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FULL PAPER

Physicochemical, functional properties, *in vitro* starch digestibility and estimated glycaemic index of composite flour influenced by resistant starch

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Abstract

There has been increasing demand for resistant starch-enriched food products with a low glycaemic index (GI) as consumers nowadays are aware to improve health status. Therefore, the present study was carried out to investigate the effect of different substitution levels of type-2 resistant starch (high-amylose maize starch) (HM) into wheat flour. In this study, wheat flour sample (control) and six test composite flour samples comprising wheat flour substituted with 5%, 10%, 15%, 20%, 25% and 30% of HM powder were analyzed to compare their physicochemical characteristics, functional properties, *in vitro* starch digestibility and expected glycaemic index (eGI). The results revealed that the incorporation of HM had resulted in increased moisture (12.70 – 13.31%) and total dietary fibre (TDF) (0.19 – 0.46%), as well as a decreased proportion of ash, fat, and protein. The carbohydrate and energy values were not significantly different upon the increasing percentage of HM ($p>0.05$). Mineral analysis showed that HM composite flour had significantly lower Mg, Ca, K, P, Fe, Zn and Se than the control. HM composite flour exhibited greater water holding, water holding capacity, oil holding capacity and swelling power than the control sample. The hydrolysis index and eGI of HM composite flour decreased with higher HM substitution. In conclusion, HM composite flours showed a good potential to be used in functional food, where positive impacts have been observed for in vitro starch digestibility and eGI characteristic.

1. Introduction

Composite flour is defined as a mixture of flour, starch, and other ingredients to replace all or part of wheat flour in bakery and pastry products (Milligan *et al.*, 1981). These starchy products are consumed as part of the diet in most countries. However, in countries where no wheat is grown, wheat must be imported from others and this affects a nation's economy and food security (Moreno-Àlvarez *et al.*, 2009). In order to reduce the importation of wheat, substituting wheat flour with local high-carbohydrate materials such as cassava flour, rice flour, sweet flour, cocoyam flour and maize flour serves as an alternative in bread and pastry making (Sanful and Darko, 2010; Hasmadi *et al.,* 2021a; Hasmadi *et al.,* 2021b).

The development of nutritious and healthier low-calorie products with acceptable functional and sensory attributes remains a major industrial challenge, seeking to fulfil the expectation of consumers (Gormley, 2018). Over the decades, there has been one such trend to increase the fibre content in food products to overcome health problems, such as diabetes, hypertension, and colon cancer (Wang *et al.*, 2018). Normal starches are easily metabolized to monosaccharides and absorbed into the body, but some are resistant to enzymatic hydrolysis, passing through the small intestine, and are left to ferment in the large intestine where they act as a dietary fibre to improve the health of the digestive system (Arp *et al.*, 2018b). This type of starch is called resistant starch (RS) which has a lower calorie count (Lockyer and Nugent, 2017).

Considering the technological use of composite flour, resistant starch has been used to substitute the amount of wheat flour to enrich the nutritional content of Chinese steamed bun (CSB) (Haini *et al.,* 2021). According to Shukri *et al.* (2017), the addition of 15% cross-linked rice-resistant starch is suitable to increase fibre in CSB with little effect on appearance and sensory

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attributes. Wang *et al*. (2017) and Fu *et al*. (2010) have incorporated high amylose maize-resistant starch (5–10%) and successfully imparted nutritional value without altering the textural, sensory, and shelf-life properties of CSB. On the contrary, the substitution of 5% resistant starch extracted from buckwheat powder in Guan (2007) has decreased the textural quality and sensory scores of CSB. Those studies have shown the applicability of resistant starch in terms of physical, sensory, and nutritional aspects in CSB that are not related to the functional properties of RS composite flour itself.

Henceforth, the present investigation aimed to study the effect of different substitution levels (5% – 30%) of type-2 RS, high-amylose maize starch, Hi-Maize® 260 (HM) into wheat flour. Given that HM is commercially available, the physicochemical, functional, nutritional, *in vitro* starch digestibility and eGI characteristics of HM composite flour were explored. All these properties are important to determine the quality of HM composite flour whether or not it shows qualities that are similar to or much better than that of plain wheat flour (Mepba *et al*., 2007). The results obtained from composite flour analysis provide useful insights for future food product development.

