LWT - Food Science and Technology 151 (2021) 112203

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Evaluation of technological properties, microstructure and predictive glycaemic response of durum wheat pasta enriched with psyllium seed husk

Niccolò Renoldi [a], Charles Stephen Brennan [b,c], Corrado Lagazio [d], Donatella Peressini [a,*]

[a] Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via Sondrio 2/A, 33100, Udine, Italy
[b] School of Sciences, RMIT University, Melbourne, Australia
[c] Department of Wine, Food and Molecular Biosciences, Lincoln University, P O Box 84, Lincoln, 7647, Christchurch, New Zealand
[d] Department of Economics and Business Studies, University of Genoa, via Vivaldi 5-16126, Genoa, Italy

ARTICLE INFO

Keywords:
Spaghetti
Dietary fibre
Starch digestion
Dough
Rheology

ABSTRACT

Psyllium seed husk (PSH) is a soluble dietary fibre with interesting health benefits, including reduction in blood glucose level in subjects with type 2 diabetes. Its supplementation in pasta represents a challenge due to a negative impact of high PSH levels on product acceptability. The aim of this work was to investigate the effects of the substitution of semolina with different levels of PSH on cooking properties, microstructure and *in vitro* glycaemic response of pasta. Dry pasta samples enriched with PSH were produced by replacing durum wheat semolina with 25, 50, 75 and 100 g/kg PSH. Cooked enriched pastas were firmer and sticker than the control. Cooking loss increased with increasing PSH levels above 25 g/kg with values below the technological acceptable limit of 80 g/kg. Semolina substitution with 50–100 g/kg psyllium was effective in lowering the predictive glycaemic response of enriched pasta in comparison with the control. Scanning electron microscopy and dough rheology (dynamic frequency and temperature sweep tests) suggested that this decrease was related to the formation of fibre aggregates limiting starch swelling. Semolina replacement with 50 g/kg PSH has the potential to provide a health benefit with minimal impact on cooking features of functional pasta.

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 (Rao, Mulvaney, Dexter, Edwards, & Peressini, 2001; Sissons, 2008).

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 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;

Abbreviations: G', storage modulus; G″, loss modulus; tan δ, 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; WA, water absorption.

* Corresponding author.
E-mail address: donatella.peressini@uniud.it (D. Peressini).

https://doi.org/10.1016/j.lwt.2021.112203
Received 27 March 2021; Received in revised form 25 June 2021; Accepted 23 July 2021
Available online 27 July 2021

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 700 g/kg of the chemical composition of psyllium seed husk, where arabinose and xylose are the most abundant monosaccharides (625 g/kg) (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, Peighambardoust, Beikzadeh, Asghari Javar-Abadi, & Homayouni-Rad, 2016; Kamaljit, Amarjeet, & Tarvinder Pal, 2011; Pejcz, Spychaj, Wojciechowicz-Budzisz, & Gil, 2018), while Brennan, Derbyshire, Brennan, and Tiwari (2012) fortified extruded snacks with psyllium. In literature, 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 150 g/kg substitution and found a reduction in glyacemic response for cooked fresh-extruded pasta and dry pasta (Foschia et al., 2015a; Peressini et al., 2020). Unfortunately, supplementation was detrimental for pasta cooking behaviour, and also for sensory acceptability (Foschia, Peressini, Sensidoni, Brennan, & Brennan, 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

PSH (particle size < 0.149 mm, 900 g/kg dietary fibre) was kindly offered by Azelis (Trezzano sul Naviglio, Milan, Italy), while durum wheat semolina (134 g/kg protein, 127 g/kg moisture, 30 g/kg dietary fibre, and 56 g/kg ash) was purchased from Molino Sgambaro (Treviso, Italy). Pepsin from porcine gastric mucosa (EC 3.4.23.1; 268 kat/mg), α-amylase from porcine pancreas (EC 3.2.1.1; 0.54 kat/mg), bile extract from porcine (B8631), pancreatin from porcine pancreas (EC 232-468-9; 0.68 kat/mg), amyloglucosidase from *Aspergillus niger* (EC 3.2.1.3; 90 kat/mL), L-(+)-arabinose, D-(−)-fructose, D-(+)-glucose, $CaCl_2(H_2O)_2$, $NaHCO_3$, NaCl, KCl, KH_2PO_4, $MgCl_2(H_2O)_6$, and $(NH_4)_2CO_3$ 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 PSH were prepared by substituting durum wheat semolina with PSH at 25 g/kg (PSH25), 50 g/kg (PSH50), 75 g/kg (PSH75) and 100 g/kg (w/w) (PSH100), while control was made using only durum wheat semolina. Blends were utilised for dough preparation and pasta making.

2.3. Pasta production

Pasta was manufactured using a pilot pasta press under processing condition similar to that of industrial scale (PST95 model, Pavan, Galliera Veneta, Padua, Italy). Semolina and PSH were blended for 15 min using a flour ribbon mixer (FM50 model, Pavan, Galliera Veneta, Padua, Italy) and transferred to the pasta press (mixer-extruder). Semolina or fibre blends (20 kg) and water (6.1–7.9 kg) were mixed for 15 min under a partial vacuum (−0.8 bar pressure relative to atmospheric pressure) to give a uniform hydration of particles. After hydration, the mass was transferred to a single screw extruder (0.09 m screw diameter; 10 L/D ratio; 0.22 m head diameter) equipped with a spaghetti-shaped die. Processing parameters were monitored during extrusion and were as follow: 10 rpm screw speed, 37 °C barrel and extrusion head temperature, and about 12 MPa head pressure. Fresh pasta was dried at 50 °C and 80 % relative humidity for 12 h in a static dryer (SD 100 model, Pavan, Galliera Veneta, Padua, Italy). Diameter and moisture of dry pasta were 1.5–1.6 mm and 110 g/kg, respectively.

