

**EVALUATION OF TECHNOLOGICAL PROPERTIES, MICROSTRUCTURE
AND PREDICTIVE GLYCAEMIC RESPONSE OF DURUM WHEAT PASTA
ENRICHED WITH PSYLLIUM SEED HUSK**

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Abbreviations: G' , storage modulus; G'' , loss modulus; $\tan \delta$, loss tangent; CL, cooking loss; d.b., dry basis; OCT, optimum cooking time; DF, dietary fibre; PSH, psyllium seed husk; IAUC, incremental area under the curve.

ABSTRACT

Dry pasta samples enriched with psyllium seed husk (PSH) were produced by replacing durum wheat semolina with 2.5, 5.0, 7.5 and 10% PSH. The aim was to investigate the effect of the substitution of semolina with different levels of PSH on cooking properties, microstructure and *in vitro* glycaemic response of pasta. Dough rheology was evaluated by dynamic tests within the linear viscoelastic region, and by farinograph. Doughs supplemented with psyllium were observed to have a reduced farinograph stability, indicating a weakening of gluten structure. Bulk elasticity and solid-like characteristics of doughs increased with PSH level, due to hydrated fibre aggregates. Changes in dough viscoelasticity between 25 and 95 °C and scanning electron microscopy on cooked pasta showed a reduction in swelling of gelatinised starch granules with an increase in PSH level, which led to a positive impact on nutritional and cooking features. Cooked samples containing psyllium were firmer than the control, while stickiness was higher for enriched pasta. Psyllium addition was effective in lowering the predictive glycaemic response of enriched products in comparison with the control. A positive linear relationship between starch digestion and a rheological index associated to starch swelling was obtained ($R = 0.898$; $P < 0.05$).

Keywords: pasta; psyllium; starch digestion; rheology; dietary fibre

1. INTRODUCTION

Dry pasta is considered one of the most staple foodstuffs in the world due to its versatility, ease of cooking and storage, good nutritional quality and low cost (Bustos, Perez, & Leon, 2015; Rakhesh, Fellows, & Sissons, 2015). Durum wheat semolina represents the ingredient for the manufacture of superior pasta products because of high content of yellow pigments (carotenoids) and proteins, and inextensible and strong gluten of doughs (Sissons, 2008; Rao, Mulvaney, Dexter, Edwards, & Peressini, 2001).

Dietary fibre (DF) has been broadly investigated for its health benefits, notably the ability to prevent a wide range of disorders, such as type 2 diabetes (Anderson et al., 2009). Pasta represents a good vehicle for functional ingredients, such as dietary fibre (DF), which partially replace durum wheat semolina (Foschia, Peressini, Sensidoni, & Brennan, 2013; Rakhesh et al., 2015). Several studies have been performed on durum wheat pasta supplemented with soluble DFs, such as β -glucans (Aravind, Sissons, Egan, et al., 2012; Cleary & Brennan, 2006;), inulin (Aravind, Sissons, Fellows, Blazek, & Gilbert, 2012; Brennan & Tudorica, 2008), guar gum and carboxymethylcellulose (Aravind, Sissons, & Fellows, 2012; Brennan & Tudorica, 2008), as well as protein ingredients (Jayawardena, Morton, Brennan, & Bekhit, 2019). It was recognized the ability of these DFs to reduce the glycaemic response of enriched pasta. Nevertheless, incorporation of these ingredients can be detrimental for pasta quality mainly due to decrease in firmness, in addition to an increase in cooking loss and stickiness (Desai, Brennan, & Brennan, 2019; Foschia et al., 2013). Consequently, the manufacture of high DF products needs a proper selection of fibre type and content, in order to satisfy sensory acceptability while delivering nutritional benefits. Replacement of durum wheat semolina with a combination of different DFs may represent

a valuable strategy to obtain high DF-enriched pastas with concomitant good cooking quality and reduced glycaemic index (GI), since some DFs work better in combination than individually added (Foschia, Peressini, Sensidoni, Brennan, & Brennan, 2015a; 2015b; Peressini, Cavarape, Brennan, Gao, & Brennan, 2020).

