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Psyllium husk gel to reinforce structure of gluten-free pasta?

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Abstract

Gluten-free pasta is a technological challenge. The effect of *Psyllium* particle size, processing temperature and gel concentration on the quality of rice-based pasta was investigated. The rheological properties, i.e. maturation kinetics and mechanical spectra, of the *Psyllium* gels were studied and optimal conditions were set: 160 – 315 μm particle size, 4 g/100 g *Psyllium* husk concentration thermally processed at 40ºC. Cooking quality parameters, texture properties, nutritional composition, antioxidants and digestibility of pasta were determined. Consequently, the use of *Psyllium* husk in gluten-free pasta showed good overall properties. Moreover, the pre-gelatinization step of rice flour can be eliminated, resulting in a final gluten-free pasta formulation with *Psyllium* gel and rice flour (50/50) with high digestibility.

Keywords: Gluten-free pasta; By-products; *Psyllium*; Maturation kinetics; Digestibility

1. Introduction

In the last decade, the worldwide gluten-free (GF) food market increased 83%, with pasta products having an estimated annual growth rate of 12.3% until 2022 (Chauvin, 2019). Despite this massive increase, GF food products are still not very sensory appealing, are nutritionally unbalanced, often show texture problems, and its price is at least 75% higher than its gluten similar (Singh & Whelan, 2011; Vici, Belli, Biondi & Polzonetti, 2016).

The main issue in GF pasta production is the lack of gluten, the structuring element of traditional *durum* wheat pasta. In the absence of this protein matrix, which promotes pasta cohesiveness, viscosity, extensibility and elasticity (Lazaridou, Duta, Papageorgiou, Belc & Biliaderiset, 2007), it is necessary to consider other *structure builders*. Previous strategies were focused on thermal and high pressure treatments to build up structure of GF flours (Jalali, Sheikholeslami, Elhamirad, Khodaparast & Karimi, 2019; Vallons, Ryan & Arendt, 2011); the use of pulses and pseudo-cereals (Burešová et al., 2017); hydrocolloids (e.g. hydroxypropyl methylcellulose (HPMC), xanthan gum, locust bean gum) and proteins (e.g. transglutaminase, casein, albumin) (Crockett, Ie & Vodovotz, 2011; Storck et al., 2013).

A gluten free pasta formulation, based on pre-gelatinized rice flour from broken grains (a by-product of the rice industry), was previously developed (Fradinho, Sousa & Raymundo, 2019a). However, this pasta had not enough mechanical resistance compared to wheat-based fresh pasta. A way to tackle this is fibre enrichment, which generally contributes positively to the preservation of the microstructure of pasta and entraps starch granules, thereby improving the dough and cooking properties (Mercier, Moresoli, Mondor, Villeneuve & Marcos, 2016).

Following consumer trends, both researchers and the food industry are focused on the development of healthy food products with clean labels (Angus & Westbrook, 2019) to fulfil consumer expectations and needs for more natural foods, made from ingredients that are recognized, sustainable, locally produced and authentic (Asioli et al., 2017). In this context, replacing synthetic (e.g. hydroxypropyl methylcellulose, HPMC) and natural hydrocolloids (e.g. xanthan gum) with alternative sources of biopolymers (flaxseed, chia, *Psyllium* husk) could be a valid technological approach to improve both texture and nutritional properties of foods.

The plants of *Plantago* genus, the source of *Psyllium* husk, have been used worldwide in traditional medicine (Samuelsen, 2000), but only in 2012 FDA recognized the positive effect of *Psyllium'* soluble fibre on coronary heart disease risk reduction (FDA, 2012). The structure of *Psyllium* husk arabinoxylan is also able to resist gut fermentation (Pollet et al., 2012), acting as a prebiotic agent with health effects, i.e. i) increases in bifidobacteria and lactobacilli, ii) production of beneficial metabolites, iii) increases in calcium absorption, iv) decreases in protein fermentation, v) decreases in pathogenic bacteria populations, vi) decreases in allergy risk, vii) effects on gut barrier permeability, and viii) improved immune system defence (Broekaert et al., 2011, Carlson, Erickson, Lloyd & Slavin, 2018). *Psyllium* husk has multifunctional applications in several industrial fields due to its unique gelling properties

(Haque et al., 1993), low cost, biodegradability and eco-friendliness (Thakur & Thakur, 2014; Belorio, Sahagún & Gómez, 2019).

Furthermore, several authors focus on the use of *Psyllium* as a structure builder that mimics the gluten matrix in bread (Ziemichód, Wójcik &Różyło, 2019).

This study aims to determine the optimal processing conditions for *Psyllium* husk, suitable for pasta dough incorporation, to build-up the structure and to enhance the rheology and nutritional properties and *in vitro* digestibility of the GF pasta, compared to a commercial reference.

2. Materials and methods

2.1. Materials

Psyllium husk (lot 047058-02, Solgar, USA) from India and rice flour (lot 3411/18, Ceifeira, Dacsa Atlantic, Portugal) came from the local market. A dried commercial rice spaghetti (lot L1223, Urtekram, Denmark) was used for comparison.

2.2. Experimental design

Gluten free pasta formulation optimized in a previous work (Fradinho et al., 2019a) with rice flour pre-gelatinised gel and rice flour (50/50) was used as control. Preliminary trials were performed to assess the range of *Psyllium* husk concentration (1 to 5 g/100 g). The following conditions were considered: i) <u>*Psyllium* particle size</u> - *psyllium* husk milled by centrifuge mill (Pulverisette 14 Premium, Fritsch, Idar-Oberstein, Germany) at 6000 rpm for 300 s and sieved. Three particle fractions (< 160 μm; 160-315 μm; 315-500 μm) separated, and the distribution of particle size established. Gels were prepared adding 3 g/100 g (d.b.) *Psyllium* husk to distilled water at 20ºC under mechanical stirring for 10 min, covered with aluminium foil to prevent evaporation. Rheology studies were performed according to the procedure described in section 2.3. The best *Psyllium* particle size was determined based on the fastest maturation gel, a technological advantage, and taking into account the amount of each particle size fraction obtained after milling and sieving; ii) Processing temperature - gels of 3 g/100 g were prepared at 20ºC, 40ºC, 60ºC, 80ºC and 90ºC under mechanical stirring for 10 min. Rheology studies were performed to exclude gels with the lowest maturation kinetics. Subsequently, pasta was prepared according to the previously studied conditions reported by Fradinho et al. (2019a), replacing a fraction of the rice flour gel with the matured *Psyllium* gel, resulting in a 10/40/50 formulation. Pasta samples were characterized in terms of cooking quality parameters and texture. The next selection step was concentration: iii) *Psyllium* concentration - *psyllium* gels were prepared with 1, 2, 3, 4 and 5 g/100 g (d.b.) at particle size and temperature conditions chosen considering the best results obtained in the previous trials. Rheology studies were performed to exclude the gels with the lowest maturation kinetics. Next followed the fine tuning of the *Psyllium* gel/Rice gel ratio: iv) *Psyllium* gel/Rice gel ratio - pasta formulations were prepared by combining *Psyllium* gel and rice flour gel at 10/40, 25/25, 40/10 and 50/0 ratios, added to the other 50% of rice flour. Pasta developed was analysed for cooking quality parameters and texture properties.

