

## Effect of partial replacement of wheat flour with flour/starch containing resistant starch on macaroni quality

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### Abstract

The present study aimed to add high resistant starch (RS) flours including black beans, mung beans, green bananas and purple sweet potatoes as well as potato starch starches into the macaroni formulation. The RS of the materials used was analyzed. The physical, chemical, textural, qualitative and organoleptic values of macaroni products were measured and determined. A significant difference ( $P < 0.05$ ) in RS content of food ingredients was obtained, in which potato starch was the highest at 56.43% and black turtle beans powder was at least 16.59%. Swelling and water absorption showed an inverse relationship with RS content. The RS content of macaroni increased significantly with the replacement of wheat flour with flours and starches of high RS content in their formulation. The  $L^*$  lightness and yellowness  $b^*(+)$  of the macaroni were significantly reduced with the high RS flours/starch in the formulation. Scanning electron microscopy images showed an increase in pore sizes in macaroni with high RS flour in comparison with the control sample. Analysing of sensory values by principal component analysis (PCA) revealed that the sample from formulation M2 with 19.2% powder containing RS substitute was a highly accepted product by the panellists with desirable properties. Food processors have incorporated various highly nutritious foods in many conventional foods, therefore, high RS flours and starch in macaroni have been shown to improve RS consumption, which essentially has many health benefits.

### Introduction

Non-communicable diseases are the major causes of high mortality rates in the current times in both developed and developing countries. According to WHO (2021), 71% of mortality is a result of non-communicable diseases. Several factors contribute to increased incidences of these diseases, including a sedentary lifestyle, poor dietary choices, and genetic factors. This challenge has prompted food technologists to develop solutions for highly nutritious foods and reduce the incidence of these conditions. Individuals have acquired knowledge on the benefits of consuming a diet high in resistant starch, which has influenced their food choices. Resistant starch (RS) consumption contributes to the proper functioning of the digestive tract, increases the content of good microbial flora, reduces blood cholesterol levels, and lowers the glycemic index of food, hence managing type 2 diabetes (Fuentes-Zaragoza *et al.*, 2010; Jyoshna and Hymavathi, 2017). The importance of functional foods has been emphasized, contributing to great awareness among

people for healthy foods. RS is the sum of starch and products of starch degradation not absorbed in the small intestine of healthy people and it enters the large bowel where it is fermented by the microflora, with SCFAs as major end products (Chung *et al.*, 2011). RS occurs naturally in many starchy food sources such as cereals, grains, seeds, tubers, and legumes, and they have been grouped into five categories based on their sources. Therefore, adding RS as an ingredient in foods will lower food's overall glycemic index value because it replaces existing readily absorbed forms of carbohydrate, increasing food manufacturers' interest (Nugent, 2005). The incorporation of high RS flours from mung beans, black turtle beans, red kidney beans, green bananas, and purple sweet potatoes increases the RS content of pasta products. However, it affects the textural, microstructural, and sensorial properties of the pasta products (Petitot *et al.*, 2010; Wu *et al.*, 2015; Sun *et al.*, 2019). Food technologists need to balance the amount of supplemented flour to achieve high RS pasta with desirable physical properties. The research aimed to incorporate high resistant starch flours from different

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starchy food sources in formulations of macaroni to increase its RS content and identify the undesirable effects of unconventional flours on the quality of macaroni.

## 2. Materials and methods

### 2.1 Raw materials and ingredients

Starchy foods including purple sweet potato (*Ipomoea batatas*) and green bananas flours were prepared in the laboratory. Black turtle beans (*Phaseolus vulgaris*), mung beans (*Vigna radiata*), potato starch and wheat flour (Baker's Choice, Vietnam) were purchased at the supermarket, Can Tho city, Vietnam. Other materials, including semolina (Bob's Red Mill, USA), salt, eggs, and xanthan gum (Bob's Red Mill, USA) were used. The purple sweet potatoes were cleaned with tap water to remove soil, peeled and sliced into thin sheets of 2 mm, and then blanched for two mins in 100°C water before drying in an oven at 65°C for 7 hrs until the moisture content of the dried sample was around 6-7% (Thuy *et al.*, 2020). Green bananas were peeled and immediately placed in a sodium metabisulfite ( $\text{Na}_2\text{S}_2\text{O}_5$ ) solution with a concentration of 200 ppm and left to soak for 0.5 hrs to prevent polyphenol oxidation from polymerizing naturally occurring polyphenols in the banana. The sliced bananas of 2 mm thickness were dried at 65°C for 7 hrs to 12% moisture content. Mung beans, black beans and red kidney beans were dried at 65°C for 4 hrs to about 12% moisture content. The dried samples were ground into flours and sieved through US standard mesh No. 70 (212  $\mu\text{m}$ ) (Thuy *et al.*, 2020). The sieved powder was placed in a sealed airtight container and stored at 25°C for further use.