Psyllium is obtained from plants of *Plantago* genus (*Plantago ovata*), which are native of Asia and Mediterranean regions. The psyllium seed husk (PSH) is rich in water-soluble fibre (hydrophilic mucilloid) (Theuwissen & Mensink, 2008). Psyllium fibre is made of a highly branched acidic arabinoxylan, with β -(1,3) and β -(1,4) linkages in the xylan backbone, substituted with minor monosaccharides and acids, as L-arabinose and D-galacturonic acid, as well described by previous authors (Fischer et al., 2004; Guo, Cui, Wang, & Young, 2008). The non-cellulosic carbohydrates represent about 70% of the chemical composition of psyllium seed husk, where arabinose and xylose are the most abundant monosaccharides (62.5%) (Van Craeyveld, Delcour, & Courtin, 2009). PSH is a highly hydrophilic fibre able to absorb large amount of water and to swell, forming a highly viscous suspension or a gel, depending on content (Kale, Yadav, & Hanah, 2016; Ren, Yakubov, Linter, MacNaughtan, & Foster, 2020). For these characteristics, psyllium has been used recently as structural component in gluten-free formulations (Fradinho, Soares, Niccolai, Sousa, & Raymundo, 2020; Mancebo, San Miguel, Martínez, & Gómez, 2015).

Several clinical studies provided strong evidence supporting the correlation between psyllium supplementation in the human dietary regime and reduction in blood glucose level in subjects with type 2 diabetes (Anderson, Allgood, Turner, Oeltgen, & Daggy, 1999; Gibb, McRorie, Russell, Hasselblad, & D'Alessio, 2015; Ziai et al., 2005). Therefore, psyllium is suggested as a functional ingredient for food products to modulate glycaemia. Incorporation of psyllium into wheat flour for baked products was investigated by several authors

(Beikzadeh, Peighambaroust, Beikzadeh, Asghari Javar-Abadi, & Homayouni-Rad, 2016; Kamaljit, Amarjeet, & Tarvinder Pal, 2011; Pejcz, Szychaj, Wojciechowicz-Budzisz, & Gil, 2018), while Brennan, Derbyshire, Brennan, & Tiwari (2012) fortified extruded snacks with psyllium. To the best of our knowledge, few studies were performed on psyllium incorporation into durum wheat pasta (Foschia et al., 2015a, b; Peressini et al., 2020). These authors added psyllium to semolina at 15% substitution and found a reduction in glycaemic response for fresh and dry pasta products (Foschia et al., 2015a; Peressini et al., 2020). Unfortunately, supplementation was detrimental for pasta cooking behaviour, and also for sensory acceptability (Foschia et al., 2015b; Peressini et al., 2020). Since both nutritional benefit and cooking quality are important in a functional product, further studies are required to evaluate if it is possible to satisfy both aspects at a lower psyllium supplementation. To address this issue, it is necessary to focus on the influence of psyllium level in a systematic way.

Therefore, this work aims to evaluate the impact of the replacement of durum wheat semolina with different levels of psyllium seed husk on cooking properties, microstructure and *in vitro* starch digestion of dry pasta (spaghetti). Moreover, a rheological characterisation of doughs under small and large deformations were performed to acquire knowledge of the effects of psyllium on dough structure in relation to cooking and functional properties of spaghetti.

2. MATERIALS AND METHODS

2.1. Materials

Psyllium seed husk (PSH, 100 mesh powder, 90% dietary fibre) was kindly offered by Azelis (Trezzano sul Naviglio, Milan, Italy), while durum wheat semolina (15.4% protein and 12.7% moisture) was purchased from Molino Sgambaro (Treviso, Italy). Pepsin from porcine gastric mucosa (P6887), α -amylase from porcine pancreas (A3176), bile extract from porcine (B8631), pancreatin from porcine pancreas (P7545), amyloglucosidase from *Aspergillus niger* (A9913), L-(+)-arabinose, D-(–)-fructose, D-(+)-glucose, $\text{CaCl}_2(\text{H}_2\text{O})_2$, NaHCO_3 , NaCl , KCl , KH_2PO_4 , $\text{MgCl}_2(\text{H}_2\text{O})_6$, and $(\text{NH}_4)_2\text{CO}_3$ were purchased from Sigma Aldrich (Milan, Italy). Acetonitrile, ethanol, HCl and NaOH were purchased from Carlo Erba Reagents (Milan, Italy).