2.3. *Psyllium* gel rheology measurements

Rheology behaviour of *Psyllium* gels was assessed by small-amplitude oscillatory shear (SAOS) measurements in a controlled stress rheometer (MARS III Haake, Thermo Scientific, Karlsruhe, Germany) with temperature control by an UTC-Peltier system. Parallel plate geometry (35 mm diameter) and 1.5 mm gap rough plates, to avoid slippage. Exposed edges of samples were covered with paraffin oil to prevent evaporation. Prepared *Psyllium* gels were immediately poured into the bottom plate of the rheometer to perform all the measurements.

Time sweeps were performed at 20ºC and 1 Hz for 180 min to obtain the maturation kinetics of the gels. For fully matured gels within the period mentioned, frequency sweep tests were performed from 0.01 to 100 Hz, at 20ºC, with a stress within the linear viscoelasticity region, previously determined by a stress sweep at 1Hz. The storage modulus (G') and loss modulus (G´´) were the highlighted parameters. Each test was run in triplicate.

2.4. Fresh pasta preparation and sampling

All pasta formulations (200 g) were prepared in triplicate, by mixing the ingredients in a food processor (Bimby TM31, Vorwerk, Wuppertal, Germany) for 3 min (speed 4) at 25ºC. Then, the dough was sheeted and laminated as "tagliatelle" using a benchtop pasta machine (Atlas 150 Wellness, Marcato, Italy) and these strands were covered by aluminium foil and allowed to equilibrate for 15 min at 25 °C.

For biochemical analysis, antioxidant activity and *in vitro* digestibility determinations pasta samples were cooked for 1 min, frozen, lyophilized (Scanvac Coolsafe 55-4, Labogene, Allerød, Denmark), crushed into powder (< 0.5 mm) and stored in a desiccator at room temperature. The assessment of the cooking quality parameters and texture properties of pasta were performed within 2 h of pasta preparation.

2.5. Pasta analysis

2.5.1. Cooking quality evaluation of pasta

Cooking time of the control pasta (50 gelatinised rice flour/50 rice flour) was assessed in the authors previous study (Fradinho et al., 2019a). This pasta shape (tagliatelle) is very thin and partly composed of gelatinised starch, so 1 min is enough to cook it, without breaking the pasta strands. Replacing gelatinised starch with Psyllium gel did not change the cooking time.

Cooking quality parameters: water absorption (WA), swelling power (SP) and cooking loss (CL), were determined as earlier reported by Fradinho et al. (2019a). At least three measurements were performed.

2.5.2. Texture analysis

Cooked pasta texture parameters were determined using a texturometer TA.XTplus (Stable MicroSystems, Godalming, UK) with a 5 kg load cell and a blade set with guillotine (HDP/BSG) and a Kieffer Dough & Gluten Extensibility Rig (A/KIE), in a 20ºC controlled temperature room. Pasta samples were cooked in boiling water for 1 min, rinsed with distilled water and drained. Cutting and extensibility tests were performed within 15 min after draining, according to the procedure earlier described in Fradinho et al. (2020). Each test was replicated eight times.

2.5.3. Proximate composition and antioxidant capacity determination of cooked pasta

Crude protein, total lipids and total carbohydrate contents were determined following Lowry, Rosebrough, Farr & Randall (1951), Marsh & Weinstein (1966) and Dubois, Gilles, Hamilton, Rebers & Smith (1956), respectively. Moisture and ash were analysed (ISTISAN Report 1996/34, method B and ISTISAN Report 1996/34). The total phenolic content (TPC) was determined using the Folin Ciocalteu assay according to Ganesan, Kumar & Bhaskar (2008).

To evaluate the radical scavenging capacity of the cooked pasta samples, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Rajauria, Jaiswal, Abu-Ghannam & Gupta, 2013) was performed. The antioxidant capacity of the samples was expressed in terms of µg of Vitamin C Equivalent Antioxidant Capacity (VCEAC) per gram of sample (ascorbic acid calibration curve: 0 to 10 mg mL^{-1}, R^2=0.992) and corresponding Radical Scavenging Activity (RSA) (%). Two blank assays, one without samples and another without reagents, were also performed. Analyses were repeated in triplicate and performed in cooked pasta samples, previously lyophilized.

2.5.4. *In vitro* digestibility tests

The *in vitro* digestibility (IVD) of cooked pasta samples was assessed according to the Boisen & Fernández method (1997) modified by Niccolai, Zittelli, Rodolfi, Biondi & Tredici (2019). Briefly, 1 g of lyophilised sample was weighed (particle size ≤ 1 mm) and transferred to 250 mL conical flasks. Then, 25 mL of phosphate buffer (0.1 M, pH 6.0) was added and mixed, followed by 10 mL of 0.2 M HCl and pH was adjusted to 2.0. A freshly prepared pepsin water solution (3 mL) containing 30 mg of porcine pepsin (0.8 FIP-U/mg) was added, and the flasks were incubated at 39 °C for 6 h with constant agitation (150 rpm). Subsequently, phosphate buffer (10 mL, 0.2 M, pH 6.8) and NaOH solution (5 mL, 0.6 M) were added to each sample and pH was adjusted to 6.8. A freshly prepared pancreatin ethanol:water solution (10 mL, 50:50 v/v) containing 500 mg of porcine pancreatin (42362 FIP-U/g) was added to each sample and the flasks were incubated at 39 °C, 150 rpm, for 18 h. A reagent blank without sample was also prepared. The undigested residues were collected by centrifugation at 18,000xg for 30 min and washed with deionised water. This procedure was repeated twice, and the final supernatant was filtered on glass-fibre membranes (47 mm Ø, pore 1.2 µm). The pellet and membranes were dried at 80 °C for 6 h, and then at 45 °C until constant weight. The dry matter, crude protein, and carbohydrate *in vitro* digestibility (%) of all pasta samples was calculated from the difference between the initial biomass and the undigested dry matter, crude protein, and carbohydrate biomass (after correction for the blank assay), expressed as percentage of the initial dry matter, crude protein, and carbohydrate biomass. Casein (Sigma Aldrich Corp., St. Louis, USA) was used as the reference material for 100% digestibility.

2.6. Statistical analysis

Experimental data is presented as average ± standard deviation (s.d.). Significant differences between samples were assessed by one-way ANOVA followed by Tukey's HSD test at 95% confidence level ($p < 0.05$) using RStudio (version 1.1.463 – © 2009-2018 RStudio, Inc.).

3. Results and discussion

3.1. *Psyllium* gel settings

3.1.1. *Psyllium* husk particle size

After milling and sieving, *Psyllium* husk showed the following particle size distribution: 12.1% with < 160 μm, 38.4% with 160-315 μm and 49.1% with 315-500 μm.

The maturation kinetic curves of *Psyllium* gels, prepared at 20ºC with different particle sizes, were monitored through the evolution of G' and G'' with time (Fig. 1). As observed, particle size impacts the rheology of *Psyllium* gel. This mucilage has a high water uptake, dependent upon a multitude of factors, such as particle size, type of milling and processing temperature (Van Craeyveld, Delcour & Courtin, 2008; Raymundo, Fradinho & Nunes, 2014), which explains the different gel profiles.

