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Preparation and characterization of corn flours with variable starch digestion

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ABSTRACT

Several grains such as wheat, rice, corn, oat, barley and rye are cultivated throughout the world. They are converted to variety of food products using a multitude of processing technologies to quench the growing organoleptic demands and consumers' preferences. Among them, corn, ranking third in wide consumption, is cost-effective and has long-term storability. Herein, ready-to-eat corn flours with variable starch digestion have been developed by processing at high temperature with shear using a twin screw continuous processor. The influence of processing temperature (121, 145 and 160°C) and moisture (25, 30 and 35%) has been studied. Results suggest both processing temperature and moisture modulate the rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) amounts of the flours. The presence or absence of oil in the flour further controls the starch digestion. The outcome is deemed to be helpful to design and develop healthy and palatable functional food products in addition to furthering the current market share for corn and other grains.

1. Introduction

Several epidemiological studies stress the beneficial role of whole grain foods, mainly for weight management (Lean, Astrup, & Roberts, 2018) and to reduce the risk of cardiovascular disease (Zhang, Zhao, Guo, Bao, & Wang, 2018), coronary heart disease (Temple, 2018), type II diabetes (Della Pepa, Vetrani, Vitale, & Riccardi, 2018) and cancer (He et al., 2019). The whole grain foods supply valuable nutrients including dietary fiber, vitamins, minerals and phytochemicals along with carbohydrates, proteins and fats. As majority of the nutrients are concentrated in the bran and germ, they get milled out during the refinement process. For example, there is a significant reduction in the phenolic content upon corn nixtamalization to flour compared to raw corn (De La Parra, Serna Saldivar, & Liu, 2007). Similarly, refined wheat flour loses 83% of phenolics, 79% of flavonoids and 93% of ferulic acid compared to the whole-wheat flour (Adom, Sorrells, & Liu, 2005). Consumption of such nutrient-poor endosperm might not be healthy. In this regard, food products with preserved nutrition along with palatable organoleptic properties are gaining high demand. Toward this end, new food processing technologies incorporating innovative preservation protocols are being explored, and continuous processing stands out as a feasible approach (Awolu, Oluwaferanmi, Fafowora, & Oseyemi, 2015; Mahasukhonthachat, Sopade, & Gidley, 2010; Mishra, Mishra, & Rao, 2012; Oliveria, Rosell, & Steel, 2015; Moussa, Qin, Chen, Campanella, & Hamaker, 2011). It is a high-temperature with shear processing by a twin-screw continuous processor that yields pre-gelatinized starchy products with low moisture content.

Herein, instant porridge flours with variable starch digestion have been prepared by taking corn as a test "whole grain". The novelty of our approach is using a twin-screw continuous processor at high temperature with shear but without the dye at the end so that there will not be any pressure to be felt by the sample at the output, and even if any it would be very subtle (Fig. 1). Thus obtained flours would possess all the intrinsic qualities of whole grains and deemed to be healthy. The choice of corn stems from its cost-effective and long-term storable property. It is also one of the widely consumed cereal grains, and is rich in vitamins, minerals and phytochemicals. In this research, the effect of processing temperature with shear and moisture level on the starch digestibility has been established. In order to expand the research outcome, oil has been extracted from the prepared flours as well. The flours have been analyzed using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Differential

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scanning calorimetry (DSC). In addition, the total starch content along with Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS) and Resistant Starch (RS) amounts in each flour have been estimated so as to understand the applicability of the prepared flours for human consumption and associated impact on the starch digestion. The research outcome could be extended to other grains.

2. Materials and methods

2.1. Materials

Corn kernels were freshly harvested and dried obtained from the Purdue Agriculture research farm. Pancreatin from porcine pancreas, amyloglucosidase from *Aspergillus niger*, pepsin from porcine gastric mucosa and thermostable α-amylase were purchased from the Sigma-Aldrich. The GOPOD kit was obtained from the Megazyme. Ethanol and hexane were from the Fisher Scientific.

2.2. Sample preparation

The 2 kg whole corn kernels (~12% moisture content) were used, and moisture content (25, 30 and 35% w/w) was achieved by adding required amount of water. Later, 5 mL thermostable α-amylase was added to partially gelatinize the starch during processing (Fig. 1a). An ultra-power kitchen aid (Model No. KSM90WW) was applied to mix the samples for 30 mins. The mixture was then fed into a twin-screw continue processor (Readco, Kurimoto, LLC, York, PA, USA) – a high temperature with shear and low-pressure dual shaft mixer to produce a partially gelatinized and low moisture flour (Fig. 1b). The processing variables of the feed include rotating speed, barrel temperature and moisture content. A rotating speed of 120 rpm was selected and the barrel input temperature was set at 121, 145 and 160°C. In order to accomplish the desired temperature, the pressure in the steam jacket was adjusted and these three are the available choices at our facility. The three selected moisture contents coupled with three temperature settings result in a total of nine distinct experiments for evaluation. The corn kernels processed at room temperature (RT), 22°C, and at the above three humidifies (25, 30 and 35% w/w) serve as the control samples. Average values from triplicate measurements are reported.

2.3. Extraction of corn oil and zein after processing

The processed corn flours passed through a 60-mesh sieve were packed in a glass column (Fig. 1c) and 190 proof (95%) and 200 proof (100%) of ethanol was added to extract oil and zein from the flours (Fig. 1d). The combination of oil and zein was then mixed with hexane to separate oil from zein, and later hexane was evaporated out from the oil. The resulting flours contain corn protein, fiber and partially gelatinized starch. There were two ready-to-eat products: de-oil flours (without zein) and with-oil flours (Fig. 1e). The final moisture content of the prepared whole grain corn flours is found to be 10%. The flours are stored at the room temperature for further analysis.