2.2. Pasta formulation

Pasta samples supplemented with 2.5, 5.0, 7.5 and 10% (w/w) PSH were prepared by substituting durum wheat semolina with PSH, while control was made using only durum wheat semolina. Blends were utilised for dough preparation and pasta making.

2.3. Pasta production

Pasta (spaghetti-shaped) was manufactured using a pasta press from Pavan (Galliera Veneta, Padua, Italy), and then dried, as reported by Peressini et al. (2020). Diameter and moisture of dry pasta were 1.5-1.6 mm and 11%, respectively.

2.4. Farinograph test

Dough water absorption (% on 14% moisture basis) and stability (min) of control and PSH blends were determined using a farinograph (Promylograph T6, Max Egger, Austria) in accordance with AACC Approved Method 54-21 (AACC, 2000).

2.5. Dough rheological properties

Dough viscoelastic properties were determined by a controlled stress rheometer equipped with a plate-plate geometry (diameter of 35 mm, gap of 2 mm) (Haake RheoStress 6000, Thermo Scientific, Karlsruhe, Germany). Doughs at a water absorption of 64% (moisture content of 47.6%) were prepared in the farinograph mixer until maximum development according to Peressini et al. (2020). The dough was immediately loaded between the rheometer plates, its excess was removed, then the air-exposed surface covered with silicon grease to avoid moisture loss. Before testing, dough was rested for 5 min to relax. Frequency sweep tests were carried out between 0.1 and 10 Hz within the linear viscoelastic region (LVR) at 25 °C. Temperature sweep tests were performed at 1 Hz between 25 and 95 °C at 1.5 °C min⁻¹ in LVR. Storage modulus G' , loss modulus G'' , and loss tangent $\tan \delta$ (G''/G' ratio) were measured. Rheological parameters were compared at 1 Hz.

2.6. Colour of pasta

Colour of both raw and cooked pasta at optimum cooking time was assessed using a tristimulus colorimeter equipped with a CR-400 head and illuminant C (Minolta Camera Co., Osaka, Japan), as described by Peressini et al. (2020). Before analysis, the colorimeter was calibrated using a standard plate ($L^* = 98.23$, $a^* = 0.10$, $b^* = 2.18$) and results were reported as L^* (lightness/darkness), and chromatic parameters a^* (greenness/redness) and b^* (blueness/yellowness).

2.7. Pasta cooking quality

Spaghetti samples were cooked to their optimum cooking time (OCT) according to the AACC Approved Method 66-50 (2000). Cooking loss (CL, %), which corresponds to the content of solid substance released in water during cooking, was evaluated according to the Approved Method 66-50 (AACC, 2000).

A Texture Analyser (TA.XT plus, Stable Micro Systems Ltd., Godalming, UK), which was equipped with a load cell of 5 kg, was used to evaluate textural properties of cooked pasta. Firmness was determined in accordance with the Approved Method 66-50 (AACC, 2000). Peak force (N) achieved during the cutting cycle of five pasta strands was used as measure of pasta firmness. Results are expressed as average of twenty-four measurements from three different cooking replications.

Pasta moisture and stickiness were assessed according to Peressini et al. (2020). Stickiness (N) corresponded to the peak force necessary to separate the probe from the surface of five

strands upon its retraction. Results are average of twelve measurements from three different cooking replications.

2.8. Scanning electron microscopy (SEM)

The raw and cooked pasta cross-section microstructure was observed using a scanning electron microscope (Stereoscan 440, Cambridge, UK). Pasta samples were cooked at OCT, drained and frozen in liquid nitrogen. Frozen spaghetti strands were freeze-dried. Dry pieces (3 mm length) were cut using a laboratory razor. Then, they were attached to the specimen holders using carbon tape and covered with gold at 1.4 kV and 10 mA for 5 min using a Polaron Sputter Coater E5400 (Polaron, Watford, UK). Sample images were captured with secondary electron mode at 5 kV at two magnifications (500x and 1000x).

