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Study and Development of New Functional Foods Containing Cereals

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Martina Foschia

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Coordinator:	Mara Lucia Stecchini, Professor Department of Food Science University of Udine, Italy
Supervisor:	Donatella Peressini, PhD Department of Food Science University of Udine, Italy
Reviewers:	Atze Jan van der Goot, PhD Food Process Engineering Group, Wageningen University, The Netherlands
	Frédéric Debeaufort, Professor UMR PAM-PAPC Université de Bourgogne/AgroSup, Dijon, France

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List of abbreviations

AUC, area under the curve
BB, Barley Balance
CB, cereal bran
CF, cassava flour
CLSM, confocal laser scanning microscopy
d.b., dry basis
DF, dietary fibre
DP, degree of polymerisation
CL, cooking loss
G', storage modulus
G", loss modulus
GI, glycaemic index
GG, Glucagel
LBG, locust bean gum
NSP, non-starch polysaccharide
OCT, optimal cooking time
OHC, oil holding capacity
PGI, predicted glycaemic response
RS, resistant starch
SI, swelling index
tan δ, loss tangent
Vs, specific volume
WAI, water absorption index
WB, wheat brans
WHC, water holding capacity

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Preface

Functional food is any healthy food similar in appearance to conventional foods, able to prevent nutrition-related diseases and enhance physical and mental well-being of consumers. In this regards, the functional ingredient considered in this research project was dietary fibre.

Cereal products are consumed daily by the majority of the population. Popular belief is that these cereal products, rich in carbohydrates, produce a high glycaemic response and may not be a contributing factor to the obesity epidemic throughout the world. Recently the food industry has investigated ways of improving the overall nutritional balance of carbohydrate rich foods and focused on increasing their dietary fibre (DF) contents at the expense of readily digestible carbohydrates. It is well documented that dietary fibre is involved in disease prevention and enhanced health of consumers. Moreover, the food industry can take advantage of the physicochemical properties of fibre to improve the viscosity, texture, sensory characteristics and shelf-life of their products. The focus of this PhD research project was on the influence of dietary fibre supplementation on the quality and nutritional aspects of common foods containing cereals.

The PhD project activities were carried out at the:

- ✓ Department of Food Science (University of Udine, Italy), in collaboration with Pavan S.r.l. (Italy), leader in the design and engineering of food industry technologies for cereal based food;
- ✓ Department of Food, Wine and Molecular Biosciences (Lincoln University, New Zealand) under supervision of Professor Charles Stephen Brennan.

The research study carried out at the University of Udine considered two kinds of food products produced by Pavan S.r.l.: extruded snacks enriched with inulin and gluten-free pasta enriched with resistant starch. Finally, a preliminary rheological study, aimed to investigate possible interactions between different DFs in a model system that represented conventional pasta formulation, was conducted. Based on the results obtained in this study, the research project, carried out at Lincoln University, focused the attention on the impact of DF (added individually and in combination) inclusion on pasta quality and glycaemic response. In order to obtain this information, a method for predicting the glycaemic content of pasta was developed.

Overall abstract

The aim of this PhD thesis was the development of staple foods enriched with compounds, which have an important impact on the nutritional and physiological aspects. Selection of ingredients with high content of dietary fibre (inulin, β-glucans and resistant starch) has been performed to formulate functional foods. In particular, the effect of the ingredients on dough rheological properties, final products quality and on glycaemic response was evaluated in order to identify optimum formulation and manufacturing process conditions for high quality functional foods.

In particular, the inclusion of inulin (short and long-chain: GR and HPX respectively) in extruded snack, at levels from 2% to 7%, lowered dough consistency due to a reduction in water absorption. Large differences in elastic properties of samples were observed between 25 and 95 °C due to incompatibility between inulin and starch and different kinetics of starch gelatinization; however, inulin GR had a greater effect than HPX. Short-chain inulin increased product expansion and hardness compared with the reference, while 7% of HPX decreased these parameters. Short-chain inulin lowered the extent of non-enzymatic browning. Snacks made with 5% inulin HPX can be used to enhance the fibre content without impacting negatively on product quality.

A level of 8% of the two types of inulin and β-glucan (Barley Balance) were added to a gluten-starch model system, which represented semolina for pasta production. Dietary fibre ingredients were added individually and in combination. In general, when added individually, inulin lowered dough consistency, while β-glucan increased this parameter compared to control (without fibre). Moreover, the differences in elastic properties and kinetics of starch gelatinization suggested that short-chain inulin weakens dough structure, while Barley Balance increases elastic effective interactions. However, when dietary fibres were coupled, dough characteristics resulted similar to the gluten-starch model (control); the results suggested that a combination of inulin and Barley Balance allowed to obtain a dough suitable for functional pasta production.

A method for predicting the glycaemic content of pasta was developed. With this purpose, a series of experiments were conducted to evaluate the effect of different sample preparation steps, prior to the *in vitro* starch digestion process, on the predictive *in vitro* glycaemic response of durum wheat pasta (control) and enriched with fibre (pea flour). The evaluation of the different methods of sample preparation illustrated that the maceration of pasta samples prior to starch digestion significantly increased the extent of starch degradation and hence the area under the curve (AUC) of reducing sugar released during the digestion process. Mastication of the samples prior to *in vitro* assessment increased the initial reducing sugar content of samples but yielded the lowest recorded AUC for all samples. The results indicate that the choice of the preparation step used prior to *in vitro* starch digestion procedures can significantly affect the predictive glycaemic response - AUC values of samples, and hence manipulate differences attributed to product composition or structure. This may have an impact in terms

of choosing the most appropriate method of glycaemic analysis for the food industry.

Based on the results obtained from the study of the gluten-starch model system and from the development of *in vitro* starch digestion analysis, pasta samples were made by replacing 15% of durum wheat semolina with inulin HPX, inulin GR, Glucagel, psyllium and oat material (added individually and in combinations). The cooking, textural, colour characteristics and predicted glycaemic response of the pastas were evaluated and compared to control sample containing exclusively durum wheat semolina. Generally, material addition to the durum wheat pasta increased the cooking losses, swelling index and water absorption, whilst reduced firmness and resistance to uniaxial extension of pastas. Raw spaghetti samples resulted significantly darker (L*) and more redness (a*) than raw control pasta. In the cooked pasta, all inulin enriched pasta samples were brighter than semolina pasta. Pasta containing 15% semolina of oat flour showed the best performance (except for the colour) compared to the other experimental pasta samples, but was significantly different to control durum wheat sample. Moreover, the inclusion of inulin GR had a less deteriorating effect when added in combination with oat flour. This illustrates that some fibre rich sources may act better in combinations than separately. In general, all enriched dietary fibre pasta sample showed a significant decrease 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.

The last product took in consideration in this research project was gluten free-pasta, which was prepared substituting rice flour (reference) with 10, 15 and 20% of resistant starch type II (RS). Farinograph test registered no changes in water absorption at any level of substitution. The presence of fibre caused an increase in optimum cooking time and firmness parameters and a decrease in stickiness and cooking loss values; however, no significant differences among all levels of substitution (10-20%) could be appreciated. The loss of resistant starch content (31%) in raw gluten-free pasta suggested that processing conditions could be a critical point for resistant starch stability. Rhelogical on doughs obtained from all raw pastas showed different G' slopes for fibre-enriched samples compared with the reference, ascribable to some modifications in resistant starch granules during pasta cooking. This observation was confirmed by polarized light microscopy analysis performed on RS granules during heating process conditions. Based on these results, pasta samples made with 20% of RS can be considered as a food product source of dietary fibre.

Chapter 1 - Introduction

Over the last decades, consumer demands in the field of fabricated food production have changed considerably. For this reason, foods today are not intended only to satisfy hunger and to provide necessary nutrients, but also to prevent nutrition-related diseases and enhance physical and mental well-being of consumers (Betoret et al., 2011; Brouns and Vermeer, 2000). In this regard, functional foods offer an outstanding opportunity to improve the quality of products. Early functional foods were characterised by fortification with vitamins and/or minerals. Subsequently, the focus has shifted to foods enriched with dietary fibre (DF) and various micronutrients such as ω-3 fatty acid, phytosterol (Betoret et al., 2011). In particular, DF have been extensively studied through the last twelve years because of their well proven beneficial physiological effects such as lowering of blood cholesterol, improvements in large bowel function and attenuation of post-prandial blood glucose and insulin levels (Brennan, 2005; Flamm et al., 2001; Greenwald et al., 2001; Mudgil and Barak, 2013). This functional ingredient has several physico-chemical functions (such as water binding and alteration of viscosity) and is usually classified as soluble (oligosaccharides, pectins, β-glucans, and galactomanan gums alginate, psyllium fibre) or insoluble (cellulose, hemicellulose and lignin), based on its solubility in water (Grajek et al., 2005; Greenwald et al., 2001; Kale et al., 2011; Rodríguez et al., 2006; Tudorica et al., 2002). Insoluble fibre primarily promotes the movement of material through the digestive system, thereby improving laxation. The majority of insoluble fibre is fermented in the large intestine, supporting the growth of intestinal microflora, including probiotic species. Soluble fibre can help to lower blood cholesterol and regulate blood glucose levels. The most widely cereals used as dietary fibre source for products containing cereals are: wheat, oat, barley, rice, rye. For this reason the main components of DF used in food industry tend to be arabinoxylans (wheat bran), β-glucans and resistant starch (Table 1.1).

Table 1.1. Summary of the main published results on the impact of insoluble and soluble dietary fibre in products containing cereals.

Article	Dietary Fibre (%)	Results
Bread		
Almeida et al. (2013)	Wheat bran (WB) Locust bean gum (LBG) Granular RS2-type corn resistant starch (RS) Combination of: 0-20 WB; 0-20 RS; 0-3 LBG	WB: reduced specific volume, crumb luminosity; increased high-speed mixing time, crumb chroma and crumb moisture content. Good results in the sensory evaluation. LBG: reduced crumb luminosity, increased crumb moisture content, but reduced high-speed mixing time. Good results in the sensory evaluation RS: increased high-speed mixing time; it was a more “inert” fibre in relation to bread quality characteristics. Good results in the sensory evaluation.
Peressini and Sensidoni (2009)	Inulin ST; DP=10 Inulin HP; DP=23 Inulin HP-gel; DP=23 Substitution: 2.5, 5 and 7	ST: lower changes in linear viscoelastic properties of dough; no negative effects on crumb hardness and volume of bread prepared with flour suitable for breadmaking. Addition of inulin ST over 5% caused sweet taste. HP: higher changes in linear viscoelastic properties. Breads with 5% inulin ST and HP: high sensory acceptance. Flour replacement at different levels by inulin change dough machinability, viscoelasticity and breadmaking performances.
Skendi et al. (2010)	Barley β-glucans Substitution: 0.2, 0.6, 1.0 and 1.4	Increase in the farinograph water absorption of the doughs with increasing fortification level. High molecular weight preparation had a greater impact. Breads containing barley β-glucans showed lower moisture loss during storage than control. Increasing the β-glucan content increased the number of gas

		cells but it produced a coarser and darker crumb structure with less rounded cells. The addition of β-glucans decreased the firmness of bread during storage.
Symons and Brennan (2004)	Barley β-glucan Substitution:1 and 5	Significant reduction of sugars during *in vitro* digestion of breads prepared by replacing 5% of wheat flour with purified β-glucans.
Cleary et al. (2007)	HMW and LMW barley β-glucan extracts Substitution: 5	
Pasta		
Aravind et al. (2012a)	Duram wheat germ (pollard); substitution: 10, 20, 30, 40, 50 and 60	Pollard at 10%: minimal impact on quality with higher antioxidant status (AO) and fibre content. Pollard above 30%: pasta had undesirable colour, sensory properties and higher starch digestion.
	Duram wheat bran; substitution: 10, 20 and 30	Bran: undesirable sensory and technological properties, especially at 30% incorporation; it does provide more dietary fibre and antioxidants than regular pasta and does not affect starch digestibility.
Aravind et al. (2012b)	Inulin Frutafit HD; DP 12-14; substitution: 2.5, 5, 7.5, 10 and 20	HD: the impact on technological and sensory properties was minimal, with deterioration in properties becoming significant only at 20% incorporation. Improvement of the GI of pasta.
	Inulin LV-100; DP 7-8; substitution: 2.5, 5, 7.5 and 10	LV-100: more negative impacts on pasta firmness, cooking loss, sensory acceptability and a significant reduction in starch hydrolysis was observed only at 10% level but pasta was of inferior quality.
Bustos et al. (2011)	Resistant starch type II (RSII) Resistant starch type IV (RSIV) Oat bran (OB)	Resistant starch is odourless, and does not considerably alter the organoleptic properties of the original product. Oat bran is a good alternative when substitution does not exceed 5 %, but some problems

	Substitution: 2.5, 5.0, 7.5, and 10	could come up due to its flavour and texture. RS II and RS IV enriched pasta presented an important improvement in nutritional quality with a significant reduction of the estimated glycaemic index and a slow release of maltose.
Manno et al. (2009)	Inulin (Fibruline XL; DP≥20); substitution: 5, 10 and 15	Inulin at 5% and 10%: pasta is acceptable by consumers and with a slightly reduced caloric value and a higher level in fibres.
Chillo et al. (2011)	Glucagel (GG) and Barley Balance (BB) Substitution: 2, 4, 6, 8, 10	BB, but not GG, significantly reduced the susceptibility of spaghetti to pancreatic digestion *in vitro* compared to the control at all concentrations. Spaghetti added with GG and BB (all the treatments) did not have different colour and hardness properties compared to spaghetti without β-glucan. All the treatments (except for the spaghetti with 2% BB): higher cooking loss and adhesiveness values than the control. BB appears to be a better treatment for reducing the glycaemic potency of spaghetti.
Cake and muffin		
Gómez et al. (2008)	Chickpea flour; substitution: 50 and 100	At increasing substitution percentage decreased cake volume and symmetry, texture became firmer, more gummy and less cohesive. The changes are minimized if white chickpea flours are used instead of whole chickpea flours.
Lebesi and Tzia (2011)	Wheat flour with dietary fibre (DF) from oat, wheat, maize, barley. Cereal bran (CB) from oat, rice and wheat. Substitution: 10, 20, 30	DF addition improved cake volume, texture, sensory characteristics in contrast to CB, and prolonged the shelf-life. Wheat fibre and oat fibre up to 30%, improved quality characteristics of cupcakes. CB yielded firm cakes that had low

		volume, low moisture, compact crumb texture and low sensory acceptability.
Sudha et al. (2007)	Apple pomace blends; substitution: 10, 20 and 30	At increasing level of substitution: water absorption, density and hardness mixing tolerance, index resistance to extension values increased significantly; the volume of the cakes, dough stability and extensibility values decreased. Apple pomace having high amount of TDF can function as a valuable source of dietary fibre in cake making.
Zahn et al. (2010)	Fibruline Instant, a native inulin (DP~10); Fibruline S20, a highly soluble inulin (DP < 10); Inulin Orafti GR (DP ≥ 10). 50, 75 or 100% margarine was removed and replaced with appropriate amounts of inulin and water	With increasing amounts inulin: product moisture and crumb density increased significantly, whereas muffin volume decreased. For all inulin preparations, the replacement of 50% baking fat significantly increased the crumb springiness, firmness of the muffins. The replacement of baking fat by inulin-water mixtures significantly affects batter flowability and cohesivity of crumb. A replacement of up to 50% baking fat by inulin is possible in muffins.
Extruded snacks		
Brennan et al. (2013)	β-glucan from barley and mushroom; Substitution: 5 and 10	At 10% β-glucan: increased the total and soluble dietary fibre content. At increasing content of β-glucan: increase product expansion and reduction in product hardness. Inclusion of β-glucan caused a significant reduction of potential glycaemic response.
Brennan et al. (2008)	Wheat bran, fine guar gum, inulin, hi-maize 1043 Swede fibre. Substitution: 5, 10 and 15	Insoluble dietary fibre: increase in bulk density and cellular structures have lower cell sizes and a higher cell density. Soluble dietary fibres: higher expansion and more favourable texture than insoluble fibres. Addition of fibre: reduction

		of the amount of readily digestible starch components and increase of the amount of slowly digestible starch.
Jin et al. (1995)	Yellow corn meal Soy fiber Substitution: 10, 20, 30, 40	Increasing insoluble dietary fibre content: increase in longitudinal expansion and in bulk density; a higher expansion results in a lower WAI and a higher WSI. The effects of salt are not significant on the WAI, WSI, air cell size distribution and cell wall thickness.
Robin et al. (2011)	Wheat bran Substitution: 12.6 and 22.4	Increasing the wheat bran concentration: reduction in both volumetric and sectional expansion, increase in the longitudinal expansion of the extruded product, finer structure consisting in a higher number of small cells with shapes closer to spheres and higher surface porosity of the samples; glass transition temperature of starch was reduced by down to 15 °C (this may induce a decrease in the viscosity of the starch phase). At the highest bran concentration: increase in shear viscosity

1.1 Common sources of dietary fibre for the food industry

Arabinoxylans and mixed linkage (1→3)(1→4)-β-D-glucans are the major non-starch polysaccharides present in various tissues of wheat, barley and oat. Arabinoxylans consist of a linear chain backbone of β-D-xylopyranosyl (Xylp) residues linked through (1→4) glycosidic linkages. Residues of α-L-Arabinofuranosyl (Araf) are attached to some of the Xylp residues at O-3, O-4, and/or at both O-2,3 positions, resulting in four structural elements in the molecular structure of arabinoxylans: monosubstituted Xylp at O-2 or O-3, disubstituted Xylp at O-2,3, and unsubstituted Xylp (Fig. 1.1). The structure and physico-chemical properties of arabinoxylans affect their functionality in product making. Water-extractable arabinoxylans form highly viscous aqueous solutions, whereas water-unextractable arabinoxylans generally process stronger water-holding capacity. Thus in breadmaking, water-unextractable arabinoxylans are detrimental, while water-extractable arabinoxylans and solubilized arabinoxylans have positive effects on dough and bread characteristics such as loaf volume and crumb structure (Trogh et al., 2007).

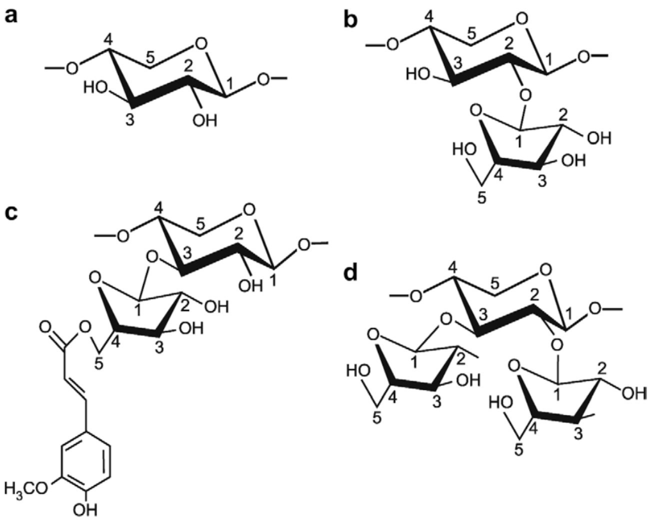

Figure 1.1. Structural elements present in arabinoxylans: (a) unsubstituted Xylp; (b) monosubstituted Xylp at O-2; (c) monosubstituted Xylp at O-3 with ferulic acid residue esterified to Araf and (d) disubstituted Xylp at O-2,3 (Izydorczyk and Dexter, 2008).

Mixed linkage (1→3,1→4)-β-D-glucans, commonly known as β-glucans, are linear homopolymers of D-glucopyranosyl (Glcp) residues linked mostly via two or three consecutive β-(1→4) linkages that are separated by a single β-(1→3) linkage. Less frequent are longer segments of consecutively β-(1→4) – linked Glcp with degree of polymerization (DP) 5-28 (Fig. 1.2). The fundamental molecular characteristics of structure and molecular weight influence viscosity of polymer solutions and gelling properties (Vaikousia et al., 2004). Moreover, one should take into account that molecular weight ranges reported for β-glucans show variability between cereals, with oat β-glucans generally having a higher upper molecular weight compared to barley (Brennan and Cleary, 2005; Colleoni-Sirghie et al., 2003). The molecular weight of the glucans has significant effects on product viscosity, which may in turn have effects on end-use potential.

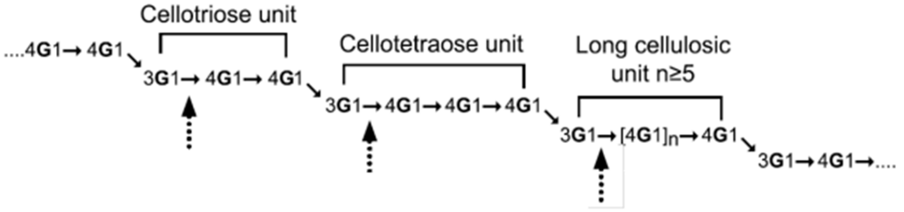

Figure 1.2. General molecular structure of β-glucans (Izydorczyk and Dexter, 2008).

In addition to the cellular components (non-starch polysaccharides) within cereals, the portion of starch and starch products which resist digestion in the small intestine has been described as resistant starch (RS) and it is now regarded as a component of dietary fibre (AACC International, 2010). Starch may become resistant to digestion due to several reasons, as it may be physically inaccessible (RS1), compact granular structure (RS2), retrograded or crystalline non-granular (RS3), chemically modified or re-polymerized (RS4) or amylose-lipid complexed (RS5) starches.

Another interesting ingredient (but not commonly extracted from cereals) widely used as dietary fiber in a variety of foods is inulin. Inulin is a polydisperse fructan consisting mainly of D-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 (Fig. 1.3). Although inulin is found in a wide range of botanical sources, cereals included, the main sources of inulin that are used in the food industry are chicory and Jerusalem artichoke (Bornet, 2008). Native chicory inulin is a non-fractionated inulin extracted from fresh roots that always contains glucose, fructose, sucrose and small oligosaccharides. The degree of polymerisation (DP) of chicory fructans varies from 2 to 60 (average DP = 12) and about 10% of the fructan chains in native DF have a DP ranging between 2 and 5 (Roberfroid, 2005). The long-chain inulin (DP: 10-60; average DP = 25) is produced by applying physical separation techniques to eliminate all oligomers with DP < 10. The physico-chemical properties of inulin are linked to the degree of polymerisation. The benefits of inulin on human health have focused the research on this ingredient, but some interesting technological properties have also been reported, such as a low-calorie sweetener, fat substitute or texture agent (Gonzalez-Tomás et al., 2008). Long-chain inulin is more stable thermally, less soluble and more viscous than the native one and it can be used to structure low-fat foods. Its structure resembles that of a network of fat crystals in oil, since this type of inulin forms small aggregates of microcrystals that occlude a great amount of water, creating a mouthfeel similar to that of fat (Meyer et al., 2011).

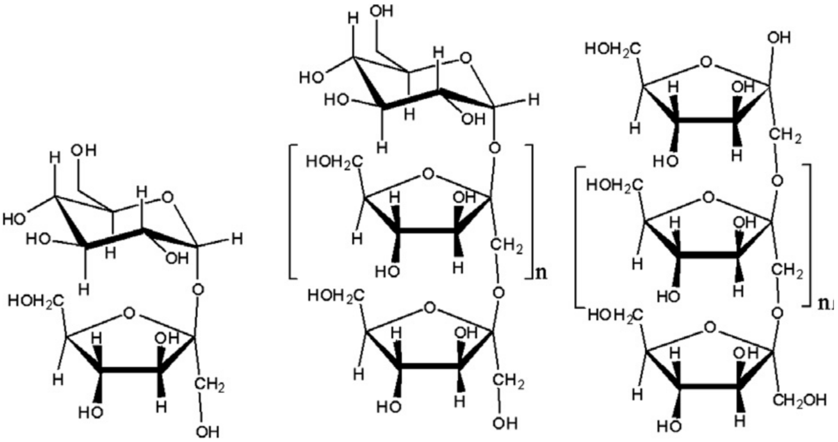

Figure 1.3. Chemical structures of sucrose (GF) and fructo-oligosaccharides (GFn and Fm). G = glucose; F = fructose. Short chain fructo-oligosaccharides are known as oligofructose (n = 1–8), while medium-chain fructo-oligosaccharides are known as inulin (n = 10–13 on average and 63 at maximum) (Morris and Morris, 2012).

1.2 Supplementation of foods with dietary fibre

Supplementation with dietary fibre can result in fitness promoting foods, low in calories, cholesterol and fat. According to current recommendations, the amount of DF daily intake per adult should be in the range of 25-38 g (Food and Nutrition Board, Institute of Medicine, 2001; Romo et al., 2008). To claim that a food is a ‘source of fibre’, the food should contain at least 3 g of fibre per 100 g (or 1.5 g of fibre per 100 kcal). To claim that a food is ‘high in fibre’ this should be at least 6 g per 100 g (or 3 g of fibre per 100 kcal) (European Commission, 2006). The US Food and Drug Administration (FDA) approved health claims for two DFs, beta-glucan (0.75 g/serving) and psyllium (1.78 g/serving), on the assumption that 4 servings/d would reduce cardiovascular disease risk (Jenkins et al., 2002). The Codex Alimentarius (2009) recommends that any product claiming to be a “source‟ of fibre should contain 3 g of fibre per 100 g of serving or 1.5 g of fibre per 100 kcal of serving or 10% of daily reference value per serving, while to claim that a food is “high” in fibre, the product must contain at least 6 g of fibre per 100 g of serving or 3 g of fibre per 100 kcal of serving or 20% of daily reference value per serving.

