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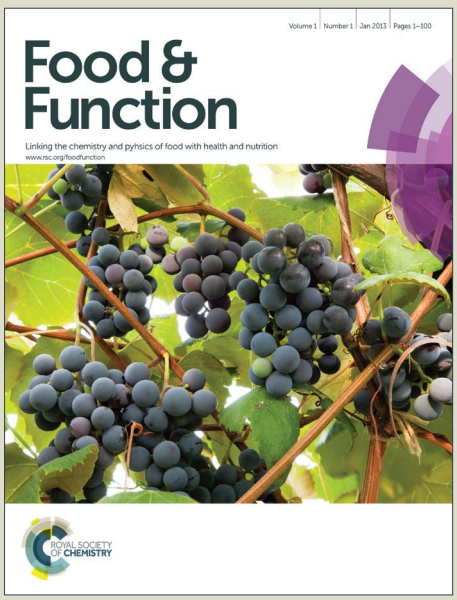

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Structural properties and digestion of green banana flour as a functional ingredient in pasta

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Abstract

Gluten free pasta was made from raw banana flour in combination with vegetable gums and protein for comparison to pasta similarly made from wheat flour. After cooking, it was found that the banana flour pasta was less susceptible to alpha-amylase digestion compared to conventional wheat flour pasta. Release of glucose by alpha-amylase digestion followed first order kinetics with an initial rapid rate of digestion and a subsequent second slower phase. The structure of green banana pasta starch at the inner and outer pasta surfaces was observed under confocal laser scanning microscopy (CLSM) and the viscosities of the flour mixtures were measured by a Rapid Visco Analyser (RVA). The digestibility of banana flour pasta was found to be related, not only to the properties of the starch granules, but also to the protein network of the surrounding food matrix. The effects of gums and proteins on pasta formation and digestibility are discussed in the context of its potential use as a gluten free lower glycaemic alternative to conventional wheat based pastas.

Key words:

Green banana flour pasta; Resistant Starch; Protein Network; Starch Digestion Rate

1. Introduction

Celiac disease is an adverse immune response to gluten and is becoming a common disorder affecting almost one in 100 of the population.[1] The increased interest in foods that address gluten intolerance is also demonstrated by the worldwide rise in demand for gluten-free foods such as bread and pasta made from non-gluten cereals and pulses.[2] Recently, Zandonadi et al[3] formulated a gluten free pasta exclusively with green banana flour as the carbohydrate source and reported that 84% of celiac and 61% of non-celiac test subjects found the sensory properties of the green banana pasta acceptable.

Nutritionally, starches in green banana have a low susceptibility to amylase digestive enzymes with more than 75% of ingested α-glucans from banana starch escaping breakdown in the small intestine of ileostomates.[4,5] Although heat treatments, such as cooking of pasta, can gelatinise the enzyme resistant starch granules making them accessible to digestive enzymes, the dense packing of pasta dough can hinder diffusion of water for gelatinisation as well as restrict the swelling of granules further hindering enzymic activity.[6,7] Apart from the presence of nutritionally beneficial starch, green bananas also have nutritionally significant contents of soluble and insoluble fibres, minerals, vitamins and phenolic compounds.[3, 8]

Economically, bananas are a major horticulture crop in tropical and subtropical areas.[8] However, about one fifth of all bananas harvested are discarded due to physical and cosmetic defects with consequential economic loss as well as environmental hazard.[9] Utilisation of reject green bananas as a raw material for functional foods thus can have both economical as well as nutritional benefits.

Although the structure and functional properties of banana starch and flour have been widely studied,[9] the micro-structural properties that lead to the lower glycemic response of banana flour in pasta have not been investigated. Zandonadi et al[3] reported the sensory and nutritional properties of green banana pasta produced with green banana flour, egg white,

xanthan and guar gum. The present research has investigated the enzyme susceptibility of cooked green banana flour pasta and its micro- and macro-structural properties in comparison to that of wheat flour pasta in order to elucidate the factors affecting the structure and enzyme susceptibility of green banana flour pasta. The output of the current work will be useful for food manufacturers to optimise the formulation of gluten-free banana flour pasta.

2. Materials and methods

2.1 Materials

Banana flour was gifted from Mt Uncle Bananas Pty (Hansen Road, Walkamin, QLD Australia). White Wings brand unbleached soft wheat flour (Goodman Fielder, NSW, Australia) with 10.50 % protein (db) was purchased from a local supermarket. Guar gum and xanthan gum were purchased from Melbourne Food Ingredient Depot (Victoria, Australia) and were used as such. Artificial saliva (Sigma A-3176), pepsin (Sigma P-6887) and pancreatin (Sigma P-1750) were obtained from Sigma-Aldrich, USA. Fungal amyloglucosidase (Megazyme E-AMGDF) was purchased from Megazyme, Ireland. All other chemicals were obtained from Sigma-Aldrich and were of analytical grade.

2.2 Pasta process

Pasta was made with green banana flour according to the formulations of Zandonadi et al.[3] In order to study the effect of each ingredient on the micro-structure and enzymic susceptibility of pasta, seven variations of the pasta dough recipe were developed, each with wheat flour and raw banana flour versions as shown in Table 1.

Table 1: Formulations of green banana and wheat flour pasta

	% of ingredient (w/w)							Pasta properties[1]	
Formulation code	Green banana flour	Wheat Flour	Xanthan Gum	Guar Gum	Whole egg	Egg white	Water	Moisture content (% db)	Starch content (% db)
A1	75	-	2.5	2.5	-	-	20	8.4	71.6
A2	-	75	2.5	2.5	-	-	20	9.2	78.8
B1	75	-	5	-	-	-	20	11.4	71.7
B2	-	75	5	-	-	-	20	9.7	78.8
C1	75	-	-	5	-	-	20	N/D	N/D
C2	-	75	-	5	-	-	20	N/D	N/D
D1	80	-	-	-	-	-	20	N/D	N/D
D2	-	80	-	-	-	-	20	10.4	84.1
E1	47	-	-	-	31	-	22	N/D	N/D
E2	-	47	-	-	31	-	22	N/D	N/D
F1	47	-	2.5	2.5	31	-	17	10.6	56.1
F2	-	47	2.5	2.5	31	-	17	9.6	60.7
G1	47	-	2.5	2.5	0	31	17	7.6	64.2
G2	-	47	2.5	2.5	0	31	17	7	62.5
Green Banana flour								10.8	76.4
Wheat flour								14.1	84.1