### 2.2 Chemical reagents

Resistant starch (RS) assay kit (Megazyme, Bray, Ireland) was used to measure the RS content of samples. Other materials included pancreatic  $\alpha$ -amylase (pancreatin, 10 g, 3 Ceralpha units/mg),

amyloglucosidase (AMG) [12 mL, 3,300 U/mL, on soluble starch (or 200 U/mL on p-nitrophenyl  $\beta$ -maltoside\*)], glucose peroxidase (GOPOD) reagent buffer (50 mL pH 7.4), p-hydroxybenzoic acid and sodium azide (0.09% w/v), GOPOD reagent enzymes (glucose oxidase and peroxidase) and 4-aminoantipyrine, D-glucose standard solution (5 mL, 1.0 mg/mL in 0.2% w/v), benzoic acid, and RS control. The chemicals used in this study were of analytical grade.

### 2.3 Formulation of macaroni

The ingredients were mixed into four formulas of macaroni while ensuring the total components are 100%. The control sample of macaroni was formulated with whole wheat flour. While, high RS flours were added to the other samples, as presented in Table 1. The quantification of the ingredients used to prepare pasta products was based on a previous study (Thuy *et al.*, 2020). The macaroni product was designed by placing the ingredients in a PHILIP pasta maker equipment (China) and stirring for five mins, followed by 30 mins of incubation. After tempering time, macaroni was extruded from pasta maker equipment. The quality of the products was analyzed.

### 2.4 Determination of resistant starch and amylose content

#### 2.4.1 Resistant starch

RS test was adapted for each matrix using the Association of Official Analytical Chemists (AOAC) Official Method 2002.02 (Ang, 2011). The absorbance of each solution was measured at 510 nm against a reagent blank using a Beckman Coulter DU-800 spectrophotometer. The reagent blank was prepared by mixing 0.1 ml of 0.1 M sodium acetate buffer (pH 4.5) and 3.0 mL of GOPOD reagent. Standards were prepared by mixing D-glucose (1 mL) with an appropriate amount of water, creating a range of D-glucose standards, to measure absorbance, the 0.1 mL of D-glucose standard (0.1 mL) was mixed with 3.0 ml of GOPOD. RS content

Table 1. Four formulations of macaroni.

Ingredients	M0(g)	M0 (%)	M1(g)	M1 (%)	M2(g)	M2 (%)	M3(g)	M3 (%)
Mung bean flour	0	0	12	2.82	16	3.76	20	4.7
Green banana flour	0	0	12	2.82	16	3.76	20	4.7
Black turtle bean flour	0	0	12	2.82	16	3.76	20	4.7
Purple sweet potato flour	0	0	12	2.82	16	3.76	20	4.7
Potato starch	50	11.74	45	10.57	40	9.39	35	8.21
Wheat flour	200	46.97	157	36.88	146	34.29	135	31.7
Semolina	40	9.39	40	9.37	40	9.38	40	9.39
Salt	2	0.47	2	0.47	2	0.47	2	0.47
Egg	65	15.26	65	15.27	65	15.27	65	15.27
Water	63	14.8	63	14.8	63	14.8	63	14.8
Xanthan gum	5.8	1.36	5.8	1.36	5.8	1.36	5.8	1.36
Total	425.8	100	425.8	100	425.8	100	425.8	100

(% dry weight basis) was calculated as follows (Equation 1).

$$RS = \text{mg/mL} \times \text{mL} \times (162/180) \times 100/\text{MC} \quad (1)$$

Where RS is the mg of RS/100 mg sample (% dry weight basis), mg/mL is obtained from calibration curve, mL is the final volume, 162/180 is the factor to convert free D-glucose, as determined, to anhydro-D-glucose as occurs in starch, and MC is the moisture content of sample.

#### 2.4.2 Amylose content

Amylose content was measured using the method of Ronoubigouwa *et al.* (2010). The absorbance was measured using a Beckman Coulter spectrophotometer, and amylose content was determined. The best linear model selected was expressed by the following Equation 2 ( $R^2 = 96\%$ ). Absorbance 620 nm as recommended by Ronoubigouwa *et al.* (2010)

$$\text{Amylose content (\%)} = (5.83539 + 1.47157x \ln (\text{Ab}_{620 \text{ nm}}))^2 \quad (2)$$

#### 2.5 Swelling and water absorption capacity

The swelling capacity of the samples and water absorption capacity (WAC) were determined according to the method of Nyam *et al.* (2014).