2.9. *In vitro* pasta digestion

The *in vitro* pasta digestion was carried out according to the INFOGEST protocol for the static *in vitro* gastrointestinal food digestion proposed by Brodkorb et al. (2019). Before starting, simulated digestion fluids, such as salivary (SSF), gastric (SGF) and intestinal (SIF) fluids, were prepared in stock solutions and aliquots were stored at -20 °C and 4 °C for analysis. Cooked product at OCT (15 g) was ground for 20 s using an electric grinder to simulate the oral chewing step. Then, 1 g of sample was transferred into a tube with 0.1 g of L-(+)-arabinose as the internal standard for glucose determination. The digestion started with the dilution of pasta sample 1:1 (w/w) with SSF and α -amylase solution prepared in distilled water to achieve 75 U/mL in the final mixture. The sample was incubated for 2 min at 37 °C

and was kept constantly rotating with a laboratory rotator. The gastric phase started with the dilution 1:1 (v/v) of the oral bolus with SGF and pepsin solution prepared in distilled water to achieve 2000 U/mL (in final mixture). Then, the sample was incubated at 37 °C for 2 h and kept constantly rotating. The intestinal phase started with the dilution 1:1 (v/v) of the gastric chyme with SIF, pancreatin and bile extract solutions prepared in SIF to achieve 100 U/mL and 10 mM, respectively in the final mixture. Once more, the sample was incubated at 37° C for 2 h and kept constantly rotating. For the final phase, the pH was modified to 4 and 100 µL of amyloglucosidase were added to the sample, then the digesta was kept at 37 °C for 2 h in rotation. The release of glucose was determined after 20, 60, 90, and 120 min by stopping the enzymatic activity with ethanol 98% (1/4, v/v).

2.10. Determination of glucose after *in-vitro* digestion

After the *in vitro* digestion, glucose released in the liquid phase was determined using a HPLC Varian ProStar (Varian Chromatography Systems, California, USA) according to Renoldi, Peighambardoust, & Peressini (2020).

The incremental area under the curve (IAUC) was calculated from the glucose release vs. digestion time, in agreement with the trapezoid method recommended by the FAO/WHO (1998).

2.11. Statistical analysis

Experiments were performed at least in triplicate ($n \geq 3$) and data are reported as mean \pm standard deviation. Pooled standard deviation is also reported. Shapiro-Wilk test was used to check the normality distribution of the data. Bartlett's test was conducted to check the homogeneity of variance. Given the very low sample size, even if all these tests were not significant we decided to couple standard one-way analysis of variance (ANOVA) with Kruskal-Wallis test, that does not depend on distributional assumptions. Only variables with a joint significance to the two tests were further analysed. In this case Tukey's HSD test was applied to determine statistically significant differences among means ($P < 0.05$) (R software package, version 3.5.1, the R Foundation for Statistical Computing, Vienna, Austria).

3. RESULTS AND DISCUSSION

3.1. Dough rheological properties

Farinograph testing (large deformation) and fundamental rheological tests under small deformation were conducted to gain information on effects of PSH incorporation on gluten network and gelatinization of starch granules. Fig. 1 and Table 1 show the farinograph results. PSH affected the water absorption (WA) and mixing curves of psyllium-enriched doughs. WA increased linearly with PSH level from 57% to 132% for control and 10% PSH, respectively ($R = 0.998$, $P < 0.0001$) (Table 1). Psyllium polysaccharides possess gelling properties and extreme water absorption capacities due to their hydrophilic nature (Belorio, Marcondes, & Gómez, 2020; Mariotti, Lucisano, Pagani, & Ng, 2009).

Fig. 1 shows farinograph curves of control and samples added with 2.5% and 10% PSH. The control sample exhibited a high tolerance to overmixing, which is consistent with a strong dough (Fig. 1A). Inclusion of 2.5% PSH decreased the mixing stability indicating a weakening effect on gluten network (Fig. 1B, Table 1). The same behaviour was observed at 5% and 7.5% PSH (not shown). The differences between control and other treatments, though evident, is not statistically significant ($P > 0.05$) (Table 1). In contrast, dough at 10% PSH reached maximum consistency and then remained constant during mixing (Fig. 1C). The recorded curve of 10% PSH showed a high variability in positive and negative excursions in consistency band compared to other samples, suggesting a heterogeneous system due to water distribution in the dough. Probably, this curve reflects more dough hydration than structure development. Two mechanisms may explain weakening of gluten structure in doughs supplemented with PSH: 1) a partial dehydration of gluten due to a

competition with DF for water; 2) fibre acts as a physical barrier, which interrupt gluten network continuity (Skendi, Papageorgiou, & Biliaderis, 2009; Zhou et al., 2021).