2.4. Morphological characteristics

Morphology of each extruded flour was analyzed using a Scanning

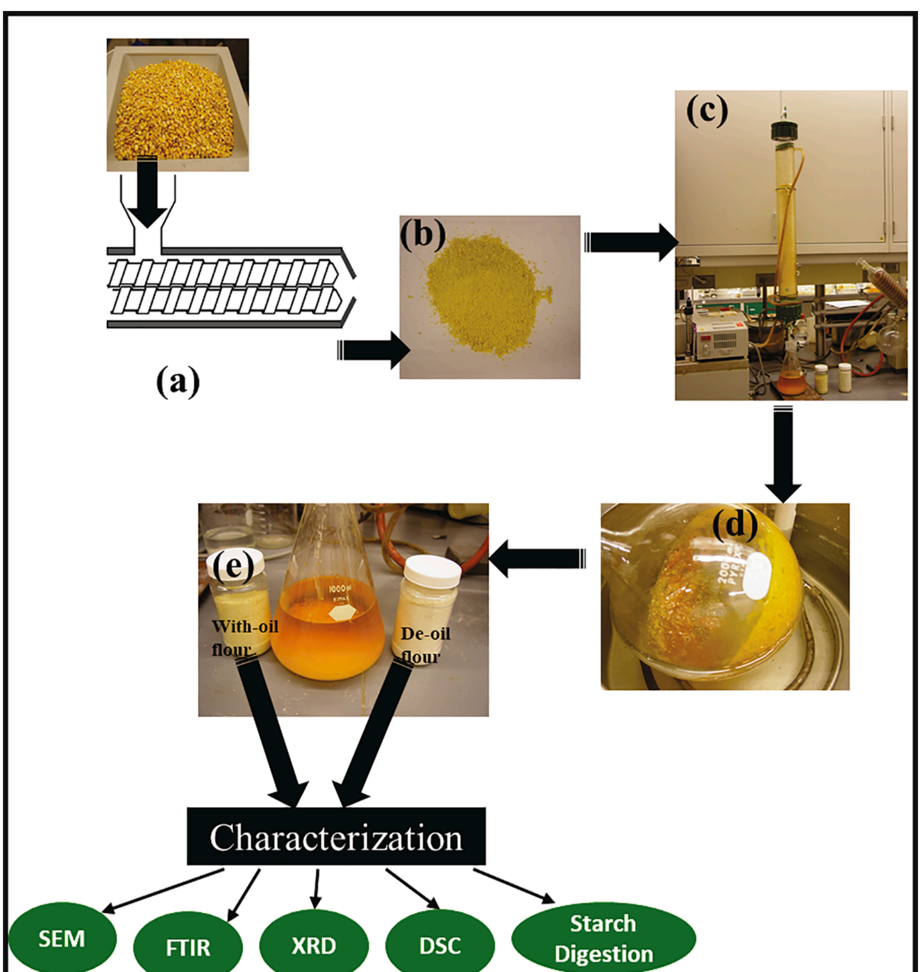

Fig. 1. Schematic representation of ready-to-eat corn flours processed from whole corn kernels. (a) The corn kernels after mixing with α-amylase were processed using a twin-screw continuous processor at high temperature with shear and at various moisture conditions. (b) The obtained flours (after passing through 60-mesh sieve) are partially gelatinized with reduced moisture content (<10%). The oil and zein were extracted by ethanol and the extracted ethanol could be recovered though rotary evaporator. (d) The combination of oil and zein was then mixed with hexane to separate oil from zein, and the hexane could be evaporated out from oil. (e) Two ready-to-eat food products: with-oil flours and de-oil flours are produced and characterized through SEM, FTIR, XRD and DSC, and subjected to *in vitro starch* digestion to estimate RDS, SDS and RS amounts.

Electron Microscope (SEM) equipped with FEI Nova Nano SEM 650. The sample was put on a double-sided electric tape held on a metal platform. To obtain the electron signal, high voltage platinum coating was applied and a layer (40–50 nm) of platinum was attached on to the sample surface, and examined at an accelerating voltage of 5 kV.

2.5. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the prepared flours were recorded using the Nicolet Nexus 670 FT-IR Spectrometer equipped with a Smart iTR diamond and ATR accessory (Thermo Scientific, Pittsburgh, PA) in range 800 to 2000 cm^{-1}. A resolution of 4 cm^{-1} with an average of 128 scans was used.

2.6. X-ray powder diffraction

About 500 mg of sample was packed in an aluminum holder and mounted on a Philips PW 3170 diffractometer interfaced to a personal computer equipped with the Automated Powder Diffraction (APD) software. Diffraction patterns were collected at room temperature using Ni-filtered CuKα ($\lambda = 1.5418$ Å) radiation while the X-ray tube operated at 40 kV and 25 mA. Intensity data were obtained in the diffraction angle range $10 - 40^{\circ}$ of 2θ in the step scan mode with 0.04° step width and 5 s collection at each step. A total of 751 intensities were collected. In order to calculate the crystallinity, in each diffraction pattern around 500 data points in the nonpeak regions were selected as background intensities. A sixth order polynomial was fitted using the OriginPro 2020b and the coefficients were used to estimate the background intensity at each diffraction angle using the Microsoft Excel. The background profile was then scaled such that it abuts the semicrystalline pattern. The percentage of crystallinity was calculated: % crystallinity = (total area – background profile area) × 100 ⁄ total area.

2.7. Thermal properties

The thermal properties of the prepared products were determined using a Modulated Differential Scanning Calorimetry mDSC Q2000 from TA instruments, New Castle, DE. In an aluminum pan, 2 mg of sample (dry basis) was mixed with distilled water to a total weight of 6 mg (1:3 w/w). The pan was then hermetically sealed, equilibrated at room temperature for 1 h and scanned in the temperature range 0 to 110°C at a heating rate of 10°C/min. A sealed empty pan was used as reference, and indium for calibrating the instrument. Average values from triplicate measurements are reported.

2.8. Total starch (TS) determination

In a vial, 100 mg of the prepared product (on dry basis) was added with 0.2 mL of 80% ethanol (ethanol:water, v/v) and 2 mL of KOH solution (2 mol/L) and stirred for about 20 min at 4°C. Later, 8 mL of sodium acetate buffer (1.2 mol/L, pH 3.8) and 0.1 mL of α–amylase (No.3306, Sigma–Aldrich) and 0.1 mL of amyloglucosidase (No. 7095, Sigma–Aldrich) were added and mixed thoroughly. It was then incubated at 50°C in a water bath for 30 min. The solution was adjusted to 100 mL with distilled water and centrifuged at 2000 rpm for 15 min. Finally, 0.1 mL of the diluted solution was mixed with 3 mL GOPOD reagent and incubated at 50℃ for 20 min. The absorbance was then read at 510 nm.