2. Materials and methods

In this study, control wheat flour sample named control flour and six test composite flour (CF) samples comprising wheat flour substituted with 5% (CF5), 10% (CF10), 15% (CF15), 20% (CF20), 25% (CF25) and 30% (CF30) of HM powder were analyzed to compare their functional, physicochemical, nutritional, *in vitro* starch digestibility and estimated glycaemic index properties. RS (Hi-Maize® 260, 12.4% moisture, 63% total dietary fibre, 80% amylose content, based on dry basis) was purchased from Ingredion (Australia). Pepsin 0.7 FIP-Umg-1 (107185); Pancreatic α-amylase (Pancreatin, 10 g, 3 Ceralpha-Umg-1); amyloglucosidase from *Aspergillus niger*, 260 UmL-1 (A-7095); were obtained from Sigma–Aldrich and used in *in vitro* digestibility test. The glucose oxidase peroxidase (GOPOD) assay kit (K-GLUC) was obtained from Megazyme International Ireland Ltd. Other chemicals used were of analytical grades.

2.1 Proximate analysis and total dietary fibre

Proximate composition analyses were conducted according to AOAC (2013) for moisture, total ash, fat, protein, and carbohydrate. Total dietary fibre (TDF) was measured using a Megazyme TDF kit (AACC, 2000). The calorific content (kcal/100 g) was calculated by multiplying crude protein, crude fat, dietary fibre and available carbohydrate contents by factors of 4, 9, 2 and 4, respectively. Total energy was expressed in terms of kilocalories (kcal) unit (Ministry of Health Malaysia, 2010).

2.2 Macro and trace mineral

Major (K, Na, Mg, Ca), trace elements (Fe, Zn, Mn, Se, Cu) and heavy metals (Cd, Cr, Pb, As) were determined using Inductively Coupled Plasma Optical Emission (ICP-OES) (Optima 5300 DV, Perkin Elmer, England). The standard of mineral elements for flame ICP-OES was obtained by diluting 1000 ppm of ICP-OES stock solution using deionized water (de la Guardia and Garigues, 2015). The calculation is applied in accordance with Beer's Law.

2.3 Water holding capacity

Water holding capacity (WHC) was determined according to Robertson *et al.* (2000). For each sample, 0.5 g was added to 5 mL distilled water and vortexed for 15 s every 5 mins. It was then centrifuged at 2100×*g* for 10 mins. The supernatant and precipitate were dried at 100°C separately. WHC (%) values were calculated as in Equation 1.

$$\text{WHC (\%)} = \frac{\text{wet precipitate (g) - dry precipitate (g)}}{\text{sample weight}} \times 100 \qquad (1)$$

2.4 Oil holding capacity

Oil holding capacity (OHC) was measured based on the method described by Jung *et al*. (2017). Sample (0.5 g) and corn oil (6.0 mL) were added into a graduated centrifuge tube. The tube was vortexed for 1 min, left for 30 mins and centrifuged for 25 min at 3000×*g*. After 25 mins the supernatant was removed, and the sediment was weighed. Oil holding capacity was calculated in Equation 2:

$$\text{OHC (\%)} = \frac{\text{initial weight (g) of sample before treatment}}{\text{final weight (g) of sample after treatment}} \times 100 \qquad (2)$$

2.5 Swelling capacity

Each flour sample (0.5 g) was dispersed in 6 mL of distilled water in a centrifuge tube. The tube was then kept at 30°C for 30 min prior to centrifugation at 2,500×*g* for 20 mins. The supernatant was poured carefully into an evaporating dish before drying at 105°C for 24 h and weighing. The remaining gel from the centrifugation was also weighed (Equation 3).

$$\text{WHC (\%)} = \frac{\text{wet sample (g) - dry precipitate (g)}}{\text{initial sample weight (g) - dried supernatant (g)}} \times 100 \qquad (3)$$

2.6 Colour analysis

Colour characteristics were studied by measuring lightness (L*), redness (a*) and yellowness (b*) with a

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Minolta CR-400 spectrophotocolorimeter (Konica Minolta Sensing, Japan). The whiteness index (WI) was calculated by using the formula (100 − L*)2 + a*^2 + b*^2)^0.5 (Zhu and Sun, 2019).