2.4. Farinograph test

Dough water absorption (g/kg, on 140 g/kg moisture basis) and stability (min) of control and PSH blends were determined using a farinograph (Promylograph T6, Max Egger, Blasen, Austria) in accordance with AACC Approved Method 54–21 (AACC, 2000). Water was added to 100 g of samples (on 140 g/kg moisture basis) to obtain a dough consistency of 500 Promylograph Units (PU). A lower sample amount (80 g) and dough consistency (400 PU) was used for PSH10 due the presence of clumps during mixing. Results are the average of three replicates.

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 640 g/kg (moisture content of 476 g/kg) were prepared in the farinograph mixer until maximum development plus 1 min (5 min for control, 6 min for PSH25, 7 min for PSH50, 8 min for PSH75 and 10 min for PSH100) (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 at a constant shear stress (5–25 Pa) 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 in LVR, immediately after frequency sweep test on the same dough sample. Storage modulus G′, loss modulus G″, and loss tangent tan δ (G″/G′ ratio) were measured. Rheological parameters were compared at 1 Hz. All measurements were conducted in triplicate, where each replicate represents a separately mixed dough.

2.6. Colour of pasta

Colour of both raw and cooked pasta was assessed using a tristimulus colorimeter equipped with a CR-400 head and illuminant C (Minolta Camera Co. Osaka, Japan). 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). Raw spaghetti strands (thirty) were put in a single layer and nine readings were performed on the surface. Then, pasta was cooked at optimum cooking time (OCT), drained and equilibrated 5 min before measuring colour as described for uncooked product. A paper towel was used to absorb water on the surface layer of cooked spaghetti just before testing. Results are the average of three replicates, where each replicate represents a separately cooking batch.

2.7. Pasta cooking quality

OCT, cooking loss (CL) and firmness of cooked pasta were evaluated according to the Approved Method 66–50 (AACC, 2000).

Spaghetti samples were cooked to their OCT, which was the time it took for the inner white core to disappear. Raw spaghetti samples (25 g) were broken (5 cm pieces) and cooked in boiling tap water (300 mL) with no added salt. Cooking and rinse water of cooked pasta were combined to evaluate CL, while cooked pasta was collected for moisture content and firmness determinations. CL (g/kg) corresponds to the content of solids released in water during cooking. Moisture content (g/kg) was determined as loss in weight of cooked samples (3 g) when heated at 105 °C for 15 h.

A Texture Analyser (TA.XT plus, Stable Micro Systems Ltd. Godalming, UK), which was equipped with a load cell of 5 kgf, was used to evaluate textural properties of cooked pasta. After cooking before being tested, the sample was rested in a covered container at 25 °C for 10 min. Firmness was determined using a light knife blade (A/LKB) at a cutting speed 0.17 mm/s. Peak force (N) achieved during the cutting cycle of five pasta strands was used as measure of pasta firmness.

Pasta strands (drained but not rinsed) were evaluated for stickiness 10 min after cooking using a pasta firmness/stickiness rig (HDP/PFS) at compression force of 1 kg for 2 s and a compression speed of 0.5 mm/s. Stickiness (N) corresponded to the peak force necessary to separate the probe from the surface of five strands upon its retraction.

Results are the average of three replicates, where each replicate represents a separately cooking batch. For each replicate the following analytical measurements were performed: one for CL, three for moisture content, eight for firmness and four for stickiness.

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 (500× 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 (g/g) with SSF and α-amylase solution prepared in distilled water to achieve 4.5 kat/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 (mL/mL) of the oral bolus with SGF and pepsin solution prepared in distilled water to achieve 120 kat/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 (mL/mL) of the gastric chyme with SIF, pancreatin and bile extract solutions prepared in SIF to achieve 6 kat/mL and 0.01 mol/L, 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 980 mL/L (1:4, mL/mL).

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 (model 230, Varian Chromatography Systems, Palo Alto, California, USA) equipped with a chromatographic column (Robusta 100A, NH2, 5 μL, 250 mm × 4.6 mm, Sepachrom Srl, Rho, Italy) and a refractive index detector (model RID-10A, Shimadzu, Kyoto, Japan) as reported by Renoldi, Peighambardoust, and Peressini (2021). L-(+)-arabinose was used as the internal standard for the glucose calibration curve according to Englyst, Englyst, Hudson, Cole, and Cummings (1999).

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). Results are the average of three replicates, where each replicate represents a separately cooking batch. For each replicate two analytical measurements were performed.