Further rheological investigations were performed using oscillatory tests in LVR. The frequency sweep test for both the control and psyllium-enriched doughs are shown in Fig. 2. A weak gel-like behaviour was observed since storage modulus (G') is frequency dependent and $\tan \delta$ values are lower than 1, as expected for wheat doughs (Peressini & Sensidoni, 2009). Viscoelastic moduli at 1 Hz were selected to compare samples (Table 1). PSH-supplemented doughs gave significantly higher storage modulus and lower loss tangent values than control, in agreement with previous results on 15% PSH-semolina dough ($P < 0.05$) (Peressini et al., 2020). The rise in PSH content increased G' and decreased $\tan \delta$ values, which correspond to more elastic and solid-like characteristics. A linear relationship was found between viscoelastic parameters and psyllium level ($R = 0.993$ for G' vs. PSH level and $R = 0.956$ for $\tan \delta$ vs. PSH; $P < 0.001$).

Wheat dough is essentially a composite material, in which starch granules are dispersed in a continuous protein phase (Bloksma, 1990). Dough rheology depend mainly on hydrated continuous phase (gluten network) (Edwards, Peressini, Dexter, & Mulvaney, 2001). Psyllium polysaccharides have the ability to produce fibrillar gels in water via self-associations (Guo, Cui, Wang, Goff, & Smith, 2009). PSH incorporation may alter dough viscoelastic properties with two concomitant mechanisms: 1) weakening gluten structure, which would decrease elastic properties; 2) replacing regions of free water with hydrated fibre aggregates, related to a higher number of elastic interactions and a rise of solid-like behaviour. Based on our results (higher G' and lower $\tan \delta$ values for psyllium-enriched doughs) (Table 1), the latter seems to have a greater effect on linear viscoelasticity. Skendi et al. (2009) recognized a similar effect on dough upon addition of long chain β -glucans.

In order to evaluate the impact of PSH on starch gelatinisation, an oscillatory temperature sweep test at a frequency of 1 Hz was conducted. Changes in G' as a function of temperature for control and fibre-enriched samples are shown in Fig. 3. When control dough was heated, a sharp rise of G' to a maximum was observed, attributed to starch granule swelling because of gelatinization (Peressini et al., 2020). Ascent of storage modulus was lower for PSH doughs compared to control, suggesting a decrease in the volume fraction of dispersed starch granules (Peressini, Melchior, Berlese, & Calligaris, 2021) (Fig. 3). A similar behaviour was observed for wheat doughs containing 15% PSH and β -glucans individually added and in combination (Peressini et al., 2020).

No significant differences in the peak temperature (T_{peak}) were detected between enriched pasta and the control (Fig. 3; Table 1). Additionally, doughs containing psyllium exhibited peak values of G' significantly lower than the control, indicating differences in swelling of starch granules ($P < 0.05$) (Table 1). From the temperature sweep results (Fig. 3), $\text{Log } G'_{\text{peak}} - \text{Log } G'_{\text{min}}$ difference was calculated to estimate the extent of starch swelling in different samples ($\Delta \text{Log } G'$), as proposed by Peressini et al. (2020). Values of $\Delta \text{Log } G'$ were significantly lower for PSH-enriched doughs and decreased with psyllium level ($P < 0.05$) (Table 1). This parameter was in the range of 0.31-0.70 for enriched samples and 1.23 for control, revealing remarkable differences in swelling of starch granules between doughs. This effect of psyllium on starch appears to be associated to high water binding capacity and structuring ability (increase in bulk elasticity) (Table 1) (Peressini et al., 2020). The latter may induce the formation of fibre aggregates, which act as physical barriers around starch granules limiting swelling.