2.9. In vitro starch digestion

The *in vitro* digestibility of the starch complexes was carried out according to the Englyst protocol (Englyst, Kingman, & Cummings, 1992) with some modifications. Initially, pepsin enzyme solution was prepared by dispersing 1.03 g of pepsin in 75 mL distilled water along with 0.312 mL of HCl and 0.375 g of guar. Later, starch sample of 550 mg (dry basis) was mixed with 10 mL of distilled water and 4 mL of pepsin enzyme solution, and incubated at 37℃ in a shaker water bath for 30 min to suppress the effect from protein. The sodium acetate buffer solution (0.25 M, pH 5.2) of 10 mL was added along with 0.05 g of guar gum to mimic the intestinal environment; guar gum provides the required viscosity to the sample. The mixture was then incubated at 37°C by mixing 5 mL of enzyme preparation that is composed of pancreatin from porcine pancreas (50 units) (No. 7545, Sigma–Aldrich) and amyloglucosidase from *Aspergillus niger* (35 units) (No. 7095, Sigma–Aldrich). At 20 and 120 min time intervals, 0.5 mL of the hydrolyzed solution was taken out and added to 20 mL of 80% ethanol to inactivate the enzyme. In order to estimate the glucose amount, the solution was centrifuged at 2000 rpm for 15 min and mixed with the GOPOD reagent followed by incubation at 50℃ for 20 min and then read the absorbance at 510 nm. The classification of starch was based on its digestibility and as described by the Englyst[35]. The rapidly digestible starch (RDS) was the fraction hydrolyzed within 20 min of incubation, resistant starch (RS) not hydrolyzed within 120 min, and slowly digestible starch (SDS) digested during 20 and 120 min. The combined SDS and RS amount was calculated by subtracting RDS from the total starch (TS).

2.10. Statistical analysis

Duncan's multiple range tests ($p < 0.05$) were carried out to analyze the differences, and the statistical analysis was performed using the SPSS 17.0 (SPSS Inc., Chicago, IL, USA). In order to understand the impact of processing parameters on the starch digestibility, 2-tailed Pearson correlation was carried out using IBM SPSS statistics 25 on TS, RDS, (SDS + RS), ΔH, and starch crystallinity were correlated with the processing temperature and moisture. The mathematical modeling was carried out using the Design Expert Version 10 (Stat-Ease, Minneapolis, MN, USA) to determine the optimum processing conditions.

3. Results and discussion

3.1. Morphology

Polyhedral, round and oval shape granules are characteristic of native flours (Fig. 2). The starch granules in both with-oil and de-oil samples appear alike and the moisture content imparts subtle changes on the granule surface. At moisture levels of 25 and 30%, granule integrity is intact; however, at 35% distorted broken fragments and multiple starch layers on the outer granule surface are spread out. On the other hand, rise in the temperature (from 121 to 145°C) causes the formation of larger agglomerates composed of spherical and polygonal shaped, damaged and rough surface granules. Granule impairment increases with the temperature, which is related to starch gelatinization caused by heat. At the highest temperature of 160°C, granule rupture is maximum and a continuous network structure filled with an increased number of flattened and sheared aggregates is noticed. These morphological changes suggest clearly the occurrence of starch granule rupture and amylose leaching, which could influence starch digestion and associated functional properties.

3.2. Spectroscopic analysis

The samples display absorption peaks at 861, 928, 994, 1022, 1077, 1103, 1124 and 1150 cm^{-1} (Fig. S1), which are fingerprints of starch (Sui, Yao, Zhao, Ye, Kong, & Ai, 2015). The first band at 861 cm^{-1} corresponds to CH deformation. The second to fifth are due to COH bending and CH_2-related modes. The sixth to eighth are from CO and CC stretching with COH contribution (Vasko, Blackwell, & Koenig, 1971). A significant decrease in the intensity of 994 and 1022 cm^{-1} is noticed, especially for samples processed at 121, 145 and 160°C. This indicates the weakening of COH bending and CH_2-related modes after heat treatment. A clear difference between the de-oil and with-oil flours is