#### 2.6 Hardness testing

Texture attributes of macaroni were analyzed using a Brookfield CT3 Texture Analyzer equipped with a 1,500 g load cell and software version 1.8 (Brookfield Engineering Laboratories, Middleboro, USA).

#### 2.7 Cooking quality analysis

The cooking quality was analyzed by measuring cooking loss (%), rehydration rate (%), and volume increase (%). A sample of 50 g of macaroni was placed in 500 mL of boiling water for 4 mins. The 4 mins was considered optimum cooking time, which was described as the time needed to obtain complete gelatinization of starch, the time it took for the white core to disappear. The cooked pasta was separated from cook water by strainer; the collected cook water was dried in an oven at 103°C for cooking loss (CL) determination using Equation (3), by Wang and Ratnayake (2016).

Cooked macaroni was dried in an oven at 103°C for 20 hrs to estimate the rehydration rate (RR) by measuring the weight of fresh cooked macaroni and its weight after drying and calculated rehydration rate according to Equation 4 by Kamolchote *et al.* (2010) and the volume increase (%) was determined by comparing the volume of cooked macaroni and fresh macaroni using Equation 5 (Thuy *et al.*, 2020).

$$CL(\%) = \frac{\text{Weight of residue from cooking water (g)}}{\text{Weight of noodle sample (g)}} \times 100 \quad (3)$$

$$RR(\%) = \frac{WCN(g) - OWN(g)}{OWN(g)} \times 100 \quad (4)$$

Where WCN is the weight of cooked macaroni and OWN is the original weight of macaroni.

$$\text{Volume increase (\%)} = \frac{\text{Volume of cooked pasta} - \text{Volume of uncooked pasta}}{\text{Volume of uncooked pasta}} \times 100 \quad (5)$$

#### 2.8 Colour analysis

The colour of both cooked and uncooked macaroni was measured on the hunter scale for L\*, a\*, and b\* using Minolta Chroma meter (CR 400 Konica Minolta, Tokyo, Japan). A white standard plate calibrated the Chroma meter. L\* values described the black to white (0 -100), a\* values showed redness (positive) and greenness (negative), and b\* values described yellowness (positive) and blueness (negative). A total of ten measurements were carried out on each sample.

#### 2.9 Sensory evaluation

Quantitative Descriptive Analysis (QDA) and Check All That Apply (CATA) were applied for sensory evaluation. Sensory evaluation was carried out by ten panellists aged 22 years old who consumed macaroni often. The CATA questions were multiple-choice, commonly used in marketing research, to reduce responses. The questions contained a list of words where the panellists select attributes that appropriately describe the samples (Espitia-López *et al.*, 2019). The data was recorded in binary format (0-attributes not ticked and 1-attributes ticked) for CATA, and the rating scale of intensities for QDA was from 0 to 5 (0 = attributes not detected and 5 = attribute detected strongly).

#### 2.10 Scanning Electron Microscopy

Cooked macaroni was dried in an oven at 60°C for 270 mins until the moisture content was about 8 to 10%. Macaroni was cut by a razor blade cut, and the sample was mounted onto brass stubs using double-sided carbon conductive adhesive tape. A gold coating of (0.5 nanometers thick) was applied under an 8-9 Pascal vacuum. The bulk sample was examined at 15 kV, the sample distance to the 7 cm ejection glass, 230× magnifications using a JEOL model J550 scanning electron microscope (Japan).

#### 2.11 Statistical analysis

Statistical analysis was carried using STATGRAPHICS Centurion XIX (USA). Results were expressed as mean ± standard deviation (SD). To compare the mean values, One way ANOVA was used. LSD was used at a 5% significance level to compare

mean individuals. Sensory analysis was carried out using XLSTAT 2014 for Principal Component Analysis (PCA) and Check All That Apply (CATA).