The steady value of G´, when the gel reaches a stable and fully developed structure, can be defined as the G'_{eq}, i.e. the value of G´ at the pseudo-equilibrium-state at infinite time (Nunes, Batista, Raymundo, Alves & Sousa, 2003).

$$G'_{eq} = \lim_{t \to \infty} G'(t) \qquad (2)$$

or alternatively

$$G'_{eq} = \lim_{1/t \to 0} G'(t) \qquad (3)$$

The experimental data can be fitted to the following second order exponential decay equation:

$$G'(k) = y_0 + A_1 e^{-k/b1} + A_2 e^{-k/b2} \qquad (4)$$

Where y_0, A_1, A_2, b_1 and b_2 are the equation parameters and k is the reciprocal time, i.e. 1/t. Table 1 presents G'_{eq} values extrapolated along with the parameters of Eq. 4, G' obtained at 180 min and the maturation index, given by the ratio (G'_{180min} / G'_{eq}) x 100 (Batista et al., 2012). The shorter maturation time of 5 h was obtained for *Psyllium* gel with 160 – 315 μm particle size, against almost 7 h and 8.5 h of 315-500 μm and < 160 μm, respectively. This range is close to a coarse flour (132 – 200 μm), recommended by de la Hera et al. (2014) and Gómez & Martínez (2016) in terms of bread quality and *in vitro* starch digestibility.

After milling, the intermediate particle size fraction had about 3 times more quantity than the lower particle size fraction. Also, there was the possibility of further milling the 315-500 μm fraction in order to increase the amount of 160-315 μm *Psyllium* fraction. For these reasons, the range of 160 – 315 μm particle size was selected.

3.1.2. *Psyllium* processing temperature

Based on previous work by Haque et al. (1993), a range of temperatures between 20 and 90ºC was selected for *Psyllium* gel processing. Gels were subjected to isothermal time sweep measurements (Fig. 2a) followed by a frequency sweep at 20ºC (Fig. 2b). Except for the *Psyllium* gel processed at 20ºC, all the other *Psyllium* gels attained full maturation almost immediately (Fig. 2a). The mechanical spectra of gels were all similar, with G' higher than G'' with some frequency dependence over the 0.01 to 10 Hz frequency range studied, typical of a weak gel-like structure, where molecular associations tolerate low-amplitude oscillation but are broken down under steady shear, giving rise to flow (Fig. 2b). An increasing G' with increasing processing temperature is also observed, which reflects the temperature dependence of *Psyllium* gels, already described by Haque et al. (1993). As expected, at 20ºC the gel structure is weaker and more frequency dependent, therefore gels processed at 40ºC, 60ºC, 80ºC and 90ºC were used to produce fresh pasta, to assess the best processing temperature for pasta incorporation.

Based on a gluten-free fresh pasta developed in a previous work (Fradinho et al., 2019a), a fraction of the rice flour gel was replaced by *Psyllium* gel, resulting in a final formulation composed by 10% *Psyllium* gel, 40% rice flour gel and 50% rice flour. All pastas presented similar water absorption (WA) and cooking loss (CL) values and comparable to the Control pasta without *Psyllium* gel (WA: 43.5-49.0 g/100 g; CL: 1.1-1.7 g/100 g). However, swelling power (SP) of *Psyllium* pastas (except 90ºC) was higher (SP: 0.85-0.92 mL/g) than of the Control (SP: 0.79 mL/g). For the texture parameters, all *Psyllium* pasta samples showed significant ($p < 0.05$) lower firmness values (1.94 – 2.04 N) than the Control (2.1 N). This could be related to *Psyllium* husk hydration properties that increased the water imprisoned into the pasta matrix, as observed in swelling values. Adhesiveness is a negative feature in pasta, lower at 40ºC ($A_{40ºC}$=0.025N; $A_{Control}$=0.109 N), maintaining a firmness value close to the Control. Although *Psyllium* gel processed at 90ºC also led to pasta with similar texture characteristics, a higher processing temperature means a higher energy input, which translates in higher processing costs. For this reason, the processing temperature of 40ºC was selected.

3.1.3. *Psyllium* husk concentration

The maturation kinetic curves of *Psyllium* gels processed at 40ºC with concentrations between 1 and 5 g/100 g were conclusive for full maturation within the time considered (180 min) and were subsequently characterized in terms of their mechanical spectra (Fig. 3).

Besides particle size and processing temperature dependence, *Psyllium* gels, as expected, also show concentration dependence with two groups of spectra: 1 and 2 g/100 g (Fig. 3a) and 3, 4 and 5 g/100 g curves (Fig. 3b). At 1 g/100 g the behaviour is similar to a suspension since G'' is close to G'. Although 2 g/100 g showed to be more structured with G' over G'' with time, both 1 and 2 g/100 g systems were not fully maturated within 180 min. For the second group of *Psyllium* concentrations the spectra are from a similar structure, as loss tangent is not affected by concentration ($\tan \delta_{1\ Hz}$ = 0.256 – 0.278), typical of a weak gel-like behaviour (Fig. 3b).

Based on the previous rheology measurements, there is no obvious reason for choosing one concentration over another, in terms of pasta production. All three *Psyllium* concentrations were used in the next trials.

3.1.4. *Psyllium* gel/rice flour gel ratio

Different *Psyllium* gel and rice flour gel ratios were tested: 0/50, 10/40, 25/25, 40/10 and 50/0 and respective formulations of rice pasta were prepared using the conditions selected in the previous trials, i.e., 50% rice flour (Fradinho et al., 2019a) and *Psyllium* husk (3 – 5 g/100 g) with 160-315 μm particle size thermally processed at 40ºC.

As observed in Fig. 4, the formulations within the grey backgrounds produced pastas with high stickiness or with evident breaking points emerged during lamination. The final step of pasta development was performed considering the other formulations (blue shadowed, in Fig. 4). All resulting pasta samples were characterised for cooking quality (Fig. 5) and texture (Fig. 6).

Pasta cooking behaviour is a critical step for its quality perception by the consumers. The replacement of gelatinized rice flour by *Psyllium* gel did not affect significantly ($p < 0.05$) the pasta hydration capacity in terms of swelling and water absorption (Fig. 5). However, *Psyllium*' addition had a significant ($p < 0.001$) positive effect on the decrease of leached solids into the cooking water (cooking loss), especially at the highest concentrations (4 and 5 g/100 g). Some works on gluten pasta with fibre addition report contrasting results, i.e. fibre addition increased the cooking loss. They relate this behaviour to the competitive hydration tendencies of the fibres, weakening the gluten network, which is responsible for retaining the solids during cooking (Tudorică, Kuri & Brennan, 2002). In fact, Foschia, Peressini, Sensidoni, Brennan & Brennan (2015) found more than 10 g/100 g cooking loss in semolina pasta with *Psyllium* husk. In the present study, due to the absence of gluten, the pasta network was mainly formed by gelatinized starch. Adding *Psyllium* in gel form, and not in powder, most likely decrease *Psyllium*' hydration competitiveness, showing a complementarity with the starch gel to build up the GF pasta internal matrix, hindering the leaching of materials into the cooking water. Gasparre & Rosell (2019) results seem to support this hypothesis referring that hydrocolloid addition (xanthan gum, inulin and carboxymethyl cellulose) to GF pasta significantly decreased the cooking loss, but still showing much higher values (13.7 – 16.5 g/100 g) compared to (0.6 – 1.5 g/100 g) of the present work.