2.11. Statistical analysis

Experiments were performed in triplicate (n = 3), where each replicate corresponds to separately mixing batch and cooking batch for rheological tests and pasta characterisation, respectively. Data are reported as mean ± 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 doughs containing psyllium. WA increased linearly with PSH level from 571 g/kg to 1320 g/kg for control and 100 g/kg 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 (A) and samples added with 25 g/kg (B) and 100 g/kg PSH (C). The control sample exhibited a

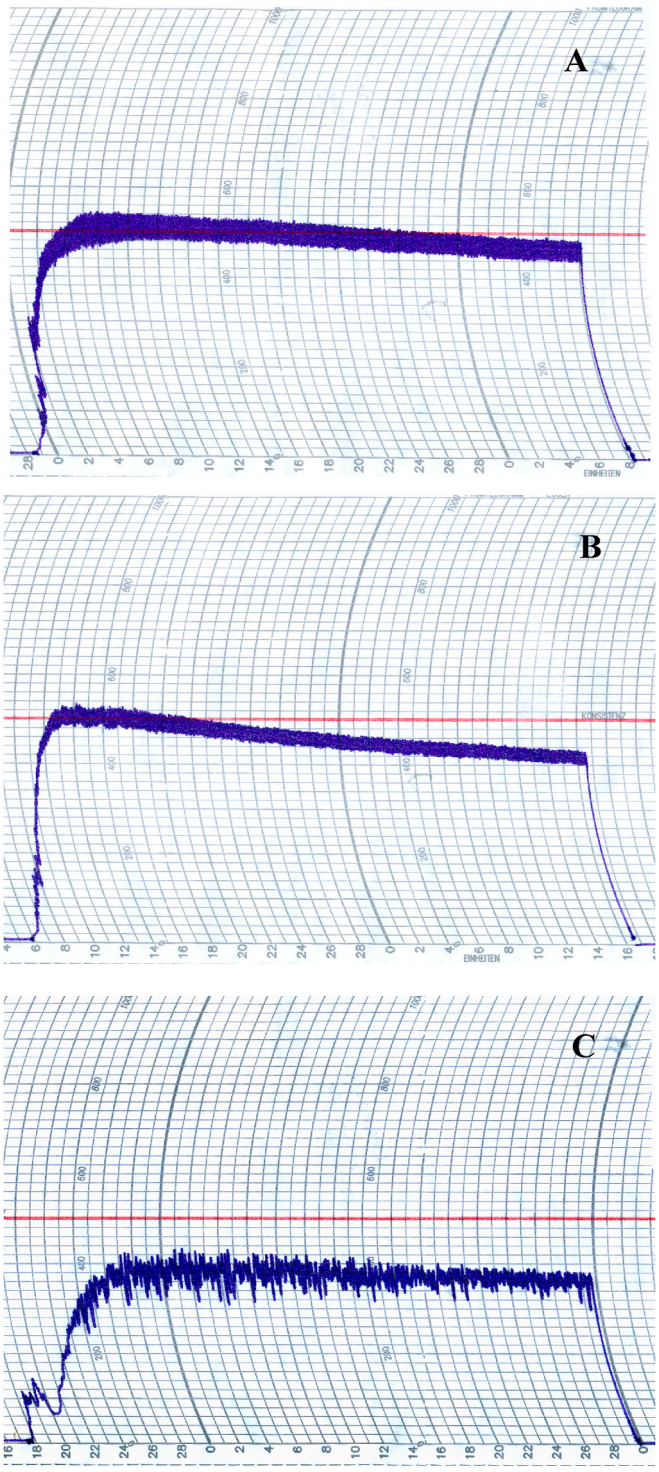

Fig. 1. Dough rheological properties from farinograph test. Consistency as a function of mixing time for doughs with and without psyllium seed husk (PSH). Semolina substitution with PSH: 0 g/kg or control (A), 25 g/kg (B) and 100 g/kg (C).

high tolerance to overmixing, which is consistent with a strong dough (Fig. 1A). Inclusion of 25 g/kg PSH decreased the mixing stability indicating a weakening effect on gluten network (Fig. 1B, Table 1). The same behaviour was observed at 50 g/kg and 75 g/kg PSH (not shown). No significant differences in stability were observed between doughs with fibre up to 75 g/kg PSH ($P > 0.05$) (Table 1). In contrast, dough at 100 g/kg PSH reached maximum consistency and then remained constant for 16 min during mixing (Fig. 1C, Table 1). The recorded curve of 100 g/kg 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 doughs containing psyllium are shown in Fig. 2. A weak gel-like behaviour was observed since storage modulus (G′) is frequency dependent and tan δ 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 150 g/kg PSH-semolina dough ($P < 0.05$) (Peressini et al., 2020). The rise in PSH content increased G′ and decreased tan δ 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 δ 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 rheological results (higher G′ and lower tan δ values for PSH 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 gelatinization, 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 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 150 g/kg PSH and β-glucans individually added and in combination in substitution of semolina (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), $\log_{10} G'_{peak} - \log_{10} G'_{min}$ difference was calculated to estimate the extent of starch swelling in different samples ($\Delta\log_{10} G'$), as proposed by Peressini et al. (2020). Values of $\Delta\log_{10} G'$ were significantly lower for PSH doughs and decreased with psyllium level ($P < 0.05$) (Table 1). This parameter was in the range of 0.31–0.70 Pa for PSH samples and 1.23 Pa 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.

Table 1
Effect of semolina substitution with psyllium seed husk (PSH) on water absorption and rheological properties of durum wheat doughs.

