Synergistic effect of different dietary fibres in pasta on in vitro starch digestion?

Martina Foschia, Donatella Peressini, Alessandro Sensidoni, Margaret Anne Brennan, Charles Stephen Brennan

Department of Food Science, University of Udine, via Sondrio 2/A, 33100 Udine, Italy
Department of Wine, Food and Molecular Biosciences, Lincoln University, PO Box 84, Lincoln 7647, Christchurch, New Zealand

Article info
Article history:
Received 23 May 2014
Received in revised form 30 July 2014
Accepted 13 September 2014
Available online 23 September 2014

Keywords:
Pasta
Dietary fibres interactions
In vitro digestion

Abstract
Pasta is traditionally manufactured using only durum wheat semolina, but it is possible to incorporate other flours or ingredients into pasta in order to increase its nutritional value to the consumer, compared to conventional pasta. For this reason, pasta was prepared substituting durum wheat semolina with 15% of enriched dietary fibre flours (Glucagel, inulin Raftiline HPX, inulin Raftiline GR, psyllium and oat). Moreover, all dietary fibres (excluded Glucagel) were added in combination in order to evaluate their possible antagonistic or synergic effect on predicted glycaemic response. In general, all enriched dietary fibre pasta sample showed a significant decrease (except for pasta containing a combination of 7.5% inulin Raftiline GR and 7.5% oat bran flour) in reducing sugars released and standardised AUC values compared to control pasta. However, this study showed that the combination of dietary fibres in pasta formulation led to an antagonistic effect on the predicted glycaemic response.

1. Introduction

The glycaemic index (GI) is a parameter that allows for the classification of foods based on their postprandial blood glucose responses (Jenkins et al., 1981). The main factor that influences GI is the rate of digestion or absorption of the carbohydrates present in a food. Several studies have demonstrated that foods with high GI are rapidly digested and absorbed resulting in marked fluctuations in blood glucose levels and greater insulin demand. On the contrary, low GI products are the ones slowly digested and absorbed, resulting in gradual rise in blood glucose and insulin levels (Augustin, Franceschi, Jenkins, Kendall, & La Vecchia, 2002). Durum wheat semolina is the traditional raw material for the production of high quality pasta that is considered to be a low to medium glycaemic index food (Granfeldt & Björck, 1991). A further reduction of the GI in starch-based foods, such as pasta, can be obtained with the utilisation of dietary fibres (Behall, Scholfield, & Hallfrisch, 2005; Chillo, Ranawana, & Henry, 2011a; Foschia, Peressini, Sensidoni, & Brennan, 2013), which have been associated with decreased risks of developing chronic diseases such as colon cancer, atherosclerosis, diabetes, hypertension and obesity (Mudgil & Barak, 2013).

Based on this knowledge, many studies were carried out in order to increase dietary fibre (DF) intake in human diet by using two different strategies:

1) substitution of durum wheat semolina with non-conventional flours such as amaranth, chickpea, broad bean, quinoa, buckwheat, oat flours (Bustos, Perez, & Leon, 2013; Fiorda, Soares Jr., da Silva, Souto, & Grosmann, 2013; Giménez et al., 2012; Wolter, Hager, Zannini, & Arendt, 2013);
2) enrichment of semolina with dietary fibre ingredients such as inulin, β-glucans and arabinoxylans (Brennan, Kuri, & Tudorica, 2004; Bustos, Perez, & León, 2011; Chillo et al., 2011a; Chillo, Ranawana, Pratt, & Henry, 2011b; De Pili, Derossi, & Severini, 2013).

Among the flours rich in dietary fibre, psyllium and oat flour have been analysed in the current study. Psyllium flour is a material prepared from the seed husk of the Plantago genus and it has been recognised as a rich source of dietary fibre, in particular arabinoxylan (Van Craeyveld, Delcour, & Courtin, 2008). Psyllium seed husk arabinoxylan is a highly branched...
polysaccharide with a main chain of densely substituted β-(1,4)-linked xylopyranose residues. Substituents include single arabinofuranose and xylopyranose residues or short side chains consisting of these monosaccharides and rhamnose and galacturonic acid residues. Psyllium has been investigated for its potential health benefits and is well recognised for its laxative activity, cholesterol lowering capacity, potential in reducing the risk of colon cancer and hyperglycemia, and possible application in the treatment of irritable bowel syndrome and in body weight control (Yu, Lutterodt, & Cheng, 2008).