Texture results (Fig. 6) show that the firmness and adhesiveness of GF pastas depend on the *Psyllium* concentration as well as on the ratio of gelatinized starch to *Psyllium* gel. As earlier reported (Bustos, Perez & León, 2013), fibre-enriched pasta has low firmness, and high adhesiveness due to amylose leaching to the cooking water. However, in this study, all *Psyllium* incorporated pastas were less adhesive than the Control, probably related to the gelling properties of this material, promoting a more cohesive structure with lower cooking loss. Similarly, Belorio et al. (2019) stated that the incorporation of pre-hydrated *Psyllium* was responsible for a more cohesive dough, contributing to reduce the oil percentage in cake formulations.

Regarding firmness results, for the same gelatinized rice flour content (e.g. 25/25/50) there seems to be a tendency for firmness decrease for the formulations with increasing Psyllium concentration. On the other hand, for formulations without gelatinized rice flour (50/0/50), increasing *Psyllium* gel concentration resulted in higher pasta firmness after cooking. This could be due to a sort of competitive phenomena between the two gels, hindering the development of the full potential of Psyllium to build up the internal structure. Likewise, Gasparre & Rosell (2019) described a rise of firmness along with a significant reduction of adhesiveness of GF pasta in the presence of hydrocolloids.

It is noteworthy that only pasta samples with 4 g/100 g *Psyllium* (25/25/50, 40/10/50, 50/0/50) allowed the performance of extensibility measurements, with Resistance to extension (R_{max}) ranging from 0.58 to 0.64 N and distance until rupture (ER_{max}) from 5.23 to 5.94 mm. Although these values were lower than the ones obtained for wheat pasta (Fradinho et al., 2020) they show a positive result for GF pastas.

Based on these results, the GF pasta composed of only rice flour (without rice gel) and *Psyllium* gel (4 g/100 g) at a 50/50 ratio was selected, which eliminates the rice flour pre-gelatinization step, making industrial processing far easier. In addition, starch gelatinization increases glycemic index (GI) of the food matrices (Parada & Aguilera, 2011), so replacing this material with *Psyllium* husk, which is a fibre, is a promising alternative to develop GF pasta with health benefits and lower GI.

3.2. Proximate composition, antioxidant capacity and *in vitro* digestibility

In Table 2 the proximate composition, the antioxidant capacity and the *in vitro* digestibility of the optimized cooked Psyllium pasta formulation (PP) against the control are shown. A commercial rice pasta (CRP) and a wheat pasta (WP) were also characterized for comparison.

In terms of proximate composition, the PP pasta showed very low lipid content, consistent with the low lipid content of the raw materials, namely *Psyllium* husk and rice flour (Raymundo et al., 2014; Fradinho et al., 2019a), value in line with WP and significantly ($p < 0.05$) lower than the commercial rice pasta (CRP).

Although PP showed a higher carbohydrate content than the other pastas, this can be attributed to the higher fibre content of *Psyllium* (Raymundo et al., 2014), rendering a pasta with around 6 g/100 g (d.b.) total fibre content, as the authors already stated in a previous work (Fradinho, Raymundo, Sousa, Domínguez & Torres, 2019b). Regarding the antioxidant activity, the results revealed that all GF pastas showed significantly ($p < 0.05$) higher antioxidant activity than wheat pasta. *Psyllium* incorporation did not affect the antioxidant activity (RSA and VCEAC) of GF pasta, neither its total phenolic content.

The *in vitro* digestibility (IVD) of the cooked pasta samples was determined by an enzymatic method using pepsin and pancreatin. Due to the well-recognised influence of fibre on starch digestion, preventing excess glucose absorption, the addition of dietary fibre to cereal-based foods has been investigated as an alternative to lower its GI (Bustos et al., 2013; Oh, Bae & Lee, 2014). To our knowledge, the *in vitro* digestion-retarding effect of *Psyllium* husk in rice-based foods has not been examined. GF food products generally have higher GI that their wheat counterparts (Foster-Powell, Holt & Brand-Miller, 2002; Berti, Riso, Monti & Porrini, 2004), which is also confirmed by the present work, when comparing WP and Control IVD values. This is due to the raw materials used in GF food production (e.g. rice, corn) which have high starch digestion rates (Toutounji et al., 2019).

As observed (Table 2), *Psyllium* addition contributed significantly ($p < 0.05$) to the decrease of dry matter and carbohydrate digestibility in PP pasta when compared to the Control. In a previous work, (Koh, Kasapis, Lim & Woo, 2009) found that the *in vitro* digestion of rice-based noodles was retarded by the alginate addition. According to Parada, Aguilera & Brennan (2011), due to the hygroscopic nature of dietary fibres, they reduce water available for starch gelatinization, consequently reducing starch digestibility. Although similar carbohydrate IVD results were obtained for PP and WP pastas, the structure of *Psyllium* husk arabinoxylan is able to withstand fermentation in the gut (Pollet et al., 2012), acting as prebiotic (Broekaert et al., 2011). *Psyllum* husk contains a high amount of arabinoxylan (Fischer et al., 2004). Arabinoxylan of *Psyllum* is highly branched non-starch polysaccharide with a main chain of densely substituted β-(1,4) linked xylopyranose residues. Single arabinofuranose and xylopyranose residues, or short side chains consisting of these monosaccharides, are attached at positions 2 and/or 3 of the main chain xylopyranose residues (Fischer et al., 2004). Arabinoxylan oligosaccharides selectively stimulate the growth and activity of beneficial colon bacteria. Bifidogenic effects in the gut include the growth of health-promoting bacteria (such as lactobacilli and bifidobacteria), the increase in production of short-chain fatty acids (such as butyric and propionic acid) which are believed to be positive for colonic health, and the decrease of toxic bacterial metabolites (such as polyamines and ammonia) (Broekaert et al., 2011).

Protein digestibility values of PP were similar to the ones of CRP and Control, i.e, all the GF samples showed similar protein digestibilities. The average protein digestibility value of GF pastas analyzed in this work (36.2%) is comparable to that of pastas made with *durum* wheat semolina + gluten powder, *durum* wheat semolina dried at different temperatures, and corn (39.2, on average 38.4%, and 34%, respectively) (Laleg, Barron, Santé-Lhoutellier, Walrand & Micard, 2016; Palavecino, Ribotta, León, & Bustos, 2018; Petitot, Abecassis & Micard, 2009). Interestingly, Laleg et al. (2016) reported a similar value of protein digestibility for wheat pasta (42%) compared to one found in the present work for CRP (40%), highlighting like GF pastas did not present alteration in protein digestibility compared to conventional wheat-based pastas. A significantly lower protein digestibility between Control and CRP pastas developed in this study and rice-based pastas developed in different studies available in the literature has been found (Obulesu & Bhagya, 2006; Rafiq, Sharma & Singh, 2017). It is worth pointing out that differences in pH, mineral type, ionic strength and digestion time, which alter enzyme activity and other phenomena, may also considerably alter digestibility results in the different studies (Minekus et al., 2014). To fully clarify these points, further studies aimed at evaluating the structure of GF pastas protein after *in vitro* enzymatic digestion compared to commercial pastas are necessary. However, the WP with higher protein content, also showed higher protein digestibility, consistent with findings from other works (e.g. Susanna & Prabhasankar, 2013).