2.7 In vitro starch digestibility and estimated index (eGI)

In vitro starch digestibility is an enzymatic analysis to measure starch hydrolysis rate for the prediction of glycaemic index (GI). The *in vitro* starch digestibility and glycaemic index (eGI) were determined based on Goñi *et al*. (1997). The glucose content was analyzed using GOPOD K-GLUC (AACC, 2000). Starch digestion rate was expressed as the percentage of total hydrolyzed starch at different time intervals (30 mins, 60 mins, 90 mins, 120 mins, 150 mins and 180 mins). The percentage of hydrolyzed starch was calculated by multiplying the glucose content by 0.9. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) contents were calculated as in Equation 4 and 5:

$$\text{RDS (\%)} = (G_{20} - FG) \times 0.9 \quad (4)$$

$$\text{SDS (\%)} = (G_{120} - G_{20}) \times 0.9 \quad (5)$$

Where G_{20} = quantity of free glucose measured after 20 min incubation with the enzyme, G_{120} = quantity of free glucose measured after 120 min incubation with the enzyme and FG = Free glucose content.

The free glucose (FG) content was carried out using a D-Glucose GOPOD assay Kit (Megazyme International K-GLUC, Ireland) (AACC, 2000). Goñi *et al*. (1997) stated that the kinetics of *in vitro* digestion followed a nonlinear model with a first-order equation of $C = C\infty (1 - e^{-kt})$, where C is the percentage of starch hydrolyzed at time t (min), C∞ is the equilibrium percentage of starch hydrolyzed after 180 mins and k is the kinetic constant. Total starch hydrolysis (%) values of samples were plotted against time (min) and the area under the curve (AUC) was calculated using Microsoft Excel. The hydrolysis index (HI) was obtained by dividing the AUC of the sample by the AUC of the standard reference. Glucose was used as the standard reference (HI=100). The eGI value was calculated using the formula established by Goñi *et al*. (1997), eGI = (0.594 × HI) + 39.71.

2.8 Statistical analysis

Statistical analysis was carried out by using version 25 of Statistical Package for Social Science (SPSS) software. All results for functional, physicochemical, nutritional, starch in vitro digestibility and estimated GI were analyzed using one-way ANOVA, followed by multiple comparisons using Tukey's B significant difference test ($p < 0.05$) and data were presented as mean ± SD.

3. Results and discussion

3.1 Proximate composition and total dietary fibre

The proximate composition for control and HM composite flour is tabulated in Table 1. In general, the