[1]Moisture content was determined using a hot air vacuum oven, heating the samples at 105 ^{o}C overnight followed by cooling and weight measurement. The starch content was determined according to the standard protocols of the manufacturer (K-TSTA, Total Starch Assay Kit (AA/AMG), Megazyme, Ireland). N/D= not determined. The amount of water was varied to compensate the moisture (water) from added ingredient (e.g. egg). Some formulation (as denoted by N/D) did not produce a sufficiently cohesive dough for sheeting and were excluded from further investigation

For the preparation of pasta, dry ingredients (banana flour, guar gum, xanthan gum) were mixed manually in a mixer bowl for 3 min before adding the egg and water. The mix was then blended in a Bench Mixer (Model P41710371, Homemaker Bench Mixer, Kmart, Australia) at low speed for 15 min. The dough was further kneaded manually for 30 min. The mixed dough was then passed between stainless steel rollers of a pasta machine (Marcato Atlas 150 Multi Pasta Machine, Italy) to form a sheet with a thickness of 5 mm. The sheet was gradually re-passed through the rollers 7 times until a final thickness of ca 1 mm was

reached before passing through the cutter to form pasta strips ca 1.5 mm wide. The fresh pasta was kept overnight at 40°C in an oven to produce dried pasta.

Among the recipe variations (Table 1), some combinations (green banana flour + guar gum + water (C1), green banana flour + water (D1), green banana flour + whole egg + water (E1)) were unable to produce a sufficiently cohesive dough for sheeting. Thus these combinations, along with their wheat flour variations, were excluded except for pasta formulated with wheat flour and water only (D2).

2.3 Confocal Laser Scanning Microscopy

The micro-structural properties of cooked pasta from various formulations were investigated using confocal microscopy (LSM 700, Carls Zeiss, Germany). A double-staining technique with a combination of FITC (1% w/v, ethanol) and rhodamine B (0.1% v/w, ethanol) was used[12]. Normally, double-staining of such systems with a mixture of rhodamine B and FITC leads to staining of proteins by rhodamine B and starch granules by FITC. However rhodamine B and FITC can also bind non-covalently with starch and protein respectively, depending upon the concentration of the components in the system. In a low protein system, such as pasta, and a high concentration of FITC (FITC 1% vs rhodamine B 0.1%), the FITC binds with both starch and protein. In contrast rhodamine B binds more specifically with protein allowing the visualisation of both starch and protein simultaneously as FITC and rhodamine excite at different wavelengths of 488 and 555nm respectively.

2.4 In vitro digestion

Pasta was cut into approximately 2 mm long strands using scissors. Pasta equivalent to 100 mg dry starch was cooked in 4mL sodium acetate buffer (0.2M, pH 6.0) in an 95°C water bath for 10 min and cooled down to 37°C prior to *in vitro* digestion. The pasta was cooked in acetate buffer to allow the appropriate buffer system to work for amylases during digestion.

The time gap between cooking, cooling, and enzyme treatment was kept to a minimum (less than 20 minutes) so as to avoid retrogradation of starch molecules. The *in vitro* digestion was carried out according to the method of Dhital et al.[10] with slight modifications as follows.

The pasta at 37°C after cooking and cooling was mixed with 1mL of artificial saliva containing porcine α-amylase (250U per mL of carbonate buffer, pH 7) for 45 s followed by 2 mL of pepsin (1mg per mL in 0.02M HCl, pH 2). The acidified mixture was incubated at 37°C for 30 min. The digesta was then neutralised (2 mL, 0.02M NaOH) followed by addition of 4mL sodium acetate buffer (pH 6, 0.2M) and 1mL of a mixture of pancreatin (2mg per mL) and amyloglucosidase (28U per mL). The mixture was incubated with continuous mixing (200 rpm) at 37°C with a 4mm x 12 mm magnetic stirrer bar. At each time interval (5, 10, 15, 20, 30, 40, 50, 60, 90, 120, 240 and 360 min), 100uL aliquots were taken and transferred into 2mL polypropylene centrifuge tube before addition of 300 μL of stop solution (0.3 M Na_2CO_3) to prevent further amylase activity in the aliquot. After centrifugation (2000×g for 5 min), the glucose concentration in the supernatant was determined using a glucose oxidase colorimetric analysis kit (TR-1511-200, Thermo Electron Noble Park, Victoria, Australia) with photometric detection at 505nm. A factor of 0.9 was used to convert the glucose concentration to starch to account for the water moiety added during hydrolysis of the glycosidic bond. Results are presented as starch hydrolysed in grams per 100 g dry starch.

2.5 Fitting to first-order kinetics

The logarithm of the slope (LOS) method proposed by Butterworth et al[11] was used to determine the rate coefficient of pasta digestion by amylase. If a digestion follows first order kinetics, a plot of $\ln(dC/dt)$, represented as the rate of conversion of starch (C) over time (t), against time (t) is linear with a slope of $-K$, where K is defined as the digestion rate

coefficient (min^{-1}). The degree of fit of first order plots can be judged from the R^2 value of the linear fit. Changes in the linearity of the LOS plot, if present, indicate different rates of the digestion reactions.

2.6 Statistical analysis

Results are expressed as means of at least 2 duplicate measurements. Initial data and linear regression fitting was carried out in Microsoft Excel. Analysis of variance (ANOVA) was used to determine the least significance at $p < 0.05$ using Minitab 16 (Minitab Inc., State College, PA).

3. Results

3.1 Micro-structure of pasta using confocal microscopy

As mentioned in Table 1, dough of formulations containing green banana flour + guar gum (C1), green banana flour (D1) and green banana flour + whole egg (F1) were fragile and crumbled during sheeting and cutting. Thus they were unable to give appropriate intact pasta strands for cooking and were excluded from further study.

The dyes, FITC and rhodamine B, were used for simultaneous observation of both starch and protein by confocal scanning laser microscopy. Rhodamine B stains proteins in red while FITC stains starch granules and proteins in green. Therefore, due to the dual staining, protein shows as yellow under the microscope. The internal structure of pasta with various formulations after cooking is shown in Figure 1.