One way to increase fibre intake is through its incorporation in staple foods. However, in doing so there is a challenge to optimise the potential health benefits derived from DF while retaining the consumer acceptability of the food. Several studies investigated the influence of DF supplementation (inulin, fructo-oligofructose, β-glucans, arabinoxylans and resistant starch) on the quality of foods containing cereals, such as pasta, bread, muffin and extruded snacks (Almeida et al., 2013; Aravind et al., 2012a, 2012b; Borneo and Aguirre, 2008; Brennan et al., 2008; Morris and Morris, 2012; Peressini and Sensidoni, 2009; Romero-Lopez et al., 2011; Tudorica et al., 2002; Wang et al., 2002).

1.3 The effect of DF enrichment on the quality of pasta

Pasta is starchy staple food widely consumed across the world and is favoured by consumers for its ease of transportation, handling, cooking and storage properties. The World Health Organization (WHO) and Food and Drug Administration (FDA) consider pasta a good vehicle for the addition of nutrients. In recent years pasta has become even more popular due to its nutritional properties and the fact that it is regarded as a product with a low glycaemic index (Bornet et al., 1997; Brennan and Tudorica, 2008; Chillo et al., 2011b; Granfeldt and Bjorck, 1991). Many studies have attempted to improve the nutritional properties of pasta by including supplementation with protein, dietary fibre, vitamins and minerals or substituting (partially or completely) the durum wheat semolina with non-conventional flours, such as amaranth, chickpea, broad bean, quinoa, buckwheat (Borneo and Aguirre, 2008; Chillo et al., 2008; Petitot et al., 2010; Sissons, 2004; Torres et al., 2007). Addition of dietary fibre has been shown to reduce the GI of pasta (Brennan and Tudorica, 2008; Chillo et al., 2011a, 2011b), introduces additional health benefits and allows developing novel functional food products.

Chillo et al. (2008) prepared pasta with combinations of buckwheat and bran, and they found that the addition of 15% and 20% bran produced a decrease of the breakage susceptibility with respect to the control, but other quality characteristics such as cooking resistance, cooking loss and instrumental stickiness were not significantly different from that of the control sample. Additionally, when subjecting the samples to consumer analysis, spaghetti samples with added buckwheat flour and bran demonstrated sensorial properties fairly similar to the spaghetti made only of durum semolina, in particular for the overall quality. Borneo and Aguirre (2008) manufactured pasta fortified with dried amaranth leaf flour (17.5%) and found it to be similar to that produced with added spinach leaves in terms of chemical composition, cooking quality, textural characteristics (firmness and adhesiveness) and sensory acceptance. More recently, spaghetti prepared with pollard, with 10% substitution of semolina, resulted in a minimal impact on sensory and technological properties, while providing higher antioxidant status (AO), TDF had no effect on starch hydrolysis (Aravind et al., 2012a). In contrast, higher levels of pollard produced pasta with undesirable colour and sensory properties and starch was digested to a greater extent, suggesting a potential higher glycaemic index. By comparison, bran inclusion at all the levels tested had negative impacts on many sensory and some technological properties. However, such products still have a place in the consumer market offering much higher fibre and AO with no impact on starch digestibility. In particular, a significant proportion of the total AO in the semolina-fibre mixtures is retained in the cooked pasta that would be consumed. The CLSM images of bran and pollard at similar doses shows greater disruption to the starch-gluten matrix by pollard than bran. This may explain the faster starch digestion in pollard pasta.

Recent research has indicated that the impact of higher molecular weight inulin incorporation on the technological and sensory properties of fibre rich foods could be minimal, with deterioration in properties becoming significant only at 20% incorporation (Aravind et al., 2012b). Interestingly, inulin, with its lower DP, had more negative impacts on pasta firmness, cooking loss and sensory acceptability. Although the data on *in vitro* starch digestibility showed great variability between samples, the lower DP inulin yielded a significant reduction in starch hydrolysis at 10% level (compared to the control), but generated pasta of inferior quality with low firmness, high cooking loss and inferior sensory scores. The *in vitro* starch digestion was reduced in pasta with higher DP inulin up to a 5% inclusion level but was increased when more inulin was added to the product (clearly evident at 20% inulin). This is of significant interest in that it illustrates that high inclusion levels of inulin could lead to pasta with elevated starch digestibility (high GI pasta), yet these levels have a minimal impact on the technological and sensory properties of the pasta (Aravind et al., 2012b). Moreover, other research has demonstrated that inulin can substitute wheat flour by up to 10%, still producing pasta acceptable by consumers and with a slightly reduced caloric value and a higher level in fibre (Manno et al., 2009). Similarly, Brennan et al. (2004) and Brennan and Tudorica (2008) showed that inulin substitution in semolina of 10% had no impact on pasta cooking loss but decreased firmness and water absorption while slightly reducing starch digestion by an α-amylase *in vitro* method. 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.

The use of RS as food ingredient typically does not change the taste or the texture, but may improve sensory properties compared with many of the traditionally used fibres, such as brans and gums (Sajilata et al., 2006). RS provides good handling in processing due to its low water-holding capacity. The incorporation of RS in pasta products improves textural properties (Fuentes-Zaragoza et al., 2010). Bustos et al. (2011) evaluated the impact that the incorporation of resistant starch type II (RSII), resistant starch type IV (RSIV) and oat bran (OB) have on sensory and nutritional quality of pasta. Oat bran is a good alternative when substitution does not exceed 5 g/100 g, but some problems could come up due to its flavour and texture. On the other hand, resistant starch resulted odourless and did not considerably alter the organoleptic properties of the original product. Moreover, resistant starch type II and type IV enriched pasta presented an important improvement in nutritional quality with a significant reduction of the estimated glycaemic index and a slow release of maltose.

The *in vitro* glycaemic impact and cooking quality of functional spaghetti made using semolina enriched with different levels (2-10%) of two types of β-glucan concentrate, Glucagel (GG) and Barley Balance (BB), was investigated (Chillo et al., 2011a, 2011b). BB, but not GG, significantly reduced the susceptibility of spaghetti to pancreatic digestion *in vitro* compared to the control at all concentrations (Chillo et al., 2011b). Results indicated that cooked spaghetti added with GG and BB (all the treatments) did not have different colour and hardness/firmness properties compared to spaghetti without β-glucan. Except for the spaghetti with 2% BB, all the other treatments demonstrated significantly higher cooking loss (CL) and adhesiveness values compared to the control. CL and adhesiveness are important parameters and they were significantly different for samples with added barley β-glucan concentrates. BB appears to be a better treatment for reducing the glycaemic potency of spaghetti and justifies further *in vivo* study. Barley by-products obtained by air classification have been used to produce barley functional spaghetti, which were compared to different commercial whole semolina samples (Verardo et al., 2011). In particular, three different functional spaghetti were produced: BS50 was produced by replacing 50% durum wheat semolina with coarse fraction air classified barley flour; BS45 was produced by replacing 45% durum wheat semolina with coarse fraction air classified barley flour and adding 5% vital wheat gluten; BS95 was produced with 95% coarse fraction air classified barley flour and adding 5% vital wheat gluten. Total, insoluble and soluble fibre and β-glucan contents of the barley spaghetti were found to be greater than those of commercial samples. Furthermore, it was proved that barley spaghetti reached the FDA requirements, which could allow these pastas to deserve the health claims "good source of dietary fiber" and "may reduce the risk of heart disease". Incorporation of barley coarse fraction as ingredient in spaghetti formulation improved the content of phenolic compounds and an increase of antioxidant activity was reported. Moreover, this study showed that BS45 and BS95 spaghetti had the same firmness, bulkiness and stickiness values. BS50 spaghetti reported the absence of bulkiness and stickiness and a good firmness. Overall, this sample showed a good quality but lower than BS45 and BS95 samples. According to the literature, addition of vital gluten in BS45 and BS95 samples decreased the stickiness and increased the firmness (Bruneel et al., 2010; Sissons et al., 2007). In general, the addition of barley fractions enriched in β-glucans in semolina or wheat flours at up to 20%, resulted in pasta with acceptable sensory properties and cooking quality despite changes in the product colour (Lazaridou and Biliaderis, 2007).

Padalino et al. (2013) focused their work on production of functional spaghetti based on maize flour and naked oat. Results showed that addition of hydrocolloids greatly enhances both chemical and functional properties of final pasta. Rheological data showed that the addition of structuring agents to dough based on maize and oat flour influenced the viscoelastic properties. Regarding the sensory properties, the best overall quality was obtained by the addition of 2% carboxymethylcellulose (CMC) or chitosan (CHIT), respectively. The authors report that the incorporation of hydrocolloids improved the cooking quality of spaghetti. In particular, samples with CMC and CHIT exerted the better quality characteristics (improved texture and cooking loss). In conclusion, pasta based on maize and oat flours with hydrocolloids that increased the insoluble-water fibres content (i.e. chitosan) could be used for reducing the glycaemic index; spaghetti with hydrocolloids that increases the soluble-water fibre content (i.e. carboxymethylcellulose and agar) could be used for reducing the blood cholesterol.

1.4 The effect of DF enrichment on the quality of bread

An increased consumer demand for healthy bread has led to considerable efforts to develop breads that combine health benefits with good sensory properties. In breadmaking applications, a careful selection of DFs with suitable physicochemical properties preventing permanent disruption of the protein matrix is a prerequisite to obtain sensorial accepted breads in highly substituted flour systems.

DFs structure, physiological and technological functional properties in a food matrix are strictly linked together. Angioloni and Collar (2011) demonstrated that few technological functional (sensory firmness and overall acceptability) and most nutritional bread properties (protein digestibility, rapidly digestible starch, slowly digestible starch, *in vitro* expected GI, total digestible starch and RS) were found to depend on dietary fibre molecular characteristics (mean particle diameter), storage modulus (G'), loss modulus (G''), complex viscosity (η^*) and lactic acid solvent retention capacity. Dietary fibres with larger particle size resulted in highly sensory acceptable breads with higher amounts of RS and slightly lower protein digestibility. Fibres exhibiting high viscoelasticity (G' and G'') and complex viscosity (η^*) in concentrated solutions yielded breads with better sensory perception, lower digestible starch and higher resistant starch contents leading to lower *in vitro* expected GI.

Almeida et al. (2013) studied the effects of adding different dietary fibre sources: wheat bran (WB), RS and locust bean gum (LBG), on process and quality parameters of pan bread. Through this study it was possible to verify that, depending on the type and quantity of the dietary fibre source used, different responses can be obtained for process parameters and final quality characteristics of pan bread. WB and LBG were the fibre sources that most interfered with most of the parameters evaluated. WB reduced specific volume and crumb luminosity and increased high-speed mixing time, crumb chroma and crumb moisture content. LBG also reduced crumb luminosity and increased crumb moisture content, but reduced high-speed mixing time. RS increased high-speed mixing time, but was a more "inert" fibre source in relation to bread quality characteristics, presenting interaction effects with the other fibre sources present in the system. Regarding sensory analyses, the acceptance of crust colour, appearance, aroma and taste was not significantly affected by addition of dietary fibre. For the attributes crumb colour and appearance acceptance, all three fibre sources had similar effects. RS and LBG had little influence on acceptability while greater additions of WB, although not significantly different from the other samples, generally yielded a higher acceptability score amongst panellists.

Much research has concentrated on the inclusion of β-glucans in bread based on the potential role of β-glucans in reducing postprandrial cholesterol levels. Generally results indicate that a significant decrease in loaf volume and height is associated with increasing amounts and molecular weights of β-glucans (Izydorczyk and Dexter, 2008; Skendi et al., 2010; Symons and Brennan, 2004). However, the effects of β-glucan addition on dough rheology and bread characteristics strongly depend on the molecular size of the polysaccharide, the supplementation level and the quality of the wheat flour used in the formulation (Skendi et al., 2010). The high molecular weight polymer showed a greater impact than its low molecular weight counterpart, presumably due to a higher water binding capacity of the former. When weak flour was used in breadmaking, β-glucan addition increased the dough resistance due to a strength effect on the dough and increased its gas retention capacity; the dough extensibility remained similar or decreased compared to the control. An increase in the β-glucan content also enhanced the number of gas cells, but gave a crumb with a coarser structure, less rounded cells and a darker colour. Relatively little work has been reported on the effects of food processing conditions (mechanical energy and temperature) on the properties of β-glucans. Processing may affect DP, polymer interactions and functional properties (viscosity, water binding capacity and solubility) which could influence the sensory, physiological and the health benefits of β-glucans (Brennan and Cleary, 2005). The increase in hardness of the bread crumb fortified with β-glucans at a higher level of addition (>1%) has been observed and various interpretations have been put forward. Rossell et al. (2001) proposed that the increase in crumb firmness may be a consequence of the thickening of walls surrounding the gas cell that occurs upon addition of hydrocolloids into bread formulas. Furthermore, an increase in bread firmness may be a consequence of a decrease of the total area of the gas cell in bread containing β-glucans; indeed, the greatest crumb firmness is usually observed in breads with the lowest loaf volume. Also water promotes starch recrystallization, and the water content of β-glucan-enriched breads is generally higher than that of control breads. However, Skendi et al. (2010) reported that crumb firmness of the breads, measured after 24 h of storage, decreased with increasing β-glucan level, reaching a minimum, and thereafter the trend was reversed, but the values always were smaller than the control (except for the bread made with good breadmaking flour Yekora). These results suggest that incorporation of β-glucans into low quality wheat flour seems to be more effective in reducing bread firmness than when it is added to good breadmaking flour. Gill et al. (2002) suggested that, in addition to a plasticization effect brought about in the composite gluten-starch matrix (higher hydration leads to softening), β-glucans added to wheat flour would compete for water with native wheat starch granules in the dough. This, in turn, might restrict swelling and solubilisation of the starch during baking, and thereby reduce firmness (Gill et al., 2002). The texture results showed that the firmness values of all β-glucan-supplemented breadcrumbs, after eight days of storage, were similar to or significantly lower than the control after storage.

The effectiveness of barley β-glucan inclusion on the *in vitro* digestibility of breads was shown by Symons and Brennan (2004) and Cleary et al. (2007). Both studies reported significant reduction of sugars during *in vitro* digestion of breads prepared by replacing 5% of wheat flour with purified β-glucans. The effects were partly attributed to the inhibition of enzyme accessibility to starch polymers due to the increased digesta viscosity and/or altered rheological properties of breads containing β-glucans. Scanning electron micrographs of *in vitro* digests of bread containing barley β-glucan illustrated the more compact structure of bread and the retention of undigested starch granules compared to control breads. Cavallero et al. (2002) demonstrated a potential to regulate, *in vivo*, the sugar release from breads containing β-glucans. A significant reduction in the area under the blood glucose curve and delay in the mean peak of blood glucose was observed in human subjects who consumed bread supplemented with 20% of barley β-glucans. Izydorczyk and Dexter, 2008 showed that substitution of wheat flour with 20% of barley fibre-rich-fractions in two-layer flat bread increased the amount of total β-glucans from 0.2 g to 3.0 g, soluble β-glucans from 0.09 g to 1.43 g, and arabinoxylans from 2.4 g to 4.2 g per serving, thus making significant contribution to the daily recommended intake of both soluble and insoluble DF (20-35 g per day for the healthy adult population) (American Dietetic Association, 1993).

Inulin contributes to development of functional food as well as its quality, in consequence to improved health of consumers. Peressini and Sensidoni (2009) found that flour replacement with up to 5% inulin of 10-23 DP produced bread with acceptable sensory quality. The main inulin/FOS impacts reported were lower bread loaf volumes, increased crumb hardness and darker crust (Morris and Morris, 2012). While inulin appears to integrate well to the gluten network, it also dilutes it, resulting in lower gas retention ability. A darker colour and increase in aroma compounds characteristic of the Maillard reaction were attributed to a larger number of reducing ends. Those in turn, may be partly due to inulin/FOS degradation upon baking as there is evidence that both yeast invertase and dry heat degrade inulin. Whether prebiotics remain fully active in the end product is still to be established. A supplementation of 5% inulin appears to be achievable and should contribute 0.7-1.2 g of inulin per slice of bread toward daily intake. The changes in the end product depend on the type of inulin, i.e. the DP and the presence of low molecular weight sugars in the formulation and the level of its contribution in the recipe (Juszczak et al., 2012; Peressini and Sensidoni, 2009). In all cases, technical challenges were apparent in terms of dough machinability resulting in end product quality slightly lower than that of the control (Brasil et al., 2011; Karolini-Skaradzinska et al., 2007).

1.5 The effect of DF enrichment on the quality of extruded snacks

Crispy extruded snack foods have become part of the dietary habits of a great part of the population. Snacks consist essentially of a cereal blend extruded with a certain amount of water. Inside the extruder, the cereal mixture is heated above the starch gelatinisation temperature leading to a cooked product that may be directly enrobed and flavoured, or needs further processing such as frying, roasting, etc. (de Cindio et al., 2002; Peressini et al., 2002). Starch is the main constituent of the extruded snacks and is responsible for most of their structural attributes. DF addition in extruded cereals most often leads to detrimental effects on product quality due to reduced expansion volume, increase in density and hardness, and decrease in crispness (Robin et al., 2012). The deleterious effects of various fibres depend greatly on DF interactions with starch, and the molecular and physico-chemical properties of the DF. This in turn has lead to differing effects being observed for varying DF's (Robin et al., 2012). In particular, insoluble fibre significantly reduces expansion volumes and increases density of extruded products, leading to harder textures. However, the effect of fibre content on expansion volume often depends mainly on the process conditions and dough water content. In fact, increasing wheat bran from 0 to 20% content has been shown to have only a limited effect on the sectional expansion of an extruded wheat flour/pinto flour bean blend at high water content in the extruder (Hernández-Diaz et al., 2007). On the other hand, at low water content, increasing wheat bran significantly decreases the sectional expansion. Moreover, increasing insoluble dietary fibre content often leads to an increase in longitudinal expansion (Jin et al., 1995; Robin et al., 2011), to an increase in bulk density (Jin et al.,1995; Brennan et al., 2008) and to cellular structures with lower cell sizes and a higher cell density (Robin et al., 2012). This increase in cell density when adding insoluble fibre can be associated with an increase in nucleation degree in the extruder.

Contrary to insoluble fibres, inclusion of soluble fibre has been shown to lead to better expansion volume while less affecting bulk density of extruded products (Robin et al., 2012). Differences in expansion volume between soluble and insoluble fibres can be attributed to differences in water absorption, viscoelastic properties of the dough at the exit of the extruder die and stabilisation of the bubble membrane during bubble growth (Brennan et al., 2008).

Limited information is available on influence of inulin enrichment on the quality of extruded snacks (Blake, 2006; Brennan et al., 2008). Soluble fibres such as inulin tend to generate a higher expansion and more favourable texture than insoluble fibres such as cereal bran (Blake, 2006; Brennan et al., 2008). However, Robin et al. (2012) reported that the effect of soluble fibre content on expansion properties of extruded cereals is unclear, while increasing insoluble fibre appeared to systematically decrease sectional expansion and increase bulk density. It is clear that the effect of fibre addition to extruded snack products does not only depend on the type of DF used and the overall inclusion rate, but also on the polymer molecular weight and structure of the DF and its ability to hydrate during the mixing and shearing stages of extrusion. What is more clear is that the addition of fibre elicits a reduction in the amount of readily digestible starch components and an increase in the amount of slowly digestible starch after adding dietary fibre

(Padalino et al., 2013). This has potential benefits in terms of attenuating the glucose response post ingestion, and may also lead to an increased feeling of satiety (Brennan et al., 2008). This attenuation of glucose response has also been shown when including by-products rich in fibre from agricultural processing (Brennan et al., 2013).

1.6 The effect of DF enrichment on the quality of cake and muffin

The interest in foods with high fibre contents has led to the development of a large market for fibre-rich ingredients and products such as muffins or types of cake that are normally consumed at breakfast. In general, when the substitution percentage of wheat flour by dietary fibre showed an increase in elasticity (G') of batters, a decrease in loss modulus (G") of batters and a decrease in cake/muffin volume and in symmetry were observed; texture became firmer, more gummy and less cohesive (de la Hera et al., 2012; Gómez et al., 2008; Gularte et al., 2012). However, the studies demonstrated that the effect depends on the type of cake being made, flour characteristics (with a preference for fine flours) and the level of substitution. In fact, Singh et al. (2012) demonstrated that a cake mixture with up to 20% corn bran replacement can be used without causing any deterioration in food quality. Similar results were obtained by Gómez et al. (2008). Moreover, enriched cakes increased significantly their dietary fibre content, which was connected to the nature of the fibres added. Fibres significantly affected the *in vitro* hydrolysis of starch fractions. In fact, Gularte et al. (2012) proved that the rapid digestible starch contents of all the fibre-enriched cakes were lower than the control and decreased with a reduction of the soluble dietary fibre ratio. The slow digestible starch increased according to a reduction of the soluble dietary fibre ratio in the 3 g fibre-enriched cake, while there was no difference in the 6 g fibre-enriched cake. The resistant starch contents were not significantly different in the 3 g and 6 g fibre-enriched cakes according to the ratio of soluble dietary fibre and insoluble dietary fibre. Nonetheless, the fibre-enriched cakes showed higher values for slow digestible starch compared to the control cakes, demonstrating the potential effectiveness of DFs on starch digestion fractions when they are added into the cake formulation. However, a high value of slow digestible starch is more desirable than rapid digestible starch since slow digestible starch is slow digested in the small intestine and induces a gradual increase of postprandial plasma glucose and insulin levels. These results agree with Gularte et al. (2012), where the most pronounced effect was the decrease in the slowly digestible starch.

Apples are a good source of soluble and insoluble dietary fibre (O'Shea et al., 2012). Sudha et al. (2007) substituted wheat flour with apple pomace in a cake formulation and found that as the level of substitution increased, the volume of the cakes decreased and the density and hardness increased. In general, apple pomace affected the elastic properties of the wheat flour dough as well as the pasting properties. Apple pomace having high amount of TDF can function as a valuable source of dietary fibre in cake making. Similar results were observed by

Rupasinghe et al. (2008, 2009). In contrast to this, Gomez et al. (2010) observed a significant increase in volume of cakes when wheat flour was substituted with wheat and oat fibres of different particle sizes. The results shown in the literature indicated that it is possible to obtain cakes with very similar physical and sensory characteristics to the control when flour is substituted by fibre in quantities higher than 20%. Optimum results were obtained with small sized fibres.

Lebesi and Tzia (2011) examined the effect of incorporating graded levels of dietary fibre from oat, wheat, maize, barley and cereal bran (CB) on the quality and sensory characteristics (appearance, texture, flavour and overall acceptability) of high ratio cupcakes. DF addition to cakes improved cake volume, texture and sensory characteristics in contrast to CB which impaired some sensory characteristics of the cakes. Cakes containing 20% and 30% wheat fibre and oat fibre had the highest volume, the lowest crumb firmness, good moisture retention after baking and high overall acceptance scores. The CB yielded firm cakes that had low volume, low moisture, compact crumb texture and low sensory acceptability. It was evident from the study that the use of DF, and in particular wheat fibre and oat fibre up to 30%, improved quality characteristics of cupcakes. Finally, DF addition prolonged the shelf-life of the cakes by delaying the moisture loss of the crumb and the increase in crumb firmness.

The replacement of baking fat in a muffin formulation by commercial inulin preparations showed that with increasing amounts of added fibre, product moisture and crumb density increased significantly, whereas muffin volume decreased (Zahn et al., 2010). Inulin preparations with the replacement of 50% baking fat significantly increased the crumb springiness of the muffins, indicating that the strength of the bonds in the three-dimensional crumb network was increased. The replacement of baking fat by inulin-water mixtures significantly increased the flowability of batter. This appeared to be dependent upon the type of inulin used for fat replacement. For instance, flowability was lower for Orafti GR and Fibruline Instant incorporated as prefabricated gel. The higher firmness observed in the inulin containing muffins was attributed to insufficient gel structure formation. Cohesivity, which is the ratio of compression work in the second compression cycle to compression work in the first cycle, was lowest for the formulation containing the gelled inulin and highest when Fibruline S20 was used. For all inulin preparations, the replacement of 50% baking fat significantly increased springiness (defined as the remaining relative sample height when an initial force was registered during the second compression) of the crumb of the muffins, indicating that the strength of the bonds in the three-dimensional crumb network is increased. The results of the study showed that a replacement of up to 50% baking fat by inulin is possible in muffins or related products. For a 50% baking fat replacement, inulin intake per muffin (50 g batter) was between 1.6 and 2.9 g, which is also consistent with nutritional recommendations (Grabitske and Slavin, 2009).

Baixauli et al. (2008) illustrated that replacing wheat flour with 15% or higher levels of RS produced muffins with decreased volume, height, number and area of gas cells. The authors also indicated that a decrease in the viscosity and in the elastic properties of the muffin batter was correlated to increased replacement by RS. The study of the rheological properties of the batters during heating by small amplitude oscillatory shear test revealed a decrease in the elastic properties of the muffin batter upon increasing the level of RS, which could be related to the decrease in the structural elements provided by wheat flour. The flow and linear viscoelastic properties of the muffin batters at 25 °C indicated a decrease in the structure complexity with the increase in RS. However, the specific gravity values did not reveal lower bubble retention capacity of the raw batter in the presence of RS. This is of interest to the bakery industry when trying to reformulate products with higher amounts of resistant starch as fibre-rich products.