4. Conclusions

The ability of *Psyllium* husk to form gel at lower temperatures was successfully employed in GF fresh pasta development with potential health benefits and lower GI. This approach led to the suppression of the flour pre-gelatinization step, a time/energy-consuming procedure. This is a strong argument for the industrial production of GF pasta. The optimized GF *Psyllium* pasta (50% *Psyllium* gel/50% rice flour) showed increased cooking and textural quality properties and carbohydrate IVD was in line with CRP and WP pastas. The *Psyllium* pasta showed very low lipid content, consistent with the low lipid content of the raw materials, and higher carbohydrate content than the other pastas, attributable to the higher healthy fibre content of *Psyllium*. Interestingly, all GF pastas showed significantly higher antioxidant activity than wheat pasta.

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Figure Captions

Fig. 1. Maturation kinetic curves of *Psyllium* gels with < 160 μm (●), 160 – 315 μm (●) and 315-500 μm (●) particle size. Close symbol (G'), open symbol (G'').

Fig. 2. Maturation kinetic curves (a) and mechanical spectra (b) of 3 g/100 g *Psyllium* gels thermally processed at 20ºC (▪), 40ºC (▪), 60ºC (▪), 80ºC (▪) and 90ºC (▪). Close symbol (G'), open symbol (G'').

Fig. 3. Maturation kinetic curves (a) and mechanical spectra (b) of gels prepared with 1 (▪), 2 (▪), 3 (▪), 4 (▪) and 5 g/100 g (▪) *Psyllium* husk. Close symbol (G'), open symbol (G'').

Fig. 4. Pasta dough formulations produced with 3–5 g/100 g *Psyllium* husk and different *Psyllium* gel/rice gel ratios (10/40, 25/25, 40/10 and 50/0). The pastas with blue background were selected for further analyses.

Fig. 5. Cooking quality parameters (swelling - ▪; water absorption - □; cooking loss - ▪) of pasta formulations produced with 3-5 g/100 g *Psyllium* husk and different *Psyllium* gel/rice gel ratios (10/40, 25/25, 40/10 and 50/0), and the control (without *Psyllium* gel). Data shown is mean ± SD, n=4. Different letters in the same parameter show significant differences (p < 0.001, one-way ANOVA post-hoc Tukey test).

Fig. 6. Firmness (▪) and adhesiveness (▪) of pasta formulations produced with 3-5 g/100 g *Psyllium* husk and different *Psyllium* gel/rice gel ratios (10/40, 25/25, 40/10 and 50/0), and the control (without *Psyllium* gel). Data shown is mean ± SD, n=6. Different letters in the same parameter show significant differences (p < 0.001, one-way ANOVA post-hoc Tukey test)

Table 1. Parameters of exponential decay and calculated G'_{eq} and $G'_{180\ min}/G'_{eq}$ x 100 of *Psyllium* gels with different particle sizes.

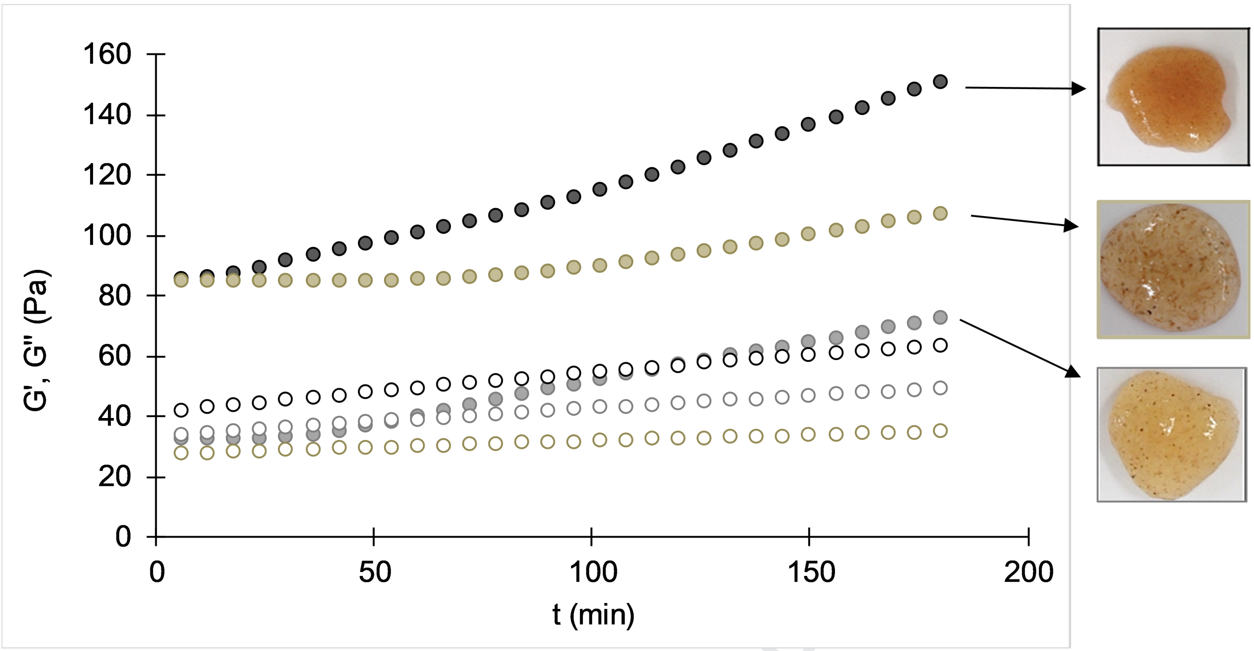
	y_0 (Pa)	A_1 (Pa)	b_1 (1/min)	A_2 (Pa)	b_2 (1/min)	G'_{eq} (Pa)	$G'_{180\ min}$ (Pa)	$G'_{180\ min}/G'_{eq}$ x 100 (%)
Particle size								
< 160 μm	85.7	37.7	0.0179	302.0	0.0027	425.4	151.0	35.5
160-315 μm	33.0	46.1	0.0063	46.1	0.0063	125.2	72.8	58.1
315–500 μm	85.1	79.3	0.0028	79.3	0.0028	243.7	107.3	44.0

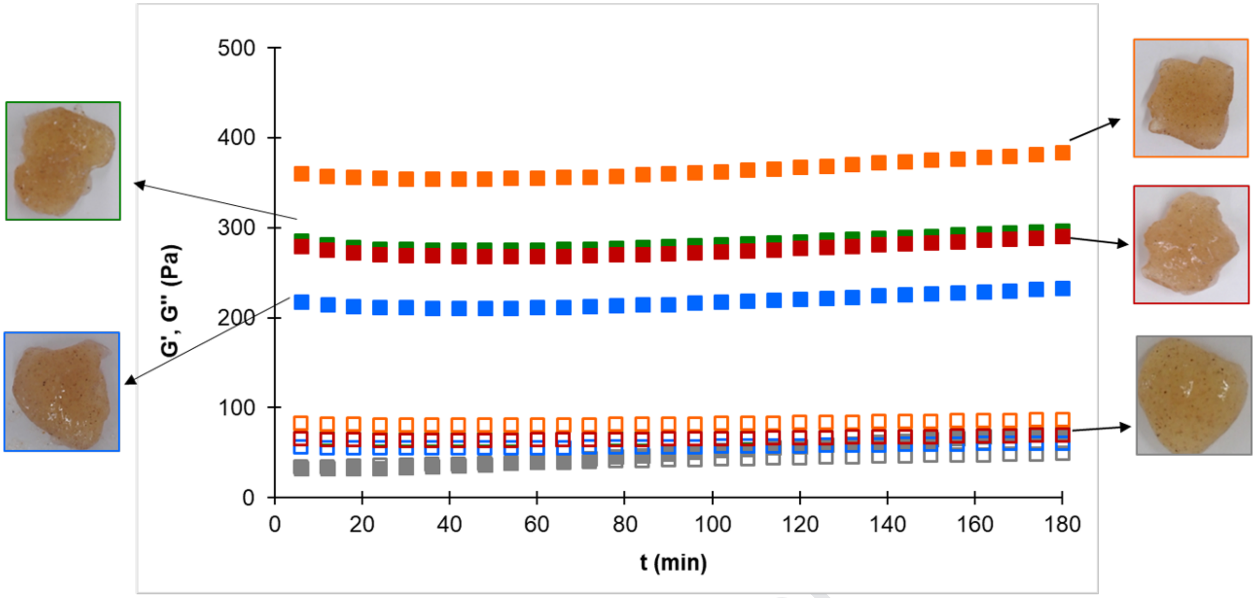
Reduced Chi-square χ^2= 1.7 – 3.0 ; R^2 = 0.997 – 1.000