Oat (Avena sativa) has been recognised positive health effects such as lowering of serum cholesterol and attenuation of blood glucose thanks to its soluble fibre compound, β-glucan (Butt, Tahir-Nadeem, Khan, Shabir, & Butt, 2008). Mixed-linkage (1 → 3), (1 → 4)-β-D-glucan, or β-glucan, is a linear and partially water soluble polysaccharide that consists only of glucose.

Another interesting ingredient widely used as dietary fiber in a variety of foods is inulin. Inulin is a polydisperse fructan consisting mainly of β-fructose joined by β-(2 → 1) linkages. Sometimes, the last fructose may be linked with a glucose by an α-(1 → 2) bond as in sucrose. The main sources of inulin that are used in the food industry are chicory and Jerusalem artichoke (Bornet, 2008). The degree of polymerisation (DP) of chicory fructans varies from 2 to 60 and the physico-chemical properties of inulin are linked to the degree of polymerisation (Roberfroid, 2005).

The food industry is always endeavouring to include high levels of DF into cereal food products from a health and nutrition standpoint so inclusion of 15% is of use from a product point of view and also a legislative viewpoint (with potential health claims in some markets as high in fibre or rich source of dietary fibre – being allowed).

The purpose of the present work was to substitute durum wheat semolina in pasta production with different types of DFs added individually and in combination in order to understand if a synergetic or antagonistic effect can be appreciated on the predicted glycaemic response.

2. Materials and methods

2.1. Materials

Commercial durum wheat semolina was obtained from Molino Borgo San Dalmazzo (Borgo San Dalmazzo-CN, Italy). The composition of the semolina as supplied by the manufacturer was 13% protein, 75% carbohydrate, 12% moisture. Two inulin products from chicory of different degree of polymerisation (DP) were supplied by Orafti Food Ingredients (Belgium): Raftiline® GR (inulin GR, DP = 23) and Raftiline® GR (inulin GR, DP = 10). Sugar content (glucose, fructose and sucrose) was 0.5% d.b. and 12% d.b. for inulin HPX and GR, respectively. Glucagel (GG) with 76.73% of β-glucan content was supplied from DKSH (Italy). Psyllium fibre (85% dietary fibre) and oat bran flours (78% dietary fibre) were purchased from Piko Wholefoods (Christchurch, New Zealand).

2.2. Pasta preparation

Pasta was produced using a fresh pasta machine fitted with a spaghetti die (2.25 mm diameter of die hole) (model: MPF15N235M, Firmar, Villa Verucchio, RN, Italy). Each blend (1.2 kg) was mixed for 4 min in order to ensure uniform moisture of semolina and DF fortified flours. The conditions applied were the following: tap water temperature 41 °C, dough moisture content 30% and mixing time 20 min according to the manufactures guidelines. Extruded fresh pasta samples (20 g) were put in sealed bag, frozen and kept at −18 °C for in vitro digestion analysis.

Ten different samples supplemented with DFs were produced substituting durum wheat semolina with the following dietary fibres: oat bran (O), psyllium (P), Glucagel (GG), inulin GR (GR) or inulin HPX (HPX). Fibre enriched formulations replacing up to 15% (w/w) semolina are detailed in Table 1. Control sample (C) was prepared using 100% durum wheat semolina.

2.3. Cooking loss

Cooking loss (CL, %) was determined according to Approved method 66-50 (AACC, 2000).

2.4. In vitro digestion analysis

Frozen pasta (20 g) was defrosted for 10 min at room temperature and cooked in boiling tap water (600 mL) to optimum time (6.30–12 min) and cut with knife in order to obtain a 2–5 mm size.