Table 1. Nutritional composition of control and HM composite flour

	Control	CF5	CF10	CF15	CF20	CF25	CF30
Proximate values (%)							
Moisture	12.70±1.01^{b}	13.21±1.12^{a}	13.23±1.06^{a}	13.26±0.67^{a}	13.28±1.67^{a}	13.29±1.21^{a}	13.31±0.91^{a}
Ash	0.97±0.06^{a}	0.93±0.05^{a}	0.86±0.03^{b}	0.82±0.01^{bc}	0.77±0.03^{c}	0.71±0.04^{cd}	0.65±0.02^{e}
Protein	9.93±0.25^{a}	9.51±0.58^{b}	8.94±0.80^{c}	8.52±0.51^{d}	7.95±0.40^{e}	7.43±0.49^{f}	6.90±0.57^{g}
Fat	0.91±0.15^{a}	0.87±0.06^{ab}	0.84±0.11^{bc}	0.80±0.10^{c}	0.73±0.06^{d}	0.69±0.08^{de}	0.64±0.05^{ef}
Dietary Fibre	0.19±0.01^{d}	0.23±0.01^{c}	0.27±0.02^{c}	0.34±0.01^{b}	0.39±0.03^{ab}	0.41±0.01^{a}	0.46±0.02^{a}
Carbohydrate	75.09±1.46^{a}	75.29±1.26^{a}	75.86±1.48^{a}	76.05±1.39^{a}	76.62±1.28^{a}	77.51±1.31^{a}	78.35±1.25^{a}
Energy (kcal)	346.80±3.28^{a}	345.74±2.87^{a}	345.60±2.36^{a}	344.56±2.09^{a}	344.10±1.88^{a}	345.41±2.55^{a}	346.40±1.77^{a}
Traceable elements (mg/100 g)							
Ca	2.31±0.01^{a}	2.11±0.04^{b}	1.82±0.05^{c}	1.73±0.09^{d}	1.60±0.08^{e}	1.55±0.07^{e}	1.35±0.06^{f}
Fe	1.34±0.07^{a}	1.23±0.06^{b}	1.21±0.10^{b}	1.20±0.04^{b}	1.18±0.05^{c}	1.16±0.07^{c}	1.11±0.01^{d}
K	1.57±0.02^{a}	1.29±0.04^{b}	1.05±0.03^{c}	0.91±0.02^{d}	0.78±0.03^{e}	0.66±0.04^{f}	0.58±0.02^{f}
Mg	2.71±0.07^{a}	2.65±0.04^{a}	2.53±0.01^{b}	2.43±0.02^{c}	2.35±0.03^{d}	2.21±0.08^{e}	2.13±0.02^{f}
Na	1.49±0.05^{a}	1.35±0.02^{b}	1.31±0.01^{b}	1.25±0.001^{c}	1.19±0.001^{c}	1.08±0.00^{d}	0.86±0.04^{e}
P	1.46±0.03^{a}	1.39±0.02^{b}	1.30±0.01^{c}	1.28±0.02^{cd}	1.22±0.01^{de}	1.13±0.02^{f}	1.04±0.01^{g}
Zn	0.67±0.01^{a}	0.56±0.02^{b}	0.47±0.01^{c}	0.40±0.06^{cd}	0.37±0.01^{d}	0.33±0.02^{d}	0.26±0.01^{de}
Se	0.43±0.01^{a}	0.36±0.02^{b}	0.32±0.01^{b}	0.30±0.06^{b}	0.27±0.01^{bc}	0.23±0.02^{c}	0.21±0.01^{c}

Values are presented as mean±SD. Values with different superscripts within the same row are significantly different ($p<0.05$). HM: represents Hi-Maize® 260, Control: represents control wheat flour without HM addition (control), CF5%–CF30%: represent wheat flour incorporated with increasing HM percentage, respectively.

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incorporation of HM (5–30%) into each formulation resulted in increased moisture (12.70–13.31%) and total dietary fibre (TDF) (0.19–0.46%), as well as the decreased proportion of ash, fat, and protein. On the contrary, the carbohydrate and energy values were not significantly different upon an increasing percentage of HM ($p>0.05$). The increased TDF in CF5–CF30 had been stipulated from the rich source of resistant starch found in HM whereby the increased moisture was collectively increased with TDF in HM composite flour which formed hydrogen bonding in the fibre structure (Rosell *et al*., 2001). There had been similar studies reported on the increased moisture and TDF upon higher substitution of fibre-like ingredients such as high-amylose maize starch (Collar *et al*., 2014; Arp *et al*., 2018; Magallanes-Cruz *et al*., 2020) and cross-linked resistant starch (Shukri *et al*., 2017).

Meanwhile, the decreased ash percentage was related to low ash content in wheat flour (0.97%; data not shown) and HM powder (0.1%, supplementary data by Ingredion). Aziah *et al*. (2012) reported that ash percentage reflected the mineral composition in a food sample, replacing wheat flour with HM could therefore reduce the ash content and cause the loss of minerals available in composite flour. Likewise, the decreased protein was attributed to the addition of HM (Zhu *et al*., 2013), suggesting that wheat flour was the main source of protein and thus formed more gluten compared to HM composite starch (Wang *et al*., 2017). Similarly, the decrease in fat content was probably due to the added HM, indicating that wheat flour was the main contributor to fat, consequently reducing the fat content in HM composite flour (Mohebbi *et al*., 2018). The value for available carbohydrates and energy were not all significant and was subjected to the outcome from the calculation, owing to the increased moisture and TDF as well as the decreased ash, fat, and protein content (AOAC, 2013). Overall, the proximate results found for HM5 to HM30 implicated that HM possessed beneficial nutrients that could serve as an alternative ingredient for calorie control in the making of food products.