### 3. Results and discussion

#### 3.1 Chemical and physical characteristics of starchy foods

The physical and chemical characteristics of starchy foods flours used were measured and presented in Table 2. The results showed that the highest content of RS was found in potato starch with 56.43%, green banana flour was second with 48.29% RS. Mung bean flour and purple sweet potato flour had an RS content of 30.47% and 22.93% while the lowest RS content was 16.59%, found in black beans flour. The RS content of potato starch obtained in the current study was lower than the 79.3% by Chen *et al.* (2010). In the present study, the RS content of green bananas was comparable with the 48.88% (Moongngarm *et al.*, 2014). However, these results varied slightly from a study that obtained 52.7% RS content in raw green bananas of Langkilde *et al.* (2002). Englyst *et al.* (1992), also got RS content of green bananas of 54.2%, which was higher than the current study. Fabbri *et al.* (2016) found a high RS content of 31%; however, Silva-Cristobal *et al.* (2010) found a slightly lower result of RS in black beans of 11.4% in comparison to this study. Moongngarm (2013) found the amount of RS in mung beans to be 2.3%, while Eashwarage *et al.* (2017) found 5.95% of RS in mung beans, lower than the results in the current study. Two studies found the native purple sweet potatoes to have 5.02% (Zheng *et al.*, 2016) and 3.06% (Yea *et al.*, 2019) RS content, which were relatively low compared to the current study (22.93%). In a general preview, the content of RS in five cultivars of sweet potatoes ranged between 13.2% and 17.2% (Senanayake *et al.*, 2013). However, in a different study carried out in Korea, the RS contents of sweet potato starches ranged from 1.76% in Pungwonmi to 30.75% in Jeonmi (Kim *et al.*, 2020); these results were in tandem with the RS content of both yellow sweet potatoes and purple sweet potatoes. Differences in RS content among starches from various sources were brought by chemical differences and physical or structural characteristics such as granule shape and size, crystallinity pattern, molecular interaction, and

arrangement (Kittipongpatana and Kittipongpatana, 2015). The difference in RS content also may have been brought by different measurements and growing regions. High RS content in food is a desirable property as it exhibits functional properties and physiological health benefits (Sajilata *et al.*, 2006; Perera *et al.*, 2010).

The amylose content was significantly different between samples. The highest amylose content was found in potato starch (21.05%) and the lowest in mung bean (14.97%). The amylose contents of green bananas, purple sweet potatoes and black beans were in between. The content of amylose in potato starch obtained was slightly higher than the 17% obtained by Rosin *et al.* (2002) and 18% by Svegmarm *et al.* (2002), but it was comparable with Vasanthan *et al.* (1999) at 19%–24%. In another study, Jansky and Fajardo (2016) obtained a 25%–30% amylose content range in several varieties of potato tubers. The amylose content of green bananas was slightly lower than the results obtained by Li *et al.* (2018), with 21.3%. However, the study carried out by Ravi *et al.* (2013) obtained different results on amylose content in green bananas, with the highest being 36.87% on one cultivar. Black beans were found to have the highest amylose content at 45.4% and red kidney beans at 32.4% (Du *et al.*, 2014), which were extremely high compared to the current study. The amylose content of mung bean flour in a study by Kaur *et al.* (2011) was high at 29.9–33.6% compared to this study. The difference in amylose content of the tested samples in this study from that reported in the previous study may have resulted from different growing regions and conditions. Figure 1 shows a relationship between the RS content and the amount of amylose in flours.

Swelling capacity was known as the ability of starch that absorb water and swell (Ojo *et al.*, 2017). It is evidence of interactions between the water molecules and the starch chains in the crystalline and amorphous region of the starch. Several factors affect the swelling power of starch powders, such as amylose, protein, lipids, amylopectin and particle size (Aprianita *et al.*, 2014). Amylose could strengthen the internal network and reduce the swelling ability of starch powder due to inter-associative forces within the amorphous and crystalline domains (Kusumayanti *et al.*, 2015). The

Table 2. The resistant starch content, amylose content, swelling capacity and water absorption capacity of some starchy foods.

Samples	Moisture content	Resistant starch (%)	Amylose (%)	SC (g/g)	WAC (g/g)
Black beanflour	9.25±0.05 <sup>ab</sup>	16.59±0.70 <sup>a</sup>	15.70±0.44 <sup>a</sup>	5.46±0.72 <sup>c</sup>	2.11±0.20 <sup>c</sup>
Purple sweet potato flour	7.73±0.11 <sup>a</sup>	22.93±0.35 <sup>b</sup>	17.04±0.41 <sup>a</sup>	7.97±0.01 <sup>c</sup>	4.63±0.10 <sup>d</sup>
Mung bean flour	8.85±0.11 <sup>a</sup>	30.47±0.80 <sup>c</sup>	14.97±1.24 <sup>a</sup>	6.96±0.70 <sup>d</sup>	1.57±0.43 <sup>b</sup>
Green banana flour	9.32±1.01 <sup>ab</sup>	48.29±0.66 <sup>d</sup>	19.60±2.34 <sup>b</sup>	3.99±0.00 <sup>b</sup>	1.75±0.00 <sup>b</sup>
Potato starch	14.72±0.04 <sup>b</sup>	56.43±0.78 <sup>e</sup>	21.05±0.71 <sup>b</sup>	1.49±0.01 <sup>a</sup>	0.00±0.00 <sup>a</sup>

