrogradation and crystallinity (Liu et al., 2016; Wang et al., 2016). Temperatures promote the conversion of amorphous starch into more ordered structures, which resist enzymatic hydrolysis (Chung et al., 2009; Wang et al., 2018). For example, Gu et al. (2024) reported an increase of SDS and RS contents in high-amylose maize starch when HMT temperature increased, a result which aligns with the findings of the present study. Whereas, the length applied during HMT is not a critical factor in influencing the formation of SDS and RS in most cases (Ye et al., 2016). As such, the variation in moisture content and temperature influenced SDS and RS formation in these maize varieties regardless the heating duration.

The maximum SDS and RS values of 46 was obtained after the HMT application for the maize variety DK777 (Fig. 1B: a_1 , b_1 , c_1). Generally, the variation of the independent variable affected the development of SDS and RS. Elliptical contour plots revealed a significant interaction between the independent variables except for the temperature and heating length where the plot is circular implying that no perfect interaction between these two independent factors (Fig. 1B: c_1 – c_2). For the maize variety Longe 10H, the variation in the independent variables significantly affected the development of SDS and RS. As shown in Fig. 1C: a_1 – a_2 , the maximum SDS and RS values reached 48 when the moisture content was high for a short duration and when the temperature was kept constant. The elliptical contour plots for this variety further revealed a significant interaction between the independent variables (Guo et al., 2010). For the variety DT Max, the elliptical contour plots revealed a significant interaction between the independent variables expected for the temperature and heating length (Fig. 1D: c_1 – c_2)

as depicted by the circular contour plot.

3.2.2. Optimal HMT conditions

Heat-moisture treatment (HMT) parameters were optimized to maximize the Slowly Digestible Starch (SDS) and Resistant Starch (RS) development in the maize varieties. These optimum conditions varied according to the variety which is in agreement with the findings of Hsu et al. (2015) which suggested that optimum conditions should be established per variety albeit for non-polished maize. This implies that the polishing of maize does not affect the HMT conditions among varieties. The optimum combinations of moisture content, heating length and temperature were (23.17 %, 4.12 h, 80.00 °C) for UH5354, (20.83 %, 5.42 h, 95.43 °C) for DK777, (34.51 %, 3.01 h, 90.46 °C) for Longe 10H and (19.24 %, 5.88 h, 97.02 °C) for DT Max (Table 3). The variation in optimum HMT conditions among the four maize varieties could be due to variations in the internal factors that affect starch digestibility (phenolic compounds, total starch and amylose) (Chang et al., 2020; Zurak et al., 2023). The four maize varieties used in the current study were from an earlier assessment of thirteen (13) varieties of maize commonly consumed in Uganda and East African countries. Considering that the four varieties used were the predominant ones from the four distinct groups based on factors that affect starch digestibility (phenolic compounds, total starch, and amylose) (Chang et al., 2020; Zurak et al., 2023), the optimized HMT conditions can be applied to other maize varieties with similar factors that affect starch digestibility.

3.3. Validation of optimal conditions

A separate validation experiment was conducted for the derived optimum conditions to verify the predictions and adequacy of the models. The heat moisture treatment experiments were carried out under optimal conditions for each variety. The results of the experimental values and the predicted values generated by the model are shown in Table 4. It was noted that the experimental values were not significantly different from the predicted values for slowly digestible starch and resistant starch for all varieties investigated except for UH5354 ($p = 0.022$). This suggests that the regression models are in line

Table 3

HMT optimum application conditions according to characteristics of maize varieties.

Varieties	Varieties characteristics		Moisture	Heating length	Temperature
UH5354	Phenolic (mg Eq Galic Acid)	201.23 ± 6.68	23.17	4.12	80.00
	Amylose (%)	22.35 ± 2.13			
	Total starch (%)	76.79 ± 2.55			
DK777	Phenolic (mg Eq Galic Acid)	191.93 ± 7.20	20.83	5.42	95.43
	Amylose (%)	23.09 ± 0.83			
	Total starch (%)	66.05 ± 2.37			
Longe 10H	Phenolic (mg Eq Galic Acid)	183.64 ± 4.66	34.51	3.01	90.46
	Amylose (%)	24.94 ± 1.93			
	Total starch (%)	76.00 ± 1.61			
DT Max	Phenolic (mg Eq Galic Acid)	217.00 ± 8.08	19.24	5.88	97.02
	Amylose (%)	24.81 ± 1.33			
	Total starch (%)	70.14 ± 3.45			

Data for varieties characteristics were obtained from the results of previous study of the correspondant authors under revision for publication.