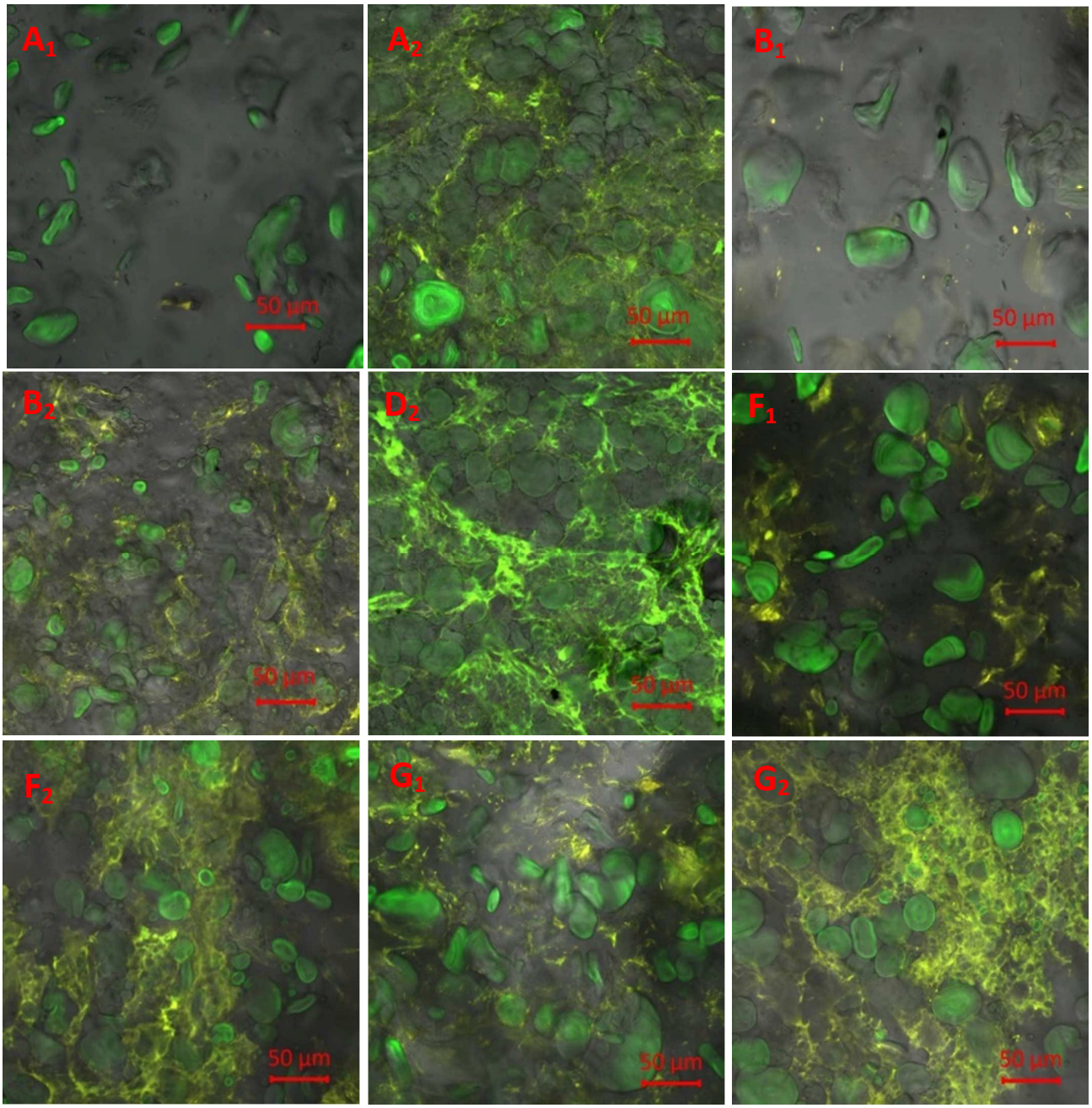

Figure 1. Confocal images of cooked pasta of different formulations. A1:Green banana flour, xanthan gum, guar gum; A2: Wheat flour, xanthan gum, guar gum; B1: Green banana flour, xanthan gum; B2: wheat flour, xanthan gum; D2: Wheat flour; F1: Green banana flour, xanthan gum, guar gum, whole egg; F2: Wheat flour, xanthan gum, guar gum, whole egg; G1:Green banana flour, xanthan gum, guar gum, egg white; G2:Wheat flour, xanthan gum, guar gum, egg white.

Pasta in formualtion A1 consisted of green banana flour, xanthan gum and guar gum without an obvious structural network. The swollen starch granules are distributed randomly and loosely within the protein matrix. In contrast, when green banana flour was replaced with wheat flour, an extensive network of protein encapsualting the starch granules is observed (Figure 1 A2).

In order to investigate the effect of viscous gums, guar gum was replaced with xanthan gum in formulation A1. The resulting pasta (B1) is also similar to A1, without a protein network and with granules loosely packed in a non-staining polysaccharide mass. However, when green banana flour was replaced with wheat flour in formulation B1, an extensive protein network is observed (Figure B2).

Figure 1, F1 shows the pasta consisting of green banana flour, xanthan gum, guar gum and whole eggs. Compared to A1 and B1, Figure F1 shows a strong protein network (observed as yellow fluorescence) derived from the additional eggs. When whole eggs were replaced with egg white (G1), a more extensive network is observed. A similar network from protein is also observed in the wheat flour pasta (D2, F2 and G2), as well as wheat flour plus xanthan gum with or without guar gum (A2 and B2).

3.2 Inner and outer surface of pasta

In order to elucidate the morphology of starch granules at the inner and outer surfaces of cooked pasta, a thin section of both sites from pasta made from green banana flour, xanthan gum, guar gum and egg white sample (G1) and pasta made from wheat flour, xanthan gum, guar gum and egg white (G2) were observed by confocal microscopy (Fig 2).

A difference in starch granule distribution is observed at the inner and outer surfaces of pasta G1 and G2. In the outer portion of green banana flour pasta (Fig 2A), the swollen banana starch granules are loosely scattered with a less dense protein network. In contrast, the inner

surface (Fig 2B) has a tight mass of starch granules surrounded by an extensive protein structural network.