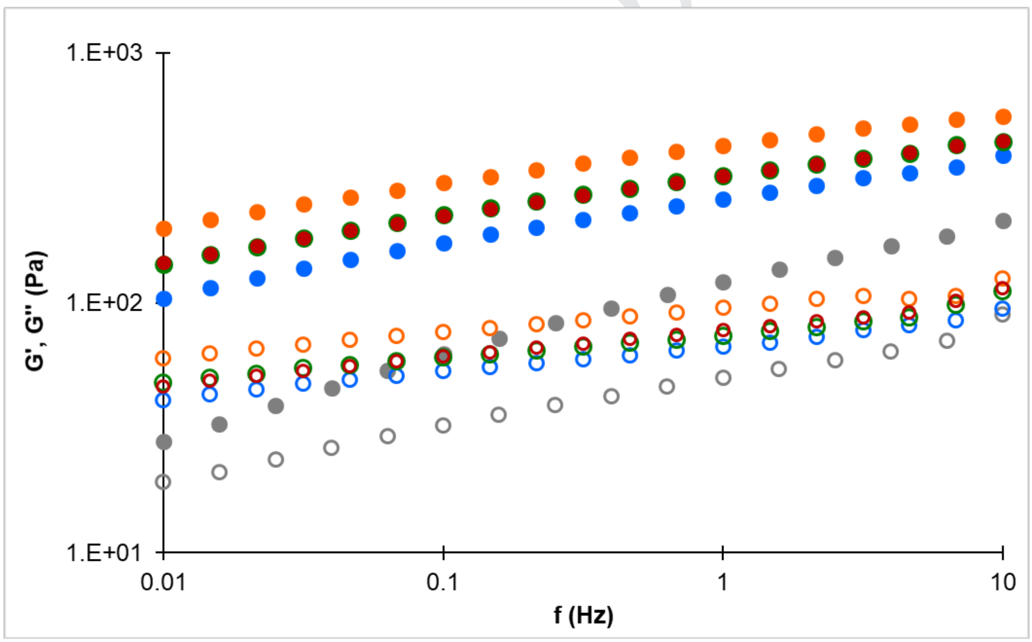
Table 2. Proximate composition, antioxidant capacity and *in vitro* digestibility of cooked pasta samples with gelatinized rice flour (Control), *Psyllium* (PP), commercial rice pasta (CRP) and wheat pasta (WP).

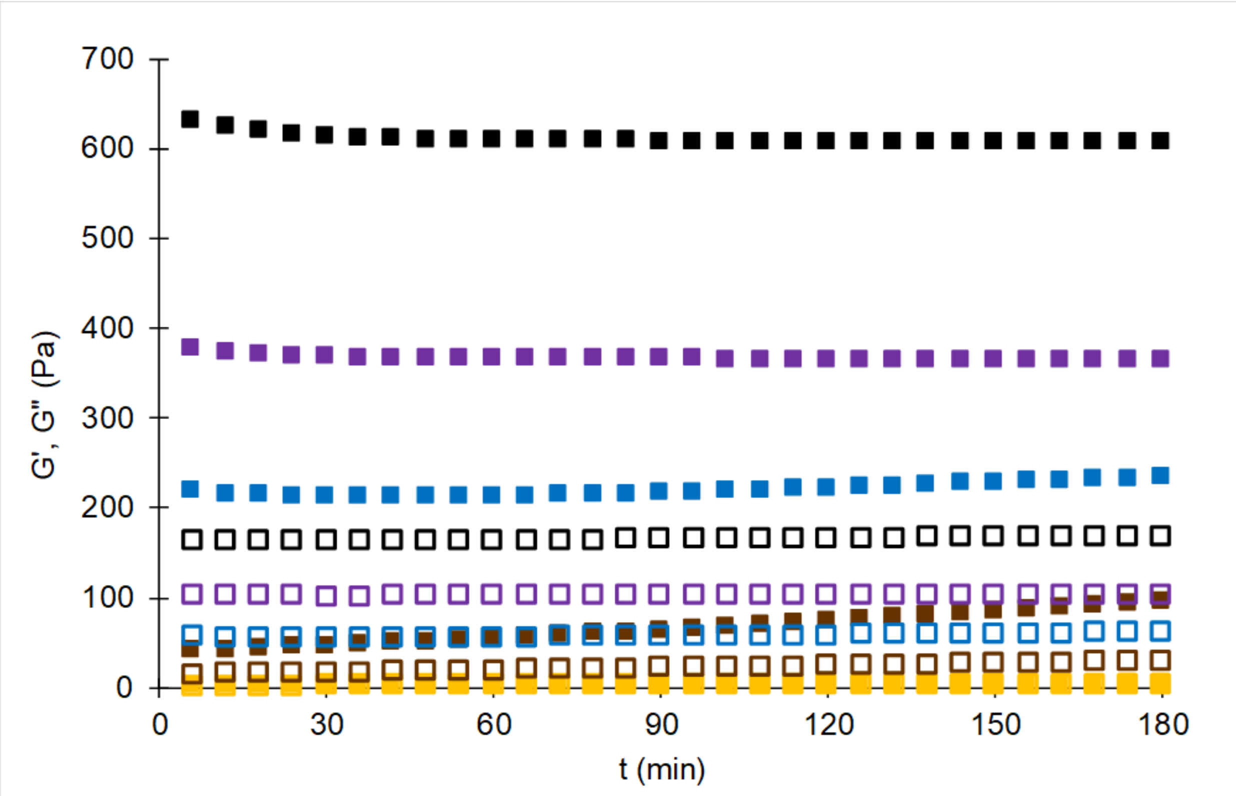
Samples	Biochemical composition				Antioxidant capacity			*In vitro* digestibility		
	Protein	Lipids	Ash	Carbohydrates	TPC	RSA	VCEAC	Dry matter	Protein	Carbohydrate
	(g/100 g, dry basis)				(mg GAE/g)	(%)	(µg/g)	(%)		
Control	3.6 ± 0.3[b]	1.4 ± 0.1[b]	0.7 ± 0.0[b]	83.8 ± 5.2[b]	0.22 ± 0.03[b,c]	52.05 ± 3.92[b,c]	0.55 ± 0.05[a]	97.61 ± 0.31[a]	32.06 ± 4.68[b]	97.45 ± 0.26[a]
PP	3.9 ± 1.0[b]	1.3 ± 0.1[b]	0.4 ± 0.4[b]	93.2 ± 4.1[a]	0.09 ± 0.05[c]	50.92 ± 2.02[c]	0.52 ± 0.02[a]	92.95 ± 1.19[b]	36.33 ± 3.07[b]	93.99 ± 0.26[b]
CRP	4.3 ± 1.4[b]	2.3 ± 0.2[a]	1.4 ± 0.1[a]	79.2 ± 3.1[b]	0.72 ± 0.10[a]	55.72 ± 5.53[a,b]	0.59 ± 0.08[a]	91.73 ± 0.23[b]	40.29 ± 1.48[b]	94.82 ± 1.10[b]
WP*	6.0 ± 1.2[a]	1.4 ± 0.1[b]	0.8 ± 0.1[b]	83.1 ± 2.7[b]	0.35 ± 0.18[b]	46.62 ± 3.19[d]	0.44 ± 0.03[b]	94.52 ± 2.10[b]	62.61 ± 8.98[a]	93.84 ± 0.51[b]