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (p<0.05). SC: Swelling capacity, WAC: Water absorption capacity.

analyzed results showed a significant difference in the swelling ability of samples. Purple sweet potatoes had high swelling powers of 7.97 g/g while black beans and green bananas recorded 5.46 and 3.99 g/g, respectively. Potato starch had the most negligible swelling of 1.49 g/g. A study by Kusumayanti *et al.* (2015) found lower swelling power in purple sweet potatoes at 3.67 g/g and yellow sweet potatoes at 3.57 g/g compared to this study. The difference resulted from different sources of sweet potatoes and pretreatment (blanching) applied to sweet potatoes in the current study before measuring the swelling. Twelve varieties of Ghanaian sweet potatoes obtained a range of swelling power between 4.6 and 5.9 g/g with a significant difference ( $p < 0.001$ ) (Tortoe *et al.*, 2017), which was still slightly lower than the results in this study. Ratnawati *et al.* (2019) obtained a slightly high swelling power for mung beans at 10.52 g/g, compared to the current study results. There was a similarity in the results obtained by Pragati *et al.* (2014) of 3.57 g/g swelling power of green bananas and 3.99 g/g in this research. The blanching of purple before grinding to powder may cause the leaching of amylose, which contributed to the high number of hydrogen bonds formed between the very long-branch chains of amylopectin and water, which led to the high swelling power of the sweet potatoes (Aprianita *et al.*, 2014) and high-water retention capacity as well as increasing the gel structure of starch powder (Pragati *et al.*, 2014). Potato starch had the lowest increase in volume making it difficult to measure the swelling capacity and WAC. Despite that, it was found that the RS content in potato starch was very high with a 56.43 g/100 g dry weight basis, therefore incorporating potato starch into food products such as macaroni could improve RS consumption in individuals. Blanched purple sweet potatoes had a higher swelling capacity compared to unblanched attributing to increased amylose and amylopectin leaching (Marta and Tensiska, 2017). The high amylose content in potato starch led to low swelling power. Swelling and WAC correlated since swelling was influenced by the ability of starch to bind with water molecules through hydrogen bonding.

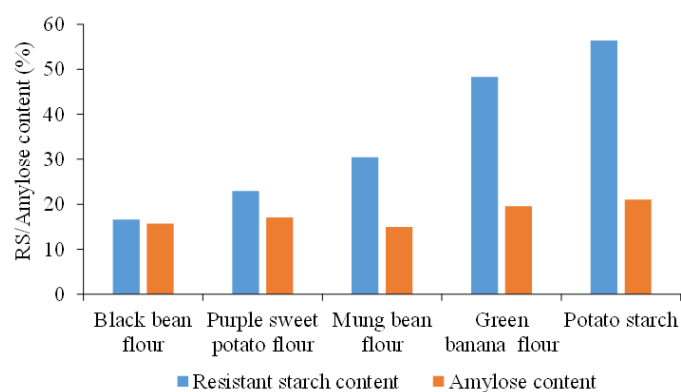


Figure 1. RS and amylose contents in starchy food sources.

### 3.2 Effect of starchy flour addition on the quality of macaroni products

The macaroni products were shown in Figure 2. According to visual appearance, control samples of macaroni (M0) maintained yellow colour caused by ingredients in the formulation. Adding starchy flour to the macaroni products altered the colour by darkening it. Macaroni in the formula M3 was darker than M1 due to the high percentage of starchy flour. The darkened colour of the macaroni was attributed to an increase in supplementary starchy flours, which have a naturally dark colour, especially black bean flour and mung bean flour. The ingredients used in macaroni products each had their specific functionality in pasta. Gluten protein in wheat flour enabled dough formation due to its viscoelastic properties, while semolina provided excellent ductility after heat treatment and a stable yellow colour (Messia *et al.*, 2021). Potato starch was a source of RS, fillers, and binders and formed a starch-protein network structure with gluten, which was conducive for improving the hardness of the noodles (Tao *et al.*, 2020). Xanthan gum was used as a thickener, as it increased the volume and improved the texture and stability of dough, as well as lightness and yellow colour in pasta and favours the process of rehydration (Javaid *et al.*, 2018). Eggs created stable emulsions, enhanced gel-forming properties, and helped increase macaroni flavour, colour, texture, and nutritional value. In addition, eggs and wheat flour formed a strong protein network.