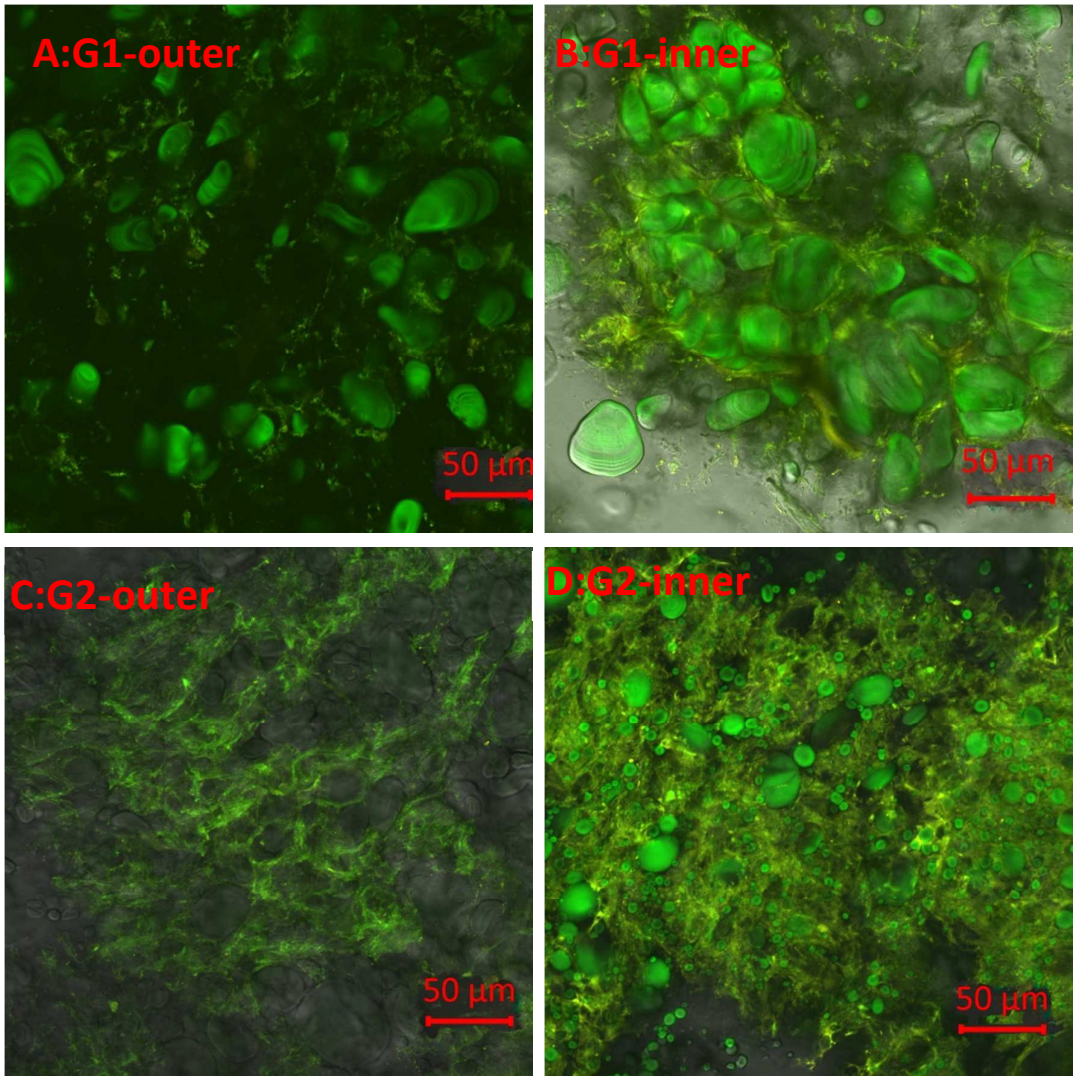

Figure *2*. Confocal images of inner and outer surfaces of cooked pasta G1 (pasta made from green banana flour, xanthan gum, guar gum and egg white) and G2 (pasta made from wheat flour, xanthan gum, guar gum and egg white). A: Outer surface of G1; B: Inner surface of G1; C: Outer surface of G2; D: Inner surface of G2.

For the external section of wheat flour pasta (Fig 2C), only a protein (gluten) network is observed with the absence of starch granules. However for the internal section of wheat pasta (Fig 2D) a highly connected and complex structural gluten network entrapping less swollen starch granules is clearly demonstrated.

214 *3.3 Enzymic susceptibility of pasta formulations*

215 Figure 3 shows the *in vitro* digestion progress curves and LOS data fits of the pasta
216 formulations. The digestion progress curve of all the formulations can be fitted to first order
217 plots (LOS plot) with two different rates, an initial fast rate followed by a slow rate. The fast
218 and slow rates (K, min-1) are presented in Table 2. The rate coefficients, normalised to the
219 rate of control wheat pasta (D2) at 100% are presented as a separate column.

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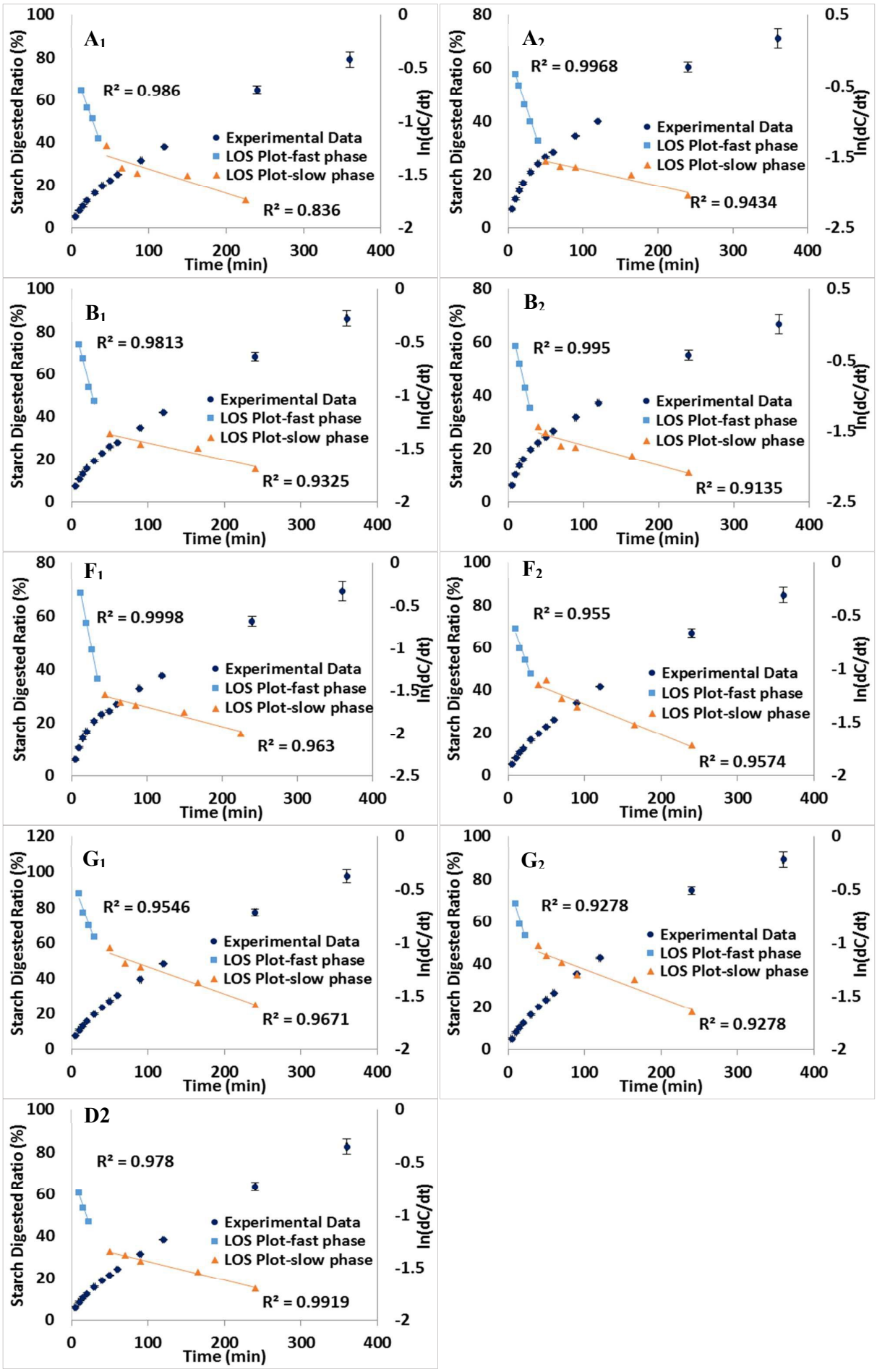