Composite flours prepared from malted and pre-gelatinized cassava through appropriate blending with cereal and/or legume and bran sources have been used for making muffins with high fibre content (Jisha et al., 2010). Cassava flour (CF), malted with amylases, followed by pre-gelatinization were used in the study to produce products having acceptable texture. The high DF content coupled with low *in vitro* starch digestibility of the baked products made from cassava-bran mixes indicated their scope in medical nutrition therapy for managing obesity-linked diseases. Indeed, the use of fibre enhanced food products in medical nutrition remains one of the major priorities of product development within the food industry.

1.7 Future trends

Since cereal products are consumed daily by the majority of the population, the food industry has focused attention on creating products which contain functional ingredients, such as dietary fibre. It is well known that DF has several physiological functions. Due to the nature of fibre having varying degrees of solubility properties and viscoelastic characteristics, the food industry can take advantage of their physicochemical properties such as water binding, gelling and structure building in creating novel food structures. It is also possible to utilise fibre-rich by-products from primary or secondary food production streams as ingredients in food products as inexpensive, non-caloric bulking agents for partial replacement of flour, fat or sugar, as enhancers of water and oil retention and to improve emulsion or oxidative stabilities (Brennan et al., 2013; Ktenioudaki and Gallagher, 2012; Martínez-Cervera et al., 2011; Zahn et al., 2010). Recently Brennan et al. (2013) have illustrated a potential use of β-glucan rich materials derived from mushroom and barley processing as ingredients in extruded snacks which reduce the glycaemic load of individuals. As mentioned previously, DFs are generally classified as soluble or insoluble, based on whether they form a solution when mixed with water (soluble) or not (insoluble). The soluble and insoluble nature of DFs involves differences in their technological functionality and physiological effects (Abdul-Hamid and Luan, 2000; Brownlee, 2011; Elleuch et al., 2011). Soluble fibres are characterised by their capacity to increase viscosity, to reduce the glycaemic response and plasma cholesterol. Insoluble fibres are characterised by their porosity, their low density and by their ability to increase faecal bulk and decrease intestinal transit. Compared with insoluble dietary fibre, in food processing the soluble fraction demonstrates greater capacity to provide viscosity, ability to form gels and/or act as emulsifiers, has neither bad texture nor bad taste and is easier to incorporate into processed food and drink. The hydration properties of dietary fibres are related to the chemical structure of the component polysaccharides, and other factors such as porosity, particle size, ionic form, pH, temperature, ionic strength, type of ions in solution and stresses upon fibres. The ability of dietary fibres to hold water is strongly related to the source of the dietary fibre.

Moreover, fibres possess the oil holding capacity (OHC), which is the amount of oil retained by the fibres after mixing, incubation with oil and centrifugation. Oil absorption of cereal derivatives, for instance wheat bran, is related mainly to the surface properties of the bran particles (Caprez et al.,1986) but may also be related to the overall charge density and to the hydrophilic nature of the constituents (Elleuch et al., 2011). Thanks to these physiological functionalities, fibres can be used as ingredients in food products. Indeed, DFs with high OHC allow the stabilisation of high fat food products and emulsions, while DFs with high WHC can be used to avoid syneresis and modify the viscosity and texture of some formulated foods. Most polysaccharide solutions exhibit non-Newtonian behaviour and an increased shear rate can increase or decrease viscosity (Sanderson, 1981). Elleuch et al. (2008) reported that DF concentrates, reconstituted with water at different concentrations (between 20 and 50 g/L) present a non-Newtonian behaviour, which is more marked when the concentration of DF is raised. The experimental results showed that the flow behaviour of DF concentrate suspensions (20-50 g/L) is pseudoplastic. This rheological behaviour was also observed in peach DF concentrates (Grigelmo-Miguel et al., 1999) and fruit juice concentrates (Hobani, 1998). The degree of the pseudoplastic behaviour can be measured by the flow index (n). All the date DF suspensions exhibit pseudoplastic behaviour ($n < 1$). This index decreases with DF concentration when the pseudoplasticity increases. The study of the physical properties has shown that the DF concentrates could be used as functional ingredients in food to avoid syneresis, to stabilise products with a high percentage of fat and emulsion and to modify the texture and the viscosity of formulated products by virtue of their high WHC and OHC and their rheological properties.

Plants containing DF and natural antioxidants have attracted increasing interest in recent years as many studies have revealed that these two species of components might be involved in disease preventive and enhance health of consumers (Zhu et al., 2010). It is interesting to note that phytochemical and antioxidants in grains have not received as much attention as the phytochemicals in fruits and vegetables. Fruits and vegetables are often cited as excellent sources of antioxidants, whereas grains tend not to be mentioned due to relatively low levels of antioxidant activity reported in literature. Most studies have reported the phenolic levels of grain using various aqueous solutions of methanol, ethanol and acetone to extract soluble phenolics (Liu and Adom, 2007). These methodologies assumed that long extraction times and/or use of finely powdered samples would ensure maximum extraction of phenolic compounds from grains. These methods at best extracted only the free or loosely attached or readily soluble phenolic compounds in the sample and did not extract phenolic compounds tightly bound to the cell wall materials. Procedures using more exhaustive extraction techniques employ digestion to release bound phytochemical from whole grains (Liu and Adom, 2007). These have yielded results that show whole grains contain more phytochemicals than was previously reported. Thus, antioxidant capacity is another important property of DF that is given by the presence of different antioxidant linked compounds. The most important groups of phytochemicals with an antioxidant role found in whole grains can be classified as phenolics, carotenoid, vitamin E compounds and lignans. DFs have additional physiological effects as related to the role played by the antioxidant compounds associated with the polysaccharides (Ajila et al., 2010; López-Vargas et al., 2013) and can offer protection against the superoxide radical, hydroxyl free radical, lipid peroxidation and exhibit good potential for reducing power and chelating ferrous ions (Zha et al., 2009). However, these antioxidant compounds make up a substantial portion of the dietary antioxidant capacity; they are not minor constituents of DF, and as such they may contribute significantly to the health effects attributed to DF and dietary antioxidants. This suggests possibilities for the use of fibres with high antioxidant activities as ingredients that allow the stabilisation of fatty foodstuffs, thereby improving their oxidative stability and prolonging their shelf life.

However, the main challenge with adding fibre in products containing cereals is the adverse effects on the end product quality (for example texture and colour) and therefore the low acceptability by consumers. Most commonly, dietary fibres are incorporated into bakery products to prolong freshness, thanks to their capacity to retain water, thereby reducing economic losses. It is evident from the review of recent literature that ingredients rich in dietary fibre will significantly affect the dough and baking properties.

The main effects, although variable, can generally be summarised below:

Dough

- ✓ Increase in the water absorption of dough during mixing;
- ✓ Increase in the development time and decrease in the mixing stability;
- ✓ Decrease in dough development during proofing;
- ✓ Change in the extensional properties i.e. decrease in dough extensibility;
- ✓ Change in the viscous and elastic moduli i.e. dough becomes stiffer, or in some cases stickiness is increased.

Baked product

- ✓ Decrease in loaf volume or height;
- ✓ Influence on texture (increases hardness of crumb, loss of crispiness);

- ✓ Changes in appearance (colour, surface properties, density);
- ✓ Influence on taste.

Pasta product

- ✓ Influence on water absorption and swelling index;
- ✓ Influence on optimum cooking and cooking loss;
- ✓ Influence on texture (rubbery, chewy, decrease in pasta firmness);
- ✓ Changes in appearance (colour, surface roughness);
- ✓ Influence on taste (floury mouthfeel).

In order to improve the acceptability of these products, many approaches have been employed (Ktenioudaki and Gallagher, 2012). One of these includes the use of enzymes such as hemicellulolytic culture filtrate, xylanase and α-amylase (Caballero et al., 2007). In general, the enzymes on bread supplemented with dietary fibre increased the loaf volume, improved the shelf-life and bread structure. Also, phytase may be used as a breadmaking improver; in fact, the supplementation of commercial fungal phytase from *Aspergillus niger* in the dough ingredients containing fiber formulation leads to an acceleration of the proofing, an improvement of the bread shape, a slight increase of the specific volume and also confers softness to the crumb. These improvements in bread quality were suggested to be associated with an indirect impact of phytase on α-amylase activity (Afinah et al., 2010). One option to improve quality of high-fibre wheat breads is through sourdough fermentation that significantly improved the volume, texture and shelf-life of wheat bread supplemented with WB.

One of the questions which remain regarding the use of enzyme technology in delivering functional DF ingredients is to what extent manipulating the molecular weights of DF and hence their molecular structure also alters their potential physiological mode of action. This could be one of the explanations as to why differing physiological responses are generated when using guar or β-glucan material isolated using different extraction techniques.

Common dietary fibre sources are represented by cereals and cereal co-products such as wheat, oat, barley and rice. However, very recently, novel sources of fibre have been discovered and/or utilized such as apple, grape, lemon, mango, orange, peach, carrot, cauliflower, onion, pea, potato, tomato (Ayala-Zavala et al., 2011; Elleuch et al., 2011; O’Shea et al., 2012). At present, up to one third of fruit and vegetables in the form of peels, pips and skins can be discarded during preparation and processing, therefore creating a ‘waste’. Utilization of fibres from exotic fruit by-products would not only open new businesses and profits, but would also contribute to give alternate uses to the huge quantities of the by-products wasted in the food industry. Currently these by-products are dispatched to animal feed, landfill or incineration, thus potentially creating negative effects on the environment. Extensive research has shown fruit and vegetable by-products to be a high source of dietary fibre. Also, their use can impart such functional benefits as gelling, thickening and water binding. These properties are advantageous and may be utilized in many fields such as bakery products.

The food industry can take advantage of the physicochemical properties of fibre to improve the viscosity, texture, sensory characteristics and shelf-life of their products (Elleuch et al., 2011). Most commonly, DFs are incorporated into bakery products to prolong freshness, thanks to their capacity to retain water, thereby reducing economic losses and at the same time to enhance digestion. Muffin batter supplemented with peach DF, and cake dough enhanced with prickly pear cladode fibre at levels up to 5% were evaluated as acceptable as the control, based on sensory scores reported by consumer panellists (Ayadi et al., 2009). Mango peel was incorporated into macaroni at three different levels (2.5, 5.0 and 7.5%) and its effect on the cooking properties, firmness, nutraceutical and sensory characteristics of macaroni was studied. The results showed that the macaroni incorporated with mango peel powder up to a 5% level resulted in products with good acceptability. Therefore, the mango peel powder enriched macaroni not only increased the nutritional quality of the product but also increased the nutraceutical property by increasing its antioxidant activity (Ajila et al., 2010). In this perspective, exotic fruits and their by-products could be seriously taken into consideration to be utilized as valuable sources of DF useful for several applications in the food industry.

1.8 Conclusions

The importance of dietary fibre in the human diet is widely accepted, and over the years extensive research has been undertaken relating to the enrichment of food products with fibre. Pasta, bread, extruded snacks, cake and muffins are staple foods that are consumed at large by the majority of the population, and can represent a convenient vehicle for imparting dietary fibre into people's diet. The introduction has reviewed the diversity of fibres that have been used for preparing high fibre products, and the generally agreed effects on dough and end-product quality. Extensive research has shown fruit and vegetable by-products to be a high source of dietary fibre. Also, their use can impart such functional benefits as gelling, thickening and water binding. These properties are advantageous and may be utilized in many fields such as bakery products, meat products, snacks and diabetic beverages. The by-products can also possess antioxidant activity. It has also emerged that DF concentrates could be used as functional ingredients in food to avoid syneresis, to stabilise products with a high percentage of fat and emulsion and to modify the texture and the viscosity of formulated products by virtue of their high WHC and OHC and their rheological properties.

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Chapter 2 - Impact of soluble dietary fibre on the characteristics of extruded snacks

Abstract

The aim of this experimental work was to evaluate the effect of inulin addition on dough rheological properties, texture and sensory quality of extruded snacks. Two commercial fructan products of different degree of polymerisation (DPn) were used at levels from 2% to 7% (DPn = 10 for inulin GR; DPn = 23 for inulin HPX). Dough rheological properties were investigated using dynamic measurements in the linear viscoelastic range (frequency sweep and time cure tests) and farinograph test. Colour, specific volume (Vs), mechanical and sensory properties of snacks were evaluated. Fibre enrichment lowered dough consistency due to a reduction in water absorption. Large differences in elastic properties of samples were observed between 25 and 95 °C due to incompatibility between inulin and starch and different kinetics of starch gelatinization. The magnitude of G' decreased with the increase in fibre content and GR had a greater effect than HPX. Inulin GR increased product expansion and hardness compared with the reference. No significant differences in Vs and mechanical properties were observed between reference and inulin HPX enriched samples up to 5%, while lower values were observed at 7%. Short-chain inulin lowered the extent of non-enzymatic browning. Snacks made with 5% inulin HPX can be used to enhance the fibre content without impacting negatively on product quality.

2.1 Introduction

Over the last decades consumer demands in the field of food production have changed considerably. For this reason, foods today are not intended only to satisfy hunger and to provide necessary nutrients, but also to prevent nutrition-related diseases and enhance physical and mental well-being of consumers (Betoret, Betoret, Vidal and Fito, 2011; Brouns and Vermeer, 2000). Functional foods play an outstanding role. In particular, dietary fibre (DF) have been the subject of considerable attention by researchers due to the potential benefit in reducing coronary heart-related diseases, diabetes incidence and gut neoplasia (Anderson et al., 2009). Recently, research attention has focused on the use of soluble DFs such as inulin-type fructans. Inulin-type fructans are a linear polydisperse carbohydrate material consisting mainly of D-fructose joined by β-(2→1) linkages (Roberfroid, 2005). 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 food industry are chicory and Jerusalem artichoke. Native chicory inulin is a non-fractionated inulin extracted from fresh roots that always contain glucose, fructose, sucrose and small oligosaccharides. The degree of polymerisation (DP) of chicory fructans varies from 2 to 60 (average DP = 12) and about 10% of the fructan chains in native DF have a DP ranging between 2 and 5 (Roberfroid, 2005). Several studies have identified beneficial attributes of inulin such as stimulation of colonic bifidobacteria and lactobacilli (prebiotic activity), improvement of bowel function, increased calcium absorption, positive effects on glucose and lipid metabolism and stimulation of immune system (Biedrzycka and Bielecka, 2004; Roberfroid, 1993, 2005).

Most studies reported the effects of inulin supplementation on dough rheological properties and on the quality of products such as pasta and bread (Aravind, Sissons, Fellows, Blazek and Gilbert, 2012; Brasil et al., 2011; Brennan, Kuri and Tudorica, 2004; Frutos, Guilabert-Anton, Tomas-Bellido and Hernandez-Herrero, 2008; Mastromatteo, Iannetti, Civica, Sepielli and Del Nobile, 2012; Morris and Morris, 2012; Peressini and Sensidoni, 2009). Limited information is available on influence of inulin enrichment on the quality of extruded snacks (Brennan, Monro and Brennan, 2008). Inside the extruder the cereal mixture is heated above the starch gelatinisation temperature leading to a cooked product, that may be directly enrobed and flavoured, or needs further processing such as frying, roasting, etc. (de Cindio, Gabriele, Pollini, Peressini and Sensidoni, 2002; Matz, 1984; Peressini et al., 2002).

Manufacturing high-DF products are directly related to technological changes and maintenance of desired sensory properties. DF addition in extruded cereals most often leads to detrimental effects on product quality due to reduced expansion volume, increase in density and hardness, and decrease in crispness (Robin, Schuchmann and Palzer, 2012). The deleterious effects of various fibres depend greatly on DF properties and inconsistent effects were frequently reported. Soluble fibres such as inulin gave a higher expansion and more favourable texture than insoluble fibres such as cereal bran (Brennan et al., 2008). Differences in expansion volume between soluble and insoluble fibres can be attributed to differences in water absorption, viscoelastic properties of the dough at the exit of the extruder die and stabilisation of the bubble membrane during bubble growth. Inulin at 10% did not induce changes in bulk density of snack products (Brennan et al., 2008). On the contrary, Robin et al. (2012) reported that the effect of soluble fibre content on expansion properties of extruded cereals is unclear, while increasing insoluble fibre appeared to systematically decrease sectional expansion and increase bulk density. Indeed, effect of fibre addition does not only depend on the content, but also on the polymer molecular weight and structure. The type of cereal ingredient to which the fibre is added, also appears important. Unlike for wheat fibre, the use of 10% inulin in extruded corn starch did not influence cell dimension and number (Brennan et al., 2008). Texture of extruded products depends mostly on starch-fibre interaction (Robin et al., 2012). An increase in breaking force of corn flour products with the increase in bran content was observed, while inulin addition gave slight changes (Brennan et al., 2008).

Extrusion-cooking of snack foods is a high temperature and shear process that can determine a loss of functional ingredients added to the formulation. Consequently, it is crucial to monitor the retention level in the final product. The impact of food processes on inulin degradation has been poorly investigated mainly in model systems (Böhm, Kaiser, Trebstein and Henle, 2005; Glibowski and Bukowska, 2011; Glibowski and Wasko, 2008; Klewicki, 2007; Matusek, Merész, Le and Örsi, 2009). The severity of functionality loss was affected by the processing conditions and aggravated by a low pH and heating. Moreover, yeast invertase induces inulin degradation to fructo-oligosaccharides or fructose.

The aim of this experimental work was to evaluate the potential use of inulin as a fibre enriching ingredient in ready to eat snack food products. The effects of various commercial inulin products on dough properties and snack quality were evaluated using rheological, physico-chemical and sensory analyses. The quantitative changes in inulin after processing (cooking-extrusion-roasting) were also determined.

2.2 Materials and methods

2.2.1 Materials

Commercial wheat flour (12.5% moisture, 11.0% protein) (Molino Munari, Italy), defatted soy flour (7.0% moisture, 57.2% protein, 12.0% starch) (Cargill, Belgium), corn starch (13.0% moisture, 0.4% protein, 86.3% starch) (Roquette, France), rice flour (12.4% moisture, 7.3% protein, 79.2% starch) (Pasini, Italy), corn grits (11.8% moisture, 8.0% protein, 69.0% starch) (NDF Atzeca Milling Europe, Italy), sugar and salt were used. Two inulin products from chicory of different degree of polymerisation (DP) were supplied by Orafti Food Ingredients (Belgium): Raftiline-HPX (inulin HPX, 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. Resistant starch (Hi-Maize 260) was provided by National Starch & Chemical Limited (UK).

2.2.2 Product formulation

The snack product formula contained wheat flour (25% w/w), defatted soy flour (25% w/w), corn starch (25% w/w), rice flour (10% w/w), corn grits (10% w/w), sugar and salt (5% w/w) (reference). Fibre-enriched blends contained 2, 5 and 7% (w/w) inulin (7.0% moisture content) were made by replacing defatted soy flour with fibre because of its low starch content. Starch is important for snack expansion and for this reason it was avoid to replace other ingredients such as wheat flour, rice flour, corn starch and grits, which contain high starch levels.

Dry flour blends containing all the ingredients were prepared by means of a traditional flour ribbon mixer and were used for rheological measurements and snack production.

2.2.3 Snack production

The dry flour blend was hydrated to 32% moisture content using a mixing vessel equipped with a micrometric pump for the water and a high speed pre-mixer. The dough was fed to a single screw, low shear cooker-extruder (30 kg/h) with a four step configuration of the screw and thermo-controlled sections of the barrel (G 55 model, Pavan, Galliera Veneta, PD, Italy). Temperatures of the four sections were 85, 145, 145 and 135 °C. Screw speed was 50 rpm. No die was put on the head and the unshaped dough went to feed a single screw former extruder (F 55 model, Pavan) with a round grids shaped die. The barrel and the head were equipped with a water cooling circuit. The screw speed was 25 rpm, head temperature 45 °C and head pressure 190 bar. Shaped product (round grids) was dried at a maximum temperature of 60 °C for 8 h in a static dryer (SD 100 model, Pavan) and roasted at 230 °C for 30 s (HTST equipment, BTO 50 model, Pavan). The moisture content of dried products was about 10.5%.

2.2.4 Mixing properties

Mixing properties of the dough were evaluated using a farinograph equipped with a 100 g bowl (T6 Promylograph Max Egger, Austria). The dry flour blend (80 g) and water (50%, on 14% moisture flour basis) were mixed for 20 min at 30 °C and changes in dough consistency (PU) were recorded during mixing.

In order to compare water absorption of defatted soy flour and inulin, the amount of water (%, on 14% moisture flour basis) required to reach a dough consistency of 500 PU (farinograph water absorption) was evaluated for the following blends: a) wheat flour (33.3%, w/w), corn starch (33.3%, w/w), rice flour (13.3%, w/w), corn grits (13.3%, w/w), sugar and salt (6.8%, w/w) (WCR); b) sample WCR (93%, on 14% moisture basis) and defatted soy flour (7%, on 14% moisture basis) (WCR-7% soy flour); c) sample WCR (93%, on 14% moisture basis) and inulin GR (7%, on 14% moisture basis) (WCR-7% inulin GR); d) sample WCR (93%, on 14% moisture basis) and inulin HPX (7%, on 14% moisture basis) (WCR-7% inulin HPX).

2.2.5 Dynamic rheological properties

Rheological measurements were carried out using a controlled stress rheometer (SR5, Rheometric Scientific, Germany) equipped with serrated parallel plate geometry (25 mm diameter, 2 mm gap). Doughs at 50% water absorption were mixed for 12 min in the farinograph until maximum development, immediately removed from the bowl and placed between the plates of the rheometer. Samples at 80% water absorption were prepared using a) RS (93% w/w) and inulin GR (7% w/w, on 7% moisture basis); b) RS (93%, w/w) and defatted soy flour (7%, w/w). Excess dough was carefully trimmed and the exposed edge coated with silicon grease in order to prevent drying. Each sample was left to rest 10 min after loading before testing. This resting time was sufficient for the dough to relax and to reach a constant temperature. A frequency sweep test was performed at 25 °C from 0.1 to 10 Hz within the linear viscoelastic range. Time cure test was carried out from 25 °C to 95 °C at 1 °C/min and 1 Hz (linear viscoelastic regime). Data obtained were storage modulus (G'), loss modulus (G") and tan δ (G"/G').

Statistical comparisons were made at 1 Hz. Results are the average of triplicates, where each replicate represents a separately mixed dough.

2.2.6 Colour

Snacks were ground using a laboratory grinder to obtain a fine powder (particle size lower than 500 μm). Colour readings were taken from seven separate points on the surface of the powder using a tristimulus colour analyser (Minolta Chroma Meter CR200, Minolta Camera Co., Japan). The illuminant C (CIE, standard, 6774 K) was used and the instrument was calibrated using a standard white tale ($L^* = 98.03$, $a^*= -0.23$, $b^* = 0.25$). Results were expressed as L^* (brightness), a^* (redness) and b^* (yellowness).

2.2.7 Inulin determination

Inulin content of snacks was obtained by a commercial test kit Megazyme International (K-FRUC 12/11, Bray, Ireland). The Megazyme assay is based on enzymatic hydrolysis of sucrose, starch and maltosaccharides present in the sample by the combined action of an enzyme mix (pullulanase, maltase and highly purified beta-amylase), followed by reduction of the sugars to the corresponding sugar alcohols by treatment with alkaline borohydride. Fructan and inulin are hydrolysed to D-fructose and D-glucose with fructanase (exoinulinase) and the released sugars are measured spectrophotometrically after derivatization with p-hydroxybenzoic acid (PAH-BAH). Data are reported as the average of three measurements.

2.2.8 Product mechanical properties

Mechanical properties of snacks were determined using a Texture Analyser (TA.XT plus, Stable Micro System, UK) equipped with a 30 kg load cell. Product (6 g) was compressed with a Kramer shear cell at a speed of 2 mm/s. A force-time curve was recorded to determine maximum peak force (hardness, N) as well as the number of fracture peaks obtained during analyses (crispness). Data are reported as the average of 15 measurements.

2.2.9 Product specific volume

Since the particular shape of the sample, the volume of snack products was calculated by using image analysis. In order to capture/acquire images by scanner (Olivetti d-color MF 2501), a single extruded snack was placed in a Petri plate. Then, the snack image was analysed by the software Image Pro Plus-6.3. In particular, the total number of pixels of the snack and the Petri plate surfaces were detected. A ratio between the pixels of the both surfaces and the knowing area of the Petri plate gave the area (cm^2) of the snacks. Specific volume (Vs, cm^3/g) was calculated using the following
equation:

$$Vs = \frac{A \times h}{m}$$

where A (cm^2) is area of snack round grid, h (cm) is product thickness and m (g) is mass. The thickness was measured by using a caliper.Ten snacks per each formulation were analysed.

2.2.10 Sensory evaluation

A nine point hedonic rating scale was used to determined acceptability of inulin-enriched snacks. The panel consisted of 20 untrained panellists, who evaluated global taste, sweetness, crispiness and overall acceptability. A score of 1 represents 'dislike extremely' and a score of 9 represents 'like extremely'. Samples were randomly coded and served with the reference.

2.2.11 Statistical analysis

All experiments were performed in a completely randomized design. Statistical differences in dough and snack characteristics were determined by one-way analysis of variance (ANOVA) and Duncan's multiple range test (P = 0.05) (Statistica software version 5, 1997).