The potential amount of glucose released over 120 min was conducted in triplicate using 2.5 g samples for each pasta type as described previously (Brennan, Derbyshire, Tiwari, & Brennan, 2012). In brief: digestions were carried out in 60 mL plastic biopsy pots placed on a pre-heated 15 place magnetic heated stirring block (IKAMAG® RT15, IKA®-WERKE GmbH & Co, Staufen, Germany). A sample (2.5 g of pasta) was mixed with 30 mL of distilled water and were held at 37 °C for 10 min. Pepsin (Acros Organics, New Jersey, USA CAS:901-75-6) was added (1 mL of 10% solution in 0.05 M HCl) in order to replicate gastric digestion. The sample was stirred at 130 rpm at 37 °C for 30 min. An aliquot was withdrawn (Time 0) and added to 4 mL absolute alcohol to stop any further enzyme reaction. Amyloglucosidase (0.1 mL) was added to the digestion pot in order to prevent end product inhibition of pancreatic α-amylase. Pancreatin (EC: 232-468-9, CAS: 8049-47-6, activity: 42362 FIP-U/g. Applichem GmbH, Darmstadt, Germany) was added (5 mL of 2.5% solution in 0.1 M sodium maleate buffer) to represent ileal digestion and an aliquot withdrawn after 20, 60 and 120 min, to which 4 mL absolute alcohol was added to arrest any further reaction. The samples were stored at 4 °C until analysis of reducing sugar content using the 3.5-dinitrosalicylic acid (DNS) method (Brennan, Derbyshire, Tiwari, & Brennan, 2013). Glucose release was plotted against time and area under the curve (AUC) was calculated by dividing the graph into trapezoids as described elsewhere (Brennan et al., 2018).
The in vitro digestion analysis was used to determine predicted glycaemic response.

2.5. Statistical analysis

All experiments were performed in triplicate unless otherwise mentioned. Statistical differences in pasta in vitro digestion values were determined by one-way analysis of variance (ANOVA) and Tukey’s comparison test \((p < 0.05)\).

3. Results and discussion

An in vitro enzymatic digestion was performed in order to mimic the behaviour of pasta when eaten. Figs. 1 and 2 represent an interpretation of the amount of reducing sugars released over 120 min in vitro digestion of all spaghetti samples. In particular, Fig. 1 shows the impact of the substitution of durum wheat semolina in pasta preparation with 15% of Glucagel, inulin GR, inulin HPX, oat and psyllium individually added. As expected, there were significantly more reducing sugars released from the control durum semolina pasta than from the DF enriched pasta samples. In particular, the amount of reducing sugars in samples containing DF was significantly lower at 20 and 60 min of time digestion. The strongest decrease was registered after addition of 15% HPX, P and O followed by GR and GG pasta samples. This trend was kept until 120 min, except for GG pasta that showed a reducing sugar release value slightly lower than control pasta. The higher values registered for GR and GG, compared to the other enriched DFs samples, could be due to the higher reducing sugar content (12%) and the lower dietary fibre content \((76.73\%)\) in GR and GG respectively. Several studies aimed to investigate the impact of DF addition in pasta on the in vitro digestion \((\text{Brennan} \& \text{Tudorica, 2008; Chillo et al., 2011a; Chillo et al., 2011b; Cleary \& Brennan, 2006; Hager, Czerny, Bez, Zannini, \& Arendt, 2013; Manno et al., 2009; Padalino, Mastromatteo, De Vita, Ficco, \& Del Nobile, 2013). Cleary and Brennan (2006) incorporated barley \(\beta\)-glucan fibre fraction into pasta between 2.5% and 10% levels. Pastas with 5%, 7.5% and 10% barley \(\beta\)-glucan fibre fraction generally exhibited a significant decrease in reducing sugars release, although not consistently until after 150 min of digestion. They attributed this delay in consistent attenuation of reducing sugars release to a slow and/or uneven hydration of the polysaccharide matrix, which delays/hinders encapsulation of the protein–starch matrix until the later stages of digestion. Similar results were reported by Chillo et al. (2011a, 2011b) who investigated the in vitro glycaemic impact, the postprandial glycaemic response and glycaemic index of spaghetti made with semolina plus the addition of one of either two types \(\beta\)-glucan barley concentrates, Glucagel (GG) and Barley Balance. In this case, Barley Balance had the most important impact. However, only 10% Barley Balance showed a significant decrease in IAUC and GI values. The functional properties of \(\beta\)-glucans have been attributed to their ability to increase lumen viscosity \((\text{Behall et al., 2005; Izydorczyk \& Dexter, 2008}).\) It has been suggested that cereal \(\beta\)-glucan, by increasing the viscosity of the gastrointestinal tract contents, delays gastric emptying and the intestinal absorption of nutrients such as digestible carbohydrates and thereby decreases postprandial glycaemia and insulin secretion \((\text{Lazaridou \& Biliaderis, 2007}).\) However, in Chillo et al. (2011a) work was observed that the digested spaghetti samples containing GG and Barley Balance did not differ greatly in viscosity and the development of viscosity did not occur until 20 min of digestion. Viscosity therefore was not a factor determining the observed differences in digestibility. It has been suggested that the presence of longer chain \(\beta\)-glucan \((650,000–700,000 \text{ Da})\) in the Barley Balance compared to GG \((150,000 \text{ Da})\) reduced the digestibility of spaghetti, perhaps because the occlusive effect of hydrated \(\beta\)-glucan reduced the rate at which enzyme penetration occurs \((\text{Cleary et al., 2006}).\) Moreover, the reduction in the digestibility of pasta added with soluble fibre can be explained by the changes in the microstructure of cereal based products \((\text{Brennan, Blake, Ellis, \& Schofield, 1996; Tudorica, Kuri, \& Brennan, 2002}).\)
and the limitation of water availability for starch gelatinisation due to the hydration of soluble non-starch polysaccharides.