Figure 3. Digestion progress curves and LOS fits of progress curves for cooked pasta of different formulations. A1: Green banana flour, xanthan gum, guar gum; A2: Wheat flour, xanthan gum, guar gum; B1: Green banana flour, xanthan gum; B2: Wheat flour, xanthan gum; F1: Green banana flour, xanthan gum, guar gum, whole egg; F2: Wheat flour, xanthan gum, guar gum, whole egg; G1: Green banana flour, xanthan gum, guar gum, egg white; G2: Wheat flour, xanthan gum, guar gum, egg white; D2: wheat flour.

As seen in Table 2, formulations with banana flour (A1, B1, F1, and G1) have lower digestion rates, compared to the analogous formulations with wheat flour. This applies for both the slow and fast phase of the digestions. The digestion rates of pasta made from both green banana flour and wheat flour are found to be hindered by addition of gums especially for the initial faster phase. For example, pasta made from green banana flour, xanthan and guar gum (A1) has a rapid digestion rate of 0.0196 min^{-1}, which was increased 1.4 times when guar gum was removed and replaced with the same amount of xanthan gum (B1). However the slower digestion rates for both formulations A1 and B1 were similar at 0.0018 min^{-1}. Similarly, when guar gum was removed from the wheat flour pasta made from wheat flour, guar and xanthan gum (A2) and replaced with the same amount of xanthan gum, the resulting pasta B2 had a 1.3 times higher initial faster digestion rate than that of A2. The effect of the addition of gums and proteins (egg) on digestion rate is also revealed when comparing the digestion rate of wheat flour only pasta (D2) with the other formulations. D2 has the highest initial rate of digestion for the faster phase compared to other formulations. However, the digestion rate for the slower phase is comparable to other formulations.

Table 2. First order rate coefficient of pasta formulations[1]

Samples	Fast phase (rate coefficient × 0.01 min^{-1})	R^2	Fast rate normalised with control pasta (D2)	Slow phase (rate coefficient min^{-1}	R^2	Slow rate normalised with control pasta (D2)
Pasta A_1	1.96 (0.27)	0.99	43.27	0.18 (0.049)	0.89	62.07
Pasta A_2	3.18 (0.24)	1.00	70.20	0.23 (0.071)	0.94	79.31
Pasta B_1	2.80 (0.74)	0.98	61.81	0.17 (0.012)	0.93	58.62
Pasta B_2	4.41 (0.17)	1.00	97.35	0.29 (0.014)	0.91	100.00
Pasta F_1	1.95 (0.09)	1.00	43.05	0.16 (0.010)	0.96	55.17
Pasta F_2	2.36 (0.28)	0.95	52.10	0.27 (0.084)	0.97	93.10
Pasta G_1	1.52 (0.15)	0.93	33.55	0.18 (0.042)	0.93	62.07
Pasta G_2	2.02 (0.84)	0.98	44.59	0.23 (0.051)	0.99	79.31
Pasta D_2	4.53 (0.62)	0.96	100.00	0.29 (0.014)	0.96	100.00

[1]Rate coefficients values express as mean with standard deviation (× 0.01) in parenthesis

3.4 Enzymic susceptibility of surface and internal sections of cooked pasta

In order to evaluate the digestion rate of both the outer part and inner part of pasta individually, thin sections representing outer and inner portions of green banana flour pasta (G1) and wheat flour pasta (G2) were manually separated with a scalpel blade (size 10) using gentle hand pressure and subjected to enzymic digestion. The first order fit of the digestion curve of the inner and outer surfaces and their rate coefficient (slope of the fit) are shown in Figure 4. It is seen that for banana flour pasta, the digestion rate of the outer surface (0.0479 min^{-1}) is more than double that of the digestion rate of the inner surface (0.0202 min^{-1}). However, in wheat flour formulations the difference is not as high compared to that of green banana flour formulations, although the inner portion of pasta is digested at a slower rate (0.0281 min^{-1}) compared to the outer portion (0.0323 min^{-1}).