Samples	WA (g/kg)[a]	ST (min)[a]	G′ (kPa)[b]	tan δ (−)[b]	T_{peak} (°C)[c]	G'_{peak} (kPa)[c]	tan δ_{peak} (−)[c]	$\Delta\log_{10}$ G′ (Pa)[c]
Control	571 ± 0^{e}	12.3 ± 0.4^{b}	15 ± 1^{e}	0.39 ± 0.01^{a}	82.4 ± 1.0^{ns}	168 ± 8^{a}	0.15 ± 0.01^{c}	1.23 ± 0.03^{a}
PSH25	729 ± 1^{d}	5.2 ± 0.4^{c}	41 ± 2^{d}	0.32 ± 0.01^{b}	82.6 ± 1.1^{ns}	133 ± 1^{b}	0.17 ± 0.01^{b}	0.70 ± 0.01^{b}
PSH50	909 ± 2^{c}	5.3 ± 0.5^{c}	52 ± 3^{c}	0.29 ± 0.01^{c}	81.4 ± 0.8^{ns}	120 ± 3^{c}	0.18 ± 0.01^{ab}	0.56 ± 0.02^{c}
PSH75	1114 ± 1^{b}	4.9 ± 0.1^{c}	80 ± 1^{b}	0.27 ± 0.00^{d}	81.7 ± 1.0^{ns}	120 ± 3^{c}	0.19 ± 0.01^{a}	0.41 ± 0.01^{d}
PSH100	1320 ± 2^{a}	15.9 ± 0.3^{a}	97 ± 3^{a}	0.25 ± 0.01^{e}	84.0 ± 0.7^{ns}	120 ± 3^{c}	0.19 ± 0.01^{a}	0.31 ± 0.01^{e}
Pooled SD	1	0.3	2.2	0.01	1.0	4.0	0.01	0.02

Mean ± standard deviation (n = 3). Values within a column followed by the same letter are not significantly different, Tukey test (P > 0.05). Values within a column followed by ns indicate that treatments are not statistically different, Kruskal-Wallis test (P > 0.05).
Semolina substitution with PSH: 0 (Control), 25 (PSH25), 50 (PSH50), 75 (PSH75) or 100 g/kg (PSH100).
[a] Farinograph water absorption (WA) and stability (ST).
[b] Values at 1 Hz obtained from the frequency sweep test at 25 °C on dough.
[c] Values obtained from the temperature sweep test on dough; $\Delta\log_{10} G' = (\log_{10} G'_{peak} - \log_{10} G'_{min})$.

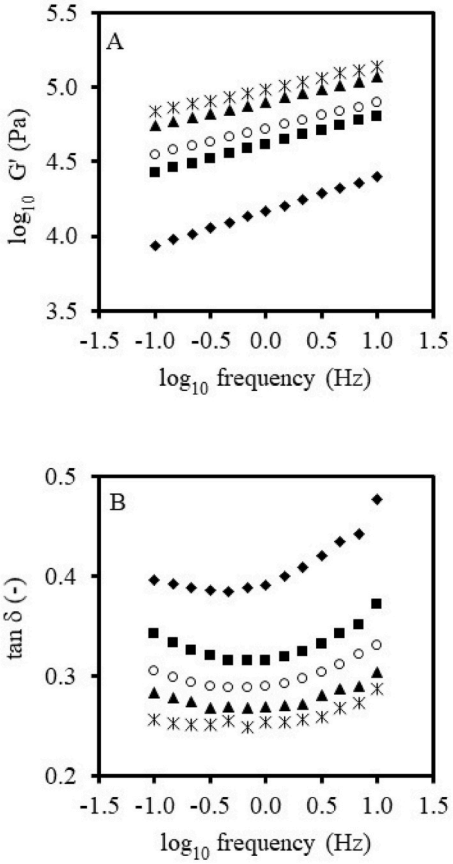

Fig. 2. Dough rheological properties from frequency sweep test. Storage modulus (G′) and loss tangent (tan δ) as a function of frequency at 25 °C for doughs with and without psyllium seed husk (PSH) at water absorption of 640 g/kg. Semolina substitution with PSH: 0 g/kg or control (rhombus), 25 g/kg (square), 50 g/kg (circle), 75 g/kg (triangle) and 100 g/kg (star). G′ (A); tan δ (B).

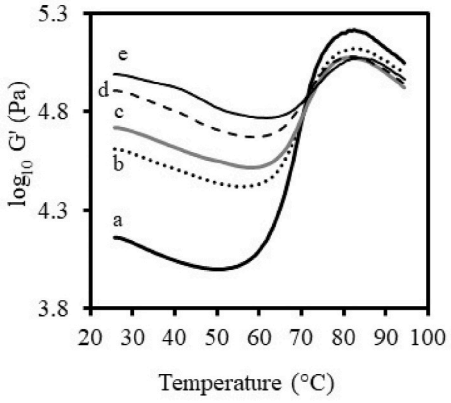

Fig. 3. Dough rheological properties from temperature sweep test. Storage modulus (G′) at 1 Hz as a function of temperature for doughs with and without psyllium seed husk (PSH) at water absorption of 640 g/kg. Semolina substitution with PSH: 0 g/kg or control (a), 25 g/kg (b), 50 g/kg (c), 75 g/kg (d) and 100 g/kg (e).