Pasta made with 15% of oat flour showed one of the lowest reducing sugars released values over 120 min (Fig. 1). Overall, the literature reported that the presence of oat flour in pasta had a significant effect on in vitro digestion values (Bustos et al., 2011; Hager, Lauck, Zannini, & Arendt, 2012; Hager et al., 2013; Krishnan, Menon, Padmaja, Sajeev, & Moorthy, 2012). Krishnan et al. (2012) demonstrated that dietary fibre sources like oat bran, wheat bran, and rice bran can reduce significantly the starch digestibility in sweet potato pasta (Krishnan et al., 2012). The rapidly digested starch fractions were much less in the fibre fortified pastas when compared to the control pasta. This indicated the slowly digestible nature of the fibre-fortified sweet potato pasta. The amount of resistant starch remaining after 120 min digestion was very high for all the fibre-fortified pastas, and such high levels indicate the potential use of the fibre-fortified sweet potato pasta as a low glycaemic food in the management of diabetes and obesity. The results on the slowly digested starch (Augustin et al., 2002; Kim et al., 2008) generally indicated a decrease with increase in the fortification levels of the three bran sources. The slow digestibility of starches in the bran-fortified pasta during 20–120 min has resulted in the low values as compared to the control sweet potato pasta in Krishnan et al. (2012) study. In this research increasing the bran content from 10% to 20% in pasta preparation further reduced the starch digestibility, and this has led to high resistant starch content after 120 min. Similar results were found when oat flour was added in fresh egg pasta preparation (Hager et al., 2013). The proportion of starch digested at different time points and the predicted GI were both significantly lower in oat pasta compared to wheat control. This may be due to the higher fibre content and/or to the higher addition level of egg white powder. In fact, it is known that the presence of protein in the food matrix influences the rate of starch digestion (Kim et al., 2008). The higher amount of protein possibly creates a stronger network, hence reducing the starch availability to enzymatic attack. Finally, among cereals, oat especially contains high amounts of soluble fibre, mainly β-glucan (Butt et al., 2008), possibly explaining the low predicted GI of the oat sample. Thanks to confocal laser scanning microscopy, Hager et al. (2013) demonstrated that the uncooked wheat pasta sample presented starch granules with two different size and shape: large lenticular and small granular ones. Starch granules of oat were relatively smaller and were sometimes organised in bigger spherical structures, the so called compound starch granules.