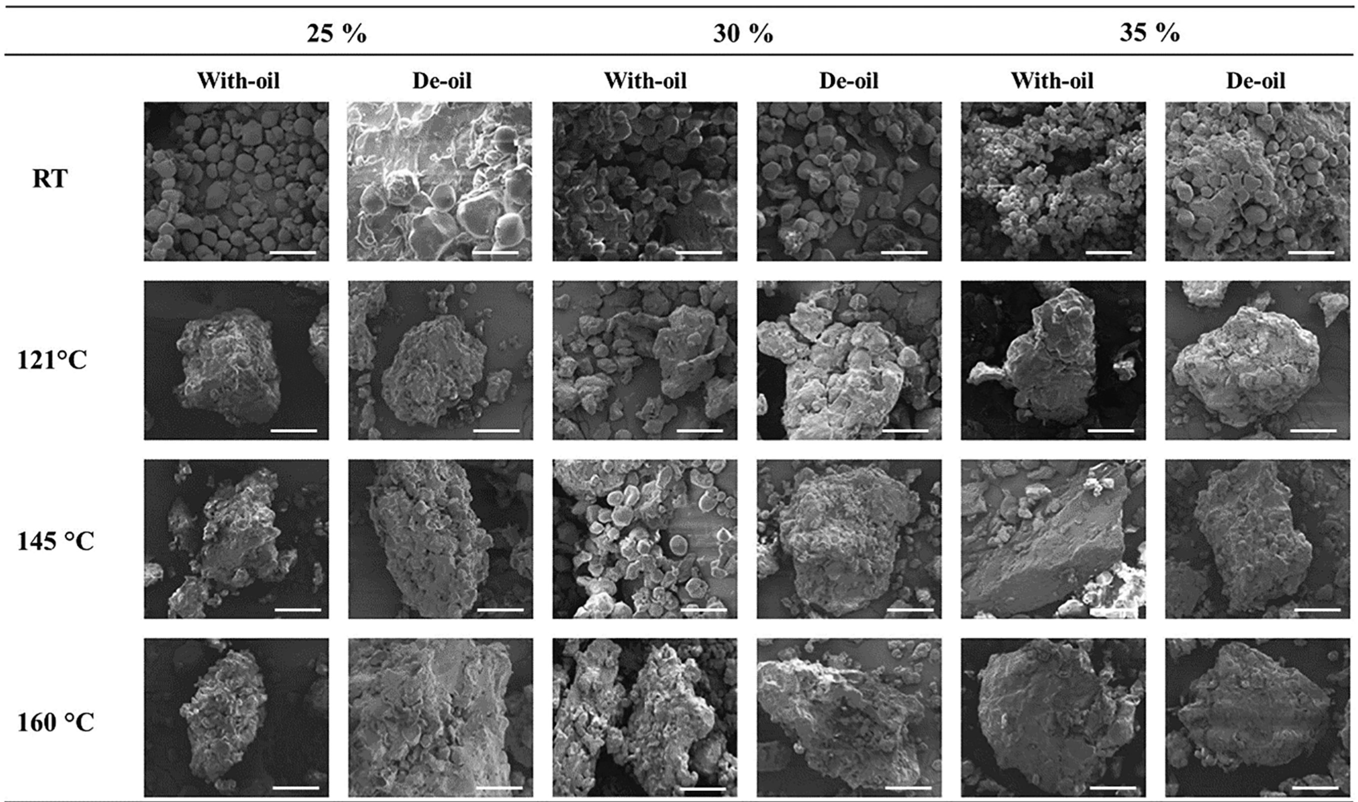

Fig. 2. Effect of processing temperature (Room temperature (RT), 121, 145 and 160°C) and the amount of moisture (25, 30 and 35%) on the morphology of corn flours with-oil and de-oil. The scale bar is 50 μm.

noticed, mainly above the 1200 cm^{-1} region. The with-oil samples display peaks at 1386, 1467, 1546, 1600, 1658 and 1747 cm^{-1}. The large peak observed at 1740 cm^{-1} is due to C═O double bond stretching. Interestingly, in the de-oil samples the 1740 cm^{-1} band diminishes gradually with the processing temperature, which might be ascribed to the loss of C═O double bond. The amide I and amide II bands of protein occur at 1660 cm^{-1} and 1550 cm^{-1}, respectively. The peak at 1550 cm^{-1} has a higher intensity than at 1660 cm^{-1} indicating that with-oil corn flours have strong absorbance of the NH stretching, which implies that they are rich in amide II protein. The regions 1550 to 1570 and 1600 to 1700 cm^{-1} are predominantly due to fat (Amir, Anjum, Khan, Khan, Pasha, & Nadeem, 2013). Indeed, spectra of with-oil flours show strong peaks in these areas but fadeout in the de-oil flours revealing decreased presence of fat and protein.

3.3. X-ray analysis

Generally, there are four starch types known as A-, B-, C- and V-starch (Wang, Bogracheva, & Hedley, 1998). Their X-ray powder diffraction patterns are quite distinct: A-type displays intense peaks at 2θ of 15 and 23° with a doublet at 17 and 18°; B-type are composed of singlets at 5, 17 and 20° along with doublets at 22 and 24°; C-types are typically a combination of A- and B-starch patterns, and V-types possess singlets at 7.2, 13.5 and 19.5°. Typically, cereal starches such as corn, wheat and rice display A–type patterns; tuber and root starches of potato and tapioca B-type patterns; pulse starches of pea type C-type patterns and starch-lipid complexes V-type patterns (Buléon, Colonna, Planchot, & Ball, 1998). Interestingly, all these four starch types are noticed in the corn flours produced in this research (Fig. S2).

The RT (control) flours are A-type starches with characteristic diffraction peaks at 15.5, 17.6, 18.6 and 23.6° of 2θ, independent of moisture content. At the high processing temperatures, the initial A-type structure still prevails but with B- and V–type as well; *e.g.* peaks at 13, 18 and 20°. Thus, upon processing, corn starch structure transforms from A- to C–starch along with the V-starch fraction. Interestingly, with- or without-oil content in the flours, the diffraction patterns are akin. Similarly, as the moisture increases from 25 to 35%, relative intensities of 13 and 20° peaks increase and the doublet at 17 and 18° merges to one along with the disappearance of 15 and 23° peaks. These observations suggest not just B-type crystallites alone but a combination of B-, C- and V-type crystallites via a partial (at lower humidity) or complete (at higher humidity) transformation of A-starch crystallites. Simple transformation of A- to B-type in normal maize through heat processing as well as B- to V-type is also noticed in high amylose maize (Zhang et al., 2016). However, with the experimental conditions adopted in our research, corn flours consisting of all the four A-, B-, C- and V-type starches is interesting and could have ramifications on the starch digestibility, long-term stability and more importantly on the organoleptic properties of the prepared flours. Further research is warranted to decipher the root cause of these differences.

The crystallinity of flours varies to a great extent and the presence or absence of oil has a significant role (Fig. S3). First of all, when processed at RT, the oil containing specimens ended up with 12% crystallinity and the de-oil ones had a marginally higher crystallinity of 13%. Second, samples with-oil processed at 25% moisture showed 8.5 to 10% crystallinity but upon oil removal the crystallinity drops to 8%. Third, at higher moistures of 30 and 35%, both with- and de-oil samples led to products with low crystallinity of 8 and 7.5%, respectively. Overall, based on the results from the 24 flour combinations, it is clear that the processing temperature and moisture content have considerable influence on the crystallinity of flours as it descents from 13 to 7.5%. This finding is in line with the reduced crystallinity of potato starch upon heat-moisture treatment (Yassaroh, Woortman, & Loos, 2019). If the drop in the crystallinity is due to starch gelatinization during processing, it might favorably be increasing the starch digestion but with subtle interfere on the shelf-life, since traces of crystallinity are adequate to extend the shelf-life of the prepared products (Sullivan, Hughes, Cockman, & Small, 2017). The correlation between these two distinct aspects is still not fully clear and further research involving shelf-life and large-scale digestion studies are in need.

3.4. Melting properties

As shown in Table 1, samples processed at the three selected temperatures 121, 145 and 160°C exhibit higher gelatinization temperatures (T_o, T_p, T_c) than the control samples prepared at room temperature for all the three moisture conditions considered in this research. This trend is similar to the increased crystallinity observed with higher processing temperature. It is in line with the increase in the gelatinization temperature of potato starch during high-temperature treatment (Varatharajan et al., 2011), mung bean starch (Barua, & Srivastav, 2017) and rice flour (Noro, Morohashi, Nakamura, Nakajima, & Ohtsubo, 2018). Another important reflection of with-oil samples is that the gelatinization temperature reaches the highest value at 35% moisture whereas in the de-oil samples the gelatinization temperature peaks at the lowest moisture level of 25%.