Table 4
Comparison of experimental with predicted values of starch digestibility.

Maize varieties	Experimental Value SDS+RS (%)	Predictive Value SDS+RS (%)	Difference (p value)
UH5354	64.09	69.94	0.022*
DK777	54.08	49.34	0.067
Longe 10H	52.46	56.73	0.655
DT Max	54.64	48.90	0.053

* Denote statistical significance different between the experimental and predicted values ($p < 0.05$).

with slowly digestible starch and resistant starch development through HMT application. Thus, it can be concluded that the models are adequately suited to assess the behaviour of heat moisture treatment conditions for the maize grains except for the variety UH5354. The variation in predicted and experimental values for the variety UH5354 can be attributed to factors such as residual errors of the model, which play a critical role in evaluating the model accuracy (Li et al., 2021).

Additionally, this variation could also be due to inherent composition such as higher proteins and lipids composition in this maize variety, which could have promoted the development of starch-lipid or starch-protein complexes (Liu et al., 2022). The starch-lipid complexes, particularly amylose-lipid complexes, can reduce the swelling of maize granules and increase the hydrophobicity (Wang et al., 2025), which influence the development of SDS and RS (Sun et al., 2021; Wu et al., 2022). In addition, the starch-protein complexes change the structural and functional properties of starch, which is known to influence the development of SDS and RS (Liu et al., 2022). As such, the lipid and protein composition of this variety could have affected the estimation of SDS and RS, hence the disparity in observed and predicted values reported in this study. In summary, the variability in SDS and RS between predicted and experimental values may be attributed to the residual errors of the regression model, as well as the potential interactions of starch components with proteins and lipids of this variety.

3.4. Effect of optimal conditions on starch digestibility

The effect on the optimal condition on slowly digestible and resistant starch of maize varieties is presented in Table 5. Quantitatively, the Slowly Digestible and Resistant starch (SDS+RS) increased significantly ($p < 0.05$) after HMT application when compared to the control/untreated sample (Table 5). Under optimal conditions, SDS+RS showed an increase ranging from 7 % to 14 % across various varieties. Specifically, the increases were 9.35 % for UH5354, 10.31 % for DK777, 7.57 % for Longe 10H, and 14.39 % for Dt Max. These results underline the effective reduction of starch digestibility in polished maize flour using the HMT process. Therefore, these optimal conditions should be promoted to enhance the quality of polished maize flour and can be applied to similar maize varieties with comparable internal factors affecting starch digestibility. Although this study has focused only on improving the SDS

Table 5
Variation in the starch digestibility component in optimal HMT treated maize varieties.

Maize varieties	HMT	RDS (%)	SDS+RS (%)
UH5354	Untreated	45.26 $\pm$ 1.46 ^a	54.74 $\pm$ 1.46 ^b
	Treated	34.13 $\pm$ 1.54 ^b	64.09 $\pm$ 1.54 ^a
DK777	Untreated	55.72 $\pm$ 4.26 ^a	44.28 $\pm$ 4.26 ^b
	Treated	45.41 $\pm$ 2.35 ^b	54.08 $\pm$ 2.35 ^a
Longe 10H	Untreated	55.11 $\pm$ 0.72 ^a	44.89 $\pm$ 0.72 ^b
	Treated	47.54 $\pm$ 1.48 ^b	52.46 $\pm$ 1.48 ^a
DT Max	Untreated	59.75 $\pm$ 2.18 ^a	40.25 $\pm$ 2.18 ^b
	Treated	41.53 $\pm$ 1.43 ^b	54.64 $\pm$ 1.43 ^a

All values are means of duplicate determination $\pm$ S.D. At varieties constant, means within columns with different superscripts are significantly different ($p < 0.05$).

and RS of polished maize flour, the effect on physicochemical and functional characteristics has not been investigated while reported by several studies in the literature. HMT affects the nutritional (proximate composition), color characteristics, functional (swelling, solubility), pasting, and thermal properties of starchy foods (Dong et al., 2021; Olayinka, Adebawale & Olu-owolabi, 2008; Pieczyk & Domian, 2021; Sandhu et al., 2020), which may impact the acceptability of HMT-treated maize flour.