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 fibre 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. 5B_2-E_2). This is consistent with rheological results ($\Delta\log_{10}$ 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 75 g/kg and 100 g/kg, swollen starch granules appeared well enveloped in psyllium network (Fig. 5D_2-E_2).

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 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 50 g/kg fibre supplementation, yellowness (b*) was about 4–5% 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 150 g/kg psyllium alone and

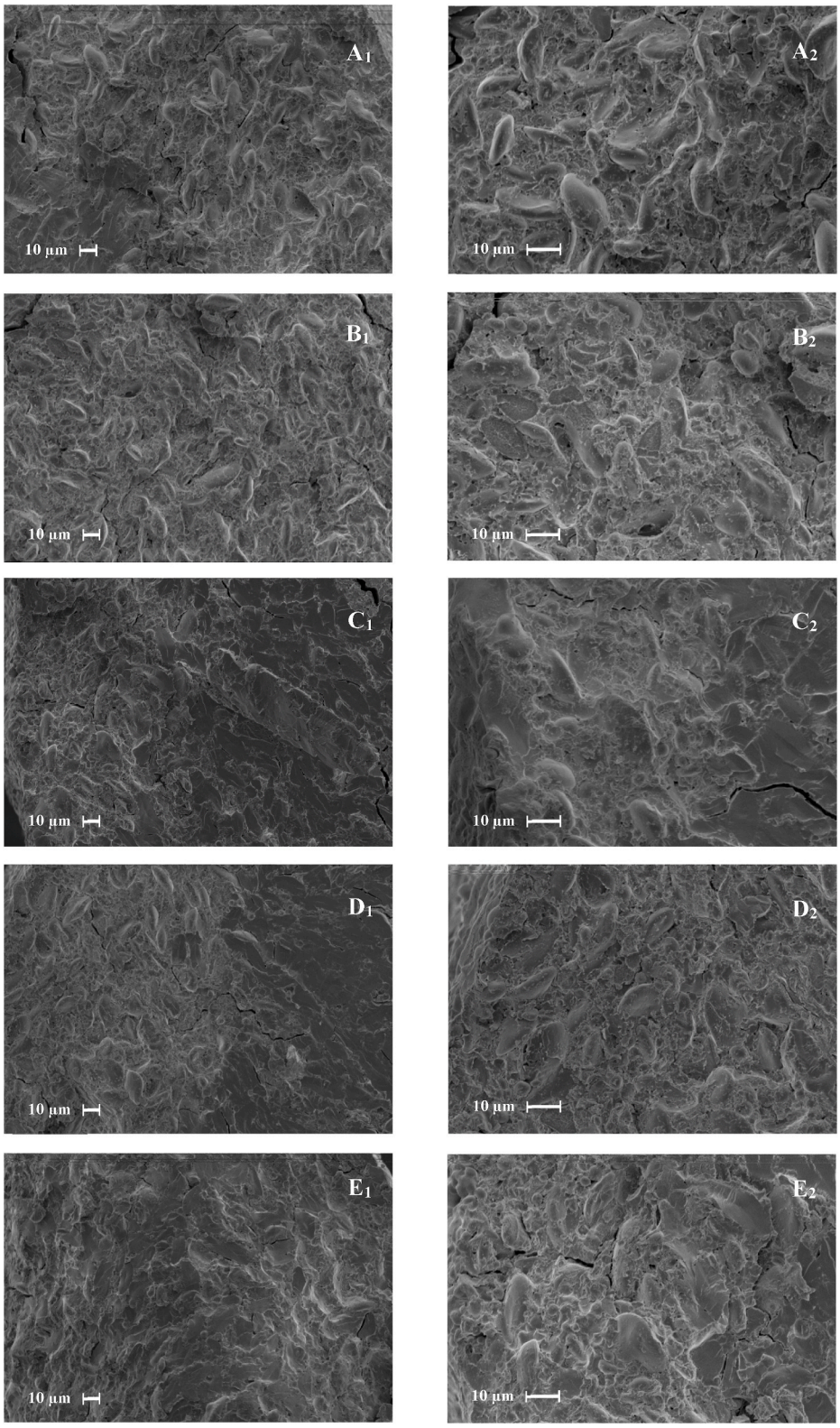

Fig. 4. Scanning electron microscopy images of raw pastas with and without PSH. Semolina substitution with psyllium seed husk (PSH): 0 g/kg or control (A), 25 g/kg (B), 50 g/kg (C), 75 g/kg (D) and 100 g/kg (E). Magnifications of 500× (A_1-E_1) and 1000x (A_2-E_2).