2.3 Results and discussion

2.3.1 Mixing properties

The farinograph results of dietary fibre-supplemented dough and the reference dough (without fibre) at constant water content are shown in Fig. 2.1 and Table 2.1. Time required for dough development, or to reach the maximum consistency during mixing, decreased as a consequence of inulin addition (Fig. 2.1). Fibre enrichment lowered dough consistency due to a reduction in water absorption in agreement with O'Brien, Mueller, Scannell and Arendt (2003), Peressini and Sensidoni (2009),Wang, Rosell and Benedito de Barber (2002). The extent of the decrease varied widely with the inulin type. The lowest dough consistency was observed with inulin GR, which decreased consistency in a linear way from 700 UP in the reference to 380 UP in the sample with 7% fibre (r = 0.97) (Table 2.1). Inulin HPX at the same level gave a firmer dough (540 UP). The influence on water absorption was higher for short-chain than long chain inulin probably due to a lubricating effect of sugars and oligosaccharides (Peressini and Sensidoni, 2009; Rouillè, Della Valle, Lefebvre, Sliwinski and van Vliet, 2005).

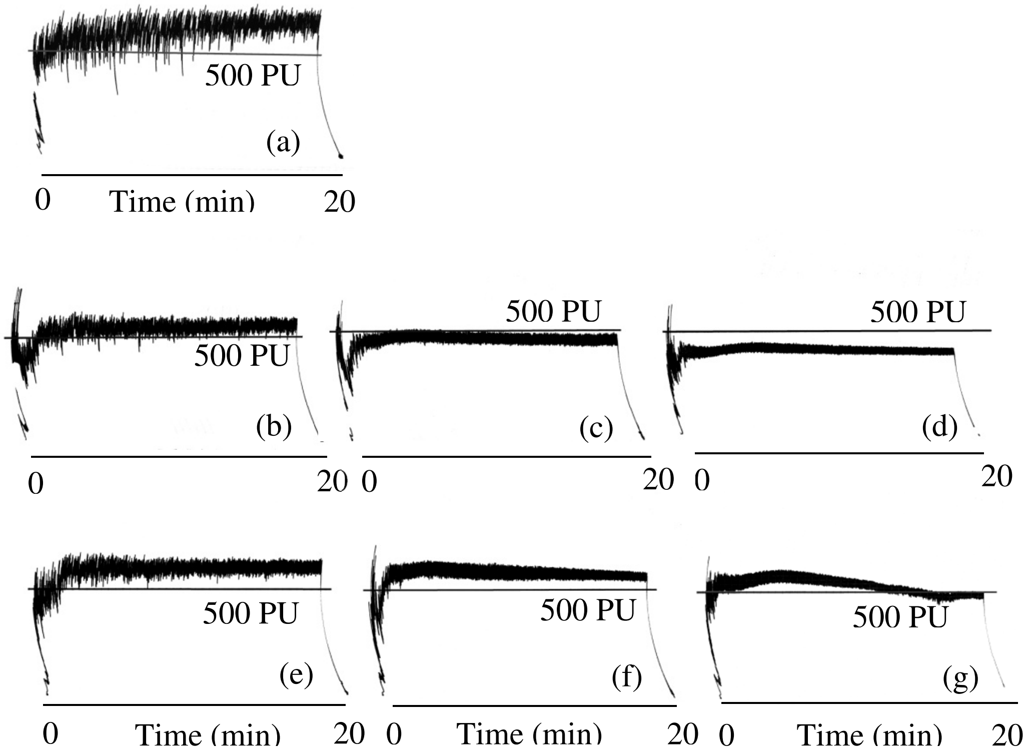

Figure 2.1. Farinograph curves of flour blend without inulin (a), and flour blend enriched with inulin GR at 2% (b), 5% (c) and 7% (d), inulin HPX at 2% (e), 5% (f), 7% (g).

In fact, farinograph water absorption of WCR was 46.5%, while WCR-7% inulin GR and WCR-7% inulin HPX gave values of 38.4% and 42.2%, respectively.WCR-7% soy flour exhibited the highest water absorption (50.9%). This is an important remark, because this means that water absorption capacity of soy flour also plays a role. On the basis of these results removing this ingredient liberates water for viscosity lowering. The effect of replacing defatted soy flour by inulin on dough consistency has important implications during processing. Dough consistency (or viscosity) expresses the tendency to resist flow as a result of internal frictions (Rao, 1999). During extrusion, shear stresses induce shear flow of dough in the extruder channel and their extent depends on material apparent viscosity. Our results indicate that stresses inside the extruder will decrease with the increase in fibre content, and inulin GR will lower their magnitude more than HPX at the same level.

Table 2.1. Effect of inulin addition on rheological properties of dough.

	Raw product base							**Extruded product**	
Inulin (%)	Consistency [a] (PU)	G' [b] (kPa)	$\tan\delta$ [b] (-)	G'_{peak} [c] (kPa)	$\tan\delta_{peak}$ [c] (-)	$\Delta G'_{peak}$ [c] (kPa)	T_{peak} [c] (°C)	G' [b] (kPa)	$\tan\delta$ [b] (-)
GR									
0	700 a	229 a	0.21 a	289 a	0.17 a	67 c	81.8 d	86 a	0.21 a
2	540 b	159 b	0.22 a	254 b	0.17 a	97 b	83.8 c	-	-
5	460 c	107 c	0.22 a	235 c	0.16 a	128 a	90.0 b	-	-
7	380 d	89 d	0.22 a	223 c	0.17 a	137 a	91.3 a	93 a	0.21 a
HPX									
0	700 a	229 a	0.21 bc	289 a	0.17 a	67 c	81.8 d	86 b	0.21 a
2	590 b	168 b	0.23 a	253 bcd	0.17 a	88 b	84.0 c	-	-
5	580 b	145 c	0.22 abc	246 c	0.17 a	97 a	88.1 b	-	-
7	540 c	140 c	0.21 bc	260 bd	0.16 a	113 a	90.3 a	122 a	0.19 b

For each inulin type values within a column followed by the same letter are not significantly different ($P > 0.05$)

[a] Farinograph measurement

[b] Values at 1 Hz obtained from the frequency sweep test at 25°C on dough

[c] Values obtained from the time cure test on dough. $\Delta G'_{peak} = G'_{peak} - G'_{25°C}$

2.3.2 Dough viscoelastic properties

The effect of inulin on the viscoelastic properties of dough was assessed by dynamic, small deformation tests. Flour blend doughs for fundamental rheological tests were prepared with constant water content and a mixing time of 12 min, which was the time to develop dough without fibre. Fig. 2.2A shows the frequency sweep test at 25 °C for flour blend doughs containing inulin GR. Similar trends were observed for inulin HPX (data not shown).

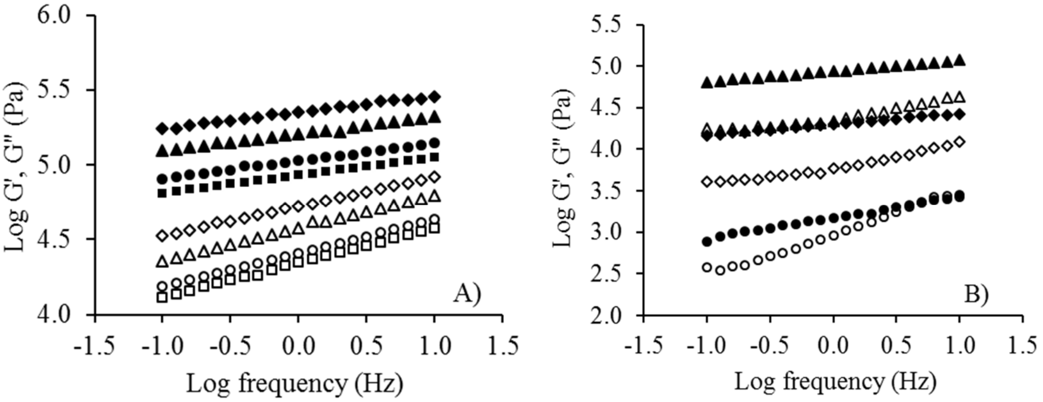

Fig. 2.2. Storage (G') and loss (G") moduli vs. frequency at 25 °C for dough. A) Flour blend dough enriched with inulin GR at levels of 0 (♦), 2% (▲), 5% (●) and 7% (■), and 50% water absorption; B) WCR (♦), WCR-7% soy flour (▲) and WCR-7% inulin GR (●) doughs at 50% water absorption. G': close symbol; G": open symbol.

Samples displayed weak gel-like behaviour because the magnitudes of G' are much higher than those of G" and viscoelastic moduli are frequency dependent. The addition of fibre considerably decreased the storage (G') and loss (G") moduli of dough. Rheological parameters at a frequency of 1 Hz were used to compare samples (Table 2.1). The magnitude of G' decreased with the increase in fibre content. Inulin GR had a greater effect than HPX. Generally, no significant differences in tan δ were observed between control and inulin doughs ($P > 0.05$). Lower G' values for samples containing fibre indicate a lower number of elastically effective interactions. This behaviour is probably due to incompatibility between inulin and starch, and the presence of small inulin crystals throughout the sample (Zimeri and Kokini, 2003a, 2003b). According to Manno et al. (2009), inulin and starch merged in the mixture without interacting chemically with each other. Low average DP and high solubility are accompanied with lower values of storage and loss moduli (Juszczak et al., 2012). This behaviour could to be connected with the presence of crystalline particles, which are especially rich in high molecular weight inulin chains (Juszczak et al., 2012). Bot, Erle, Vreeker and Agterof (2004) observed that aggregates of crystals may contain significant amounts of fluid phase. At the same level, HPX samples exhibited higher G' values than GR samples and no differences in tan δ. Since G"/G' ratio did not change, we assume that inulin HPX addition did not contribute to increase dough structure in comparison to GR inulin, but simply determined a concentration of the system due to inclusion of water between crystals. Another explanation for changes in rheological properties could be the decrease in defatted soy flour by replacing with fibre. Rheological measurements of flour blend doughs (Fig. 2.2A) do not allow to decouple the effects of soy flour and fibre. For this purpose, the linear viscoelastic properties of WCR-7% soy flour (without inulin) and WCR-7% inulin GR (without soy flour) doughs were evaluated (Fig. 2.2B). The storage and loss moduli of WCR-7% soy flour was higher than WCR dough indicating that soy flour is able to contribute to the overall dough elasticity and strength. WCR-7% inulin GR decreased both moduli and increased liquid-like behaviour (G"/G' ratio) in comparison with WCR due to incompatibility between inulin and starch, and probably because inulin absorbs less water (Fig.2.2B). Results confirm that changes in rheological properties of blend flour doughs for snack production (Fig. 2.2A) are due to both inulin addition and soy flour lowering. Temperature-dependence of the dough rheological properties during extrusion-cooking was determined by a dynamic temperature ramp test at constant frequency (Fig. 2.3) (Peressini et al. 2002). Changes in G' profile reflects structural transitions associated with heating of the system above the starch gelatinization temperature inside the extruder. During the initial heating, G' decreased as the dough temperature increased indicating a softening of the material, while a sharp increase in the storage modulus to a maximum was observed above 60 °C, as a consequence of starch gelatinisation in agreement with previous findings (Dreese, Faubion and Hoseney, 1988; Peressini et al., 2002). Peak of G' can be considered as a parameter directly linked to the swelling of starch granules (Peressini, Pin and Sensidoni, 2011; Rolee and LeMeste, 1999). In order to demonstrate that there was not a contribution of inulin or soy flour to peak of G' , a dough containing resistant starch granules (RS) and 7% inulin GR or 7% soy flour was analysed (Fig. 2.3C). RS is more stable and the magnitude of granule swelling should be lower than regular starch granules. Fig. 2.3C shows changes in G' vs. temperature for RS and flour blend doughs enriched with 7% inulin GR.

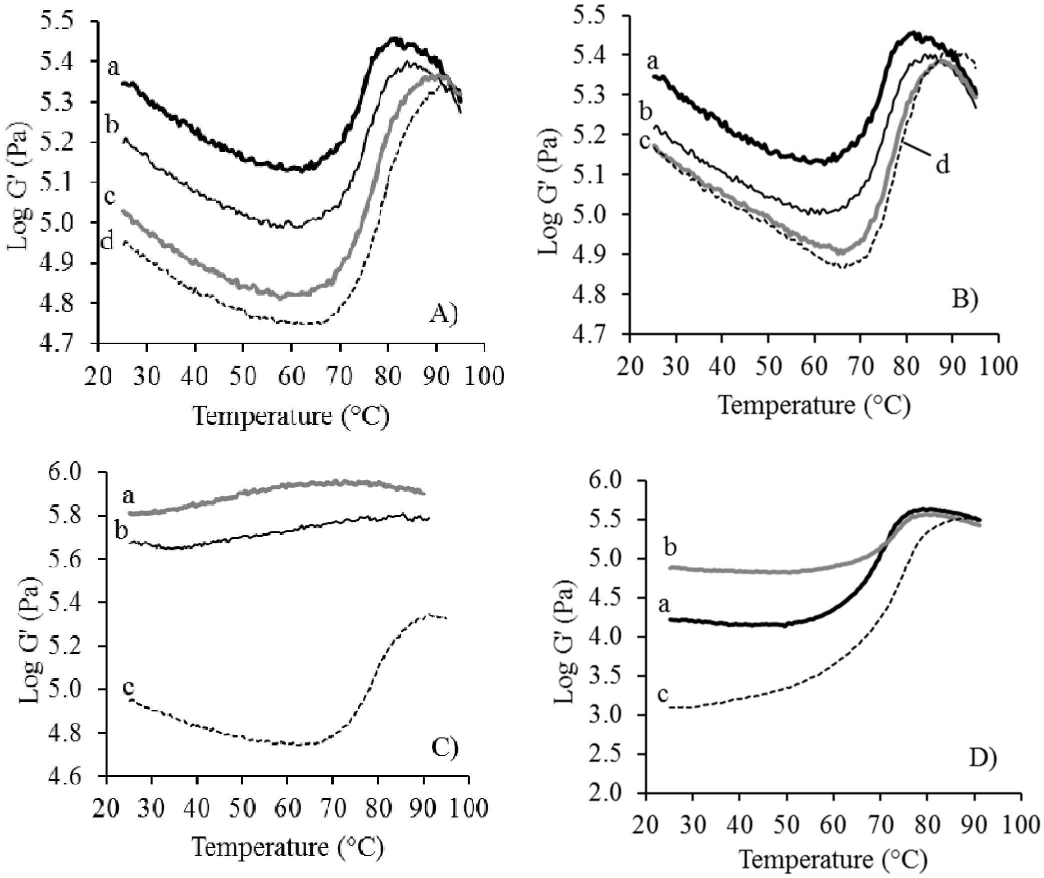

Fig. 2.3. Storage modulus (G') vs. temperature at 1 Hz for dough. A) Flour blend dough enriched with inulin GR at levels of 0 (a), 2% (b), 5% (c) and 7% (d), and 50% water absorption; B) flour blend dough enriched with inulin HPX at levels of 0 (a), 2% (b), 5% (c) and 7% (d), and 50% water absorption; C) resistant starch granules-7% inulin GR dough at 80% water absorption (a), resistant starch granules-7% soy flour dough at 80% water absorption (b) and flour blend dough enriched with 7% inulin GR at 50% water absorption (c); D) WCR (a), WCR-7% soy flour (b) and WCR-7% inulin GR (c) doughs at 50% water absorption.

The absence of important changes in storage modulus for RS samples supported the hypothesis that inulin and soy flour did not contribute to enhance in G' values above 60 °C, which is mainly due to starch gelatinisation. Inulin enrichment caused lowering of curves and a peak shift to higher temperatures (T_{peak}) (Fig. 2.3A, B; Table 2.1). The values of T_{peak} increased with the increase in inulin content (Table 2.1). The reference exhibited a T_{peak} of about 82 °C, while it rose to 90-91 °C for 7% inulin samples. Inulin-enriched doughs resulted in lower peak values of G' than that recorded for the reference (Fig. 2.3A, B; Table 2.1). Similarly, Brennan et al. (2008) and Juszczak et al. (2012) observed that the inclusion of inulin into flour and starch-based doughs resulted in a decrease in peak viscosity determined from the pasting profile. This effect increased with increasing levels in fibre and was higher for inulin GR than HPX, as observed at low temperature, but this was not simply due to the initial change in G'. Above 60 °C, ascent of G' was higher for fructan samples than that of the reference. This observation indicates that inulin supplementation seems to promote the swelling of starch granules. Since fibre was added by replacing soy flour, the effect of each ingredient on dough thermo-mechanical properties was studied (Fig. 2.3D). Ascent of G' was lower for WCR-7% soy flour and higher for WCR-7% inulin GR than WCR dough. The different behaviour of the samples is probably due to large differences in dough water absorption (38.4-50.9%).

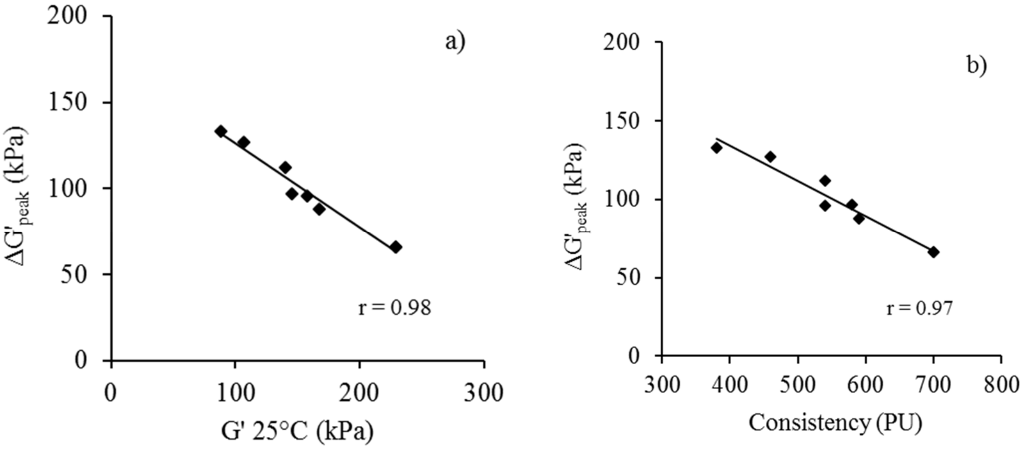

Fig. 2.4. Values of $\Delta G'_{peak}$ vs. G' 25 °C from time cure test (a) and $\Delta G'_{peak}$ vs. farinograph consistency (b) for flour blend doughs with and without inulin at 50% water absorption. - linear correlation.

Water binding properties of soy flour is probably one of the factors responsible for the decrease in swelling of starch granules, by limiting the amount of available water for amorphous regions in starch granules. On the contrary, inulin GR might favour swelling due to lower water absorption than soy flour. Besides the competition between swelling starch granules and ingredients for water, other factors such as bulk viscosity or elasticity may be important. This is clearly indicated by the fact that increasing fibre level, which leads to reduced viscosity and elasticity, resulted in a higher ascent of G' during heating. Linear correlations between dough elasticity or viscosity and difference of G'_{peak} - G' 25 °C ($\Delta G'_{peak}$) were found (r = -0.98 for $\Delta G'_{peak}$ vs. G' 25°C; r = -0.97 for $\Delta G'_{peak}$ vs. consistency) (Fig. 2.4). It seems that with inulin addition, dough exhibits low resistance and consistency, which allow for a larger increase in starch swelling. In contrast, previous investigations suggested an inhibition effect of inulin on starch swelling (Brennan et al., 2004; Juszczak et al., 2012). A possible explanation for this discrepancy could be related to differences in the rheological methods used and product type. Most investigations were performed using Amylograph and Rapid

Visco Analyser, which provide the pasting properties of an aqueous flour suspension (AACC, 2000). This requires more water than dough, and water excess probably decreases the viscosity and elasticity of the system to lower values, which are too low to give the effect observed in our experimental work. Nevertheless, the time cure test showed large differences in elastic properties of samples, which imply differences in the magnitude of shear stresses acting on the dough during the extrusion-cooking process (Fig. 2.3). The highest and lowest elasticity values (or shear stresses) were observed for the reference and 7% inulin GR dough, respectively. Structure of the extruded product is the result of the combination of high temperature and shear stress inside the extruder. In order to study extensively the structure, extruded products of reference and 7% fibre were ground and used to produce dough, which was analysed by a frequency sweep test. The storage modulus and tan d at 1 Hz are reported in Table 2.1. The extruded product from 7% inulin HPX showed significant higher G' and lower tan d values than the reference ($P < 0.05$). Rheological properties for 7% inulin GR were essentially the same as that of reference. A higher G' and a lower tan d for inulin HPX indicate a more elastic and solid-like material. This could be due to assembly of polymer chains, which differ from the original, when cooling from above the melting temperature (Sereno, Hill and Mitchell, 2007). Aggregation is strongly influenced by DP. Kim, Faqih and Wang (2001) have found that suspensions of long-chain inulin give sol-gel transition due to polymer association. Smaller chains with relatively low DP remain in the liquid portion without association with the gel structure (Kim et al., 2001).

2.3.3 Snack quality

The impact of different levels of inulin GR and HPX on snack properties is presented in Table 2. No significant differences in specific volume (Vs) were observed between reference and inulin HPX enriched samples at 2% and 5% levels, while a slightly lower value was observed at 7% ($P = 0.05$) (Table 2.2). Inulin GR increased product Vs compared with the reference sample. Differences in viscoelastic properties between extruded samples would explain these results (Table 2.1). Probably, high number of elastic interactions and solid-like behaviour of extruded products containing HPX prevent expansion during roasting (Tables 2.1 and 2.2). It is also possible that the structure collapsed a bit more due to higher water availability due to reduction of soy flour content. Since this phenomenon was not observed for inulin GR, low Vs of inulin HPX samples at 7% is mainly attributed to high elastic interactions of extruded product. Similar results have been described by Brennan et al. (2008), who observed an increase in density value after addition of 10% and 15% inulin. The cause was ascribed to a reduction in the porosity of the products. However, the effect of soluble fibre content on expansion properties of extruded cereals is unclear. Indeed, the effect does not only depend on the content of fibre. It may also depend on the molecular weight and the structure of the hydrocolloids. Additionally, the effect of soluble fibre on the expansion of extruded products appears to depend on the type of cereal to which the fibre is added (Brennan et al., 2008; Robin et al., 2012).

The moisture content of a control snack was found to be slightly higher than those supplemented with inulin HPX. No significant differences in moisture content were observed between reference and inulin GR enriched samples (Table 2.2). The moisture content has a large effect on mechanical properties of cereal products. Its range was from 2.62 to 4.38 and these values guarantee independence with the crispness characteristics (Peleg, 1994). The effect of fibre supplementation on product mechanical properties depends on inulin type and content (Table 2.2). Addition of short-chain inulin showed significantly higher values of maximum force achieved during fracturing. This implies that instrumentally inulin GR enriched snacks were harder than reference. No significant differences in hardness were observed between reference and inulin HPX up to 5% level. A lower value of maximum force was obtained at 7% inulin HPX according to Brennan et al. (2008) and Laguna, Primo-Martin, Salvador and Sanz (2013), who showed that addition of inulin decreased hardness in breakfast cereals and short-dough cookies, respectively. It is an unexpected result since 7% inulin HPX product exhibited lower Vs value, which should increase hardness (Table 2.2). The reason for the behaviour of inulin HPX at the highest content can be explained through the formation of polymer networks, which do not interact with gelatinised starch (Manno et al., 2009) and consequently weaken the cellular structure of the solid foam. Crispness was measured as the number of force drops or peaks observed during the compression revealing the presence of a brittle cellular structure (Dogan and Kokini, 2007; Vincent, 1998). No significant differences in crispness were observed between reference and inulin enriched samples ($P = 0.05$) in agreement with what reported for cookies by Laguna et al. (2013). In contrast, Brennan et al. (2008) observed that fructan addition to extruded breakfast cereal products increased the number of peaks compared with the reference.

Table 2.2. Effect of inulin addition on snack properties.

					Sensory evaluation					
Inulin (%)	Vs (cm^3/g)	Moisture (%)	Max force [a] (N)	Force drops [b] (-)	Global taste (-)	Sweetness (-)	Crispness (-)	Acceptability (-)	Fructan (%)	Fructan loss (%)
GR										
0	3.93 b	3.38 a	185 d	9.65 a	4.0 a	2.0 a	7.4 a	5.0 a	1.31 d	15.5
2	5.04 a	4.38 a	238 a	9.91 a	3.9 a	2.3 a	6.5 a	5.1 a	3.06 c	13.9
5	5.15 a	3.89 a	214 c	10.32 a	3.8 a	2.4 a	6.1 a	4.7 a	4.62 b	29.5
7	5.15 a	4.16 a	224 b	9.21 a	3.6 a	2.7 a	6.2 a	4.7 a	7.30 a	14.7
HPX										
0	3.93 ab	3.38 a	185 a	9.65 a	4.0 a	2.0 a	7.4 a	5.0 a	1.31 d	15.5
2	4.04 a	2.62 d	174 b	8.70 a	3.9 a	2.2 a	6.7 a	4.8 a	3.11 c	12.4
5	3.74 bc	2.97 c	177 ab	9.97 a	3.8 a	2.3 a	6.6 a	4.9 a	5.70 b	13.0
7	3.62 c	3.27 b	171 b	9.88 a	3.7 a	2.4 a	6.7 a	4.6 a	6.52 a	23.7

For each inulin type values within a column followed by the same letter are not significantly different ($p > 0.05$)

[a] snack hardness; [b] instrumental snack crispness

The effect of fibre addition on snack colour is summarised in Fig. 2.5. Colour was not affected by long-chain fibre enrichment. Addition of inulin GR gave higher L* and lower b* and a* parameters than reference. These changes in colour are associated to different extent of non-enzymatic browning (NEB) (Barbanti, Mastrocola and Lerici, 1990; Sensidoni, Peressini and Pollini, 1999, 2003). In particular, high extent of NEB is related to a decrease in brightness (L*) and increase in redness (a*) and yellowness (b*). On the basis of these results short-chain inulin appears to reduce the kinetic of Maillard reaction. Previous researches reported a higher crust browning in bread for all levels of addition and different types of inulin (Morris and Morris, 2012; Peressini and Sensidoni, 2009). The darker colour of fibre-enrichment bread has been explained by a higher content of reducing sugars involved in Maillard reaction. Gennaro, Birch, Parke and Stancher (2000) did not observed high reducing activity of long and short chain inulin, but some polymer chains were degraded during breadmaking leading to the formation of glucose and fructose (Morris & Morris, 2012). It is important to consider that processing affects the availability of reagents (reducing sugars) for Maillard reaction. Extrusion-cooking is known to modify starch at all structural levels: the granular structure disappears, the crystals melt and macromolecules depolymerize (Barron, Buleon, Colonna and Della Valle, 2000). van den Einde et al. (2004) reported that the maximal shear stress during heating-shearing treatment was found to be the key parameter determining the degree of macromolecular degradation of starch. The degree of macromolecular degradation during extrusion processes can be reduced by decreasing the maximal shear stresses. On the basis of rheological data increasing inulin content also decreases the shear stresses during processing and this effect is higher for inulin GR than HPX (Table 2.1). Lower starch degradation in samples containing inulin GR were assumed. Soy flour contains both proteins and reducing sugars. Removing this ingredient did not influence browning since control and inulin HPX breads did not give significant differences in colour parameters.