3.2. Pasta microstructure

Microstructure of raw and cooked pasta was investigated using SEM technique. Fig. 4 shows micrographs of raw pasta samples at two magnifications. For control (Fig. 4A), starch granules were clearly visible and embedded into a dense and continuous protein phase. Disperse and continuous phases were less discernible for enriched samples, especially at high PSH levels (Fig. 4C-E). Probably, starch granules were covered and hidden by the continuous phase (proteins and psyllium polysaccharides). Micrographs of cooked pasta samples are shown in Fig. 5. As expected, cooked control pasta exhibited larger starch granules compared to uncooked pasta, surrounded by the coagulated protein matrix (Fig. 5A). A characteristic of this sample is the presence of voids around swollen granules, attributed to their shrinkage due to dehydration (Pagani, Gallant, Bouchet, & Resmini, 1986). The more the starch granules are swollen during pasta cooking, the greater will be their deformation (shrinkage).

For PSH enriched pasta (Fig. 5B-E), starch granules appeared to be less swollen compared to control. Besides, it is evident the decrease in size of swollen starch granules with PSH level (Fig. 5B₂-E₂). This is consistent with rheological results ($\Delta \text{Log } G'$) (Table 1), which indicated a decrease in swelling of starch granules with PSH content. Psyllium appeared clearly as a gel network in the continuous phase, which surrounded starch granules. When PSH was added at 7.5% and 10%, swollen starch granules appeared well enveloped in psyllium network (Fig. 5D₂-E₂).

3.3. Pasta quality

Pasta quality was assessed in terms of colour and cooking behaviour (optimal cooking time, release in solids, firmness, and stickiness), which are the most significant attributes for consumers (Bustos et al., 2015).

Colour of raw and cooked pasta is given in Table 2. For raw spaghetti, PSH-enriched products displayed significantly lower L^* and higher a^* parameters compared to control ($P < 0.05$). Redness (a^*) increased and lightness (L^*) decreased with PSH level. Above 2.5% fibre supplementation, yellowness (b^*) was about 2-5% lower than control ($P < 0.05$). Similar effects on dry pasta colour were reported upon addition of β -glucans (Aravind, Sissons, Egan, et al., 2012) and 15% psyllium alone and added in combination with β -glucans or inulin (Peressini et al., 2020). For cooked pasta, colour parameters confirmed trends obtained before cooking (Table 2). Similar findings were reported for fresh pasta containing 15% psyllium (Foschia et al., 2015b). Previously, colour appearance score of cooked pasta was not changed upon addition of 7.5% PSH plus 7.5% Barley Balance (source of β -glucan) to durum wheat semolina (Peressini et al., 2020). Consequently, fibre-supplemented pasta was not evaluated of lower quality and less desirable due to colour alteration.

PSH incorporation at 2.5% and 5% did not give significant differences in OCT in comparison with control, above 5% enriched pasta showed a decrease in OCT ($P = 0.05$) (Table 3). This effect of psyllium supplementation on OCT, could be related to starch-protein matrix alteration and reduction in gluten content, which allowed more rapid water migration from outer layers to core of spaghetti strands during cooking (Rakhesh et al., 2015). Besides, no

significant differences in moisture content were observed between treatments ($P > 0.05$) (Table 3).

Cooking loss (CL) was not influenced by PSH addition up to 5%, above this value a significant increase in CL of about 7% was observed ($P < 0.05$) (Table 3). CL represents starchy solids released into the cooking water, which should not go over 7–8% (on dry weight of the product) for a pasta of good quality (Sissons, Abecassis, Marchylo, & Cubadda, 2012). In our work, CL was below the acceptable limit for all pasta samples. Foschia et al. (2015b) and Peressini et al. (2020) reported 9-10% CL for pasta added with 15% psyllium. Therefore, functional pasta should contain a PSH level up to 10% for a good quality. Aravind, Sissons, Egan, et al. (2012) observed an increase in CL above 7.5% Barley Balance enrichment. For pasta containing inulin, undesirable values of solids loss were obtained at 7.5-10% addition (Aravind, Sissons, Fellow, et al., 2012; Tudorica & Brennan, 2002).

Based on rheological results (Table 1), psyllium addition reduced starch swelling due to the rise of bulk elasticity and WA, and weakened gluten network. In spite of the deleterious impact on gluten, PSH addition up to 5% had no effect on losses during cooking (Table 1). We assume that lower starch granule swelling prevented breakdown of protein network preserving structural integrity of spaghetti (Delcour, Vansteelandt, Hythier, & Abecassis, 2000). In pasta samples with 7.5% and 10% PSH, increased loss of solids could be mainly attributed to leaching of psyllium polysaccharides.