Pasta represents a limited-water system and hence, even after cooking, a great proportion of starch is still present in its granular form. It was observed that in the outer layer of cooked wheat pasta, gelatinisation had occurred and starch showed a cloud like appearance. Cooked oat pasta showed a continuous mass of gelatinised starch, but no clear outer layer could be observed. The scanning of different locations in the spaghetti samples highlighted the presence of a higher number of air holes and cracks in oat pasta compared to the wheat sample, which can be explained by the lack of the viscoelastic gluten protein (Hager et al., 2012; Bustos et al. (2011, 2013) reported that more than 5 g/100 g of oat fibre addition to pasta formulation generated a disruption of the protein starch matrix so starch granules become more accessible, and hence more susceptible to enzyme degradation (Fardet et al., 1998). Protein content in oat flour is significantly lower than wheat flour (Hager et al., 2013). However not only the amount but also the quality has to be considered. Protein found in oat is known to be superior to that of wheat, due to higher lysine contents, a limiting amino acid in cereals (Laszlo, 1995). These findings are confirmed by our previous study that demonstrated that the presence of oat flour led to a pasta firmer more similar to control than the other enriched DF pasta samples (Foschia, Peressini, Sensidoni, Brennan, & Brennan, 2014).

Our study demonstrated that the inulin addition in pasta led to a decrease in reducing sugars released. However, inulin with higher DP (HPX) had significantly lower values than inulin with lower DP (GR) at 20, 60 and 120 min. This discrepancy can be due to the higher reducing sugar content in inulin GR (12%) than inulin HPX (0.5%) and also GR has a greater disruptive effect on starch–protein matrix, and the lower DP will make it less likely to form a cohesive encapsulating layer (Aravind, Sissons, Fellows, Blazek, & Gilbert, 2012). Brennan et al. (2004) and Brennan and Tudorica (2008) found that 10% of inulin substitution in semolina slightly reduced the starch digestion. At lower levels of inulin, no reduction in starch digestion relative to the control pasta was observed. Brennan and Tudorica (2008) hypothesised that inulin acts either by competing for available water with the starch or forming a protective matrix around the starch granules limiting water movement, gelatinisation and accessibility to starch-degrading enzymes. Further, Manno et al. (2009) showed that inulin caused a lowering in crystallinity, altering the continuity of the protein–starch matrix. Hence, the role of inulin in controlling glucose release may be related to the way inulin becomes incorporated into the structure of pasta (Tudorica et al., 2002). For this reason, it must be taken in account that the lower glucose released with the inclusion of inulin may result from a loss of starch from pasta during cooking process; in fact GR sample presented a cooking loss value (10.70 ± 0.14%) significantly higher than control (4.85 ± 0.18%). This behaviour was registered for all dietary fibres enriched samples (data not shown). This in turn may be due to the weakening of the starch–protein matrix in the overall pasta structure. However, the effect on starch digestion can also be attributed to interaction between protein network, starch and fibre at the microscopic level (Fardet et al., 1998). In fact, previous reports showed that non starch polysaccharides can form a matrix with proteins forming a barrier around the starch granules, reducing the digestive enzymes activity (Tudorica et al., 2002). Tolstoguzov (2003) based the explanation of the interaction between the starch and non-starch polysaccharide in the pasta matrix on the theory of thermodynamic incompatibility. This theory affirms that the reduction in starch degradation within the samples containing inulin would result from the inulin preferentially hydrating, aggregating, and forming a matrix, encasing starch granules in a semisolid gel. This encasing of the starch granules would possibly limit water movement to the starch granules in the pasta, reducing gelatinisation events. Reduction in water movement may also interfere with the accessibility of starch-degrading enzymes to the partially gelatinised starch granules.