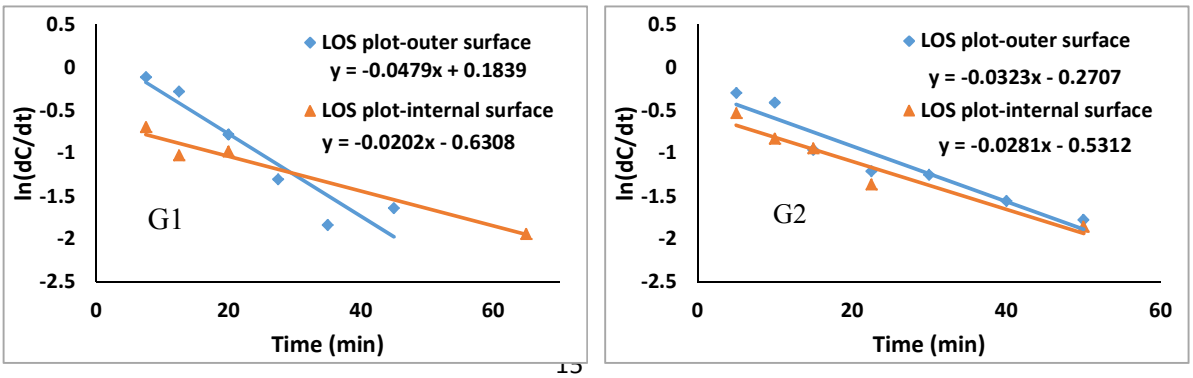

Figure 4. First order fit of inner and outer surface of pasta G1 (pasta made from green banana flour, xanthan gum, guar gum and egg white) and G2 (pasta made from wheat flour, xanthan gum, guar gum and egg white).

3.5 Pasting properties of formulations measured with Rapid-Visco Analyser

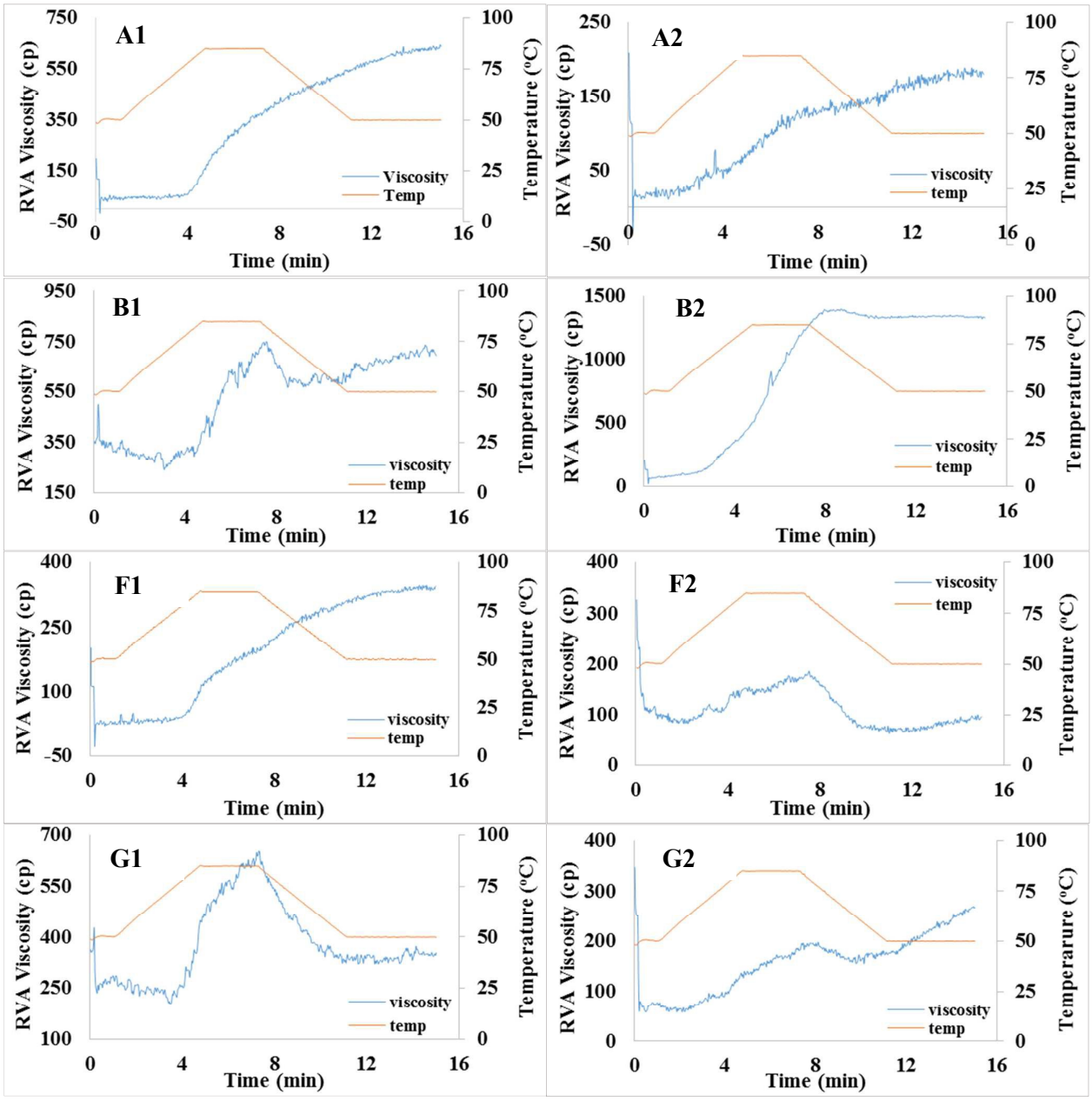

Figure 5. Pasting profiles of different pasta formulations. A1: Green banana flour, xanthan gum, guar gum; A2: Wheat flour, xanthan gum, guar gum; B1: Green banana flour, xanthan gum; B2: Wheat flour, xanthan gum; F1: Green banana flour, xanthan gum, guar gum, whole egg; F2: Wheat flour, xanthan gum, guar gum, whole egg; G1: Green banana flour, xanthan gum, guar gum, egg white; G2: Wheat flour, xanthan gum, guar gum, egg white

The pasting profiles, as measured by Rapid-Visco Analyser, were found to be dependent upon the pasta formulation (Fig 5). More specifically, in formulations A1 and A2 the swelling of granules was hampered and the viscosity kept increasing with time irrespective of temperature and stirring. The viscosity at the end of 15 min was higher for banana flour formulation (A1) compared to wheat flour formulation. This suggested that protein (gluten) in the formulation A2 hindered the swelling of the granules during the measurement. There is however no clear breakdown and set-back but instead a shear-thickening behaviour is observed (Fig 5, A1, A2). On removal of guar gum from formulations A1 and A2 and replacement with xanthan gum as shown in B1 and B2, a more definitive pasting profile was observed, possibility due to more swelling and breakage of granules exhibiting peak, trough and raised final viscosities. When the protein of whole egg was added in formulations A1, the resulting formulation F1 showed a similar trend but with a slight decrease in final viscosity. However, compared to A2, F2 showed a different and irregular pasting profile. The fluctuations in the pasting profile might be due to hindrance in swelling and breakdown of swollen granules during measurement and/or localised aggregation of egg proteins within gluten networks. The pasting profile with egg white (formulations G1 and G2), also showed a non-smooth response. Replacing whole egg with egg white in banana pasta formulation resulted in a marked difference in pasting profile being observed as seen in Fig 5 F1 and G1 whereas almost similar behaviour was observed in wheat flour formulations (Fig 5 F2 and G2).