The enthalpy of gelatinization (ΔH) is a measure of the unraveling of double helices in the starch granule during gelatinization and represents the disorder of double helices (Gunaratne, & Hoover, 2002). It can be seen from Table 1 that for both with-oil and de-oil samples, high temperature treated flours show lower ΔH values than the control samples. Overall, there is a negative correlation of enthalpy (-0.632 for de-oil and −0.817 for with-oil samples) with the processing temperature (Table 2). A similar correlation does not exist with the moisture content. Although this behavior could suggest that temperature has a more important role on the products processed, the intrinsic role of moisture to this process cannot be judiciously discounted.

3.5. Effect of temperature and moisture on the total starch (TS)

The oil containing control kernels possess 72.1 to 77.8% of TS depending on the moisture content but slightly lower values of 69.3 to 73.4% with the oil removal (Table 1). Marginally higher TS amounts are obtained at the three distinct moisture conditions: 74.3 to 85.7% in the presence of oil but are subtly higher 75.0 to 90.7% after oil removal. Interestingly, for the de-oil flours at 30% moisture, the TS steadily increases from 75% at 121°C to 83% at 145°C and 87.9% at 160°C; when the moisture is raised to 35%, the TS gradually increases from 86.3 to 87.5 and 90.7% with the ascending temperature. In contrast, at the lowest 25% moisture, surprisingly the three TS values are lower: 83.2, 86.6 and 81.3%. The "higher the temperature – higher the TS amount" trend is broken. This propensity prevails in samples with oil as well. As shown in Table 2, TS is positively correlated with the processing temperature and the correlation coefficients are 0.859 and 0.714 for the de-oil and with-oil samples, respectively. Thus it appears that moisture and temperature significantly influence the amount of available starch in the flours. The TS is negatively correlated with flour crystallinity suggesting that flours appears to be in the amorphous state, and is in line-with the thermal properties.

3.6. Effect of temperature and moisture on the RDS

Moisture during processing is an important parameter that regulates the starch functional properties. Control kernels processed at room temperature and at 25, 30 and 35% moisture, are found to have 16.4, 17.6 and 17.5% RDS, respectively (Fig. 3a). When the oil is removed, these values are higher: 18.0, 21.2 and 23.3%. This observation of relatively more RDS values after oil elimination implies that starch digestibility is reduced in the presence of oil. This is due to the fact that oil precludes enzymes reaching starch chains and thus retards the starch digestion (Farooq, Dhital, Li, Zhang, & Huang, 2018; Lau, Zhou, & Henry, 2016). Indeed, removing oil from Kodo millet flour increases the

Table 1
The gelatinization temperature (T_o, T_p, T_c), enthalpy (ΔH) and total starch of with-oil and do-oil corn flours. The values in each column with the different superscript are significantly different ($p < 0.05$).

With-oil samples Moisture (%)	Temperature (°C)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	Total starch
25	RT	69.94 ± 0.68^{c}	72.63 ± 0.37^{e}	77.19 ± 1.47^{d}	1.13 ± 0.25^{a}	72.13 ± 0.93^{e}
	121	70.99 ± 0.73^{bc}	76.44 ± 0.65^{cd}	81.83 ± 0.68^{c}	0.22 ± 0.05^{d}	85.05 ± 0.54^{a}
	145	70.87 ± 1.78^{bc}	77.95 ± 1.72^{bc}	86.54 ± 2.53^{a}	0.51 ± 0.24^{c}	85.71 ± 0.98^{a}
	160	70.95 ± 0.97^{bc}	76.36 ± 2.07^{cd}	84.90 ± 1.62^{ab}	0.26 ± 0.03^{d}	85.59 ± 3.80^{ab}
30	RT	70.18 ± 0.01^{c}	74.67 ± 0.21^{d}	82.85 ± 0.65^{bc}	0.80 ± 0.06^{b}	77.83 ± 0.52^{c}
	121	ND	ND	ND	ND	74.36 ± 0.91^{d}
	145	74.70 ± 0.62^{a}	79.72 ± 0.76^{ab}	84.94 ± 0.28^{ab}	0.19 ± 0.01^{d}	80.50 ± 0.98^{b}
	160	72.11 ± 0.80^{b}	77.65 ± 1.23^{c}	83.45 ± 1.14^{bc}	0.22 ± 0.01^{d}	82.14 ± 0.60^{b}
35	RT	71.56 ± 0.09^{bc}	74.51 ± 0.10^{d}	79.23 ± 2.29^{d}	0.72 ± 0.04^{b}	75.19 ± 0.71^{d}
	121	ND	ND	ND	ND	84.85 ± 0.73^{a}
	145	74.59 ± 2.34^{a}	79.61 ± 0.98^{ab}	87.55 ± 1.83^{a}	0.22 ± 0.01^{d}	80.61 ± 0.88^{b}
	160	74.83 ± 0.24^{a}	80.33 ± 0.99^{a}	86.86 ± 2.27^{a}	0.10 ± 0.01^{d}	81.93 ± 0.62^{b}
De-oil samples Moisture (%)	**Temperature (°C)**	**T_o (°C)**	**T_p (°C)**	**T_c (°C)**	**ΔH (J/g)**	**Total starch**
25	RT	69.88 ± 0.10^{d}	73.49 ± 0.49^{d}	82.07 ± 1.23^{cd}	1.53 ± 0.34^{a}	69.32 ± 0.14^{e}
	121	72.84 ± 0.87^{b}	77.50 ± 1.17^{c}	82.15 ± 1.26^{cd}	0.15 ± 0.01^{b}	83.21 ± 1.08^{c}
	145	69.97 ± 0.06^{d}	76.42 ± 0.30^{c}	83.97 ± 0.54^{c}	0.41 ± 0.19^{b}	86.60 ± 0.73^{b}
	160	80.37 ± 0.05^{a}	87.76 ± 0.53^{a}	95.92 ± 1.68^{a}	0.17 ± 0.03^{b}	81.33 ± 1.47^{c}
30	RT	ND	ND	ND	ND	73.28 ± 1.65^{d}
	121	ND	ND	ND	ND	74.96 ± 1.37^{d}
	145	71.94 ± 0.51^{bc}	77.42 ± 1.16^{c}	84.10 ± 3.20^{c}	0.25 ± 0.12^{b}	83.00 ± 0.88^{c}
	160	71.96 ± 0.04^{bc}	77.43 ± 0.20^{c}	83.03 ± 0.21^{cd}	0.25 ± 0.01^{b}	87.86 ± 0.98^{b}
35	RT	70.04 ± 0.06^{d}	72.99 ± 0.02^{d}	77.81 ± 1.31^{e}	1.36 ± 0.47^{a}	73.43 ± 0.88^{d}
	121	72.51 ± 0.45^{b}	76.37 ± 0.73^{c}	81.59 ± 1.22^{d}	0.26 ± 0.03^{b}	86.31 ± 0.43^{b}
	145					