Data shown is mean ± SD, n=3. Different letters in the same parameter show significant differences ($p < 0.05$, one-way ANOVA *post-hoc* Tukey test). * Fradinho et al. (2020).

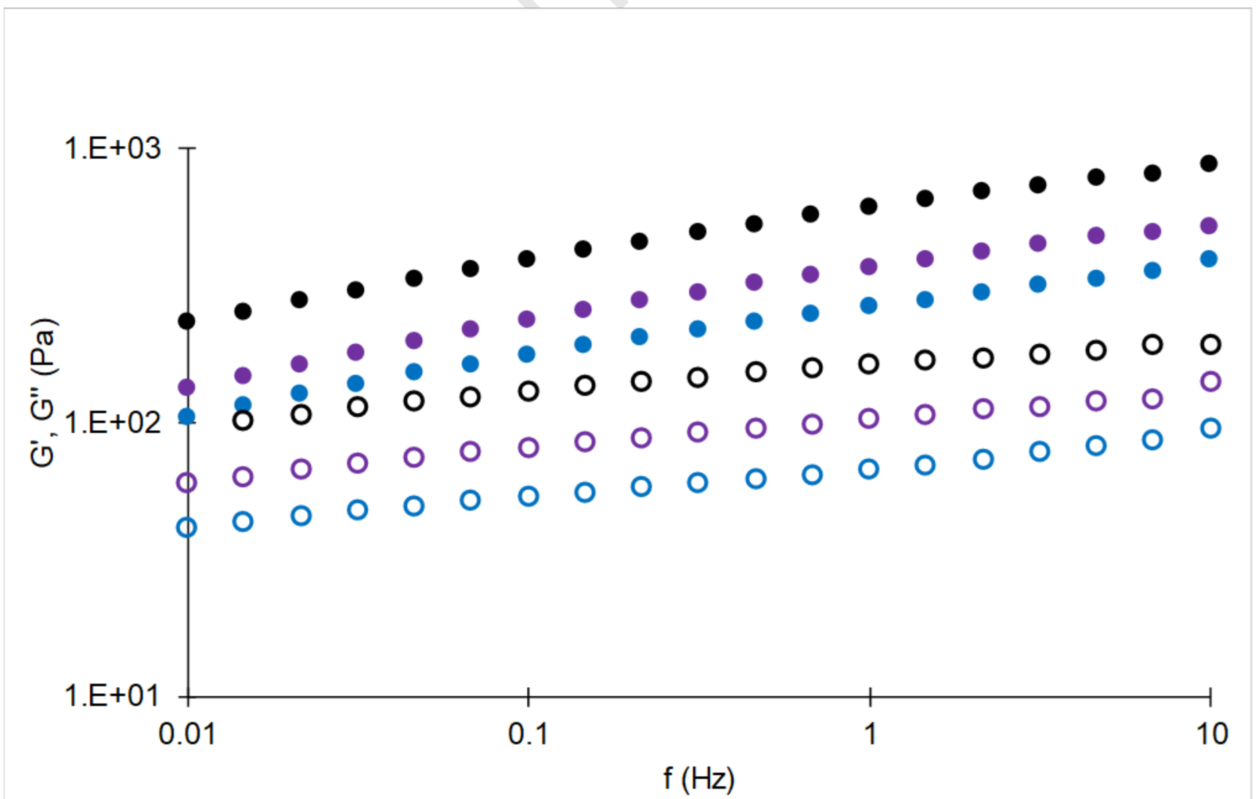

160
140
120
100
80
60
40
20
0
G', G'' (Pa)
0
50
100
150
200
t (min)


G', G'' (Pa)
500
400
300
200
100
0
0
20
40
60
80
100
120
140
160
180
t (min)