4 Discussion

Raw banana starch, in its natural granular form, is regarded as a good source of enzyme resistant starch.[5] The enzyme resistant properties, however are normally lost once the starch or flour is heat processed beyond gelatinisation temperature such as during cooking or baking. Dense packing and physical entrapment of starch in networks that inhibit both

enzyme diffusion and swelling of granules, such as in pasta, can be a way to retain the enzyme resistant properties of starch[6,7] even after cooking. Due to the lack of gluten in green banana flour, a network forming additional additives such as gums and egg proteins were required in the pasta formulation. In the current paper, we investigated the role of different ingredients in green banana flour pasta and also reported the enzyme susceptibly and structural properties of pasta formulations.

Both the green banana flour and wheat flour pasta exhibited a biphasic digestion with a fast phase followed by a slow phase. Such a biphasic digestion rate has been previously reported by Butterworth et al[11] for raw (un-cooked) wheat and pea starch. The fast phase was hypothesized to be due to readily available starch molecules formed from physical damage during processing whereas the slow phase was proposed to be due to dis-entanglement of molecules tightly packed as semi-crystalline starch granules. The authors further showed that, due to swollen and leached molecules, the cooked starch exhibited only a monophasic digestion curve. More recently, Zou et al[13] also showed the bi-phasic digestion of pasta made from wheat flour and semolina and suggested that readily available swollen granules at the outer surface are responsible for the initial faster digestion rate and that the strong gluten network is primarily responsible for the slower digestion phase of pasta compared to cooked wheat flour and semolina.

The confocal microscopic images of outer and inner surfaces of formulations with green banana flour (G1) and wheat flour (G2) (Fig 2) and the corresponding digestion plots (Fig 4) clearly show that the outer surface of green banana pasta is less condensed compared to the inner surface and that both have varying proportions of intact granules. This unique food structure developed from the protein network coupled with dense packing during sheeting and gradual reduction leading to hindrance of swelling of banana granules during cooking can synergistically interfere the enzyme access towards the inner portion of green banana

flour pasta lowering enzyme digestion rate by more than two fold. In wheat pasta, prominent protein networks are observed at both inner and outer surface (Fig 2, C-D). The overall structure of pasta is almost similar although the starch granules are more intensely labelled in the inner surface (Fig 2, D). Comparing the rate coefficient among pastas (banana flour and wheat flour) for each outer and inner section is further challenging due to difficulties in quantifying the exact proportion of outer and inner layers in the cooked pasta. Thus the rate coefficients of two separate layers of pasta are indicative only and comparing them with rate coefficient of intact pasta (Table 2) may give inconsistent information.

Considering the various formulations (Table 1) and comparing between wheat flour and banana flour pastas, a strong gluten network in wheat flour formulations provided a physical barrier limiting both enzyme access as well as swelling of the granules. This barrier is primarily responsible for the slow amylolysis kinetics of wheat pasta which is in agreement with previous reports.[13,14,15] Apart from the amount of protein required to form physical network, the quality and modification of protein can also affect starch hydrolysis in pasta.[16,17] In an another work, we found that alpha amylase has a strong affinity towards wheat gluten and is effectively bound to gluten even under very dilute conditions (ca. 1% solutions; unpublished observations) analogous to amylase binding reported for cellulose and wheat bran.[18] Thus, both physical barriers (dense packing during sheeting and protein networks) and non-specific binding of enzymes can act synergistically to limit the starch hydrolysis in cooked wheat flour pasta. In contrast, the enzymic susceptibility of green banana flour pasta is more affected by starch properties and packing during sheeting.

Considering the rate coefficients (Table 2), it is found that pasta formulations with green banana flour had lower rates of digestion compared to those for pasta made from wheat flour formulations. The lower digestion rate of green banana flour pasta, in spite of the absence of a gluten network, showed the high enzyme resistance of green banana starch compared to that

385 of wheat starch in cooked pasta. Although raw banana starch is nutritionally known as a rich
386 source of enzyme resistant starch,[4,5] we now report that the enzyme resistant properties of
387 banana starch is partially retained in cooked foods such as pasta. Similarly, in a low moisture
388 system Bello-Perez et al[19] reported a higher enzyme resistant starch content in banana starch
389 cookies compared to cookies made with conventional flours. The enzymic susceptibility of
390 starch in processed foods are affected by the ability of enzymes to access the substrate
391 (physical barrier) as well as the surface and internal structural features of substrates limiting
392 the binding and subsequent catalysis (micro- and macro structural barrier). These barrier
393 properties in relation to amylase susceptibility of starch and starchy foods have been recently
394 reviewed.[6,7]

395 In regards to individual formulations, banana pasta with gums only and no added protein (A1
396 and B1) (Figure 1) has very weak networks compared to formulations F1 and G1 that contain
397 additional whole egg and egg white, respectively. In spite of the presence of a protein
398 network, the formulations F1 and G1 has similar enzyme susceptibility to A1 and B1 in both
399 the rapid and slow digestion phases. This suggests that the protein network is not the only
400 factor controlling the digestion rate of pasta when banana flour is used, in contrast to the case
401 with wheat flour pasta. As gluten and egg protein are different in nature, it might be possible
402 that protease are more active against the egg protein compared to wheat protein thus the egg
403 protein may not provide equivalent barrier properties compared to gluten. We thus
404 hypothesise that in banana pasta, the presence of banana starch has more pronounced effect
405 on enzymic susceptibility compared to presence of egg protein network. However more
406 focused research is necessary to support the hypotheses. Compared to wheat flour only pasta
407 (D_2), the addition of external gums and eggs reduced the initial faster digestion rate of wheat
408 flour pasta but the later slower digestion rate is almost similar. This suggests that the
409 additional network formed by gums or non-gluten protein in pasta formulations can hinder

the swelling of granules on the outer surface of pasta but will not necessarily control the swelling of granules in the internal part of pasta or the diffusion of enzymes inside the pasta.