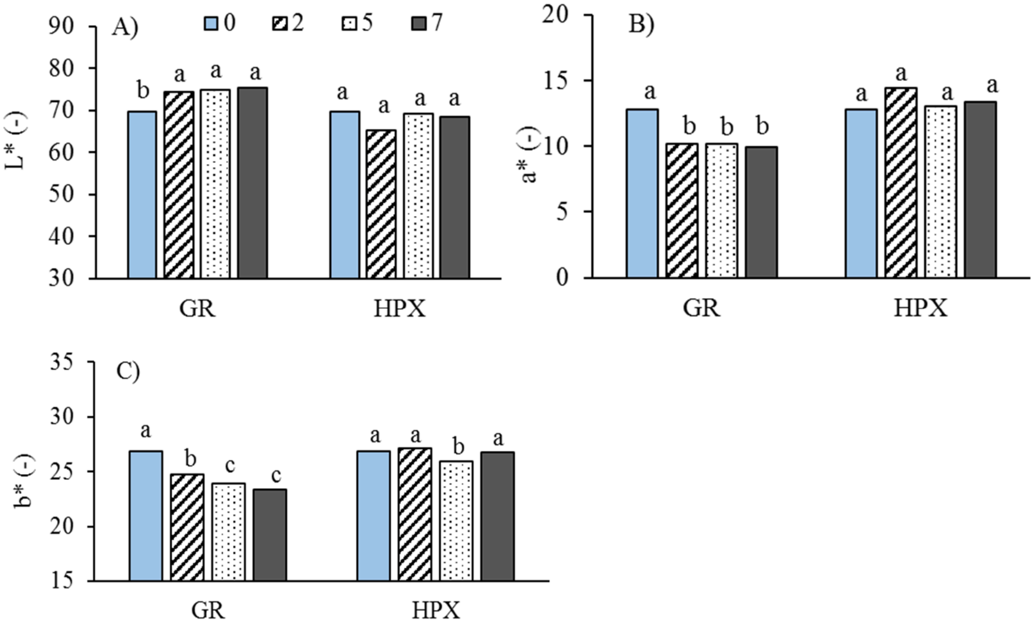

Fig. 2.5. Values of L* (A), a* (B) and b* (C) for snack products enriched with inulin GR and HPX at different levels.

Sensory evaluation did not give significant differences in global taste, sweetness, crispness and overall acceptability between reference and fibre samples ($P = 0.05$) (Table 2.2). On the basis of these attributes assessors were unable to distinguish inulin fortified snacks from the control sample.

2.3.4 Inulin degradation

Very limited information exists on the stability of inulin during a process of cooking-extrusion and roasting. Inulin degradation must be taken into account when fructan is used as a functional ingredient in thermally treated foods like many cereal products. Table 2.2 shows fructan content of different snack products. Loss in fibre ranged from about 12.4% to 29.5% compared with the initial amount added to the dough. This degradation of inulin chains occurred partially during extrusion-cooking leading to low molecular weight products, which probably determined a further reduction in dough consistency and elasticity. However, this degradation did not induce higher extent of Maillard reaction in snack containing fibre. Processing reduced fructan content by an average of 17.5%. On the basis of these results dough is subjected to heating and shearing conditions not too severe for the stability of inulin.

2.4 Conclusions

The results of the present study show that the inclusion of inulin in an extruded flour-based product significantly modified dough rheological properties and characteristics of snacks. The changes depend on the level and type of inulin in terms of degree of polymerisation and presence of low molecular weight sugars. Fibre enrichment lowered dough consistency and elasticity due to a lubricating effect of sugars and oligosaccharides, and different kinetics of starch gelatinization. Inulin GR had a greater effect than HPX. Short-chain inulin lowered the extent of non-enzymatic browning. Inulin GR increased product expansion and hardness compared with the reference, while no significant differences in volume and mechanical properties were observed between reference and inulin HPX enriched samples up to 5%. On the basis of these results, inulin HPX up to 5% can be used to enhance the fibre content of extruded snacks without impacting negatively on product texture or taste.

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Chapter 3- Rhelogical behaviour of a dough model system enriched with soluble dietary fibres

Abstract

Rheological properties of a gluten-starch dough model added with dietary fibre were evaluated. Short-chain inulin (GR), long-chain inulin (HPX) and β-glucan (Barley Balance) were used at 8% level of substitution. Moreover, all dietary fibre ingredients were added in combination. In general, when added individually, inulin lowered dough consistency, while β-glucan increased this parameter compared to control (without fibre). Moreover, the differences in elastic properties and kinetics of starch gelatinization suggested that short-chain inulin (GR) weaken dough structure, while Barley Balance increased elastic effective interactions. However, when dietary fibres were coupled, dough characteristics resulted similar to the semolina model system (control); the results suggested that a combination of fibres allowed to obtain a dough with characteristics comparable with the control. In particular, samples made with 4% of inulin GR and 4% of Barley Balance might be used with the purpose to improve nutritional value of a hypothetical pasta sample, without negatively affecting dough characteristics, which in turn influence textural characteristics of the end product.

3.1 Introduction

Pasta is starchy staple food widely consumed across the world and is made by using durum wheat semolina for delivering a product with unique rheological properties, which highly influence pasta quality and consumer acceptability. Many studies have attempted to improve its nutritional properties by including into formulation functional ingredients, such as dietary fibre (DF) (Foschia, Peressini, Sensidoni, Brennan and Brennan, 2013). Indeed, it has been demonstrated that the addition of DF introduce additional health benefits (Mudgil and Barak, 2013) and allows developing novel functional food products. DFs are generally classified as soluble or insoluble, based on whether they form a solution when mixed with water (soluble) or not (insoluble). The soluble and insoluble nature of DFs

involves differences in their technological functionality and physiological effects (Abdul-Hamid and Luan, 2000; Brownlee, 2011; Elleuch, Bedigian, Roiseux, Besbes, Blecker and Attia, 2011). Soluble fibres are characterised by their capacity to increase viscosity, to reduce the glycaemic response and plasma cholesterol. Insoluble fibres are characterised by their porosity, their low density and by their ability to increase faecal bulk and decrease intestinal transit. Compared with insoluble dietary fibre, in food processing the soluble fraction demonstrates greater capacity to provide viscosity, ability to form gels and/or act as emulsifiers, has neither bad texture nor bad taste and is easier to incorporate into processed food and drink. The food industry can take advantage of the physicochemical properties described above to improve the viscosity, texture, sensory characteristics and shelf-life of their products. However, the percentage of fibre that may be added is finite, because it can cause undesirable changes to the colour and texture of foods.

In particular, soluble fibres, such as β-glucans and inulin, can attenuate postprandial blood glucose and insulin levels, lower serum cholesterol levels and promote prebiotic activity in the host health (Gibson, Probert, van Loo, Rastall and Roberfroid, 2004; Gibson and Roberfroid, 1995; Wood, 2007).

The (1→3), (1→4)-β-D-glucan, or β-glucan, is a linear polysaccharide that consists only of glucose. β-glucan is known as one of the most valuable dietary fiber present in cereal crops such as oat and barley (Butt, Thair-Nadeem, Khan, Shabir and Butt, 2008; Dodevska, Djordjevic, Sobajic, Miletic, Djordjevic and Dimitrijevic-Sreckovic, 2013; Skendi, Biliaderis, Lazaridou and Izydorczyk, 2003; Stuart, Loi and Fincher, 1988). However, molecular weight ranges reported for β-glucans show variability between cereals, with oat β-glucans generally having a higher upper molecular weight compared to barley (Brennan and Cleary, 2005; Colleoni-Sirghie, Kovalenko, Briggsa, Fulton and White, 2003). The molecular weight of this DF has significant effects on product viscosity and gelling properties of a food system (Vaikousia, Biliaderisa and Izydorczyk, 2004). Chillo, Ranawana and Henry (2011a, 2011b) investigated the impact on the *in vitro* glycaemic response and on cooking quality of fresh pasta made with semolina plus the addition of one of either two types β-glucan barley concentrates, Glucagel® and Barley Balance®. The results revealed that the addition of up to 10% of both types of β-glucan does not significantly alter its overall cooking quality compared to the control. Finally, Barley Balance wass better able to reduce the glycaemic potency of spaghetti than Glucagel.

Inulin-type fructans are linear polydisperse carbohydrate material consisting mainly of D-fructose joined by β-(2→1) likages (Roberfroid, 2005). 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 influences their physico-chemical properties (Roberfroid, 2005), which in turn affect technological characteristics (Gonzalez-Tomás, Coll-Marqués and Costell, 2008). Moreover, long-chain inulin is more stable thermally, less soluble and more viscous than the native one and it can be used to structure low-fat foods (Meyer, Bayarri, Tárrega and Costell, 2011).

In the present research project, two types of inulin and a β-glucan concentrate products were added in a model system, which represented durum wheat semolina for pasta making. The effects of these soluble dietary fibres, added individually and in combination, on viscoelastic and mixing properties of the dough were evaluated with the purpose of developing high quality functional pasta.

3.2 Materials and methods

3.2.1 Materials

Gluten (Vital Wheat Gluten, Roquette, Italy) and starch (Wheat Starch, Roquette, Italy) ingredients were used. Two inulin products from chicory of different degree of polymerization (DP) were supplied by Orafti Food Ingredients (Belgium): Raftiline® HPX (inulin HPX, 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. Barley Balance^{TM} (BB, ~ 25% β-glucan) was provided by DKSH (Italy).

3.2.2 Product formulation

The dry blend of gluten (15% w/w) and starch (85% w/w) was used to simulate the semolina for pasta production. Fibre enriched blends contained 8% of DF were made by replacing semolina model system (control) with the following products: inulin GR, inulin HPX and Barley Balance (Table 3.1).

Dry flour blends containing all the ingredients were prepared using a mixing vessel and were used for farinograph test and rheological measurements.

Table 3.1. The percentage weight fraction of raw materials used in the preparation of the model system with and without dietary fibre.

Formulation (%)	**Sample code**					
	Control	**GR**	**HPX**	**BB**	**GR-BB**	**HPX-BB**
Semolina model	100	92	92	92	92	92
Barley Balance	-	-	-	8	4	4
Inulin GR	-	8	-	-	4	
Inulin HPX	-	-	8	-	-	4

3.2.3 Mixing properties

Mixing properties of the dough were evaluated using a farinograph equipped with a 100 g bowl (T6 Promylograph Max Egger, Austria). The dry flour blend (80 g) and water (49.8%, on 14% moisture flour basis) were mixed for 20 min at 30 °C and changes in dough consistency (PU) were recorded during mixing.

3.2.4 Dynamic rheological properties

Rheological measurements were carried out using a controlled stress rheometer (SR5, Rheometric Scientific, Germany) equipped with serrated parallel plate geometry (25 mm diameter, 2 mm gap). Doughs at 49.8% water absorption were mixed in the farinograph until maximum development (12 min), immediately removed from the bowl and placed between the plates of the rheometer. Excess dough was carefully trimmed and the exposed edge coated with silicon grease in order to prevent drying. Each sample was left to rest 10 min after loading before testing. This resting time was sufficient for the dough to relax and to reach a constant temperature. A frequency sweep test was performed at 25 °C from 0.1 to 10 Hz within the linear viscoelastic range. Time cure test was carried out from 25 °C to 95 °C at 1 °C/min and 1 Hz (linear viscoelastic regime). Data obtained were storage modulus (G'), loss modulus (G") and tan δ (G"/ G'). Statistical comparisons were made at 1 Hz. Results are the average of triplicates, where each replicate represents a separately mixed dough.

3.2.5 Statistical analysis

All experiments were performed in a completely randomized design. Statistical differences in model dough systems characteristics were determined by one-way analysis of variance (ANOVA) and Tukey's multiple range test (p = 0.05) (StataSoft Inc. version 8.0, Tulsa, USA, 2007).

3.3 Results and discussion

The farinograph results of the semolina model dough (control) and dietary fibre-supplemented doughs at constant water content are shown in Fig. 3.1 and Table 3.2.

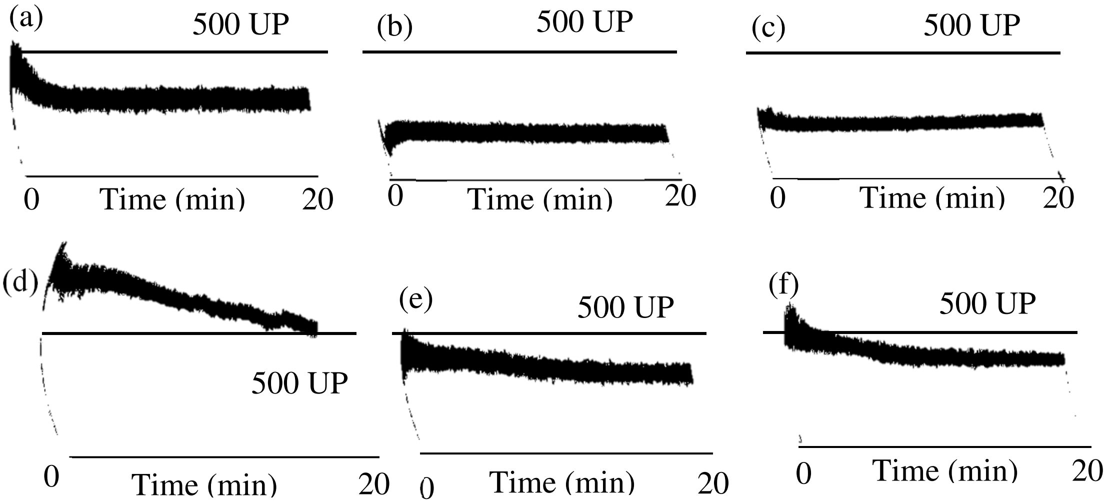

Fig. 3.1. Farinograph curves of Control (a), GR (b), HPX (c), BB (d), BB-GR (e) and BB-HPX (f) at 49.8% water absorption.

It is evident that the presence of 8% of DF in the model system formula caused changes in dough consistency compared to the control (Fig. 3.1 and Table 3.2). In particular when both types of inulin were added individually (Fig. 3.1b-c), time required for dough development during mixing decreased compared to control. Inulin enrichment lowered the dough consistency due to a reduction in water absorption in agreement with O'Brien, Mueller, Scannell and Arendt (2003), Peressini and Sensidoni (2009), Peressini et al. (2015) and Wang, Rosell and Benedito de Barber (2002). The extent of the decrease varied with inulin type. The lowest dough consistency was observed with inulin GR, which decreased consistency from 350 UP (control) to 200 UP in the sample with exclusively 8% of inulin GR (Table 3.2). Inulin HPX at the same level gave a firmer dough (250 UP) than inulin GR. The influence on water absorption was higher for short-chain than long-chain inulin probably due to a lubricating effect of sugars and oligosaccharides (Rouillè, Della Valle, Lefebvre, Sliwinski and van Vliet, 2005). The effect of adding exclusively inulin to semolina model on dough consistency has important implications during processing. Dough consistency (or viscosity) expresses the tendency to resist flow as a result of internal frictions (Rao, 1999). During extrusion, shear stresses induce shear flow of dough in the extruder channel and their extent depends on material apparent viscosity. Our results indicate that stresses inside the extruder will decrease in presence of inulin GR and HPX.

Table 3.2. Effect of dietary fibre addition on rheological properties of dough.

Sample	**Consistency** [a] **(UP)**	**G'** [b] **(kPa)**	**tan δ** [b] **(-)**	**T_{peak}** [c] **(°C)**	**G'_{peak}** [c] **(kPa)**	**$\Delta G'_{peak}$** [c] **(kPa)**
Control	350	36.19 bc	0.30 ab	64 e	246 cd	214 c
GR	200	7.85 d	0.37 a	82 a	269 c	263 b
HPX	250	30.65 c	0.21 b	73 b	496 a	464 a
BB	760	75.79 a	0.30 ab	68 d	241 cd	169 d
BB-GR	350	34.94 bc	0.29 ab	72 bc	221 d	188 cd
BB-HPX	380	45.62 b	0.28 ab	71 c	319 b	276 b

For each sample, values within a column followed by the same letter are not significantly different ($p > 0.05$)

[a] Farinograph measurement

[b] Values at 1 Hz obtained from the frequency sweep test at 25°C on dough

[c] Values obtained from the time cure test on dough. $\Delta G'_{peak} = G'_{peak} - G'_{25°C}$

Different behaviour was registered for the sample containing 8% of Barley Balance. In fact, from Fig. 3.1d and Table 3.2 it can be noted that the dough consistency reached a higher value (760 UP) than all the others samples. The peak of the dough consistency might be explained by the increase in the water binding capacity of β-glucan (Lazaridou et al., 2007). Finally, time required for reaching stability of dough consistency and a disturbing signal of the curve during mixing resulted higher for BB than the other samples. This could due to the addition of non-glutinous material, which might reduce the gluten strength and slowed down the rate of hydration and development of gluten (Sudha et al., 2012). Knowing that oat contains high amounts of soluble fibre (Butt, Tahir-Nadeem, Khan, Shabir and Butt, 2008; Dodevska, Djordjevic, Sobajic, Miletic, Djordjevic and Dimitrijevic-Sreckovic, 2013) mainly β-glucan, mixing characteristics of BB can reflect oat flours behaviour.

When both types of inulin were combined with Barley Balance (Fig. 3.1e-f), farinograph curves and dough consistency resulted similar to the control (Table 3.2). These results suggested that a combination of Barley Balance, rich in β-glucans, with inulin GR or HPX might improve nutritional value of pasta without negatively affecting dough characteristics.

The effect of dietary fibre on viscoelastic properties of model dough was assessed by dynamic, small deformation tests. Flour blend doughs for fundamental rheological tests were prepared with constant water content and a mixing time of 12 min, which was the time to develop BB dough sample. Fig. 3.2 shows the frequency sweep test at 25 °C for model blends with and without dietary fibre. Samples displayed weak gel behaviour because the magnitude of G' are much higher than magnitude of G" and viscoelastic moduli are frequency dependent. Rheological parameters at a frequency of 1 Hz were used to compare samples (Table 3.2). The magnitude of G' decrease when 8% of inulin GR were added, while inulin HPX did not exhibit a storage modulus significantly different from control (Fig. 3.2A). The higher influence on the viscoelastic properties of the dough for short-chain than log-chain inulin was probably due to the lubricating effect of sugars and oligosaccharides (Peressini and Sensidoni, 2009; Peressini et al., 2015; Rouillè, Della Valle, Lefebvre, Slinwiski and van Vliet, 2005). Significant lower G' value for sample containing inulin GR indicate a lower number of elastically effective interactions. This behaviour is probably due to incompatibility between inulin and gluten matrix and the presence of small inulin crystals throughout the model system (Zimeri and Kokini, 2003a, 2003b). According to Manno et al. (2009) and Robin et al., 2012, inulin GR and starch merged in the mixture without interacting chemically with each other. In particular, previous studies have demonstrated that in terms of pasta texture, spaghetti became softer upon incorporation of FH-D inulin (DP 12-14) only at the highest level of substitution of 20% compared to its control, whereas fortification with LV-100 inulin (DP 6-7) resulted in firmness deterioration and above relative to its control (Aravind, Sissons, Fellows, Blazek and Gilbert, 2012). In this case, the scanning electron microscopy highlighted that the control sample had a well-developed protein network with most of the starch gelatinised but having few intact granules. At high levels (20%) of inulin addition, many holes appeared in the pasta surface suggesting a less integrated structure.

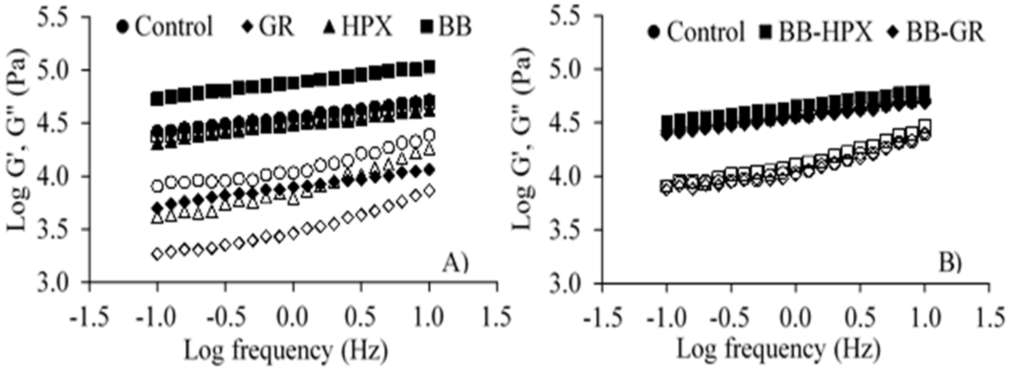

Fig. 3.2. Storage (G') and loss (G") moduli vs. frequency at 25 °C for dough control and enriched with: A) DF added individually; B) DF added in combination. G': close symbol; G": open symbol.

Opposite performance was registered for Barley Balance when added individually (Fig. 3.2A; Table 3.2). Indeed, the magnitude of G' resulted significantly higher (75.79 kPa) than all the other dough samples (range between 7.85 and 36.19 kPa). Higher G' values, indicating higher number of elastically effective interactions, confirmed the previous mixing properties results and was ascribed to Barley Balance capability at structuring the dough model. In fact, Aravind, Sissons, Egan et al., (2012), observed that the substitution of durum wheat semolina with Barley Balance led to a firmer cooked product compared with control. In addition, CLSM images of uncooked and cooked BB-fortified (7.5%) pasta showed a structure with more vacant regions compared with the control, indicating a more irregular structure. The authors suggested that BB substitution in pasta induced an uneven distribution of starch and protein or that β-glucan-protein complex occupied a larger volume than gluten alone, altering the structure. The intensity of protein fluorescence seemed to be significantly greater in BB-fortified samples than control. The protein-rich and starch-rich regions within the BB-enriched samples, as opposed to the more uniform protein network in control, may be indicative of an interaction between protein and fibre. Finally, pasta enriched with 7.5% of Barley Balance appeared to be suitable for the food industry, as it displayed minimal deterioration of its sensory and technological properties and provided lower potential glycaemic index (Aravind, Sissons, Egan et al., 2012).

From Table 3.2 and Fig. 3.2B is evident that the combination of β-glucans and inulin GR or HPX generated a dough with viscoelastic characteristics similar to semolina model system (Control).

Generally, no significant differences in tan δ (Table 3.2) were observed between control and all DF enriched doughs ($p > 0.05$).

Based on these results, it can be hypothesized that the utilization of β-glucans coupled with inulin allows the development of dough probably suitable for pasta production.

Cooking quality characteristics and consumer acceptance of pasta are manly affected by fibre incorporation in pasta formulation because uniform diffusion of cooking water is influenced by the integrity of the protein matrix (Fardet et al., 1998), which in turn is affected by protein content (Cleary and Brennan, 2006; Sissons, Soh and Turner, 2007). For this reason, temperature-dependence of the dough rheological properties during pasta cooking was determined by a dynamic temperature ramp test at constant frequency (Fig. 3.3). Changes in G' profile reflects structural transitions associated with heating of the system above the starch gelatinization temperature. The G'peak is considered as a parameter directly linked to the swelling of starch granules (Peressini, Pin and Sensidoni, 2011; Rolee and LeMeste, 1999). Fig. 3.3 shows that during initial heating G' decreased (except for GR sample) as the dough temperature increased indicating a softening of the material, while a sharp increase in the storage modulus to a maximum was observed above 60 °C, as a consequence of starch gelatinisation in agreement with previous findings (Dreeese, Faubion and Hoseney, 1988; Peressini et al., 2015). Peak of G' can be considered as a parameter directly linked to the swelling of starch granules (Peressini, Pin and Sensidoni, 2011; Rolee and LeMeste, 1999).