Textural parameters are important to define the quality of cooked pasta product, which should be firm and not sticky to guarantee high acceptance by consumers (Sissons et al., 2012). Inclusion of PSH significantly increased firmness of cooked pasta ($P < 0.05$) (Table 3). However, no significant differences in firmness were observed between enriched samples

up to 7.5% PSH, while above this level cooked pasta became firmer ($P < 0.05$). Control showed the lowest firmness and the highest $\Delta\text{Log } G'$ values among samples, whereas 10% PSH gave the opposite (Table 1) (Peressini et al., 2020). For DF-enriched pasta, a decrease in starch swelling would lead to a firmer pasta due to a more compact structure (Aravind, Sissons, Egan, et al., 2012; Rakesh et al., 2015). Our results are consistent with earlier research on dry pasta enriched with 15% PSH (Peressini et al., 2020). In contrast, Foschia et al. (2015b) did not observe differences in firmness between fresh pasta with and without 15% psyllium. Other investigations reported that pasta firmness increased with incorporation of Barley Balance (Aravind, Sissons, Egan, et al., 2012; Peressini et al., 2020) and decreased with addition of inulin (Peressini et al., 2020).

Stickiness was significantly higher for PSH-enriched pasta than control samples (Table 3). For durum wheat pasta, superficial stickiness arises from material (mainly amylopectin) exiting from the gluten network and sticking to the outer layer of pasta during cooking (Sissons et al., 2012). Increased stickiness for enriched pasta samples could be associated to the high water-absorbing capacity of psyllium, which produces a viscous layer on cooked pasta surface, as reported for other soluble fibres (Rakesh et al., 2015).

Based on a previous sensory evaluation on pasta added with 7.5% PSH in combination with 7.5% Barley Balance, the enriched sample was perceived firmer and stickier than control, but overall acceptability was similar (Peressini et al., 2020). For this supplemented pasta, the authors reported a stickiness value of 1.79 ± 0.15 N. We found stickiness values, which were 17-43% lower compared to PSH-Barley Balance (Table 3). Therefore, it is expected that increased stickiness for all PSH-enriched samples should be not detrimental for their acceptability.

In conclusion, CL and textural results showed that psyllium supplementation at 2.5-10% appears suitable to manufacture functional durum wheat pasta of good quality.

3.4. Effect of psyllium on *in-vitro* digestion of pasta

Effect of PSH inclusion on starch assimilation of cooked pasta was evaluated using a simulated *in-vitro* gastrointestinal digestion method. Fig. 6 shows the amount of glucose released during the last intestinal phase. Rapidly available glucose (RAG) corresponds to the glucose concentration after 20 min of digestion in response to the addition of amylase, as proposed by Englyst, Englyst, Hudson, Cole, & Cummings (1999). Psyllium-enriched samples showed RAG values from 92.5 mg/g d.b. for 10% PSH to 97.9 mg/g d.b. for 5% PSH, which were significantly lower than control (108.6 mg/g) ($P < 0.05$). Enrichment of PSH caused a lowering of 10-15% in starch digestion at 20 min in comparison with control. Standardised incremental area under the curve (IAUC) results of control and PSH-supplemented pasta are reported in Fig. 7. Samples containing psyllium gave IAUCs significantly lower than control ($P < 0.05$). No significant differences were observed between enriched products up to 7.5% ($P > 0.05$). The lowest IAUC value was obtained for 10% PSH incorporation (26% reduction compared to control). Earlier works observed a functional benefit of psyllium supplementation on predicted glycaemic response at 15% for both dry and fresh pasta, but this level was detrimental for product quality (Foschia et al., 2015a; Peressini et al., 2020).

Several mechanisms have been proposed in literature to explain the attenuation of glycaemic response due to DF enrichment: 1) swelling restriction of starch granules during cooking, limiting α -amylase access (Cleary & Brennan, 2006); 2) formation of a physical barrier

protecting starch granules from enzymatic degradation (Brennan & Tudorica, 2008; Cleary & Brennan, 2006); 3) increase in viscosity of the digesta system, reducing glucose diffusion rate and enzymes motility (Ou, Kwok, Li, & Fu, 2001). Based on rheological results (Table 1), PSH incorporation decreased swelling of starch granules ($\Delta\text{Log } G'$), rendering them more compact. A positive linear relationship between IAUC and the rheological parameter $\Delta\text{Log } G'$ was found ($R = 0.898$; $P < 0.05$) as previously established by Peressini et al. (2020) (Table 1). Results stress the importance of structure alteration to modulate the glycaemic response of pasta samples.