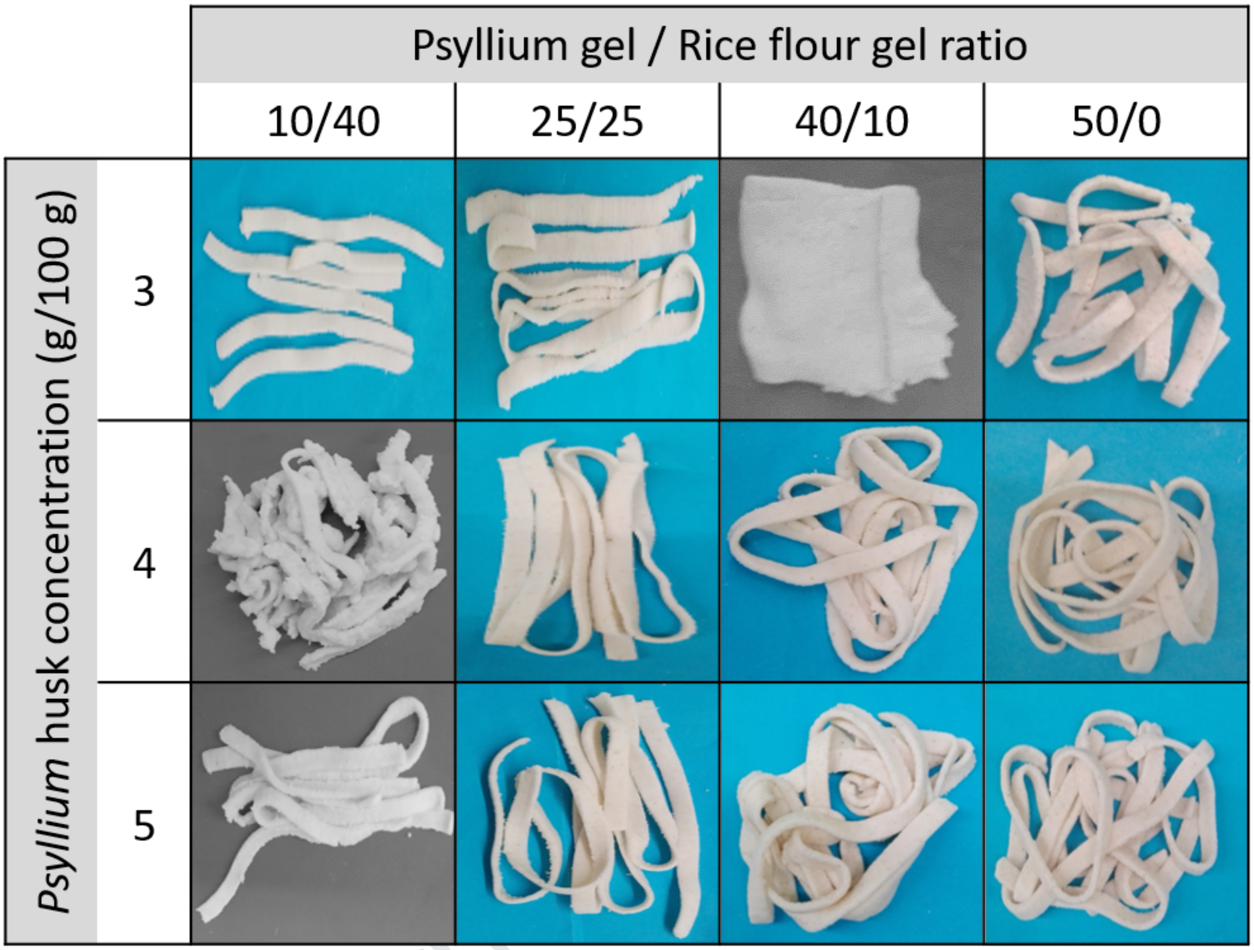

G', G'' (Pa)
1.E+03
1.E+02
1.E+01
0.01
0.1
1
10
f (Hz)

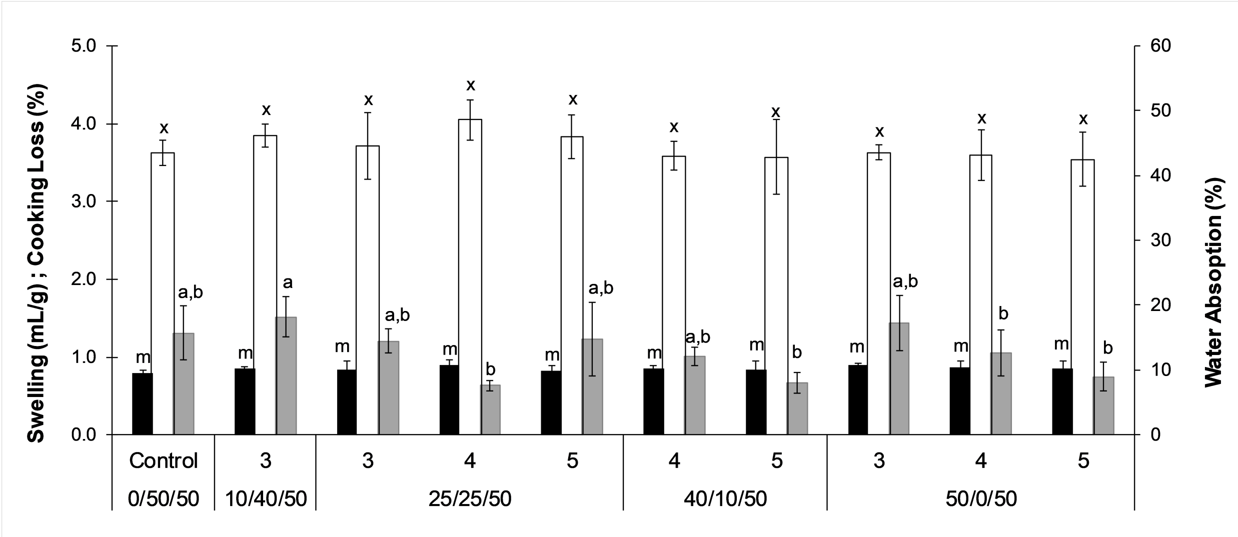


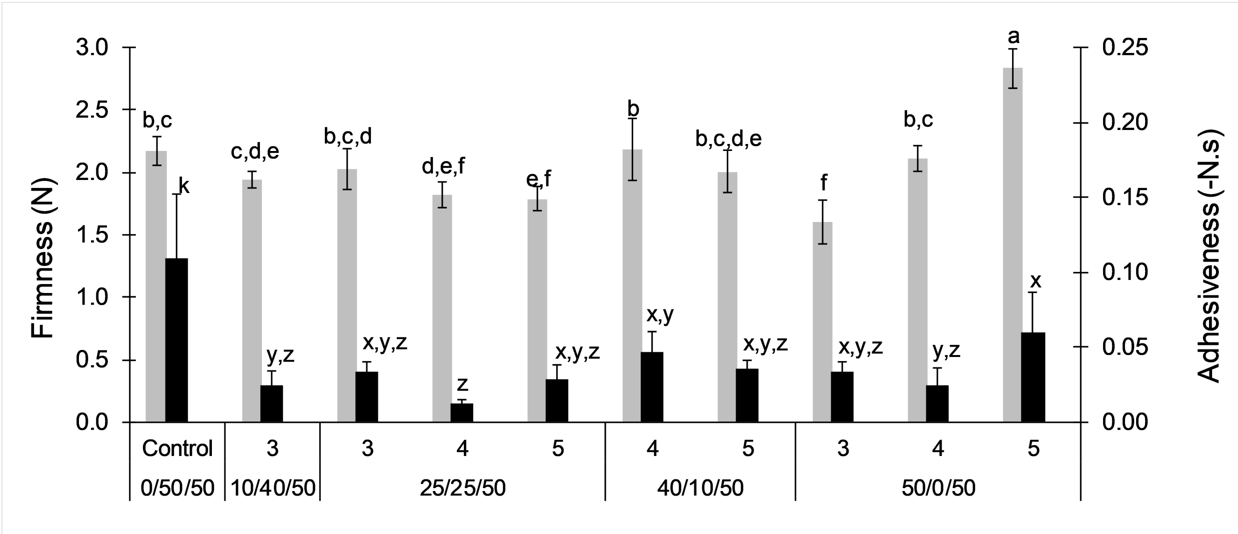
700
600
500
400
300
200
100
0
G', G" (Pa)
0
30
60
90
120
150
180
t (min)



1.E+03
1.E+02
1.E+01
G', G" (Pa)
0.01
0.1
1
10
f (Hz)


Psyllium gel / Rice flour gel ratio
10/40
25/25
40/10
50/0
Psyllium husk concentration (g/100 g)
3
4
5


Swelling (mL/g) ; Cooking Loss (%)
Water Absoption (%)
5.0
4.0
3.0
2.0
1.0
0.0
60
50
40
30
20
10
0
Control
3
3
4
5
4
5
3
4
5
0/50/50
10/40/50
25/25/50
40/10/50
50/0/50



Firmness (N)
Adhesiveness (-N.s)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.25
0.20
0.15
0.10
0.05
0.00
b,c
k
c,d,e
y,z
b,c,d
x,y,z
d,e,f
z
e,f
x,y,z
b
x,y
b,c,d,e
x,y,z
f
x,y,z
b,c
y,z
a
x
Control
3
3
4
5
4
5
3
4
5
0/50/50
10/40/50
25/25/50
40/10/50
50/0/50

Highlights

- *Psyllium* husk formed a weak-gel structure at low temperatures
- A gluten-free pasta without pre-gelatinised rice flour was developed
- Pasta *with Psyllium* gel exhibited lower adhesiveness than the control
- Gluten-free pasta showed high *in vitro* digestibility

Conflict of Interest:

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.