(continued on next page)

Table 1 (*continued*)

With-oil samples Moisture (%)	Temperature (°C)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	Total starch
		70.85 ± 1.73 [cd]	77.09 ± 1.33 [c]	84.19 ± 1.21 [c]	0.16 ± 0.04 [b]	87.50 ± 0.95 [b]
	160	71.93 ± 0.56 [bc]	78.78 ± 0.64 [b]	86.22 ± 0.59 [b]	0.26 ± 0.02 [b]	90.73 ± 0.23 [a]

starch digestion (Annor, Marcone, Bertoft, & Seetharaman, 2013).

The processing temperature (121, 145 and 160°C) and moisture (25, 30 and 35%), in each of 9 pair-wise combination, yield a much higher amount of RDS. First of all, at 25% moisture, RDS is 68.5% at 121°C (Fig. 3b); drops to 66.8 and 65.6%, respectively when the temperature is stepped up to 145 and 160°C. Interestingly, even after oil removal RDS amount stays virtually the same (68.3, 68.9 and 69.8%). Second, when moisture is elevated to 30%, RDS measures 68.3% at 121°C and higher of 72.4% at 145°C and 72.3% at 160°C, in the with-oil flours (Fig. 3c) but remain unchanged upon removing the oil. Third, at 35% moisture, RDS reaches 80.0, 76.4 and 77.5% with-oil, and 83.3, 77.4 and 73.7% without oil, in the same temperature order (Fig. 3d). These results taken together suggest that at a given moisture, higher the processing temperature higher will be the RDS amount with or without the presence of oil. However, in order to fully expand the research outcome involving additional processing temperature needs to be carried out. The same result could be achieved at a given temperature by increasing the moisture. As highlighted in Table 2, a strong positive correlation between the RDS and processing temperatures exists with coefficients of 0.945 and 0.949 for de-oil and with-oil cases, respectively. However, RDS is negatively correlated (Table 2) with crystallinity (coefficients of –0.942 for de-oil and −0.973 with-oil). These results clearly show that processing the corn grains yielded more gelatinized starch (less crystallinity) with higher RDS amounts that would be easier to digest. Certainly, presence or absence of oil in these flours offers further value but sensory analysis needs to be carried out to prove this research concept.

3.7. *Effect of temperature and moisture on the SDS and RS*

Foods with higher amounts of SDS and RS are considered to be beneficial to humans. The SDS is linked to stable glucose metabolism, diabetes management, mental performance and satiety. On the other hand, RS is believed to improve colon health and prevent colon cancer apart from a host of other health benefits. Herein, control samples display the (SDS + RS) amount of 55.7, 60.2 and 57.7% in the presence of oil and 51.3, 52.1 and 52.2% without the oil at 25, 30 and 35% moisture, respectively (Fig. 3e-h). Thus, removing oil in the flours lessens the (SDS + RS) amount. This finding is in line with the reduced RDS and further highlights the role of oils on modulating the starch functional properties. Interestingly, at higher processing temperatures the amount of (SDS + RS) wilts as a function of moisture content. Compared to RDS, the correlation coefficients of (SDS + RS) with crystallinity and processing temperature are opposite in sign. For example, (SDS + RS) is highly correlated with crystallinity with coefficients of 0.928 and 0.958 for the de-oil and with-oil samples, respectively (Table 2); while the corresponding numbers for RDS are −0.942 and −0.973. Indeed, presence of more crystallites, which increases crystallinity, will reduce the amount of starch that could be digested and hence increases SDS as well as RS amounts.

3.8. *Optimal processing conditions of corn complex samples*

In order to determine the optimum processing conditions for producing RDS and (SDS + RS) and their dependence on moisture and temperature, a mathematical modeling has been carried out using the Design Expert Version 10. The resulting three-dimensional response surface plots that highlight the variation of RDS, SDS and RS with processing temperature (T) and moisture (M) are depicted in Fig. 4. The regression analysis yielded the following second order equations:

$$RDS(with-oil) = 71.94 + 4.69 \times T + 3.33 \times M + 0.25 \times TM + 1.08 \times T^2 - -0.77 \times M^2 - -4.94 \times T^2M + 1.33 \times TM^2 \left(R^2 = 0.9736, p = 0.0012\right) \quad (1)$$

$$\mathrm{RDS}(de-oil) = 71.65 + 4.90 \times T + 2.47 \times M - -2.76 \times TM + 2.44 \times T^2 - -0.55 \times M^2 - -4.54 \times T^2M - -0.18 \times TM^2 \left(R^2 = 0.9950, p = 0.0001\right) \quad (2)$$

$$(SDS + RS)(with-oil) = 7.28 - -7.03 \times T + 0.86 \times M - -1.13 \times TM + 3.84 \times T^2 + 0.60 \times M^2 \left(R^2 = 0.9991, p = 0.0001\right) \quad (3)$$

$$(SDS + RS)(de-oil) = 10.12 - -4.99 \times T + 3.86 \times M + 4.34 \times TM + 2.25 \times T^2 - -0.28 \times M^2 - -1.10 \times T^2M + 3.38 \times TM^2 \left(R^2 = 0.9761, p = 0.0009\right) \quad (4)$$

RDS increases with the moisture level from 25 to 35%. In the case of de-oil flours (Fig. 4b, eq. (2)), RDS positively correlates with the temperature climbing from 121 to 160°C for moisture lower than 30% but negatively correlates at higher moisture of 35%. Thus, it appears that the

Table 2
Pearson correlations among Total starch (TS), Rapidly digestible starch (RDS), sum of Slowly digestible starch (SDS) and Resistant starch (RS), melting enthalpy (ΔH), starch crystallinity and the amounts of moisture and temperature. For each pair of correlation, there are two results corresponding to: (1) the first line, de-oil and, (2) the second line, with oil.