4. CONCLUSIONS

Incorporation of 2.5-10% PSH in durum wheat semolina induced changes in rheological properties of doughs, which had important implications for cooking and nutritional properties of enriched pasta. The rise in PSH increased bulk elasticity and solid-like characteristics of doughs due to the structuring ability of psyllium. The latter and its high water binding capacity restricted swelling of gelatinised starch granules as indicated by dynamic temperature sweep test and SEM of cooked pasta samples. The mechanisms by which psyllium influences dough rheological properties seems to be complex. PSH addition weakened gluten structure and concomitantly promoted the formation of a gel network constituted by hydrated fibre aggregates. Due to reduced swelling of starch granules, cooked spaghetti product containing psyllium was firmer compared with control, and solids released in cooking water was not affected (up to 5% PSH) or increased to CL values below the acceptable limit. Stickiness was higher for enriched pasta samples, but values should be not

detrimental for their acceptability. Based on these results, psyllium incorporation at 2.5-10% appears suitable to manufacture fibre-enriched pasta of good quality. PSH addition lowered predictive glycaemic response compared to control providing 26% reduction at the highest level. A positive linear relationship between IAUC and the swelling index $\Delta\text{Log } G'$ was obtained ($R = 0.898$; $P < 0.05$), indicating that attenuation of glycaemic response is related to a more dense structure of gelatinised starch granules.

In conclusion, results suggest that pasta enriched with psyllium up to 5% gave minimum deterioration of cooking properties and a reduction in starch digestion. However, it is allowed to incorporate 10% PSH to maximize the health benefit since alteration of cooking properties may be considered acceptable.

CRedit authorship contribution statement

Niccolò Renoldi: Investigation, Formal analysis, Visualization, Writing - original draft.

Charles-Stephen Brennan: Writing – review & editing. **Corrado Lagazio:**

Donatella Peressini: Supervision, Conceptualization, Methodology, Resources, Writing – review & editing.

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Declaration of interests

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CAPTIONS OF FIGURES

Figure 1. Farinograms of durum wheat semolina (A), 2.5% (B) or 10% PSH blends (C).

Figure 2. Storage modulus and loss tangent as a function of frequency at 25 °C for doughs at water absorption of 64%. Control (rhombus) and dough enriched with 2.5% (square), 5% (circle), 7.5% (triangle) or 10% PSH (star). G' (A); $\tan \delta$ (B).

Figure 3. Storage modulus at 1 Hz as a function of temperature for doughs at water absorption of 64%. Control (a) and dough enriched with 2.5% (b), 5% (c), 7.5% (d) or 10% PSH (e).

Figure 4. SEM images of raw pastas. Control (A) and pasta enriched with 2.5% (B), 5% (C), 7.5% (D) or 10% PSH (E). Magnifications of 500x (A₁-E₁) and 1000x (A₂-E₂).

Figure 5. SEM images of cooked pastas. Control (A) and pasta enriched with 2.5% (B), 5% (C), 7.5% (D) or 10% PSH (E). Magnifications of 500x (A₁-E₁) and 1000x (A₂-E₂).

Figure 6. Glucose release as a function of *in vitro* digestion time of pasta. Mean \pm SEM (n=3). Control, pasta containing 2.5% or 5% PSH (A); Control, pasta containing 7.5% or 10% PSH (B).

Figure 7. IAUC values for pasta samples containing various levels of PSH. Mean \pm SEM (n=3). Means with the same letter are not significantly different ($P > 0.05$).

CAPTIONS OF TABLES

Table 1. Effect of PSH inclusion on water absorption and rheological properties of durum wheat doughs.

Table 2. Effect of PSH inclusion on colour of raw and cooked spaghetti.

Table 3. Effect of PSH inclusion on pasta cooking characteristics at optimum cooking time (OCT).