3.2 Traceable element

Table 1 demonstrates the concentration of traceable elements found in control and composite flour. In general, control flour contained magnesium (Mg) and calcium (Ca) as the most abundant element, followed by potassium (K), sodium (Na), phosphorus (P), iron (Fe), Zinc (Zn) and selenium (Se). These concentrations were relatively comparable to Norhaizan and Nor Faizadatul Ain (2009) who studied the mineral content of wheat flour in Malaysia. The control flour had the highest element concentration given that wheat flour could be the main source of minerals in the composite flour (Aziah *et al*., 2012). In contrast, the HM composite flour had significantly lower Mg, Ca, K, P, Fe, Zn and Se than that of the control. These elements decreased with higher substitution of HM indicating the HM powder accounted for the decrease in Mg, Ca, K, P, Fe, Zn and Se concentration.

From a nutritional standpoint, these Mg, Ca, K, P, Fe, Zn and Se represent minerals that are essential for humans and are normally found in cells as components of the active site of enzymes or as regulators of enzymatic activity (de la Guardia and Garigues, 2015). Given that the amount of trace elements shown in HM composite flour was within the range of recommended daily serving (Akta Makanan, 2011), HM powder could serve as a good alternative for minerals and be appropriate to substitute wheat flour in bakery product making. Besides, traces of heavy metals were also not detected in any of the composite flour samples and thus considered safe for human consumption (de la Guardia *et al*., 2015).

3.3 Water holding capacity

The water holding capacity (WHC) of control and HM composite flour is shown in Table 2. WHC is the volume of water that remains attached to the hydrated fibre after an external centrifugal gravity force or compression (Hasmadi *et al*., 2020). The results showed that the WHC of HM composite flour (3.53–4.95%) was significantly ($p<0.05$) higher than that of the control flour (3.17%). The HM composite flour had shown greater water holding capacity (3.53–4.95%), indicating that it retained more water than the control (Collar *et al.,* 2014; Arp *et al.,* 2018) and eventually preserved the moisture in HM composite flour. The findings could be related to higher RS and TDF content in HM composite flour. Both RS and TDF constituents in HM retain water by adsorption within the fibre matrix, preventing the structure from degrading. Based on this functional characteristic, HM composite flour could be used in functional food that requires freshness preservation and viscosity growth (Magallanes-Cruz *et al*., 2020).

3.4 Oil-holding capacity

Table 2 demonstrates the results for the oil-holding capacity (OHC) of control and HM composite flours. Oil -holding capacity (OHC) is a functional property that is related to the physical entrapment of oil (Hasmadi *et al*., 2020) and is affected by the thickness, surface properties, total charge density, and hydrophobicity of fibre particles (Viuda-Martos *et al*., 2012). In this study, the OHC had been depleted with higher HM substitution (5 – 30%) whereby the control flour, on the contrary, presented the highest OHC (2.78). The low OHC of HM composite

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Table 2. Functional and physicochemical properties of HM composite flour