Figure 2. Macaroni products: (A) Uncooked macaroni and (B) cooked macaroni. M0: control sample, M1: 21.84%, M2: 24.42%, and M3: 28.18% high RS flours.

### 3.3 Effect of starchy flour addition on the resistant starch content of macaroni

Table 3 shows the proportion of starchy flour added to pasta formulations and the RS content before and after cooking pasta. A significant difference ( $P < 0.05$ ) in the RS content between macaroni samples was shown with the RS content had an increasing trend as the content of starchy flour increased. The M0 control sample had an RS content of about 11.40%, while the samples M1, M2 and M3 tended to gradually increase to 14.06%, 18.62% and 23.23%, respectively. In this study, amylose content

has been shown to correlate with the resistant starch content of starchy food positively. Analysis of amylose content in macaroni indicated an increasing trend as starchy flour percentage increased.

Table 3. The RS and amylose content of pasta according to the proportion of starchy flour.

Formulation	RS content (%)	Amylose content (%)
M0	11.40±0.06 <sup>a</sup>	16.59±0.16 <sup>a</sup>
M1	14.06±0.14 <sup>b</sup>	17.36±0.10 <sup>b</sup>
M2	18.62±0.38 <sup>c</sup>	17.71±0.08 <sup>b</sup>
M3	23.23±0.38 <sup>d</sup>	19.00±0.16 <sup>c</sup>

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

### 3.4 Effect of starchy flour on physical properties of macaroni

The results showed significant differences in the hardness with the highest recorded in M3 with 57.08 g force, formulation M2 had 44.83 g force, third was M1 with 40.25 g force and the least controlled sample M0 with 31.0 g force of hardness (Table 4). The results indicated supplementation of starchy flour in macaroni impacted the hardness of macaroni. Our results were in agreement with the study of Wu *et al.* (2015) where the hardness increased with the addition of starchy flour.

Starch characteristics, protein content, and quality play a significant role in the texture of cooked pasta (Smewing, 2016). The content of amylase has influenced the hardness of macaroni. As the percentage of RS increased so did amylose content and consequently the hardness of macaroni (Baik and Lee, 2003; Wu *et al.*, 2015). Another reason for the increase in hardness could be high levels of insoluble fibres in the flours incorporated from high RS foods. When incorporated into the dough system, there was a competition in water absorption between fibre and wheat flour. It interfered with the gluten network and increased the hardness of macaroni (Victoriano *et al.*, 2020).

Cooking losses in macaroni were also measured (Table 4). It was observed that there were not many significant differences in the cooking loss of macaroni. The cooking loss in macaroni ranged between 4.51% in the M0 control sample and 4.95% in M3. The higher cooking loss shown in M3 may result from the leaching

of many soluble solids from macaroni into the cooking water. Kuen *et al.* (2017) reported that the addition of starchy flours increased cooking loss due to the inability of less gluten to hold too much dietary fibre particles, resulting in some fibre dissolving in water during the cooking process. Since the cooking loss was also less than 12%, according to Fu (2008), therefore the macaroni produced had acceptable cooking quality among consumers.

There was no significant difference ( $P<0.05$ ) in rehydration rate among samples M0, M1 and M2, however, sample M3 was significantly different from the rest of the samples. Sample M0 presented the highest rehydration rate with 58.76%, followed by M2 with 58.35%, the third was M1 with 58.25%, while the least rehydration rate was in M3 with 55.12%. The linear structure of amylose in M1, M2 and M3 may have decreased the breakdown viscosity, requiring a longer time for water penetration (Tangthanantorn *et al.*, 2021).

The results obtained in the current study showed that the formulas M0 and M2 of macaroni had a 100% increase in volume while M1 and M3 had 93.75% and 93.53%, respectively. The high-volume increase is a desirable property of pasta products by consumers. The low volume increase shown in M1 and M3 may have resulted from the high cooking loss. The degree of volume increase during boiling of pasta was affected by starch gelatinization and protein hydration related to the size of starch (Kang *et al.*, 2017).

The effect of a subsequent percentage increase in the amount of starchy flour supplemented on the colour of macaroni was shown in Table 5. There was a significant difference in the mean L\* of the four formulations of macaroni. The control M0 had the highest lightness with L\* of 70.5±5.23, while a decrease in L\* was evident in formulations M1 which had 55.4±4.30, M2 had 53.3±5.12, and lastly, M3 was the least with 50.0±6.68. The bright colour of the control sample resulted from wheat flour and eggs used in the formulation. In contrast, the starchy flour incorporated in M1, M2, and M3 resulted in the darker colour of macaroni. The redness recorded with a\* in raw macaroni increased with an increase in high RS flour, for yellowness recorded by b\*, control formulation (M0) had the highest value with 26.7±2.58, and the least of 8.8±1.99 was in formulation 3

Table 4. The hardness, cooking loss, rehydration rate and volume increase of macaroni.