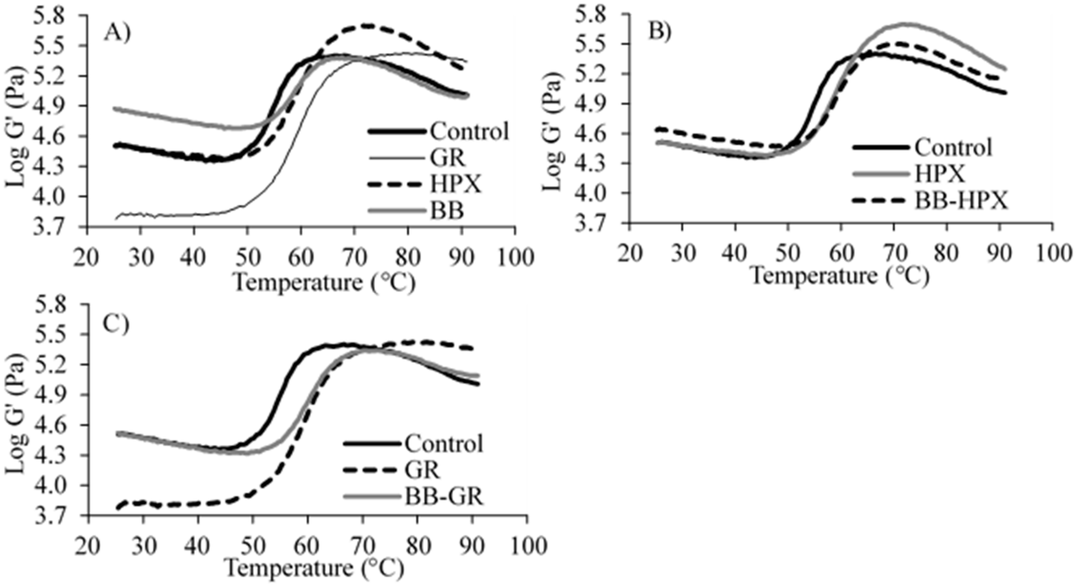

Fig 3.3. Storage modulus (G') vs. temperature at 1 Hz for dough. A) Flour blends without (control) and with 8% of different DFs added individually; B) Flour blends without (control), 8% of inulin HPX added individually and coupled with Barley Balance; C) Flour blends without (control), 8% of inulin GR added individually and coupled with Barley Balance.

When added individually, dietary fibre caused a significant peak shift to higher temperatures (T_{peak}) (Fig. 3.3A and Table 3.2) compared to the control sample in agreement with previous findings (Aravind, Sissons, Egan, Fellows, Blazek and Gilbert, 2012; Peressini et al., 2015). Several mechanism have been proposed in literature to explain the delay in the starch swelling temperature in pasta containing DF (Aravind et al., 2012; Bornet, 1993; Colleoni-Sirghie, Kovalenko, Briggsa, Fulton and White, 2003; Lazaridou, Biliaderis, 2007; Tudorica, Kuri, Brennan, 2002): 1) entrapment of starch granules in a well developed protein-fibre matrix that would act as a physical barrier, thus reducing the effectiveness of digestive enzymes; 2) increased viscosity of the digested food slowing the absorption of glucose in human intestine; 3) limited availability of water for amorphous regions in starch granules, resulting from higher water binding capacity of β-glucans.

The time cure test showed that G'_{peak} values resulted higher for HPX and BB-HPX dough samples than the control (Table 3.2). However, it can be noted from Fig. 3.3 that the doughs presented a different ascent of G'. The difference of G'_{peak}-$G'_{25°C}$ ($\Delta G'_{peak}$) clearly indicated that inulin addition, which produced dough with low resistance and consistency, allowed for a larger increase in starch swelling (Table 3.2). In addition, BB sample showed a ΔG'peak value significantly lower than control; this confirmed that β-glucan addition led to obtain a dough with high resistance and consistency, limiting the starch swelling.

As expected, Barley Balance coupled with inulin (GR and HPX), which presented an opposite behaviour, led to obtain a dough with rheological characteristics similar to the control (Fig. 3.2B). Indeed, GR-BB and HPX-BB showed lower Tpeak and $\Delta G'_{peak}$ than when added individually (Table 3.2) in the model system.

3.4 Conclusions

Farinograph and rheological analyses conducted in this work highlighted that the addition (8%, w/w) of inulin or β-glucan in a gluten-starch model changed significantly viscoelastic and mixing properties of the dough. These changes depend on type of DF in terms of degree of polymerisation, presence of low molecular sugars and water binding/absorption properties. In particular, both types of inulin (short-chain and log-chain inulin) lowered dough consistency, while Barley Balance (rich in β-glucan) increased this parameter compared to control (without fibre). Moreover, different kinetics of starch gelatinization were registered. However, when inulin were coupled with Barley Balance, mixing and rheological characteristics of dough resulted similar to the semolina model system (control). In conclusion, these results suggested that a combination of Barley Balance, rich in β-glucans, with inulin GR or HPX might improve nutritional value of hypothetical pasta sample without negatively affecting dough characteristics, which in turn influence textural characteristics of the end product.

In particular, BB-GR resulted the formula that could better fit for pasta production. For this reason, substitution of DF (added individually and in combination) in a real semolina pasta sample would be necessary in order to confirm the possibility to obtain a high quality functional product. Moreover, this could be conducted utilising not only dietary fibre concentrate, but also flours obtained from cereal crops, such as oat and barley, which are a good source of β-glucans.

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Chapter 4 - Mastication or maceration: does the preparation of sample affect the predictive in vitro glycaemic response of pasta?

Abstract

A series of experiments were conducted to evaluate the effect of different sample preparation steps, prior to the *in vitro* starch digestion process, on the predictive *in vitro* glycaemic response of durum wheat pasta (control), and pasta made with durum wheat semolina and pea flour combinations. The physico-chemical, textural and cooking quality of the pastas were also assessed. The evaluation of the different preparations processes illustrated that the maceration of the samples prior to starch digestion significantly increased the extent of starch degradation and hence the area under the curve (AUC) of reducing sugar released during the digestion process. Mastication of the samples prior to *in vitro* assessment increased the initial reducing sugar content of samples but yielded the lowest recorded AUC for all samples. The replacement of durum wheat semolina with pea flour significantly reduced the samples AUC compared to the control samples when prepared by mastication. This difference was not apparent for the samples prepared by maceration. The results indicate that the choice of the preparation step used prior to *in vitro* starch digestion procedures can significantly affect the predictive glycaemic response - AUC values of samples, and hence manipulate differences attributed to product composition or structure. This may have an impact in terms of choosing the most appropriate method of glycaemic analysis for the food industry.

4.1. Introduction

The glycaemic index (GI) is a scale commonly used in the food industry, and among nutritionists, to depict the increase in blood glucose, which occurs after eating a set portion of available carbohydrate from a test food compared to a reference food (Jenkins et al., 1981). Tools and methodology aiming to establish the predictive digestibility of starches, and hence the predictive GI/response of carbohydrate rich foods, have been studied extensively over many years (Brennan, Blake, Ellis and Schofield, 1996; Woolnough, Monro, Brennan and Bird, 2008; Brennan, Derbyshire, Tiwari and Brennan, 2013; Englyst and Cummings, 1986; Granfeldt and Bjorck, 1991; Southgate, 1969). *In vitro* starch digestibility tests are relatively successful at predicting the GI of high starch food. These methods of *in vitro* analysis focus on variations in the initial breakdown procedure, amounts and types of enzyme used, and incubation using non-restricted (test tube) versus restricted (dialysis) systems. The origin and development of these methods has been described in detail by Woolnough et al. (2008 and 2010). What remains of interest is whether the form in which the food is presented during the initial stages of the *in vitro* system affects the overall digestion process and how milling or maceration of the food prior to digestion mimics mastication in the mouth (Woolnough et al., 2008). Human chewing, similar to that achieved *in vivo*, to initiate *in vitro* testing has been proposed as the most appropriate way to achieve food breakdown. However, as with all human studies, recruitment of subjects can be difficult and variability may occur due to intra-subject physiological differences, therefore alternatives to chewing are required. The chewing process has been mimicked in procedures involving *in vitro* analysis by the use of sieves, mincers and food processors in the past (Bringhetti, Pellegrini, Casiraghi and Testolin, 1995; Eglyst, Eglyst, Hudson, Cole and Cummings, 1999; Symons and Brennan, 2004). The current research compares human chewing to initiate *in vitro* testing with maceration and grinding methods and explores how these different procedures may affect the predictive glycaemic response of pasta. Pasta products were made from durum wheat semolina as well as pea flour enrichment (to mimic high fibre and protein flours). Physico-chemical properties of the pasta products are illustrated to indicate the extent of changes the addition of pea flour may have on the physical properties of pasta.

The use of dietary fibres/non-starch polysaccharides in pasta manufacture is of interest to the industry and the consumers in terms of the production of pasta with a reduced glycaemic load (Tudorica, Kuri, and Brennan, 2002). Padalino et al. (2013) described the effects of different dietary fibres on the chemical properties and cooking quality of pasta, finding that the inclusion of dietary fibres/non-starch polysaccharides altered the rheological nature of the pasta compared to a control. At the same time, the inclusion of dietary fibres into pasta alter the physical characteristics of the product for instance by increasing cooking loss (CL) and increasing the swelling index of the pasta, hence altering the macrostructure and texture of the pasta (Foschia, Peressini, Sensidoni and Brennan, 2013).

Fibre rich foods are attracting interest in terms of potential reduction of the glycaemic response of individuals (Bustos, Perez and Leon, 2013; Sayago-Ayerdi, Tovar, Zamora-Gasga and Bello-Perez, 2014) and also in the alleviation of constipation (Gelinas, 2013). The laxative effect of dietary fibres and fibre rich foods is well known and is associated with the water absorption capacity of dietary fibres and non-starch polysaccharides (Gelinas, 2013). Fiorda et al. (2013) investigated the use of protein rich flours (amaranth and cassava) in manipulating the physical properties of pasta. Their work illustrated that a combination of protein and fibre also increased optimum cooking time and overall CL. Again, this was reported to be due to the effects the protein and fibre had in increasing water absorption and the hydration dynamics of pasta. Pasta products made from semolina generally contain grain protein concentrations of around 12% (at 14%

moisture basis) and considerable efforts have been made in determining the link between protein concentrations and product quality (AbuHammad et al., 2012). The role of water hydration in determining the predictive GI/response of foods is highly relevant as hydration dynamics affect starch gelatinization and overall food structure. What is of further interest is the role of water mobility in amylase activity during starch digestion. The review of Butterworth, Warren and Ellis (2011) clearly illustrates the importance of α-amylase in the initial step of starch digestion. Publications from this group of researchers indicate that the importance of salivary α-amylase is often overlooked when designing *in vitro* determinations of predictive GI values as the presence of salivary α-amylase may affect the rate of reaction of starch hydrolysis (Butterworth et al., 2011; Tahir, Ellis and Butterworth, 2010). In conducting the current research, we aimed at investigating if the overall predictive GI/response is affected by the presence of salivary α-amylase or whether the disruption of the food matrix itself through mechanical means (maceration) has a greater impact. An additional objective of the present work was to evaluate the effect of the durum semolina substitution with 20 and 40% of pea flour on the pasta quality/physico-chemical characteristics and potential glycaemic loading of pasta.

4.2 Materials and methods

4.2.1 Pasta making

A control pasta containing exclusively durum wheat semolina (Molino Borgo San Dalmazzo-CN, Italy) was produced using a fresh pasta machine (Firmar S.p.a.-Villa Verucchio (RN), Italy; model: MPF15N235M). In addition, two experimental pasta samples were prepared with 20 and 40% durum wheat semolina replacement using pea flour (Piko Whoolefoods, Christchurch, New Zealand). Samples were mixed in the pasta maker for 4 min in order to ensure uniform blending of the ingredients. Pasta batches (1.2 kg) were mixed with 30% of tap water (41°C) for 20 min using the pasta maker (according to the manufactures guidelines). After 20 min, the resulting dough was extruded as spaghetti through a 2.25 mm diameter die face. Pasta samples of 20 g were put in sealed

bag and frozen at -18°C until analysis. Prior to analysis, pasta was defrosted for 10 min at room temperature.

4.2.2 Optimal cooking time (OCT)

Spaghetti strands (20 g) were cut into equal lengths of 40 mm and cooked in 300 mL of boiling water. During cooking the optimal cooking time (OCT) was evaluated by taking a sample strand of pasta every 30 s and observing the time of disappearance of the core of spaghetti, by squeezing it between two transparent glass slides, according to the AACC approved method 66-50 (2000). The time at which the core completely disappeared was taken as the OCT. The analyses of spaghetti were made in triplicate.

Additionally, 10 g of spaghetti were cooked in 600 mL of boiling water at OCT, rinsed with 100mL of cold water, strained for 30 s to determine the CL and swelling index of the pasta samples. These analyses were made in triplicate.

4.2.3 Cooking loss (CL)

The CL, the amount of solid substance lost in the cooking water, was determined according to the AACC approved method 66-50 (2000). The cooking water was collected in an aluminium vessel, placed in an air oven at 105°C and evaporated until constant weight was reached. The residue was weighed and reported as a percentage of starting material. These analyses were made in triplicate.

4.2.4 Swelling index (SI) and water absorption index (WAI)

The SI of cooked pasta (grams of water per grams of dry pasta) was determined according to the procedure described by Cleary and Brennan (2006). Spaghetti (10 g) was weighed after cooking and dried at 105°C until a constant weight was reached. The swelling index was expressed as:

$$SI = \frac{Wc - Wd}{Wd}$$

where Wc is weight of cooked pasta (g) and Wd is weight of pasta after drying (g).

To measure the degree of spaghetti hydration, the WAI was determined as the difference between spaghetti weight before and after cooking (AACC, 2000). The water absorption index (WAI, g /100 g) of drained pasta was determined as:

$$WAI = \frac{Wc - Wr}{Wr} \cdot 100$$

where Wc is weight of cooked pasta (g) and Wr is weight of uncooked pasta (g). These analyses were made in triplicate.

4.2.5 Textural characteristics

Pasta (20 g) was cooked to the OCT as determined previously in 2 L of water containing 5 g of NaCl. Pasta was then strained, rinsed with 100mL of distilled water, and allowed to equilibrate at room temperature for 10 min in a covered container before texture analysis (10 replicates per sample). The Texture Analyzer TA.XT2 (Stable Micro Systems, UK) was equipped with a 5 kg load cell. Elasticity

was determined by tension using the A/SPR spaghetti/noodle rig (settings: pre-test speed, 3 mm/s; test speed, 3 mm/s; post-test speed, 5 mm/s; initial distance, 10 mm; final distance, 100 mm; trigger type, auto 5 g; rate for data acquisition, 200 pps).

Firmness was measured according to the approved method 66-50 (AACC, 2000), with some modifications. A noodle blade was used to compress the cooked pasta samples. On the basis of preliminary trials, texture parameters were set as test speed = 0.2 mm/s, post-test speed = 10 mm/s, and distance of 5 mm. Five strands of cooked strands pasta were placed parallel to each other on a flat metal plate and were compressed by the blade until the base plate was contacted.

Each analysis was conducted 10 times.

4.2.6 Colour

Colour readings were taken from nine separate points on the surface of the uncooked/raw and cooked spaghetti (OCT and equilibrated for 5 min at room temperature before analysis) using a tri-stimulus colour analyser (Minolta Chroma Meter CR210, Minolta Camera Co., Japan). The illuminant C (CIE, standard, 6774 K) was used. Results were expressed as L* (brightness), a* (redness), and b* (yellowness). The instrument was calibrated using a standard white tile (L* = 98.03, a* = -0.23, b* = 0.25). These analyses were made in triplicate.

4.2.7 Sample preparation for *in vitro* digestion analysis

Each pasta type was cooked to OCT and prepared for *in vitro* digestion analysis using four different initial breakdown procedures, as followed described:

(1) Chewed by one volunteer.

(2) Macerated with an appliance grinder (Sunbeam Grinder, Sydney, Australia) on medium setting for 30 s.

(3) Cut with knife in order to obtain a 2-5mm size and squeezed 30 times with a pestle (cut-pestle).

(4) Cut with knife in order to obtain a 2-5mm size.

The chewing of pasta was conducted by one volunteer with normal dentition. Only one volunteer was used to reduce any person-to-person variation in terms of amylase content of saliva and perceptions of mastication. Each sample (2.5 g) was chewed 15 times before being expectorated into the digest pots. To evacuate any remaining food particles, the volunteer rinsed their mouth thoroughly with 20 mL of distilled water, which was also expectorated into the digest pots. Pots containing chewed samples were immediately acidified to gastric pH (~2.5) to stop salivary α-amylase activity. All pasta samples were then digested as described below.

4.2.8 *In vitro* digestion analysis

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 and 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 Gmblt & Co., Staufen, Germany). A sample (2.5 g of pasta) was mixed with 30mL of distilled water and was held at 37°C for 10 min. Pepsin (Acros Organics, New Jersey, USA CAS:901-75-6) was added (1mL of 10% solution in 0.05M HCl) in order to replicate gastric digestion. The sample was stirred at 130 rpm at 37 °C for 30 min. An aliquot (1 mL) 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 a-amylase. Pancreatin (EC: 232-468-9, CAS: 8049-47-6, activity: 42 362 FIP-U/g, Applichem GmbH, Darmstadt, Germany) was added (5 mL of 2.5% solution in 0.1M $C_4H_4Na_2O_4$ 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 et al., 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, Derbyshire, Tiwari and Brennan, 2012).

4.2.9 Statistical analysis

All analysis were conducted in triplicate unless otherwise mentioned. Statistical differences in pasta characteristics were determined by one-way analysis of variance (ANOVA) and Tukey's comparison test ($p<0.05$).

4.3 Results and discussion

4.3.1 Effect of pea flour inclusion on physico-chemical characteristics of pasta product

The effects of pea flour addition on technological properties of pasta are shown in Table 4.1. The OCT of spaghetti containing pea flour was greater than that of the control sample (Table 4.1). It is unclear why an increased OCT was observed with the addition of pea flour, however it may be due to the structure and composition of the dietary fibre contained in the pea flour. Similar results were obtained by Zhao et al. (2005) and Gallegos-Infante et al. (2012) who found that the OCT increased in pasta enriched with bean, green and yellow pea, lentil and chickpea flour.

The SI increased with increasing pea flour content. This is in agreement with literature (Dexter, Dronzek and Matsuo, 1978; Sudha and Leelavathi, 2012). The higher SI obtained can be explained by the higher capacity of fibre and protein contained in pea flour to absorb and retain water within the starch-protein-polysaccharide network in comparison to the control. One also has to remember that pea starches are of a different structure to wheat starch and thus may also exert an effect. Table 3.1 illustrates that WAI and CL values increased at increasing amount of pea flour; this aspect may be due to the nature of interaction of legume starch with fibre and protein (Urooj and Puttaraj, 1994).

Table 4.1. Effect of pea flour addition on technological and quality characteristics of pasta.

Measurements	Control	Pea flour 20%	Pea flour 40%
DM raw pasta (g/100 g)	75.68 ± 0.13^{a}	72.26 ± 0.05^{c}	74.13 ± 0.14^{b}
DM cooked pasta (g/100 g)	34.78 ± 0.27^{a}	29.32 ± 0.15^{b}	29.17 ± 0.34^{b}
OCT (min)	6.5	13.0	14.0
SI (g water/g dry pasta)	1.88 ± 0.00^{c}	2.41 ± 0.00^{b}	2.43 ± 0.00^{a}
WA (g/100 g)	98.11 ± 0.43^{c}	125.88 ± 0.76^{b}	133.23 ±3.68^{a}
CL (g/100 g)	4.85 ± 0.18^{c}	6.69 ± 0.15^{b}	7.36 ± 0.21^{a}
Firmness (N)	2.82 ± 0.20^{a}	2.86 ± 0.24^{a}	2.94 ± 0.14^{a}
Elasticity (mm)	42.61 ± 0.38^{a}	26.96 ± 2.65^{b}	18.23 ± 1.56^{c}
Colour raw pasta			
L*	96.69 ± 0.21^{a}	94.79 ± 0.22^{b}	91.75 ± 0.64^{c}
a*	-12.62 ± 0.25^{a}	-10.81 ± 0.54^{b}	-9.33 ± 0.19^{c}
b*	32.38 ± 0.85^{b}	34.35 ± 0.31^{a}	34.62 ± 0.41^{a}
Colour cooked pasta			
L*	95.54 ± 0.17^{a}	94.89 ± 0.25^{b}	92.85 ± 0.37^{c}
a*	-12.51 ± 0.12^{a}	-11.74 ± 0.10^{b}	-10.82 ± 0.56^{c}
b*	32.13 ± 0.65^{b}	31.01 ± 0.21^{c}	32.64 ± 0.15^{a}

DM, dry matter. Values within rows with different superscript letters are significantly different from each other.

Previous research has illustrated that the substitution of semolina flour with legume flour attributed to the structural changes in the protein network, which in turn was reflected in greater cooking losses (Gallegos-Infante et al., 2012; Sudha and Leelavathi, 2012; Torres, Frias, Granito and Vidal-Valverde, 2006; Zhao et al.,2005). This increase in CL is related to a disruption of the protein-starch matrix and the uneven disruption of water within the pasta matrix due to the competitive hydration tendency of the fibre. Previously, a clear link has been demonstrated between the protein content and composition of durum wheat and the cooking quality of pasta (Fardet et al., 1998). In a review, Sissons (2008) explained that in pasta, semolina proteins are linked together by disulfide, hydrogen and hydrophobic bonds to form a matrix, which gives cooked pasta its viscoelastic properties. The continuity and strength of the protein matrix is dependent on the nature of inter- and intra-molecular bonds. During the cooking process, this matrix gradually disintegrates, releasing exudates during starch granule gelatinization, which in turn contributes to an increase in cohesiveness and stickiness on the cooked pasta surface. The results obtained in our research work are similar to those obtained by Chillo et al. (2008) and Zhao et al. (2005) who illustrated that the addition of lentil, green pea, and chickpea flour, among other leguminous flours, results in an increase in the solid loss during cooking.

However, Wood (2009) reported lower losses for spaghetti enriched with 30% of chickpea than those presented by exclusively durum wheat semolina (control). Additionally, Petitot, Boyer, Minier and Micard (2010) found no significant differences in the losses for spaghetti fortified with 35 g/100 g faba beans compared to a control sample of durum wheat semolina. Both authors reported lower losses than shown in the current research. This could be due to the different types of flours used and the effect of addition of broad bean flour on the gluten structure (Gimenez et al., 2012).

Textural properties of pasta samples are reported in Table 4.1. Pasta firmness increased with the addition of 40% of pea flour compared to the control; however, this difference was not statistically significant. Bahnassey and Khan (1986) and Zhao et al. (2005) reported that a fortification of pasta with legume flour (navy bean, pinto bean, lentil and green pea) or protein concentrates caused an increased in pasta firmness. Table 4.1 also illustrates that the elastic nature of the pasta significantly decreased with the addition of pea flour. This result, in combination with the CL values, indicates the significant structural changes in protein network occurring with the addition of pea flour to pasta. Previous results from Petitot et al. (2010) and Tudorica, Kuri and Brennan (2002) also observed a lower breaking energy in split pea and pea flour enriched pasta, respectively. Table 4.1 shows the L*, a*, and b* values for spaghetti with and without pea flour before and after cooking. Spaghetti samples with added pea flour showed a significantly lower lightness (L* value) and higher redness colour (a* value) than semolina control. This is in accordance with previous studies on pasta fortified with green pea flour and split pea (Zhao et al., 2005; Sudha et al., 2012; Petitot et al., 2010).

4.3.2 Effect of different processing preparation steps on the predictive glycemic loading values obtained from control durum wheat flour pasta and pasta made with pea flour inclusion

Figs 4.1 and 4.2 show the impact of the different simulating chewing methods used during the predictive analysis on *in vitro* digestion for pasta. Fig. 4.1 illustrates the amount of reducing sugars released during the 120 min *in vitro* digestion process.

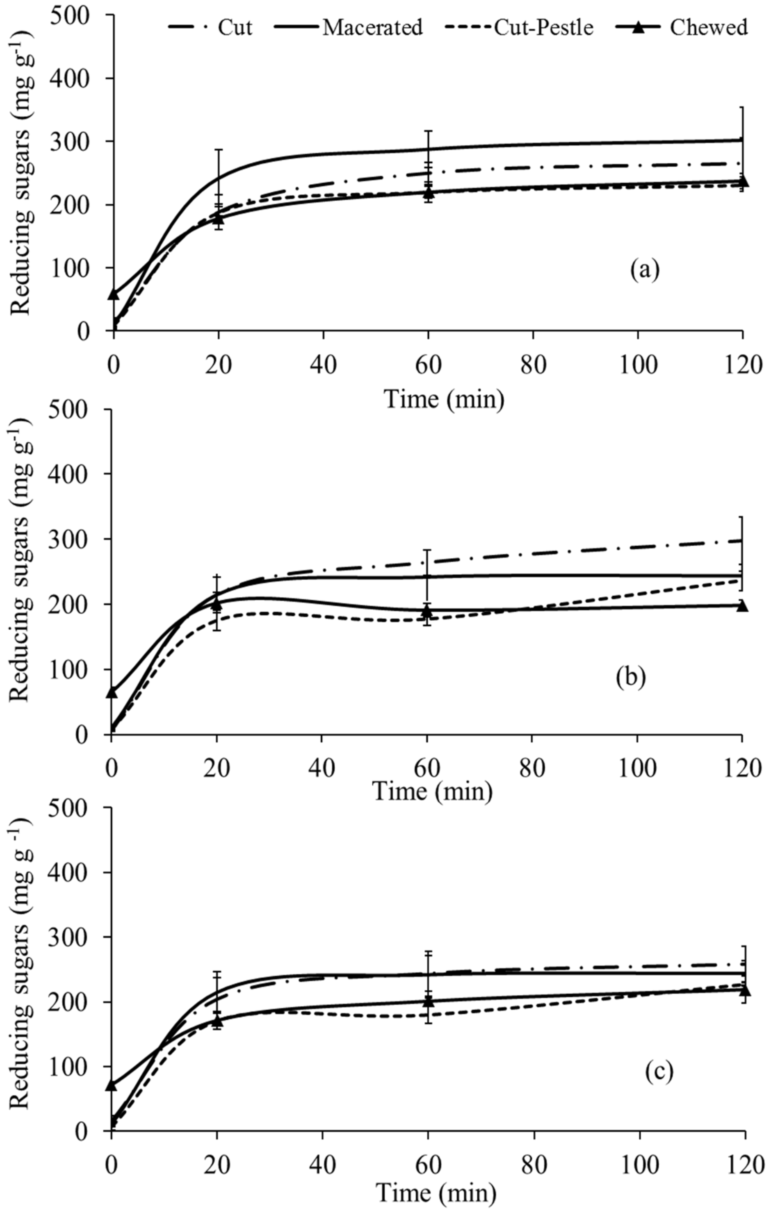

Fig. 4.1. Amount of reducing sugars released during in vitro digestion for control (a), pea flour 20% (b) and pea flour 40% (c).