	Control	CF5	CF10	CF15	CF20	CF25	CF30
WHC	3.17±0.20^{g}	3.53±0.20^{f}	3.86±0.20^{e}	4.05±0.20^{d}	4.26±0.20^{c}	4.51±0.20^{b}	4.95±0.20^{a}
OHC	2.78±0.03^{a}	2.54±0.03^{b}	2.29±0.03^{c}	2.03±0.03^{d}	1.86±0.03^{e}	1.72±0.03^{f}	1.61±0.03^{g}
SWC	1.80±0.17^{g}	2.33±0.17^{f}	2.91±0.17^{e}	3.72±0.17^{d}	4.36±0.17^{c}	4.98±0.17^{b}	5.65±0.17^{a}
Colour							
*L**	94.83±0.44^{f}	95.49±0.17^{ef}	96.08±0.39^{e}	96.88±0.33^{d}	97.61±0.04^{c}	98.67±0.29^{b}	99.60±0.21^{a}
*a**	-3.89±0.03^{e}	-3.85±0.01^{de}	-3.80±0.02^{cde}	-3.67±0.10^{cd}	-3.51±0.01^{bc}	-3.47±0.03^{ab}	-3.31±0.16^{a}
*b**	+11.50±0.20^{e}	+12.27±0.11^{d}	+12.73±0.28^{cd}	+13.03±0.12^{c}	+13.35±0.22^{c}	+14.31±0.25^{b}	+14.96±0.48^{a}
WI	12.78±0.53^{a}	12.82±0.29^{a}	12.85±0.67^{a}	12.40±0.15^{a}	12.20±0.29^{a}	12.20±0.41^{a}	12.15±0.33^{a}

Values are presented as mean±SD. Values with different superscripts within the same row are significantly different ($p<0.05$). HM: represents Hi-Maize® 260, Control: represents control wheat flour without HM addition (control), CF5%–CF30%: represent wheat flour incorporated with increasing HM percentage, respectively, WHC: Water-holding capacity, OHC: Oil-holding capacity, SWC: Swelling capacity, WI: Whiteness index, WA: Water activity.

flour might be attributed to RS content in HM as it could not absorb oil (Zhu *et al*., 2013; Jung *et al*., 2017). The mechanism could be also elucidated through physical factors such as smaller particle size and structural complexity in HM composite starch that restricts oil absorption and hence results in low OHC (Lin *et al.,* 2012).

3.5 Swelling capacity

The swelling capacity (SWC) of control and composite flour is shown in Table 2. SWC is the capacity of DF to increase the bulk after absorbing water and is measured as settled bed volume (Hasmadi *et al*., 2000). The results showed that the SWC values of HM composite flour were significantly increased (2.33–5.65%) in comparison to control flour (1.80%). The increased SWC could be related to the increase of TDF in HM composite flour which formed hydrogen bonding in the fibre structure (Rosell *et al*., 2001). From the technological and physiological standpoint, the hydration characteristics of DF are important as it is capable to restrict access to starch digestion and reducing glucose response-ability (Ng *et al*., 2017).

3.6 Colour properties

Table 2 shows the CIE-Lab colour values (*L**, *a**, *b**) for control and HM composite flour. Colour is one of the important technological properties of foods as its changes could imply nutritional modification and sensory acceptance of foods (Zhu *et al*., 2016). The incorporation of HM significantly increased the brightness (*L**) in composite flour (95.49–99.60). The yellowness (*b**) appeared significant from CF5 (+12.27) to CF30 (+14.96), whereby the whiteness index was not significantly different from CF5 to CF30 (12.15–12.82). Those findings could be possibly attributed to the natural white colour of HM that did not give many colour changes to the composite flour (Shukri *et al*., 2017). The same findings on increased brightness (*L**) and yellowness (*b**) were observed when HM was incorporated into wheat flour (Collar *et al*., 2014; Wang *et al*., 2017; Arp *et al*., 2018).

3.7 In vitro starch digestibility and eGI

Table 3 shows the starch nutritional fraction, hydrolysis index (HI) and eGI of control and HM composite flour. The values for starch fractions, HI and eGI in HM composite flour had been decreased with higher HM substitution, except for the increased RS (0.38–22.08%). The replacement of HM with composite flour also reduced the total starch hydrolysis as depicted in Table 3. For instance, incorporating HM at 30% significantly reduced the eGI for control from 73.12 to 57.09 to make the composite flour a medium GI food. Moreover, the control flour containing the least amount of RS had shown the fastest starch hydrolysis, suggesting the absence of HM was associated with lower RS which eventually increased both HI and eGI in composite flour (Arp *et al*., 2018b). Eventually, there have been several factors that contributed to the reduced *in vitro* starch digestion and eGI. The presence of RS and dietary fibre in HM could intervene with the starch digestibility through their physicochemical interactions with HM composite starch (Zhu, 2019). Furthermore, the HM composite flour that lacks starch content could also have lower enzyme susceptibility than that wheat starch (Zhu *et al*., 2013).