The inclusion of gums in pasta may have various types of effects. Studies have shown that xanthan gum is able to interact with galactomannans such as guar gum, resulting in increases in viscosity and shear-thinning property of starch mixtures.[20] These gums may also interact with starch granules creating a film layer surrounding the starch granules, which can provide a physical barrier for enzymes to access the starch granules. Additionally, amylase can form a complex with gums and the resulting gum–amylase complex may lack catalytic efficiency, an example of non-competitive inhibition as postulated by Slaughter et al.[21] The presence of both gums and egg protein has a synergistic effect as it generated a strong dough giving better banana flour pasta strands (sheets).

We aimed to link the pasting properties of green banana flour formulations and wheat flour formulations with enzyme susceptibility and pasta microstructure. However, the RVA profiles (Fig 5) are not definitive enough to make explicit conclusions, although it is clear that addition of gums and external proteins inhibited both the swelling of granules and the disintegration of swollen granules.

4. Conclusion

In this work, we have studied the enzyme susceptibility and micro-structure of pasta made from green banana flour with addition of gums and egg protein and compared them with wheat flour pasta. The results showed that green banana flour pasta is more resistant to enzyme digestion compared to wheat flour pasta. For wheat flour pasta, the restricted enzymic hydrolysis was related to the formation and intactness of a gluten network. However, in the green banana flour pasta, although the presence of a protein network was important for processing of pasta, it had comparatively small effects on enzyme

susceptibility, suggesting that the inherent enzyme resistant properties of green banana starch[4,5] are responsible for the lower enzyme susceptibility of green banana flour pasta. Thus there is potential for value addition by conversion of green bananas to banana flour for functional foods having lower starch digestion rates as well as being gluten free.

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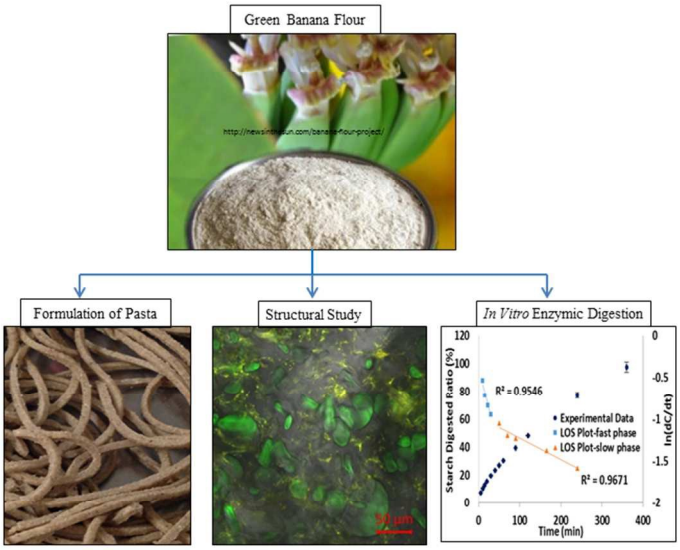
Notes

The authors declare no competing financial interest.

References

1. K. Mustalahti, C. Catassi, A. Reunanen, E. Fabiani, M. Heier, S. McMillan, L. Murray, M.-H. Metzger, M. Gasparin and E. Bravi, *Ann. Med.,* 2010, **42**, 587-595.
2. J. O'Neill, *Cereal Foods World*, 2010, **55**, 220-223.
3. R. P. Zandonadi, R. B. A. Botelho, L. Gandolfi, J. S. Ginani, F. M. Montenegro and R. Pratesi, *J. Acad. Nutr. Diet.,* 2012, **112**, 1068-1072.
4. N. Faisant, A. Buleon, P. Colonna, C. Molis, S. Lartigue, J. Galmiche and M. Champ, *Br. J. Nutr.,* 1995, **73**, 111-123.
5. H. N. Englyst and J. H. Cummings, *Am. J. Clin. Nutr.,* 1986, **44**, 42-50.
6. Zhang, S. Dhital and M. J. Gidley, *Trends Food Sci. Tech.*, 2015, **43**, 18-31.
7. S. Dhital, F. J. Warren, P. J. Butterworth, P. R. Ellis and M. J. Gidley, *Crit. Rev. Food Sci. Nutr.*, 2015, doi 10.1080/10408398.10402014.10922043
8. G. Aurore, B. Parfait and L. Fahrasmane, *Trends Food Sci. Tech.*, 2009, **20**, 78-91.
9. Zhang, R. L. Whistler, J. N. BeMiller and B. R. Hamaker, Carbohydrate polymers, 2005, **59**, 443-458.

10. S. Dhital, A. K. Shrestha and M. J. Gidley, *Carbohydr. Polym*., 2010, **82**, 480-488.
11. P. J. Butterworth, F. J. Warren, T. Grassby, H. Patel and P. R. Ellis, *Carbohydr. Polym*., 2012, **87**, 2189-2197.
12. S. Peighambardoust, A. Van der Goot, R. Hamer and R. Boom, *Cereal Chemistry*, 2004, **81**, 714-721.
13. P. Colonna, J. L. Barry, D. Cloarec, F. Bornet, S. Gouilloud, and J. P. Galmiche, *J Cereal Sci.*, 1990, **11**, 59-70.
14. C. Cunin, S. Handschin, P. Walther, and F. Escher, *LWT-Food Sci. Technol*., 1995, **28**, 323-328.
15. W. Zou, M. Sissons, M. J. Gidley, R. G. Gilbert and F. J. Warren, *Food Chem.,* 2015, **188**, 559-568.
16. N. Aravind, M. Sissons, and C. Fellows, Food Chem., 2011, 124, **3**, 816-821.
17 P. Feillet, O. Ait-Mouh, K. Kobrehel, and J. C. Autran, 1989, *Cereal Chem*., **66**, 26-30.
18. S. Dhital, M. J. Gidley and F.J. Warren, *Carbohydr. Polym*., 2015, **123**, 305-312
19. L. A. Bello-Perez, S. G. Sayago-Ayerdi, G. Mendez-Montealvo and J. Tovar, *Plant Foods Hum. Nutr*., 2004, **59**, 79-83.
20. N. Linlaud, M. Puppo and C. Ferrero, *Cereal Chem*., 2009, **86**, 376-382.
21. S. L. Slaughter, P. R. Ellis, E. C. Jackson and P. J. Butterworth, *BBA-Gen. Subjects*, 2002, **1571**, 55-63.



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