It is noticeable that for the control sample there is a significant difference between the ground/macerated preparation stage and the cut and pestle or chewed samples preparation (Fig. 4.1a). While this difference may not be so obvious for the samples containing pea flour there is a general trend that pasta, which was either ground mechanically or cut prior to the digestion stage gave higher starch digestibility than the same foods that were cut-pestle or chewed prior to the *in vitro* digestion (Fig. 4.1b and c). Similar trends have been observed in previous research works and could be attributed to a dilution of starch or the presence of fibre and protein altering the activity of the starch degrading enzymes (Woolnough et al., 2008; Brighenti et al., 1995).

Fig. 4.1 also illustrate that samples, which had been chewed by a human volunteer prior to further digestion had been subjected to a higher level of starch degradation at Time 0 (recorded as an elevated level of reducing sugars present at this point). Since the mechanical preparation sample prior the *in vitro digestion* does not involve enzymatic breakdown of starch by salivary amylase such result was to be anticipated. The results do indicate that the α-amylase catalyses during the first step of the starch digestion could be of great importance for the early part of glucose release and absorption during digestion. However, despite the initial higher level of starch digestibility, it is of interest to note that the curves relating to the chewed samples for pasta semolina and pasta enriched pea flour samples yielded less total reducing sugar released (and hence lower starch digestion) than the same pasta samples that were cut or macerated for the analysis (Fig. 4.2 indicates the significant differences between the samples when comparing AUC values). This result highlights that the presence of salivary α-amylase may not have an important role across the entire duration of *in vitro* digestion analysis. In fact, it is argued that most of the digestion of starch occurs in the small intestine in presence of pancreatic enzymes (Lebenthal, 1987).

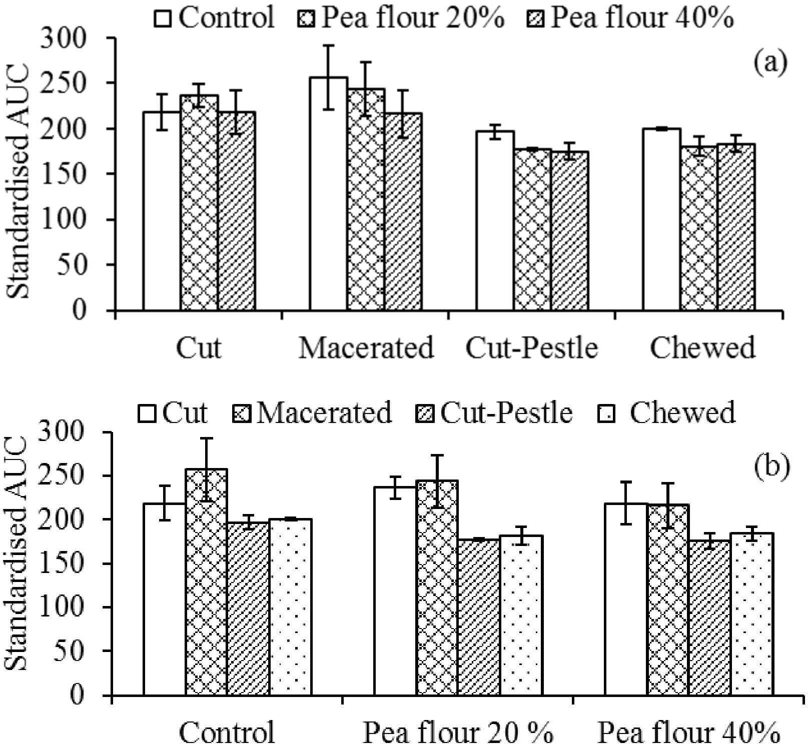

Fig. 4.2. Values for area under the curve (AUC) comparing: control and enriched pea flour pasta samples (a) and the different preparation techiques (b).

The standardized AUC values for all samples and treatments are shown in Fig. 4.2. Figure 4.2a represents the effects of pea flour 20 and 40% addition on the AUC reducing sugar levels of pasta. The trend showed a decrease (except for cut samples) in AUC values after addition of pea flour. No significant differences were observed between pasta samples containing 20 and 40% of pea flour. Similarly, no statistical difference could be observed between the control and the pea flour samples, which were macerated or only cut, whereas significant differences existed between control and pea flour samples, which were chewed or subjected to cutting and grinding prior to the digestion step. The maceration of the sample appears to have minimized any glycaemic difference, which may has existed in the sample, whereas the chewing preparative step appears to retain the glycaemic differences inherent in the product. Many researchers have studied the effect of different legume flours on starch digestibility in a range of food products (Gallegos-Infante, Bello-Perez, Rocha-Guzman, Gonzalez-Laredo and Avila-Ontiveros, 2010; Liu, Peng, Zhang, Zou and Zhong, 2013; Petitot and Micard, 2010). Their results are similar to our observations from the chewed samples in that spaghetti made with semolina generally showed a significantly higher enzymatic hydrolysis rate compared to samples made with legume flours. The decrease in AUC values may be linked to the dietary fibre content of pea flour.

Figure 4.2b represents the effects of using the four different initial breakdown procedures on *in vitro* digestion analysis. There was no significant difference in the AUC values of control samples, which had been subjected to chewing prior to digestion or the samples prepared by cutting or the cut and pestle step. However, the AUC values for the macerated samples were significantly higher than the values obtained after mastication or preparation using cutting and pestle grinding on a like for like basis. This result would indicate that the method used for the preparation of the sample prior to in vitro starch digestion procedures is an important consideration and that maceration or homogenization of the sample during preparation may result in an elevated predictive glycemic response (index) value compared to the processes which mimic chewing of the product (both the chewed sample and the samples prepared by the combination of cutting and the use of pestle and mortar).

4.4 Conclusions

The replacement of durum semolina with 20 and 40% of pea flour in pasta making caused an increase in OCT, WAI, and CL. Moreover, the cooked and uncooked spaghetti added with pea flour appear darker (lower lightness and higher redness colour) than the spaghetti made only with durum semolina.

The evaluation of the different preparation processes applied to samples prior to *in vitro* digestion demonstrated that different initial breakdown procedures of pasta sample prior to digestion will give different results for *in vitro* digestion. In particular, all samples cut or ground mechanically gave higher starch digestibility than the same foods that were cut-pestle or chewed prior to the *in vitro* digestion. Pea flour enriched pasta samples subjected to the different procedures showed lower AUC values than the sample made with durum semolina (except the ground/macerated samples). The data highlights two important aspects to be noted when considering results obtained from *in vitro* analysis of samples. Firstly, that the presence of α-amylase in the form of saliva does not significantly affect the overall *in vitro* digestion analysis and predictive glycaemic loading of the pasta used. Secondly, that maceration of a sample to a homogenous consistency results in a product with high starch digestibility and masks any significant differences between samples based on either composition or food structure. The preparation steps, which mimic the chewing process, reveal differences in starch digestibility values affected by food structure and form, therefore care needs to be taken when choosing the most appropriate predictive glycaemic loading analysis method for the food industry and aligned disciplines.

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Chapter 5 - Effect of different dietary fibres on pasta quality and predicted glycaemic response

Abstract

The production of high quality functional pasta from non-conventional raw materials represents a challenge. A partial substitution (15%) of durum wheat semolina with long-chain inulin (HPX) and short-chain inulin (GR), Glucagel, psyllium and oat material (added individually and in combinations) was performed in order to increase the level of dietary fibre intake. The cooking, textural, colour characteristics and predicted glycaemic response of the pastas were evaluated and compared to control sample containing exclusively durum wheat semolina. Generally, material addition to the durum wheat pasta increased the cooking losses, swelling index and water absorption, whilst reduced firmness and resistance to uniaxial extension of pastas. Raw spaghetti samples resulted significantly darker (L*) and more redness (a*) than raw control pasta. In the cooked pasta, all inulin enriched pasta samples were brighter than semolina pasta. Pasta containing 15% of oat flour showed the best performance (except for the colour) compared to the other experimental pasta samples, but was significantly different to control durum wheat sample. Moreover, the inclusion of inulin GR had a less deteriorating effect when added in combination with oat flour. This illustrates that some fibre rich sources may act better in combinations than separately. In general, all enriched dietary fibre pasta sample showed a significant decrease (except for pasta containing a combination of inulin GR and 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.

5.1 Introduction

Pasta is considered a healthy food being relatively low in fat and sodium levels, high in carbohydrate, and having good protein content (Malcolmson, 2003). Commonly, traditional pasta is made by using durum wheat semolina to deliver a protein rich food product with unique quality properties (Marconi, Graziano and Cubadda, 2000). In order to enhance the nutritional value of pasta, several studies have focused on the possibility of adding functional ingredients into pasta, such as novel non-durum wheat varieties (Fiorda, Soares Júnior, da Silva, Souto and Grosmann, 2013; Bustos, Perez and Leon, 2013). Dietary fibres (DFs) perform many physiological functions and can play an important role in reducing the potential glycaemic index (Chillo, Laversea, Falcone, Protopapa and Del Nobile, 2008; De Pilli, Derossi and Severini, 2013; Ovando-Martinez, Sáyago-Ayerdi, Agama-Acevedo, Goñi and Bello-Pérez, 2009; Mudgil and Barak, 2013). 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 and La Vecchia, 2002).

Pasta cooking quality and textural characteristics, such as firmness, are determined by the physical competition between protein coagulation in a continuous network and starch swelling with exudate losses during cooking. If the former prevails, starch particles are trapped in the network alveoli promoting firmness of cooked pasta; whereas if the latter prevails, the protein coagulates in discrete masses lacking a continuous framework and consequently pasta will show softness and usually stickiness (Cunin, Handschin, Walther and Escher, 1995; Grzybowsky and Donelly, 1979; Del Nobile, Baiano, Conte and Mocci, 2005). Even, a partial substitution of durum wheat semolina with DF ingredients and/or DF enriched flours can negatively affect the product palatability and consumer acceptance of the end product (Foschia, Peressini, Sensidoni and Brennan, 2013). The inclusion of DF in pasta can negatively alter the tenacity of protein-starch product, affecting the integrity of the protein-starch network and hence pasta quality in terms of water absorption, swelling index, optimum cooking time, cooking loss, texture, appearance and taste (Foschia et al., 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 plants of the Plantago genus and it has been recognised as a rich source of dietary fibre, in particular arabinoxylan (Van Craeyveld, Delcour and 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 and 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 and 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 D-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).

In the present work, pasta was made using oat bran (O), psyllium fibre (P), Glucagel (GG), inulin GR (GR) or inulin HPX (HPX). The purpose of the study was to determine the possibility of producing high quality functional foods and investigate the impact of different DFs, added individually and in combination, on pasta quality and predicted glycaemic response.

5.2 Materials and methods

5.2.1 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: protein 13%, carbohydrate 75%, moisture 12%. Two inulin products from chicory of different degree of polymerisation (DP) were supplied by Orafti Food Ingredients (Belgium): Raftiline® HPX (inulin HPX, 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 Whoolefoods (Christchurch, New Zealand).

5.2.2 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 mixture 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 5.1. Control sample (C) was prepared using exclusively durum wheat semolina.

Table 5.1. The percentage weight fraction of raw materials used in the preparation of pasta samples and dietary fibre content estimated in fresh pasta.

	SAMPLE CODE										
Formulation (%)	**C**	**O**	**P**	**GG**	**GR**	**HPX**	**GR-P**	**GR-O**	**HPX-P**	**HPX-O**	**P-O**
Semolina	100	85	85	85	85	85	85	85	85	85	85
Oat bran	-	15	-	-	-	-	-	7.5	-	7.5	7.5
Psyllium	-	-	15	-	-	-	7.5	-	7.5	-	7.5
Glucangel	-	-	-	15	-	-	-	-	-	-	-
Inulin GR	-	-	-	-	15	-	7.5	7.5	-	-	-
Inulin HPX	-	-	-	-	-	15	-	-	7.5	7.5	-
FRESH PASTA											
DF content estimation (g /100 g)	1.05	9.7	10.7	9.9	11.7	12.4	11.1	10.4	11.9	11.1	10.0

5.2.3 Cooking procedure

Optimal cooking time (OCT, min) was determined as the time when the inner white core of the pasta disappeared according to Approved Method 16-50 (AACC, 2000). Cooked pasta was analysed for cooking loss, swelling index, water absorption index and textural properties.

5.2.4 Cooking loss

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

5.2.5 Swelling index and water absorption index

The swelling index (SI) of cooked pasta (g water / g dry pasta) was determined according to the procedure described by Cleary & Brennan (2006). Pasta (10 g) was weighed after cooking and dried at 105 °C to a constant weight. The swelling index was expressed as:

$$SI = \frac{Wc - Wd}{Wd}$$

where Wc is weight of cooked pasta (g) and Wd is weight of pasta after drying (g).

The water absorption index (WAI, %) was determined as:

$$WAI = \frac{Wc - Wr}{Wr} \cdot 100$$

where Wc is weight of cooked pasta (g) and Wr is weight of uncooked pasta (g).

5.2.6 Textural characteristics

Cooked pasta textural properties were determined using a Texture Analyser (TA.XT2, Stable Micro System, UK) equipped with a 5 kg load cell. Samples were rested for 10 min after cooking before testing. Firmness, expressed as maximum cutting force (N), was determined according to Approved Method 66-50 (AACC, 2000), with some modifications as reported in a previous study (Foschia, Peressini, Sensidoni, Brennan & Brennan, 2014).

Resistance to uniaxial extension of the cooked pasta was determined by tension test using the A/SPR spaghetti / noodle rig (settings: pre-test speed, 3 mm/s; test speed, 3 mm/s; post-test speed, 5 mm/s; initial distance, 10 mm; final distance, 100 mm; trigger type, auto 5 g; rate for data acquisition, 200 pps). The results are expressed as maximal breaking strength (N).

Data are mean of twelve measurements from three different cooking replications.

5.2.7 Colour

Colour readings were taken from nine separate points on the surface of the uncooked and cooked spaghetti (OCT and equilibrated for 5 min at room temperature before analysis) using a tri-stimulus colour analyser (Minolta Chroma Meter CR210, Minolta Camera Co., Japan). The illuminant C (CIE, standard, 6774 K) was used. Results were expressed as L* (brightness), a* (redness) and b* (yellowness). The instrument was calibrated using a standard white tile (L* = 98.03, a* = -0.23, b* = 0.25).

5.2.8 *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 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 and 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 Gmblt & 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 and 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., 2012). The *in vitro* digestion analysis was used to determine predicted glycaemic response.

5.2.9 Statistical analysis

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

5.3 Results and discussion

5.3.1 Pasta quality

Quality indices such as optimal cooking time (OCT), cooking loss (CL), swelling index (SI) and water absorption index (WAI) were used as quality parameters of pasta. All combination of fibre-enriched pasta showed a significant increase in OCT compared to the control pasta made with exclusively durum wheat semolina (Table 5.2). In particular, HPX-O enriched pasta showed the highest increase and GR-P, GR-O and O pasta samples showed the lowest increase. Zhao, Manthey, Chang, Hou and Yuan (2005) evaluated the quality characteristics of spaghetti made with 5-30% of different kinds of legume flours; the OCT value in pasta made with green pea flour was significantly higher than control. On the contrary, the literature reported a decrease in OCT pasta at increasing level of semolina substitution with DF such as inulin, durum bran and bean flour (Aravind, Sissons, Egan and Fellows, 2012; Aravind, Sissons, Fellows, Blazek and Gilbert, 2012; Gallegos-Infante et al., 2012). This behaviour could be associated to the formation of a weaker gluten network as the result of a dilution effect on gluten. Chillo, Ranawana and Henry (2011a) observed a minor impact on cooking time for β-glucan fortification. However, the OCT for pasta with Barley Balance increased with increasing concentrations of β-glucan. SI of the spaghetti samples are reported in Table 5.2. All DF enriched spaghetti showed higher SI values than spaghetti made with semolina. In particular, the highest values were registered for GR, P and HPX-P pasta samples, in agreement with previous studies (Brennan and Tudorica, 2007; Chillo et al., 2011a; Cleary and Brennan, 2006). The increase in SI can be explained by the higher capacity of DFs to absorb and retain water within the starch-protein-polysaccharide network in comparison to the control. Conversely, some research works showed a significant decrease in SI at increasing concentrations of inulin or β-glucan in pasta (Aravind, Sissons, Fellows, Blazek and Gilbert, 2012; Aravind, Sissons, Egan, Fellows, Blazek and Gilbert, 2012; Brennan, Kuri and Tudorica, 2004). The differences in OCT and SI results between this study and what was found in literature could be due to different type and content of ingredient used and the different processes followed (de Noni and Pagani, 2010; Del Nobile, Baiano, Conte and Mocci, 2005; Dexter and Matsuo, 1977).

CL is one of the parameters most affected by fibre incorporation in pasta formulation because uniform diffusion of cooking water is influenced by the integrity of the protein matrix (Fardet, Hoebler, Baldwin, Bouchet, Gallant and Barry, 1998), which in turn is affected by protein content (Cleary and Brennan, 2006; Sissons, 2008) and type of fibre incorporated (Foschia et al., 2013; Tudorica, Kuri and Brennan, 2002). From Table 5.2, it is evident that CL in pasta enriched with dietary fibre was higher than pasta semolina sample. O, GR-O, HPX-O and GR-P samples presented cooking losses below 8%, the value above which pasta quality is considered unacceptable (Dick and Youngs, 1988).

Table 5.2. Effect of dietary fibre addition on pasta cooking quality characteristics.

Samples	**Moisture content (%)**	**OCT (min)**	**SI (g water/g dry pasta)**	**WAI (%)**	**CL (%)**
C	65.22^{e}	6.5 ± 0.6	1.88^{m}	98^{e}	4.85^{f}
O	67.84^{d}	8.0 ± 0.3	2.11^{h}	111^{d}	6.28^{e}
P	73.34^{a}	11.0 ± 0.3	2.75^{a}	138^{a}	10.39^{a}
GG	68.31^{b}	10.0 ± 0.3	2.16^{g}	113^{d}	9.06^{b}
GR	71.96^{b}	10.0 ± 0.3	2.57^{c}	138^{a}	10.70^{a}
HPX	70.17^{c}	10.5 ± 0.3	2.35^{d}	123^{c}	8.84^{b}
GR-P	69.32^{c}	8.0 ± 0.3	2.26^{f}	118^{cd}	7.83^{c}
GR-O	67.68^{d}	8.0 ± 0.3	2.09^{i}	110^{d}	7.16^{d}
HPX-P	72.17^{b}	10.5 ± 0.6	2.59^{b}	132^{ab}	10.09^{a}
HPX-O	70.12^{c}	12.0 ± 0.5	2.35^{d}	126^{bc}	7.33^{cd}
P-O	70.01^{c}	9.5 ± 0.3	2.33^{e}	115^{cd}	9.18^{b}

Values within a column followed by the same letter are not significantly different ($p > 0.05$).

The highest CL values were recorded in pasta samples made with 15% of P, GR and HPX. Several researches have illustrated that DF fortified pasta can exhibit higher cooking losses (Aravind, Sissons, Fellows, Blazek and Gilbert, 2012; Czuchajowska, Paszczynska and Pomeranz, 1992; Gallegos-Infante et al., 2012; Kaur, Sharma, Nagi and Dar, 2012; Sudha et al., 2012; Zhao et al., 2005). This increase may be related to the presence of water-soluble components and weakening of gluten network, which is responsible for retaining the amylose during cooking. CL is related to a disruption in the protein-starch matrix and the uneven distribution of water within the pasta matrix due to the competitive hydration tendencies of the fibre. In a review, Sissons (2008) explained that in pasta, semolina proteins are linked together by disulphide, hydrogen and hydrophobic bonds to form a matrix, which gives cooked pasta its viscoelastic properties. The continuity and strength of the protein matrix is dependent on the nature of inter- and intra - molecular bonds. During the cooking process this matrix gradually disintegrates, releasing exudates during starch granule gelatinization, which in turn contributes to an increase in cohesiveness and stickiness on the cooked pasta surface. Furthermore, the higher loss during cooking in DF enriched pasta samples may be attributed to a disruption of the protein-starch continuum (Cleary and Brennan, 2006).

Table 5.2 illustrates that the substitution of semolina flour with dietary fibre caused a significant increase in WAI. The highest values were measured in P, GR and HPX-P enriched pasta and the minor change in this parameter was observed for GR-O, O and GG pasta samples. This substantial increase is due to higher dietary fibre content that has a strong water-absorbing capacity (Czuchajowska et al., 1992; Giménez, Drago, De Greef, Gonzalez, Lobo and Samman, 2012; Kaur et al., 2011; Yu, Lutterodt and Cheng, 2009). In general, the differences in WAI values between the different kinds of DFs utilized in this research could be due to the structural difference or to the larger particle size of fibres (Chen, Rubenthaler, Leung and Baranowski, 1988). Disruptions in the protein matrix would promote water absorption and expose starch granules to swelling and rupture (Sosulski and Wu, 1988). Although, Aravind, Sissons, Fellows, Blazek and Gilbert (2012) found that pasta water absorption was unaffected by inulin with 12-14 DP (FH-D). In the case of inulin with 7-8 DP (LV-100), pasta water absorption decreased at levels greater than 2.5% substitution. This decrease could be partly explained by a decrease in SI at 7.5% and 10% substitution as decreased swelling would reduce pasta water absorption. If the inulin is competing with the starch for water during pasta formation, this would reduce starch swelling and consequently pasta water absorption, as observed. Brennan et al. (2004) added commercial inulin (similar to FH-D) to spaghetti over range of 0-10% and found a decrease in WAI. In summary, the cooking characteristics of pasta indicate that P, GR and HPX-P pasta samples have the highest CL, WAI and SI values. The data analyses suggest an improvement in cooking characteristics when oat bran flour is added in combination with another DF; on the contrary, a negative effect in presence of psyllium fibre was registered. Higher moisture contents and SI (Table 5.2) associated with the presence of DF in the formulation will result in lower firmness of the end product. For this reason, the effects of dietary fibre addition on textural characteristics of spaghetti were also investigated (Table 5.3).

Table 5.3. Effect of dietary fibre addition on textural properties of cooked pasta samples.

Samples	Firmness (N)	Maximal breaking strength (N)
C	2.81 ± 0.16^{abc}	0.39 ± 0.01^{a}
O	2.98 ± 0.28^{ab}	0.31 ± 0.03^{c}
P	2.78 ± 0.29^{abc}	0.32 ± 0.02^{bc}
GG	3.03 ± 0.18^{a}	0.40 ± 0.03^{a}
GR	1.28 ± 0.09^{e}	0.16 ± 0.02^{f}
HPX	2.75 ± 0.21^{abc}	0.40 ± 0.02^{a}
GR-P	2.27 ± 0.18^{d}	0.22 ± 0.02^{e}
GR-O	2.57 ± 0.26^{cd}	0.23 ± 0.02^{e}
HPX-P	2.87 ± 0.24^{abc}	0.30 ± 0.01^{cd}
HPX-O	2.69 ± 0.10^{bc}	0.36 ± 0.02^{ab}
P-O	2.87 ± 0.30^{ab}	0.26 ± 0.02^{de}

Values within a column followed by the same letter are not significantly different ($p > 0.05$).