Formulation	Hardness (g-force)	Cooking loss (%)	Rehydration rate (%)	Volume increase (%)
M0	31.0±8.24 <sup>a</sup>	4.512±0.20 <sup>a</sup>	58.76±0.31 <sup>a</sup>	100.00±0.00 <sup>a</sup>
M1	40.25±4.24 <sup>ab</sup>	4.795±0.05 <sup>a</sup>	58.28±0.59 <sup>b</sup>	93.75±1.76 <sup>a</sup>
M2	44.83±7.8 <sup>b</sup>	4.605±0.30 <sup>a</sup>	58.35±0.66 <sup>b</sup>	100.00±0.00 <sup>a</sup>
M3	57.08±7.4 <sup>c</sup>	4.953±0.10 <sup>a</sup>	55.12±0.89 <sup>b</sup>	93.53±5.61 <sup>a</sup>

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

Table 5. Colour measurement of uncooked and cooked macaroni.

Sample	Formulation	L*	a*	b*
Uncooked macaroni	M0	70.5±5.23 <sup>c</sup>	4.6±1.65 <sup>a</sup>	26.7±2.58 <sup>c</sup>
	M1	55.4±4.30 <sup>ab</sup>	7.0±1.15 <sup>b</sup>	11.0±2.31 <sup>b</sup>
	M2	53.3±5.12 <sup>ab</sup>	6.8±0.63 <sup>b</sup>	11.6±1.71 <sup>b</sup>
	M3	50.0±6.68 <sup>a</sup>	9.3±1.25 <sup>c</sup>	8.8±1.99 <sup>a</sup>
Cooked macaroni	M0	89.8±5.07 <sup>c</sup>	-1.2±0.79 <sup>a</sup>	25.8±3.30 <sup>b</sup>
	M1	78.5±3.34 <sup>b</sup>	3.4±0.70 <sup>b</sup>	6.40±1.07 <sup>a</sup>
	M2	76.0±3.62 <sup>ab</sup>	3.9±1.29 <sup>b</sup>	5.70±1.34 <sup>a</sup>
	M3	72.6±6.43 <sup>a</sup>	5.40±0.52 <sup>c</sup>	4.80±1.14 <sup>a</sup>

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ( $p<0.05$ ) for uncooked and cooked macaroni. L\*: lightness, a\*: redness (+) and green (-), and b\*: yellowness (+) and blue (-).

(M3). The yellowness was from eggs, wheat flour in M0, and the darker colour in M3 was from starchy flour. Cooking of macaroni resulted in increased lightness L\* to 89.8±5.07 in M0 and 72.6±6.43 in M3. The redness generally decreased after cooking, while yellowness slightly reduced after cooking.

Colour is an important parameter of the acceptability of food products by consumers (Morris, 2018). Lightness is an indispensable characteristic of macaroni that is known as a perception of high quality. Colour parameters (L\*, a\* b\*) of uncooked and cooked macaroni changed significantly with the addition of these flours. According to Zhao *et al.* (2005) and Wood (2009), the lightness L\* of all spaghetti products containing legume flours decreased significantly ( $P<0.05$ ), which is in agreement with the results in this study. As a result of cooking macaroni, the lightness increased while redness and yellowness decreased. Similar results were obtained by Petitot *et al.* (2010). The dark colour of the subsequent macaroni formulations resulted from the specific colour of the bean flours and purple sweet potatoes used in their formulations. An increase in protein content in noodles darkens the noodles, explaining the darkening of macaroni in the current study (Morris, 2018).

### 3.5 Effect of starchy flour addition on the organoleptic value of macaroni

Principle component analysis (PCA) was used to outline the relationship between the various attributes used in the study of macaroni and the effect of starchy flour addition on the pasta products (Figure 3). The analysis was carried out on cooked macaroni. The PCA revealed that three principal components (PCs) (F1-F3) explained the variance among the data, and the first two PCs (PC1 and PC2) accounted for 93.01% of the total variation (Figure 3A). PC1 accounted for 75.25%, and PC2 accounted for 17.76% of the variance. A control sample of macaroni was characterized by a yellow colour, salty and egg smell, while M1, M2, and M3 were

characterized by green colour, purple colour, cooked bean smell, and sweetness. Acceptability lies between M0 and M2, as shown in Figure 3B.