The second part of our work was focused to evaluate the effect of including DFs combination in pasta on the predicted glycaemic response. In Fig. 2a reducing sugars released values of control, GR, GR–P and GR–O pasta samples were compared. Inulin GR enriched pasta showed the lowest value, followed by GR–P. No significant difference was observed in the in vitro glycaemic response when inulin GR is added in combination with O, compared to the control (p < 0.05). Fig. 2b illustrates the combinations of inulin HPX with psyllium and oat flours, respectively. In this case, DF enriched pasta samples produced the same decrease in reducing sugars released all over the 120 min. The combination of P and O flours in pasta preparation led to significantly higher values in predicted glycaemic response than P or O flours when individually added to semolina flour (Fig. 2c). However, P–O pasta samples retarded the starch hydrolysis between 20 and 60 min and released significantly less reducing sugars at this point compared to pasta made with 100% semolina flour. At 120 min P–O sample had a value not significant different from control pasta. The results in Fig. 2 suggest that oat flour can retard the release of reducing
sugars (and hence the speed of starch hydrolysis) when added in combination to the other DFs (except for inulin HPX). Psyllium flour significantly changed the behaviour of all DFs during the in vitro digestion analysis.

Fig. 3 illustrates this more clearly. The effects of substituting semolina flour with oat and psyllium flours and Glucagel, inulin HPX and inulin GR ingredients individually and in combination on standardised AUC values are shown as comparisons against the control (100% durum wheat semolina) sample. In all samples (except GR–O) a clear decrease in AUC reducing sugars levels after addition of dietary fibre is observed. In particular, the substitution of semolina with 15% HPX, P or O in pasta production caused the major decrease in standardised values. What is of interest is the effect of the combinations of P or O with other DFs did not lead to further reduction on in vitro digestion values compared to the O or P samples individually. Therefore, the results obtained in this research work seem to indicate an antagonistic effect in including DFs’ combination in pasta on the predicted glycaemic response. However, it can be hypothesised that changing the ratio of the DFs used and increasing the substitution rate from 7.5% of the DFs with the best performance (oat bran flour, psyllium fibre and inulin HPX) could reduce the predictive glycaemic response of the extruded products thus creating a reduction in the calorific content of the food. This in turn may have positive effects in terms of weight management and potential glycaemic impacts of readily digestible starchy foods.

The present study did not focused the attention on the impact of incorporation of DF on the sensory quality of pasta; however, several research projects evaluated this aspect (Aravind et al., 2012; Bustos et al., 2011; Fiorda et al., 2013; Hager et al., 2013). Overall, the consumer acceptance depended on the type, content and DP of dietary fibre added in pasta formulation. In the matter of this, Bustos et al. (2011) found that incorporation of oat bran (10 g/100 g) decreased firmness, chewiness and stickiness values compared with control, obtaining the lowest overall acceptability: on the other hand, resistant starch addition, at the same level of substitution, into pasta recipes did not modify overall acceptability by consumers. Sensory results carried out by Aravind et al. (2012) indicated that testers were generally unable to distinguish pasta fortified with inulin LV-100 (DP = 7–8) up to a 7.5% level of substitution from the control pasta, although the instrumental data shows a negative impact at 5% LV-100.

4. Conclusions

In vitro digestion analysis conducted in this study has highlighted that the substitution of durum wheat semolina with DF in pasta can reduce the predicted glycaemic response of pasta material. In particular, pasta samples containing 15% of inulin GR, inulin HPX, Glucagel, psyllium and oat showed significant lower AUC values compared to control pasta sample. Many factors have been suggested to explain the slow digestion in DF enriched pasta. However the reduction in the predicted glycaemic response of pastas was not further improved when DFs were used in combination. This suggests that combining the functionality of different DFs in pasta may not be of as much importance as the overall concentration of fibre in the pasta, potentially illustrating an antagonistic behaviour of some pasta combinations. Since the positive effect of DF addition in pasta on in vitro digestion is well documented, at this stage it would be interesting to have the confirmation from in vivo starch digestion analysis. Finally, additional research would be necessary in order to evaluate the acceptance of DF enriched pasta from consumers.

References


Fig. 3. Values for area under the curve (AUC) comparing control and all dietary fibre enriched pasta samples.