	TS	RDS	SDS + RS	ΔH	Crystallinity	Moisture	Temperature
TS		0.854**	−0.735**	−0.530	−0.791**	0.266	0.859**
		0.691*	−0.572	−0.545	−0.686*	−0.135	0.714**
RDS			−0.981**	−0.672*	−0.942**	0.135	0.945**
			−0.988**	−0.910**	−0.973**	0.147	0.949**
SDS + RS				0.675*	0.928**	−0.076	−0.906**
				0.917**	0.958**	−0.195	−0.925**
ΔH					0.654*	−0.047	−0.632*
					0.889**	−0.327	−0.817**
Crystallinity						−0.348	−0.842**
						−0.096	−0.933**

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

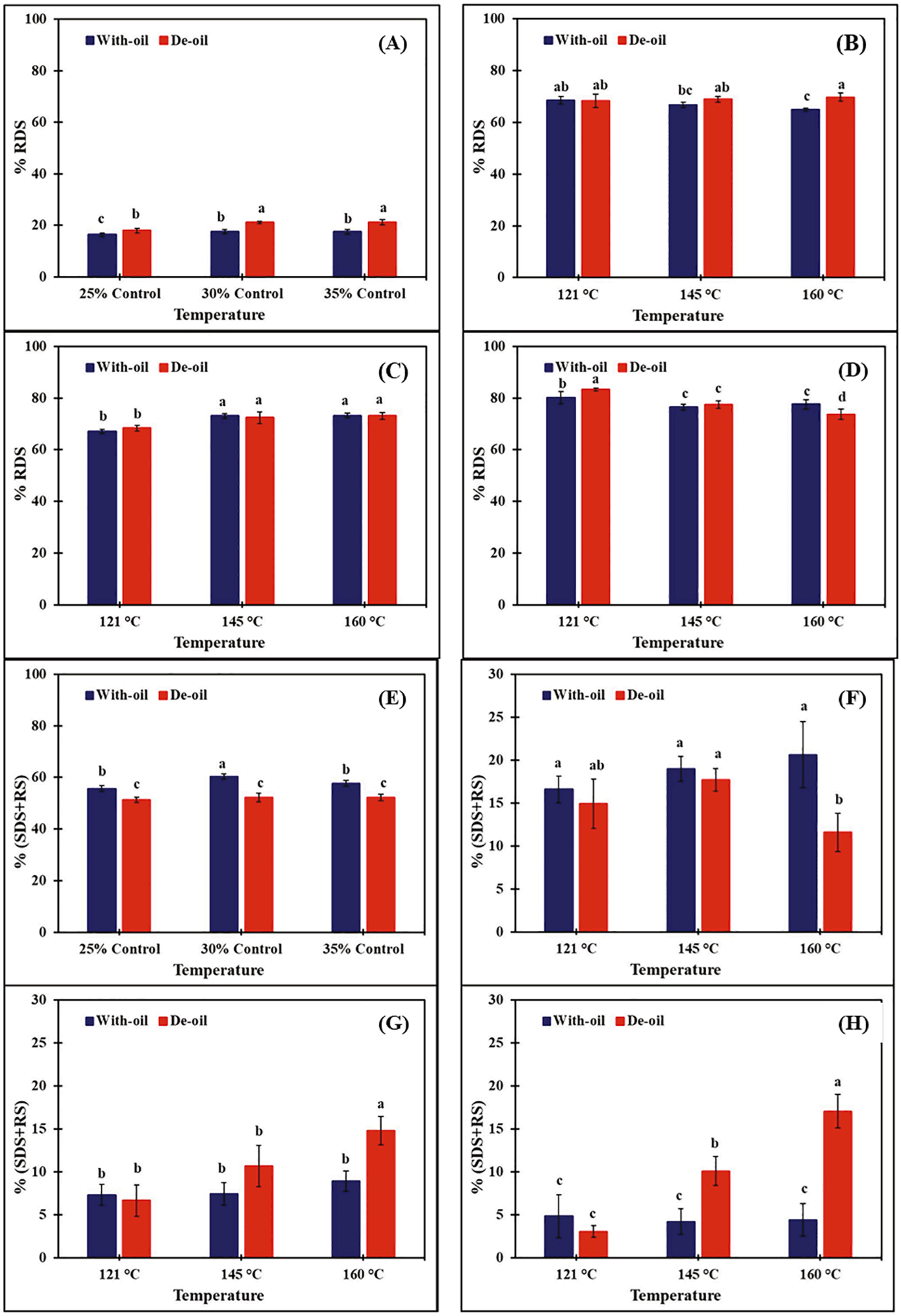

Fig. 3. The variation of Rapidly Digestible Starch (RDS) fraction of corn flours (A) processed at room temperature and as a function of moisture, and at different processing temperatures at (B) 25, (C) 30 (D) 35% moisture. The variation of Slowly Digestible Starch (SDS) and Resistant Starch (RS) amount of corn flours (E) processed at room temperature and as a function of moisture, and at different processing temperatures at (F) 25, (G) 30 (H) 35% moisture.

highest RDS occurs at low temperature and high moisture levels while the lowest RDS at low temperature and low moisture amount. On the other hand, for with-oil samples (Fig. 4a, Eq. (1)), moisture has a more significant effect on the response value than temperature; RDS is minimum at high temperature and low moisture while RDS is maximum at low temperature and high moisture. Similarly, after oil removal, (SDS + RS) declines as moisture surges from 25 to 35% and temperature is lower than 145°C but at higher temperature of 160°C it is positively correlated with the moisture raise (Fig. 4d, Eq. (4)). The minimum (SDS + RS) level occurs at low temperature and high moisture level while the maximum