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 150 g/kg psyllium (Foschia et al., 2015b). Previously, colour appearance score of cooked pasta was not changed upon addition of 75 g/kg PSH plus 75 g/kg 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 did not give significant differences in OCT and

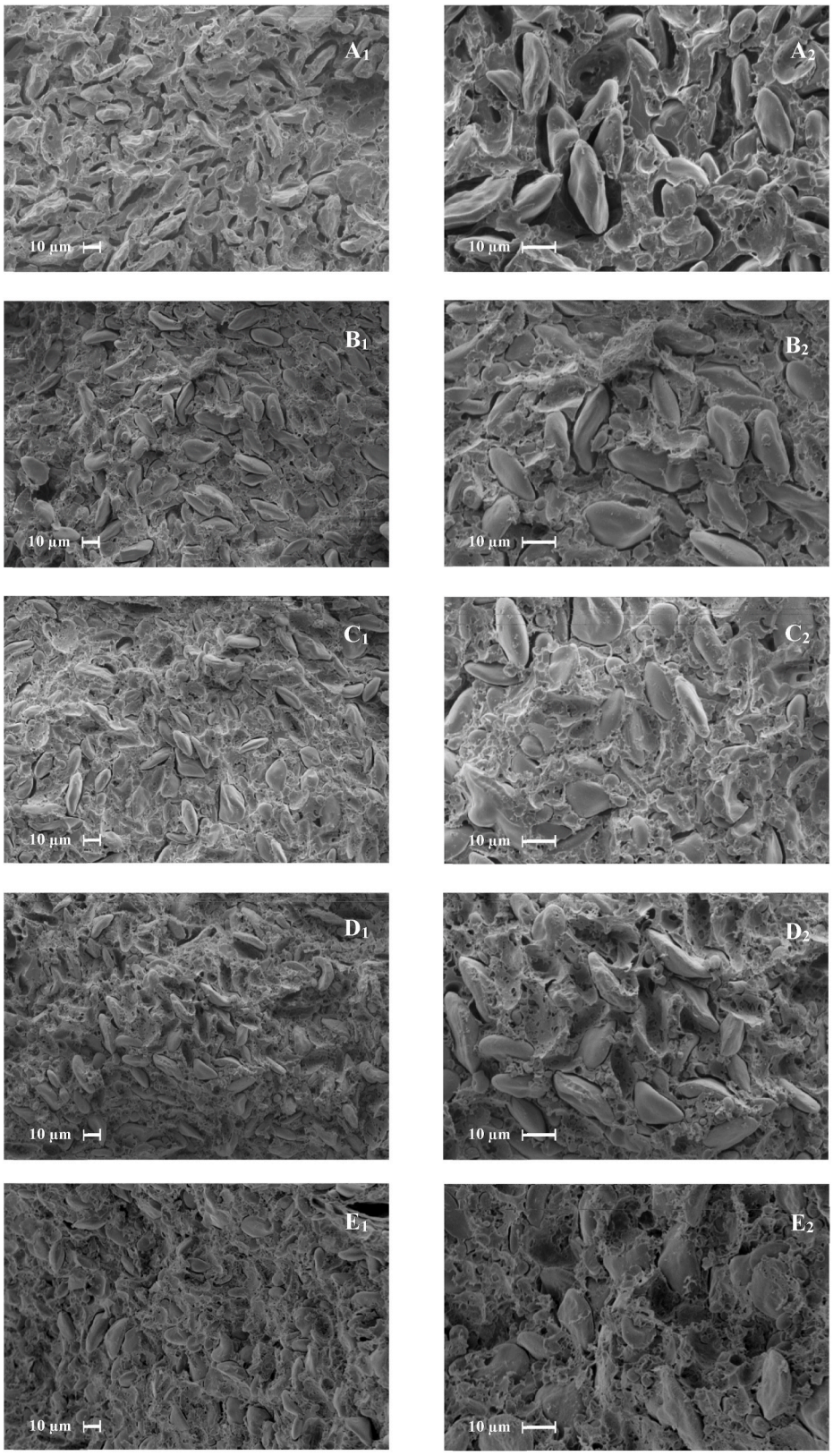

Fig. 5. Scanning electron microscopy images of cooked pastas with and without psyllium seed husk (PSH). Semolina substitution with PSH: 0 g/kg or control (A), 25 g/kg (B), 50 g/kg (C), 75 g/kg (D) and 100 g/kg (E). Magnifications of 500× (A_1-E_1) and 1000x (A_2-E_2).

Table 2
Effect of semolina substitution with psyllium seed husk (PSH) on colour of raw and cooked spaghetti.

Samples	L^*_{raw}	a^*_{raw}	b^*_{raw}	L^*_{cooked}	a^*_{cooked}	b^*_{cooked}
Control	68.6 ± 0.2^{a}	1.3 ± 0.1^{e}	40.1 ± 0.6^{a}	72.0 ± 0.2^{a}	−2.2 ± 0.1^{e}	25.1 ± 0.2^{a}
PSH25	66.9 ± 0.3^{b}	2.4 ± 0.2^{d}	39.6 ± 0.7^{a}	70.9 ± 0.3^{b}	−1.2 ± 0.1^{d}	25.1 ± 0.6^{a}
PSH50	65.5 ± 0.5^{c}	4.1 ± 0.2^{c}	39.4 ± 0.3^{ab}	70.7 ± 0.2^{b}	−0.7 ± 0.1^{c}	24.2 ± 0.2^{b}
PSH75	64.4 ± 0.2^{d}	4.6 ± 0.1^{b}	38.4 ± 0.4^{b}	70.0 ± 0.4^{c}	0.4 ± 0.2^{b}	23.7 ± 0.4^{bc}
PSH100	63.8 ± 0.3^{e}	5.1 ± 0.2^{a}	38.1 ± 0.3^{b}	69.6 ± 0.2^{d}	0.7 ± 0.1^{a}	23.3 ± 0.3^{c}
Pooled SD	0.2	0.1	0.4	0.2	0.1	0.3

Mean ± standard deviation (n = 3). Values within a column followed by the same letter are not significantly different, Tukey test (P > 0.05). Values within a column followed by ns indicate that treatments are not statistically different, Kruskal-Wallis test (P > 0.05). Semolina substitution with PSH: 0 (Control), 25 (PSH25), 50 (PSH50), 75 (PSH75) or 100 g/kg (PSH100).

moisture content in comparison with control (P > 0.05) (Table 3). Moisture content did not change with increasing PSH addition probably due to the decrease in swelling of starch granules.