Another possible factor contributing to the reduced starch digestibility and eGI is the increased WHC and SWC of HM composite CSB (Table 2). The increased WHC and SWC might contribute to DF-like physiological properties that may interfere with the physical interactions of HM composite starch (Zhu, 2019). The mechanism could be further elucidated through the formation of viscous composite starch thereby reducing starch susceptibility to digestive

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Table 3. *In vitro* starch digestibility and estimated glycaemic index (eGI) of control and HM composite flour

	TS (%)	RDS (%)	SDS (%)	RS (%)	HI	eGI
HM	83.18±0.61[e]	10.5±0.14[h]	15.40±0.39[c]	57.30±0.27[a]	12.83±0.10[i]	46.75±0.18[i]
Control	61.08±1.09[a]	38.79±0.46[a]	21.21±0.11[a]	1.08±0.02[h]	56.25±0.44[b]	73.12±0.91[b]
CF5	57.20±0.64[b]	35.30±0.28[b]	17.81±0.35[b]	4.09±0.02[g]	50.21±0.56[c]	69.53±0.83[c]
CF10	55.02±0.92[c]	30.46±0.16[c]	15.52±0.21[c]	9.04±0.04[f]	44.60±0.34[d]	66.20±0.36[d]
CF15	54.10±0.88[c]	27.65±0.39[d]	12.26±0.42[d]	14.19±0.01[e]	41.14±0.27[e]	64.15±0.19[e]
CF20	53.01±0.95[c]	23.28±0.58[e]	11.17±0.15[e]	18.56±0.16[d]	37.15±0.31[f]	61.77±0.26[f]
CF25	49.53±0.91[d]	20.16±0.13[f]	9.18±0.11[f]	20.19±0.05[c]	32.55±0.29[g]	59.04±0.32[g]
CF30	48.83±0.76[d]	17.55±0.27[g]	7.20±0.17[g]	24.08±0.01[b]	29.26±0.62[h]	57.09±0.65[h]
Glucose	-	-	-	-	100.00±0.00[a]	99.11±0.00[a]

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different ($p<0.05$).

HM: represents Hi-Maize® 260, Control: represents control wheat flour without HM addition (control), CF5%–CF30%: represent wheat flour incorporated with increasing HM percentage, respectively, TS: Total starch, RDS: rapidly digestible starch, SDS: slowly digestible starch, RS: resistant starch, HI: hydrolysis index, eGI: estimated glycaemic index

enzymes *in vitro* (Ng *et al*., 2017). The findings agreed with Ahmed and Urooj (2015) that the substitution of psyllium (10%), oat (15%) and barley (10%) had significantly reduced both the starch digestion and eGI in composite flour. The authors had reported that the increased RS, WHC and SWC were positively correlated with the reduction of eGI, therefore, suggesting that the HM composite flour could be useful in controlling glucose intake. Previous human studies have also reported that HM possessed anti-hyperglycemic properties as it delayed the rise of postprandial blood glucose (Gower *et al*., 2016; Bindels *et al*., 2016; Maziarz *et al*., 2017; Marlatt *et al*., 2018; Stewart *et al*., 2018). Based on these results, it could be concluded that the HM composite flour, particularly CF30, was markedly more resistant to digestion compared to the control flour (without HM).

4. Conclusion

In summary, this study demonstrated that the use of 30% HM powder is an innovative approach to developing a desirable high-RS and medium-GI composite flour. The medium GI of this composite flour could be attributed to the high RS level, which, reduced starch digestibility, reduced estimated glycaemic index as well as increased WHC and SWC after the incorporation of HM into composite flour. The formulated HM composite flours have the potential to be used in functional food, particularly to study the effect of HM on starch digestibility *in vitro* and eGI characteristics.

Conflict of interest

The authors declare no conflict of interest.

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