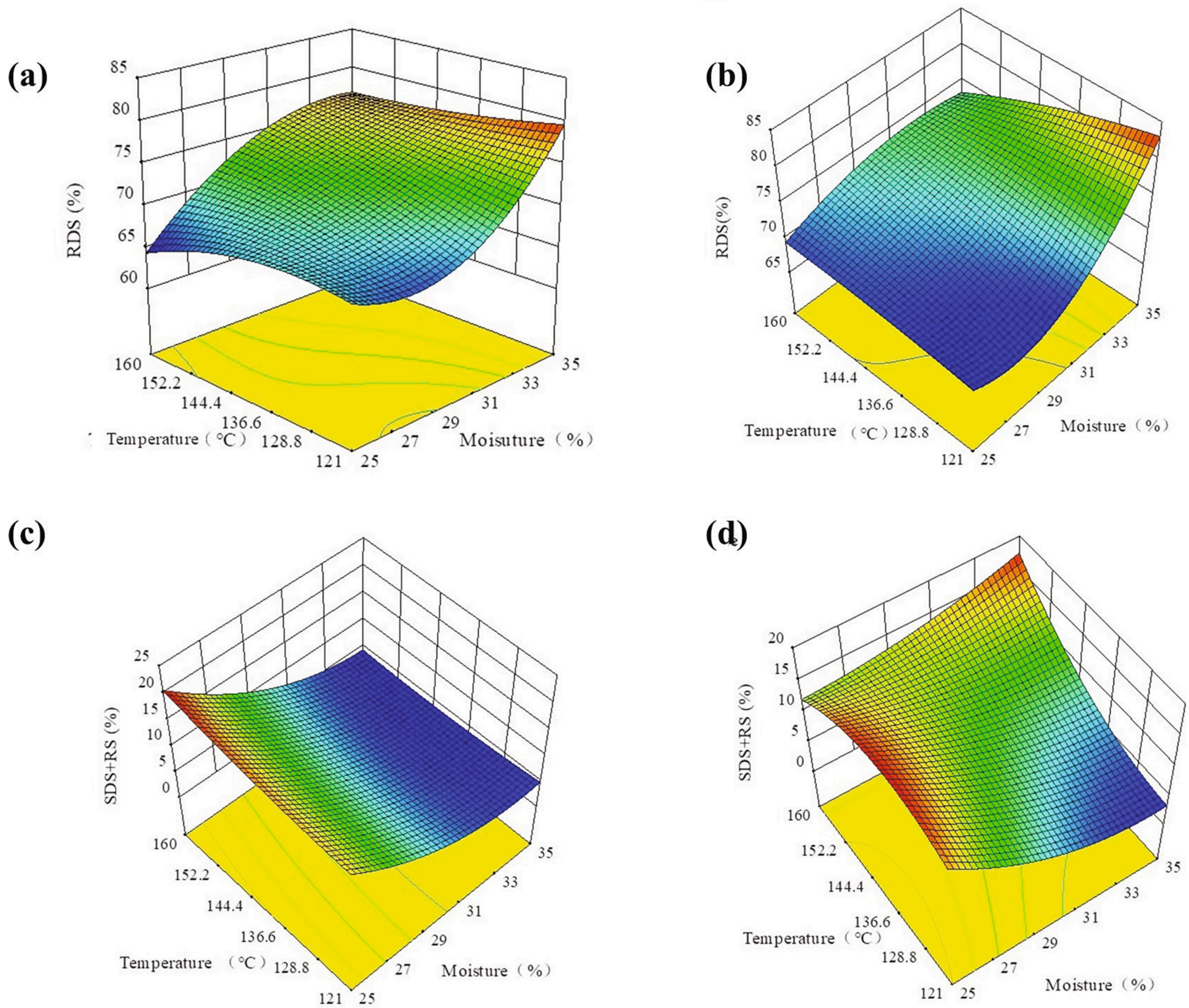

Fig. 4. 3-D surface graphs highlighting the effect of moisture amount and processing temperature on RDS values of (a) with-oil and (b) de-oil corn flours, and the (SDS + RS) of (c) with-oil and (d) de-oil corn flours.

is when both temperature and moisture are high. Interestingly, for with-oil samples, (SDS + RS) amount decreases with the increase in the moisture content from 25 to 35% (Fig. 4c, Eq. (3)). The minimum of (SDS + RS) occurs when the moisture level reaches 35% and the maximum at low moisture content and high temperature.

The RDS and (SDS + RS) yields obtained for corn flour as a function of temperature and moisture in this study are somewhat similar to the behavior of pre-gelatinized high amylose maize flour that displays higher RS coupled with lower SDS and RDS amounts (Zhang et al., 2016). The type of processing such as autoclaving, microwaving and parboiling has effect on the maize, potato, cocoyam, plantain, yam and rice flours that yield variable amounts of RDS, SDS and RS (Noro, Morohashi, Nakamura, Nakajima, & Ohtsubo, 2018). Autoclaving and parboiling increase the RDS and SDS levels while microwaving has an opposite effect. Heat-moisture treatment (HMT) and annealing are hydrothermal protocols that are being used to modify starch digestion (Zavareze, & Dias, 2011). The HMT takes place at restrained moisture level (<35%) whereas annealing at 40–80% level. Temperatures well above >80°C are being used for HMT for shorter durations (1 to 24 h) whilst lower temperatures (25–80°C) with prolonged time periods (h to days) are employed in annealing. These two modifications are performed above the glass transition temperature and below the starch gelatinization temperature. Indeed, both these protocols yield starches with varied RDS, SDS and RS amounts. The processing temperatures used in our research are much higher than the HMT or annealing. Overall, moisture coupled heat processing appear to significantly change the starch digestion.

4. Conclusions

In this research, a high temperature with shear processing method has been exemplified as a feasible contrivance to produce high-quality, ready-to-eat whole corn flours. The presence of oil, processing temperature and moisture level are observed to alter the physiochemical properties of corn flours and the starch digestibility. The amounts of RDS and (SDS + RS) could be controlled by adjusting the processing parameters, suggesting that flours with optimized digestibility could be prepared to meet the consumer demands.

The outcome of this research promises to improve the design and development of novel functional foods with controlled starch digestion. For example, oil containing flours could maintain flavor and taste compared to de-oil flours and gain consumers acceptability and utility. They could be processed with high moisture and be used to boost energy in short intervals for babies, patients and athletes. The de-oiled flours could be processed with high moisture and temperature to prepare weight controlling food products. The low moisture processed products could be sold as healthy foods as they not only provide fiber but also could help to control blood glucose. The proposed methodology would be equally applicable to other grains such as wheat, rice, oat, barley and rye. Such developed food products could as well aid to address chronic diseases namely diabetes, obesity and cardiovascular disease. In order to fully realize these benefits further research is warranted.

CRediT authorship contribution statement

Yu Peng: Conducted experiments and drafted the original manuscript. **Tianming Yao:** Conducted experiments and drafted the original manuscript. **Qin Xu:** Writing - review & editing. **Srinivas Janaswamy:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.130609.

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