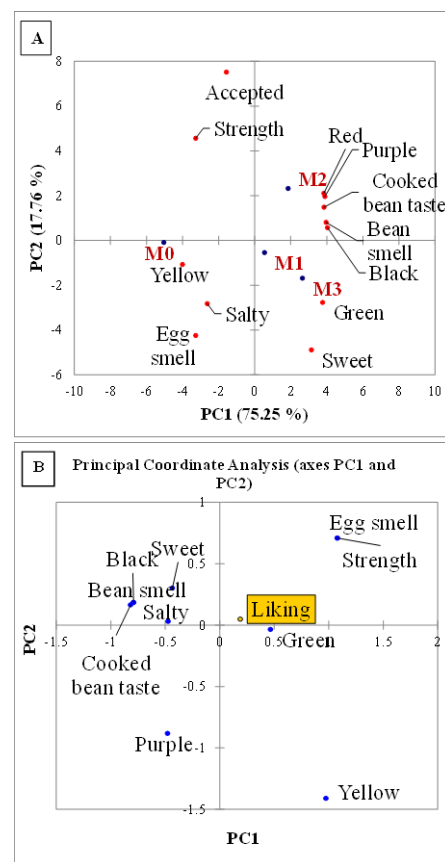


Figure 3. Principal Component Analysis (PCA) of (A) various attributes of macaroni formulated with high RS flour and (B) Check All That Apply (CATA) analysis of macaroni. M0: control sample, M1: 21.84%, M2: 24.42%, and M3: 28.18% high RS flours.

### 3.6 Morphological properties of macaroni by Scanning Electron Microscopy

The microstructure of pasta was observed using SEM to expound the effects of high RS flour addition on macaroni (Figure 4). The formulation M2 was the preferred sample based on sensory analysis. The SEM showed that in the control sample, M0 majority of the starch was still visible on the surface of macaroni, while in M2, due to the addition of high RS flour, some starch

escaped showing perforations, making the pore sizes of the control M0 sample (Figure 4A) smaller than the M2 sample (Figure 4B).

The obtained morphological structure of macaroni may result in loosely bound fibres from starchy flours dissolving in water, leaving air spaces (Kuen *et al.*, 2017). The variation in the appearance of structures between the control sample M0 and M2 because of the difference in pasting degrees of starches, the ratio of amylose and amylopectin from the starchy flour supplemented (Guo *et al.*, 2006). Gluten and starch in M0 of macaroni supported protein network responsible for stability hence smaller pores, while in M2, reduced gluten due to starchy flour addition resulted in starch being exposed, irregular shape, and larger pores (Sun *et al.*, 2019).

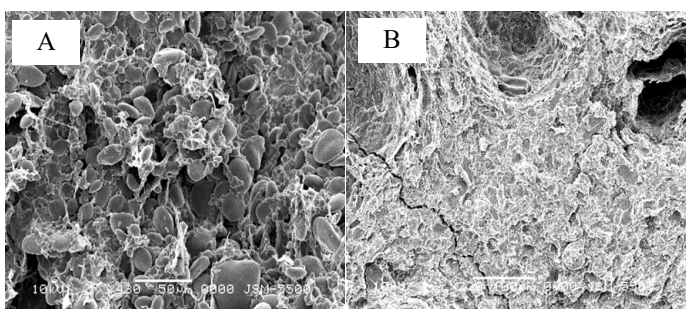


Figure 4. Scanning electron microscopy (SEM) of (A) control macaroni sample (M0) with 100% wheat flour and (b) M2 with 24.42% high RS flour.

#### 4. Conclusion

The variation in the amount of RS in starchy foods was due to differences in size, shape, and arrangement of granules in the specific raw food. High amylose content in the foods indicated high resistant starch content, while low swelling and water absorption capacity was a typical characteristic of high resistant starch foods. The high resistant starch food improves RS consumption but also affects the quality of foods that incorporate them. The addition of high starchy flour in noodle and macaroni formulations increased the content of resistant starch in the pasta products. Achieving desirable pasta quality entailed an appropriate proportion of high RS flour in the corporation in the formulation. The future works will be expounded on the application of high RS flour in various other food products and their effect on nutrient content of the food. In addition, *in vitro* and *in vivo* digestibility of starchy foods with high RS flour should also be carried out to determine the effect of high RS flour on the glycaemic index of the food products, in an environment that resembles the human digestive system.

#### Conflict of interest

The authors declare no conflict of interest.

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