CL was not influenced by PSH addition at 25 g/kg, above this value a significant increase in CL of about 4–8% was observed (P < 0.05) (Table 3). CL represents starchy solids released into the cooking water, which should not go over 70–80 g/kg (on dry weight of the product) for a pasta of good quality (Sissons, Abecassis, Marchylo, & Cubadda, 2012). The results indicate that CL was below the acceptable limit for all pasta samples. Foschia et al. (2015b) and Peressini et al. (2020) reported 90–100 g/kg CL for pasta added with 150 g/kg psyllium. Therefore, functional pasta should contain a PSH level up to 100 g/kg for a good quality. Aravind, et al. (2012) observed an increase in CL above 75 g/kg Barley Balance enrichment. For pasta containing inulin, undesirable values of solids loss were obtained at 75–100 g/kg 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 50 g/kg had no or minimal effect on losses during cooking (Table 1). The lower starch granule swelling prevented breakdown of protein network preserving structural integrity of spaghetti (Delcour, Vansteelandt, Hythier, & Abecassis, 2000). In pasta samples with 75 g/kg and 100 g/kg PSH, increased loss of solids could be mainly attributed to leaching of psyllium polysaccharides.

Table 3
Effect of semolina substitution with psyllium seed husk (PSH) on pasta cooking characteristics at optimum cooking time (OCT).

Samples	OCT (min)	Moisture (g/kg)	CL (g/kg)[a]	Firmness (N)	Stickiness (N)
Control	7.2 ± 0.3^{ns}	684 ± 4^{ns}	59.3 ± 0.8^{c}	1.63 ± 0.05^{c}	1.11 ± 0.08^{d}
PSH25	7.0 ± 0.0^{ns}	678 ± 7^{ns}	58.7 ± 0.8^{c}	1.76 ± 0.04^{b}	1.25 ± 0.04^{c}
PSH50	6.7 ± 0.3^{ns}	681 ± 4^{ns}	61.7 ± 0.6^{b}	1.81 ± 0.03^{b}	1.32 ± 0.03^{bc}
PSH75	6.3 ± 0.3^{ns}	679 ± 8^{ns}	63.5 ± 1.0^{ab}	1.81 ± 0.02^{b}	1.37 ± 0.04^{b}
PSH100	6.5 ± 0.0^{ns}	691 ± 6^{ns}	64.0 ± 2.2^{a}	1.93 ± 0.02^{a}	1.53 ± 0.03^{a}
Pooled SD	0.2	4.1	1.2	0.03	0.05

Mean ± standard deviation (n = 3). Values within a column followed by the same letter are not significantly different, Tukey test (P > 0.05). Values within a column followed by ns indicate that treatments are not statistically different, Kruskal-Wallis test (P > 0.05). Semolina substitution with PSH: 0 (Control), 25 (PSH25), 50 (PSH50), 75 (PSH75) or 100 g/kg (PSH100).

[a] Cooking loss.

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 fibre samples up to 75 g/kg PSH, while above this level cooked pasta became firmer (P < 0.05). Control showed the lowest firmness and the highest $\Delta \log_{10} G'$ values among samples, whereas 100 g/kg 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; Rakhesh et al., 2015). Results are consistent with earlier research on dry pasta with 150 g/kg PSH as semolina substitute (Peressini et al., 2020). In contrast, Foschia et al. (2015b) did not observe differences in firmness between fresh pasta with and without replacement of semolina with 150 g/kg 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 (Rakhesh et al., 2015).

Based on a previous sensory evaluation on pasta obtained by partial semolina replacement with 75 g/kg PSH in combination with 75 g/kg Barley Balance, the enriched sample was perceived firmer and sticker 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 samples added with PSH should be not detrimental for their acceptability.

In conclusion, CL and textural results showed that psyllium supplementation at 25–100 g/kg 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 intestinal phase. Since partial replacement of semolina with PSH decreases the potential glucose release of pasta, the amount of glucose was expressed per gram of semolina (d.b.) in pasta matrix. 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 et al. (1999). Psyllium samples showed RAG values from 98.2 mg/g semolina d.b. for 25 g/kg PSH to 103.5 mg/g semolina d.b. for 75 g/kg PSH, which were significantly lower than control (108.6 mg/g semolina d.b.) (P < 0.05). Enrichment of PSH caused a lowering of 5–10 % 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 at 50 g/kg, 75 g/kg, and 100 g/kg PSH (P < 0.05). No significant differences were observed between PSH products below 100 g/kg (P > 0.05). The lowest IAUC value was obtained for 100 g/kg PSH incorporation. Earlier works observed a functional benefit of psyllium supplementation on predicted glycaemic response at 150 g/kg for both dry and fresh cooked pasta, but this level was detrimental for product quality (Foschia et al.,