Marta et al. (2022) investigated the effects of HMT at 30 % moisture content for 16 h at 100 °C of maize and found there is no significant change in protein, fat, ash, and total starch content, while the amylose content increased from 26.70 % to 28.81 % (dry basis). In contrast, Dong et al. (2021) showed that HMT at 30 % moisture content, 110 °C for 90 min affects the total fat content, amylose and amylopectin contents of Whole Quinoa. Sindhu et al. (2019) investigated HMT effect at different temperatures on starch properties of buckwheat (*Fagopyrum esculentum*) and found considerable changes in the color characteristics certainly due to the Millard reaction between reducing sugars and amino groups in the protein. Otherwise, several researchers have shown that the swelling and solubility power of cereals such as sorghum (Olayinka, Adebawale & Olu-Owolabi, 2008), maize (Sui et al., 2015) and rice (Hormdok & Noomhorm, 2007) reduced after HMT. Marta et al. (2022) found that the functional properties of the maize grains mainly the solubility, water absorption capacity, and gel strength significantly increased after HMT while swelling volume decreased.

Maior et al. (2021) found that HMT increased the pasting temperature, and final viscosity while the peak viscosity and breakdown of maize starch. Similar results were reported by Marta et al. (2022) for maize flour except for final viscosity which decreased after HMT. Kunyane and Luangsakul (2022) evaluated the effects of HMT on rice flour thermal properties and found that the non-treated flour had lower onset, peak and conclusion temperature than treated flour but the gelatinization temperatures decreased after the treatment. Otherwise, Kim et al. (2017) investigate the effect of HMT of brown rice flour on the sensory quality of Korean rice cake. They found that the color and flavor score of HMT-treated brown rice cakes increased as the HMT temperature and time increased. However, the hardness and overall acceptability score of these HMT-treated brown rice cakes decreased with an increase of HMT temperature and time. Fathi et al. (2016) investigate the effect of heat-moisture treatment of proso-millet flour on the organoleptic quality of the gluten-free cake. These authors also found that the odor and taste scores of cakes decreased with increasing of HMT proso-millet flour.

These studies demonstrate that HMT can sometimes affect the chemical, functional, and pasting properties of cereals, which eventually impact the overall acceptability of HMT-derived products. Therefore, for better adoption of these HMT conditions, future research is needed to assess the nutritional and functional properties, as well as the effects of processing conditions on the aforementioned parameters.

4. Conclusion

Response surface methodology was successfully utilized to optimize the HMT conditions to reduce starch digestibility (yielding a high content of SDS and RS) for four maize varieties predominantly consumed in Uganda. The optimum moisture, temperature, and heating time varied according to the maize variety. The optimal conditions could be used as a strategy to mitigate non-communicable diseases associated with consumption of high starch digestibility polished maize flour. The decision-makers should advocate for the adoption of optimal HMT conditions to mitigate health risks associated with the high starch digestibility of polished maize flour. Given that maize flour production and distribution in Uganda, and other East African countries, is predominantly managed by industrial processors, promoting these optimal HMT conditions within the industry could improve the quality of polished maize flour available for commercialization and community consumption. Whereas

the current study developed optimum HMT conditions that reduced the starch digestibility among selected maize varieties, future studies should consider the impact of HMT on the physicochemical, functional, pasting and thermal properties, and acceptability of the flour for widespread utilization among households. Furthermore, the impact of storage conditions and traditional cooking processes on the long-term stability of SDS and RS contents of the HMT-treated maize flour could be investigated to recommend the optimal use of these flours.

CRedit authorship contribution statement

Finagnon Toyi Kevin Fassinou: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Solomon Olum:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Lawrence Okidi:** Writing – review & editing, Validation, Methodology. **Duncan Ongeng:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they do not have any conflict of interest.

Ethical statements

The study did not involve any human or animal testing. However, ethical clearance was obtained from the Gulu University Research Ethics Committee (Approval number: GUREC-2023–507) and the Uganda National Council for Science and Technology (Approval A338ES).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.afres.2025.101023](https://doi.org/10.1016/j.afres.2025.101023).

Data availability

Data will be made available on request.

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