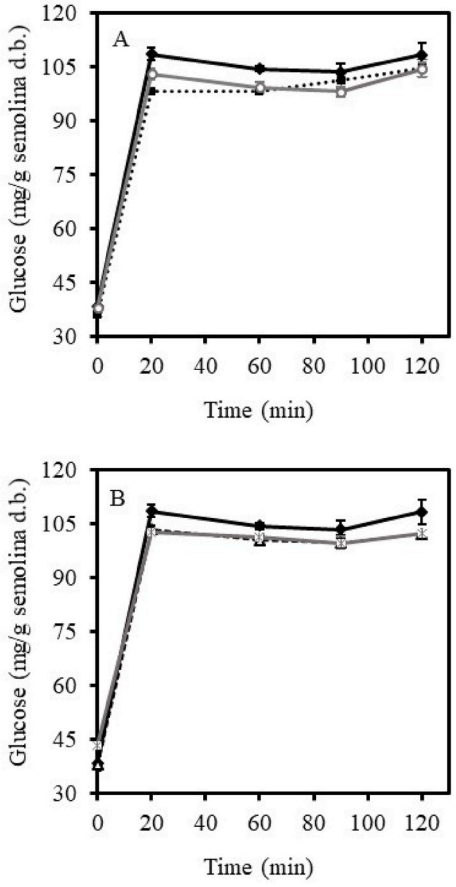

Fig. 6. *In vitro* digestion of cooked pastas with and without psyllium seed husk (PSH). Glucose release as a function of digestion time. Mean ± SEM (n = 3). Semolina substitution with PSH: 0 g/kg or control (rhombus), 25 g/kg (square) and 50 g/kg (circle) (A); 0 g/kg (rhombus), 75 g/kg (triangle) and 100 g/kg (star) (B).

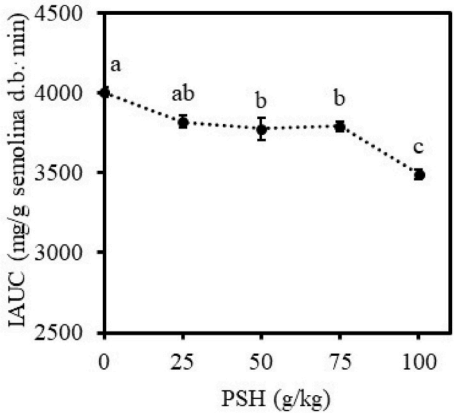

Fig. 7. *In vitro* digestion of cooked pastas with and without psyllium seed husk (PSH). IAUC values of pasta samples as a function of semolina substitution with PSH. Mean ± SEM (n = 3). Means with the same letter are not significantly different (P > 0.05). IAUC: incremental area under the curve.

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 ($\Delta\log_{10}$ G′), rendering them more compact. A positive linear relationship between IAUC and the rheological parameter $\Delta\log_{10}$ G′ was found (R = 0.86; 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

This is the first study investigating the impact of semolina replacement with different levels of PSH on physicochemical, microstructural and nutritional properties of dry pasta. Moreover, both empirical and fundamental rheological tests on the dough were carried out to obtain information on the structuring ability of PSH and changes in starch gelatinization. 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 in the dough and cooked pasta, resulting in a firmer cooked pasta with minimal or acceptable increase in solids released in cooking water. Nutritional results highlighted that semolina substitution with at least 50 g/kg is required to attenuate predictive glycaemic response compared to control. Considering other soluble fibres, the glycaemic response decreased for pasta with semolina substitution up to 50 g/kg inulin (polymerisation degree of 12–14), which was not detrimental for cooking properties, but above this value starch digestion increased (Aravind, Sissons, & Fellows, 2012). Moreover, replacement of 75 g/kg semolina with Barley Balance (a source of β-glucans) was able to lower starch digestion without changes in CL and stickiness of cooked pasta (Aravind, Sissons, Egan, et al., 2012).

Dynamic temperature sweep test allowed to identify a rheological index of gelatinised starch swelling, which gave a positive linear relationship with the glycaemic response of pasta. This result is particularly remarkable for the formulation of a functional product indicating that alteration in food structure is important to modulate the glycaemic response. More compact starch granules in cooked pasta seems to be related to a lower glycaemic response.

Semolina replacement with 50 g/kg has the potential to provide a health benefit with minimal impact on cooking characteristics of pasta appearing suitable for the food industry. The highest level of substitution maximized the health benefit and induced acceptable alterations in cooking properties.

In conclusion, PSH seems to be an interesting fibre for the production of functional pasta.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Niccolò Renoldi: Investigation, Formal analysis, Visualization, Writing – original draft. **Charles Stephen Brennan:** Writing – review & editing. **Corrado Lagazio:** Formal analysis. **Donatella Peressini:** Supervision, Conceptualization, Methodology, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Pavan S.p.a. (Galliera Veneta, Padua, Italy) is gratefully acknowledged for making plants available to this research. Authors thanks Dr. Luciano Mondardini and technologists from Pavan for pasta-making. Psyllium fibre was kindly donated by Azelis (Trezzano sul Naviglio, Milan, Italy).

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