Substitution of semolina with GR-P and GR led to a significant decrease of firmness compared to control. Maximal breaking strength and distance values decreased significantly for all dietary fibre enriched samples, except for GG, HPX and HPX-O pastas (Table 5.3). Chillo et al. (2011a) reported that the addition of β-glucan did not have a notable effect on firmness. On the other hand, Cleary and Brennan (2006) demonstrated that the inclusion of β-glucan makes the pasta softer. The same trend was appreciated in vermicelli firmness values after 5-20% addition of wheat bran or oat bran (Sudha, Rajeswari and Rao, 2012) and in fresh pasta enriched with inulin, pea fibre and β-glucan (Brennan and Tudorica, 2007). Previous studies have demonstrated that in terms of pasta texture, spaghetti became softer upon incorporation of FH-D inulin (DP 12-14) only at the highest level of substitution of 20% compared to its control, whereas fortification with LV-100 inulin (DP 7-8) resulted in firmness deterioration at levels of 7.5% and above relative to its control (Aravind, Sissons, Fellows, Blazek and Gilbert, 2012). In this case, the scanning electron microscopy highlighted that the control sample had a well-developed protein network with most of the starch gelatinised but having a few intact granules. At high levels (20%) of inulin addition many holes appeared in the pasta surface suggesting a less integrated structure. Based on the literature (Juszczak, Witczak, Ziobro, Korus, Cieslik and Witczak, 2012; Peressini, Foschia, Tubaro and Sensidoni, 2015; Rouillè, Della Valle, Lefebvre, Sliwinski and van Vliet, 2005), this may be due to a protective action on starch granules by inulin that can cause a competition inulin/starch for binding with protein, resulting in a low number or a weak starch-protein binding that can also delay the starch gelatinization. Inulin and DFs tend to show a hygroscopic nature and may reduce water available for starch gelatinization, therefore reducing starch digestibility (Parada, Aguilera and Brennan, 2011; Tudorica et al., 2002). In fact, it has been reported that non-starch polysaccharide can build a matrix with proteins surrounding the starch in cereal foods that would act as a physical barrier, thus reducing the effectiveness of digestive enzymes (Bornet, 1993; Parada et al., 2011); inulin hydrates more quickly than the starch and protein components of wheat flour, in turn leading to starch and protein fractions of the pasta being more discrete and less incorporated in a matrix, as evident from SEM observation in Aravind, Sissons, Fellows, Blazek and Gilbert (2012). Recent research has indicated that the impact of higher molecular weight inulin incorporation on the technological and sensory properties of fibre rich foods could be minimal, with deterioration in properties becoming significant only at 20% incorporation (Aravind, Sissons, Fellows, Blazek and Gilbert, 2012). Brennan et al. (2004) added commercial inulin (similar to FH-D) to spaghetti and found a decrease in firmness only at 10% level of substitution. However, Peressini and Sensidoni (2009) found that lower DP inulin (comparable to FH-D) was more suited to bread making than the higher DP inulin because of greater changes in dough viscoelastic properties impacting on bread quality. On the basis of these results, it can be concluded that pasta structure depends not only on the type and amount of DF added but also on the manufacturing conditions (Juszczak et al., 2012). Observing pasta firmness and elasticity characteristics, GR can be considered as the worst pasta sample from a textural point of view.

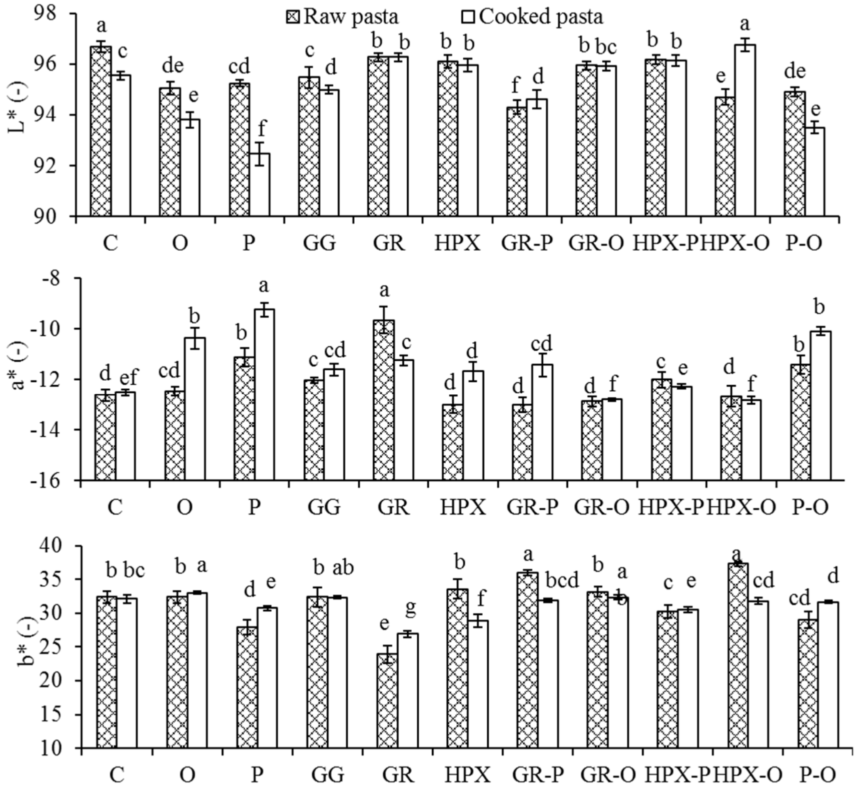

Fig. 5.1. Colour characteristics of raw and cooked pasta expressed as L*, a* and b* values.

The visual appearance of spaghetti is certainly one of the main factors responsible for consumer liking. Fig. 5.1 shows the L*, a* and b* values for all spaghetti samples before and after cooking. Raw spaghetti samples with added DF showed a significantly lower lightness (L* value) than pasta control. Redness parameter (a*) in HPX, GR-P, GR-O and HPX-O samples was not significantly different from raw control pasta; the other enriched DF samples presented a* values significantly higher than raw spaghetti control. Relating to yellowness parameter (b*), raw GR-P and HPX-O spaghetti showed higher values than control, instead P, GR, HPX-P and P-O samples showed lower values than pasta made with durum semolina. In the cooked pasta this trend changed; in fact, all inulin enriched pasta (except for GR-P samples) showed higher L* value than semolina pasta. The majority of cooked pasta samples containing DF presented a* values higher then the control except for GR-O, HPX-P and HPX-O (lower or similar to control). Chillo et al. (2011a) found that colour parameters for the spaghetti (uncooked and cooked) added with two types β-glucan barley concentrates were not statistically different to those of the semolina control. The absence of differences may be due to the low percentages of barley β-glucan concentrates used. Overall, results obtained in the present study are in agreement with data reported in literature; specifically, pasta enriched with DF was significantly darker (lower L*) when compared with reference (Gajula, Alavi, Adhikari and Herald, 2008; Hager, Czerny, Bez, Zannini and Arendt, 2013; Knuckles, Hudson, Chiu and Sayre, 1997).

5.3.2 Predicted glycaemic response

An *in vitro* enzymatic digestion was performed in order to mimic the behaviour of pasta when eaten. Figs. 5.2 and 5.3 represent an interpretation of the amount of reducing sugars released over 120 min *in vitro* digestion of all spaghetti samples. In particular, Fig. 5.2 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 for 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 released 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 (Brennan and Tudorica, 2008; Chillo et al., 2011a; Chillo et al., 2011b; Cleary and Brennan, 2006; Hager, Czerny, Bez, Zannini and Arendt, 2013; Manno et al., 2009; Padalino, Mastromatteo, De Vita, Ficco and Del Nobile, 2013).

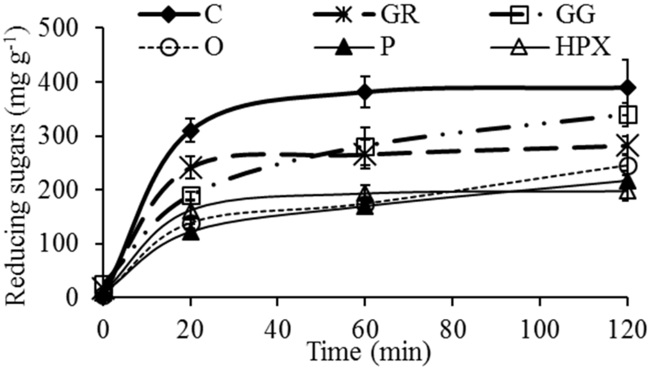

Fig. 5.2. Amount of reducing sugars released during *in vitro* digestion for control (C), and pasta containing 15% of Glucagel (GG), inulin GR (GR), inulin HPX (HPX), psyllium (P) and oat (O) flour respectively.

Cleary and Brennan (2006) incorporated barley β-glucan fibre fraction into pasta between 2.5 and 10% levels. Pastas with 5, 7.5 and 10% of barley β-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 β-glucan barley concentrates, Glucagel (GG) and Barley Balance. In this case, Barley Balance had the most important impact. However, only 10% of Barley Balance showed a significant decrease in IAUC and GI values. The functional properties of β-glucans have been attributed to their ability to increase lumen viscosity (Behall et al., 2005; Izydorczyk and Dexter, 2008). It has been suggested that cereal β-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 (Lazaridou and 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 β-glucan (650,000-700,000 Da) in the Barley Balance compared to GG (150,000 Da) reduced the digestibility of spaghetti, perhaps because the occlusive effect of hydrated β-glucan reduced the rate at which enzyme penetration occurs (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 (Brennan, Blake, Ellis and Schofield, 1996; Tudorica, Kuri and 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. 5.2). 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 and Arendt, 2012; Hager et al., 2013; Krishnan, Menon, Padmaja, Sajeev and 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. 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% 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 (Lasztity, 1995). These findings are confirmed by a 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 and 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 and Gilbert, 2012). Brennan et al. (2004) and Brennan and Tudorica (2008) found that 10 g/100 g 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 significantly higher than control (Table 5.2). This behaviour was registered for all dietary fibres enriched samples. This in turn may be due to the weakening of the protein-starch 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. 5.3a 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. 5.3b 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. 5.3c). 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 exclusively with semolina flour. At 120 min P-O sample had a value not significant different from control pasta. The results in Fig. 5.3 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.

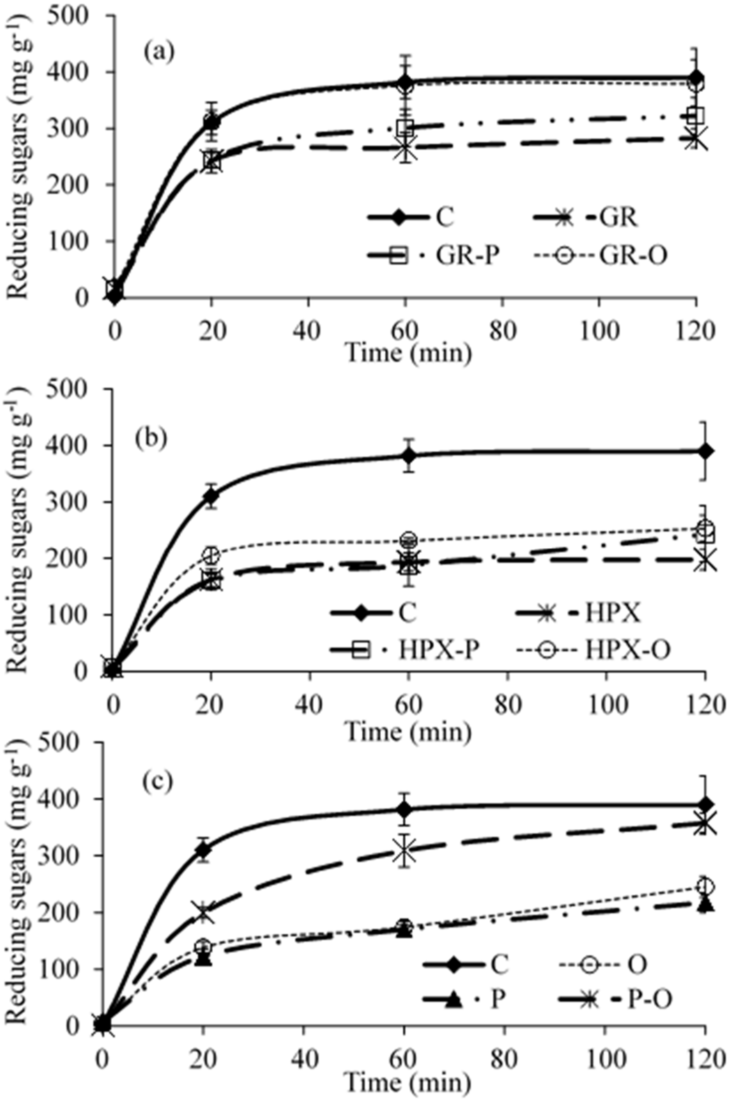

Fig. 5.3. Amount of reducing sugars released during *in vitro* digestion comparing control (C) to: GR, GR-P and GR-O (a); HPX, HPX-P and HPX-O (b); O, P and O-P (c).

Fig. 5.4 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% of HPX, P or O in pasta production caused the major decrease in standardised values. What is of interest is that 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 DF's 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.

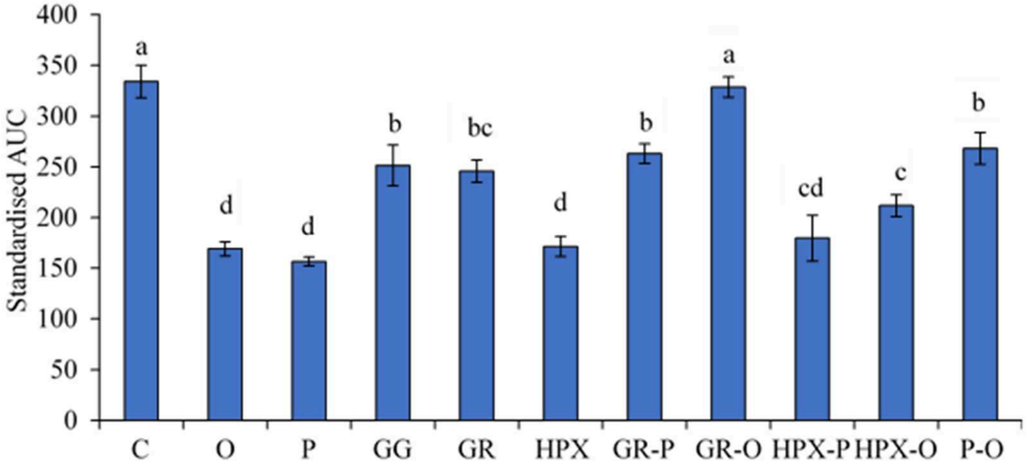

Fig. 5.4. Values for area under the curve (AUC) comparing control and all dietary fibre enriched pasta samples.

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%) 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.

5.4 Conclusions

The results illustrate that the use of DF to replace durum wheat semolina may be possible in order to obtain pastas with high DF contents. However, the addition of DF individually and in combination affected cooking, textural and colour characteristics. Over all, the presence of DF in pasta led to an increase of OCT, CL, WA and SI and to deterioration in textural characteristics. Moreover, raw pasta enriched with DF showed a darker colour than semolina pasta control. In the cooked pasta this trend changed; in fact, all inulin enriched pasta (except for GR-P samples) showed higher L* value than semolina pasta. Our data suggests an improvement in cooking characteristics when oat bran flour is added in combination with another DF; whereas a negative effect is observed when psyllium fibre was added in combination with DFs.

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, GR, HPX, GG, P and O 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.

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Chapter 6-Effects of substitution of rice flour with resistant starch in gluten-free pasta

Abstract

Gluten free-pasta was prepared substituting rice flour (reference) with 10, 15 and 20% of resistant starch type II (RS). Farinograph test registered no changes in water absorption at any level of substitution. The presence of fibre caused an increase in optimum cooking time and firmness parameters and a decrease in cooking loss and stickiness values; however, no significant differences among all levels of substitution (10-20% of RS) could be appreciated. The loss of resistant starch content (31%) in raw gluten-free pasta suggested that processing conditions could be a critical point for resistant starch stability. Moreover, doughs obtained from all raw pastas were subjected to fundamental rhelogical measurments. In particular, the dynamic temperature ramp test showed different G' slopes for fibre-enriched samples compared with the reference, ascribable to some modifications in resistant starch granules during pasta cooking. This observation was confirmed by polarized light microscopy analysis performed on RS granules during heating process conditions; in fact, the thermal energy seemed to promote the mobility of the molecules, which reduces their radial orientation and cause a loss of birefringence of resistant starch. Based on these results, pasta samples made with 20% of RS can be considered as a food product source of dietary fibre.

6.2 Introduction

Cereals may cause food intolerance, which is a specific reaction to cereal components without the occurrence of specific IgE antibodies but with increased T-cell activity (Comino et al., 2011; Gilissen, van der Meer and Smulders, 2014). Under normal physiological conditions, the immune system in the gut develops a tolerance against the vast majority of food proteins, including gluten. Individuals that express the human leucocyte antigen (HLA) DQ2 and/or DQ8 (Troncone, Auricchio and Granata, 2008) can be intolerant to gluten proteins and may develop coeliac disease (CD), a chronic inflammation of the small intestine that occurs as a response to continuous consumption of gluten, which leads to a variety of symptoms ranging from bowel to skin, bone, nerve and muscle complaints.

To not incur in these disorders, coeliac patients have to follow strictly a gluten-free based diet, which brings generally a low intake of nutrients important for the wellbeing of consumers.

During the last decades, gluten-free products have been received considerable attention by researchers due to the increasing number of coeliac disease diagnosed in patients. The cereals utilized at large by the majority of industrial production of gluten-free pasta are rice and maize (Rosell, Barro, Sousa and Mena, 2014). Rice flour is the most suitable commodity for bakery applications due to its bland taste, white colour, digestibility and hypoallergenic properties. Nevertheless, this type of flour is lack of proteins and other important nutrients, such as dietary fibre (DF). For these reasons, particular processing techniques and ingredients/additives are necessary in order to improve the textural properties and nutritional characteristics of the end product (Lai, 2001; Lazaridou, Duta, Papageorgiou, Belc and Biliaderis, 2007; Marti, Seetharaman and Pagani, 2010; Yalcin and Basman, 2008).

It is well documented that DF performs several health benefits such as reducing coronary heart-related diseases, decrease in blood glucose levels, facilitating good colonic health and weight management (Anderson, et al., 2009). According to current recommendations, the amount of DF daily intake per adult should be in the range of 25-38 g (Food and Nutrition Board, Institute of Medicine, 2001; Romo et al., 2008). To claim that a food is a ‘source of fibre’, the food should contain at least 3 g of fibre per 100 g (or 1.5 g of fibre per 100 kcal) (European Commission, 2006). Among the different types of dietary fibres, the physical properties of resistant starch, particularly its low water-holding capacity, make it a functional ingredient that provides good handling and improves texture in the final product (Baixauli, Salvador, Martinez-Cervera and Fiszman, 2008; Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata and Pérez-Álvarez, 2010). A wide range of foods has been enriched with RS including bread, cakes, muffins, pasta and battered foods (Sanz, Salvador and Fiszman, 2008).

The fraction of starch that resists digestion in human being and is fermented in the colon is referred to as resistant starch, which has been categorized into four main types: physically inaccessible starch (RS type I), native starch granules (RS type II), retrograded starch (RS type III) and chemically modified starch (RS type IV). RS type II exhibits a certain granular form and resists to enzymatic hydrolysis. In raw starch granules, starch is packed tightly in a radial pattern and is relatively dehydrated. This compact structure limits the accessibility of digestive enzymes (amylases) and is responsible for the resistant nature of RS type II (Sajilata et al., 2006).

No works about addition of resistant starch in gluten-free pasta were found in literature. Only Flores-Silva, De J. Berrios, Pan, Osorio-Dıaz and Bello-Perez (2014) determined the chemical composition, cooking quality and starch digestibility of gluten-free spaghetti prepared with mixtures of chickpea, unripe plantain and maize flours which are rich in resistant starch content. All formulations presented higher protein, fat and ash contents, but lower amount of glycaemic carbohydrates than control spaghetti made with semolina. Cooking loss was higher in the gluten-free pasta and the values were at the limit of acceptability to be considered as good cooking quality. The lower available starch and higher RS contents in the gluten-free spaghetti were associated with their lower rate of hydrolysis and predicted glycaemic index.

The aim of this work was to investigate the potential use of RS as a fibre enriching ingredient in gluten-free pasta. The effect of RS addition on dough rheological properties and pasta quality, and RS degradation due to the process were evaluated.

6.1 Material and methods

6.1.1 Raw materials

Commercial rice flour (Pasini, Mantova, Italy), propylene glycol alginate (Manucol® Ester M, ISP Alginates, Surrey, UK), distilled monoglycerides (Dimodan®, Danisco Italy Ltd., Milan, Italy), resistant starch Hi-Maize® 260 (RS type II, National Starch & Chemical Limited, Manchester, UK). Tap water was used for making dough samples.

6.1.2 Gluten-free pasta formulation

The gluten-free pasta formula contained rice flour (99.5%), propylene glycol alginate (0.25%) and distilled monoglycerides (0.25%) (RS0, reference). Fibre-enriched blends contained 10, 15 and 20% of RS were made by replacing rice flour with fibre (RS10, RS15 and RS20).

6.1.3 Gluten-free pasta production

Spaghetti-shaped pasta was produced in a semi-industrial scale pilot plant made of a mixer, an extruder and a dryer (Pavan, Galliera Veneta, Padua, Italy). The dry flour blend (10 kg) and water were mixed for 10 min for RS0 and 12-15 min for RS10-RS20 on the basis of farinograph characterization to produce a mixture with a moisture content of 33%. Hydrated mass was heat-treated in the mixer fed with steam at about 130 °C in order to induce starch gelatinization. The dough was fed to a single screw, low shear extruder with a spaghetti-shaped die (FP70 model, Pavan) under vacuum conditions. The barrel and the head temperatures were 30 and 34 °C respectively. Fresh pasta was dried at 50 °C and 76% relative humidity for 12 h in a static dryer (SD 100 model, Pavan). The diameter of the dried spaghetti was 1.6-1.7 mm.

6.1.4 Mixing properties

Mixing properties of the dough were evaluated using a farinograph equipped with a 100 g bowl (T6 Promylograph Max Egger, Austria). The dry flour blend (80 g) and water (water absorption of 59%) were mixed for 30 min at 30 °C and changes in dough consistency (PU) were recorded during mixing.

6.1.5 Pasta cooking quality

Pasta cooking quality was evaluated at optimum cooking time and overcooking (10 min past optimum time).

Optimum cooking time

Pasta (10 g) was broken into pieces of 5 cm and cooked in boiling tap water (300 mL) with no salt added. Optimum cooking time (OCT) was determined according to Approved Method 16-50 (AACC, 2000) with some modifications. The time (t_c) when the inner white core of the pasta disappeared were determined. A trained panel of three assessors tasted cooked pasta samples at cooking times around t_c and identified OCT on the basis of pasta firmness ("al dente").

Cooking loss (CL)

Cooking loss (CL, %), the amount of solid substance lost in the cooking water, was determinate according to the Approved Method 66-50 (AACC, 2000).

Moisture content

After cooking, pasta strands were cool down at room temperature by using 200 mL of distilled water. Moisture content (%) was evaluated by drying cooked pasta (3 g) samples to constant weight at 105 °C. Data are the mean of nine measurements from three different cooking replications.

Mechanical properties

Mechanical properties of cooked pasta samples were evaluated using a Texture Analyser (TA.XT plus, Stable Micro Systems Ltd., Godalming, UK) equipped with a 5 kg load cell. Pasta was cooked, cooled for 1 min under running distilled water at 20 °C to arrest the cooking process, transferred to a covered container and rested at 25 °C for 10 min after cooking before testing.

Pasta firmness, expressed as maximum cutting force (N), was determined according to the approved method 66-50 (AACC 2000). To determine firmness, the force required to cut five pasta strands was measured using a light knife blade (A/LKB) (speed 0.17 mm/s) was measured. Data are mean of twenty-four measurements from three different cooking replications.

Pasta stickiness of ten strands was evaluated using a pasta firmness/stickiness rig at a compression speed of 0.5 mm/s and compression force of 1000 g for 2 s. Stickiness (N) was defined as the maximum peak force to separate the probe from the sample's surface upon probe retraction. Results were expressed as mean of twelve measurements from three different cooking replications.

6.2.6 Dynamic rheological properties

Rheological measurements were carried out using a controlled stress rheometer (SR5, Rheometric Scientific, Germany) equipped with serrated parallel plate geometry (25 mm diameter, 2 mm gap). In order to study extensively the structure, raw pasta samples were ground (particle size < 500 µm) and used to produce doughs at water absorption of 98% which were placed between the plates of the rheometer. Excess dough was carefully trimmed and the exposed edge coated with silicon grease in order to prevent drying. Each sample was left to rest 10 min after loading before testing. This resting time was sufficient for the dough to relax and to reach a constant temperature. A frequency sweep test was performed at 25 °C from 0.1 to 10 Hz within the linear viscoelastic range. Time cure test was carried out from 25 °C to 95 °C at 1 °C/min and 1 Hz (linear viscoelastic regime). Data obtained were storage modulus (G'), loss modulus (G") and tan δ (G"/G'). Statistical comparisons were made at 1 Hz. Results are the average of triplicates, where each replicate represents a separately mixed dough.

6.2.7 Polarized light microscopy

Suspensions of RS in distilled water (1/2, w/v) were heated in a temperature-controlled glycerol bath at different temperatures (60, 70 and 90 °C). Once the sample reached the desired temperature, it was held at that temperature for 5 min. Resistant starch granules were examined for the presence of birefringence by using polarized light microscope (Leica DMRB, Leica Microsystems GmbH, Germany) and images acquired by a digital camera (Digital Sight DS-Fi2, Nikon, Japan) using a DS Camera Control Unit DS-L3 (Nikon, Japan).

6.2.8 Resistant starch determination

RS contents of raw gluten-free pasta were obtained by a commercial test kit Megazyme International (K-RSTAR 08/11, Bray, Ireland). In brief, samples were incubated with pancreatic α-amylase and amyloglucosidase (AMG) in a shaking water bath (sw-205 Julabo GmbH, Germany) with 200 strokes/min at 37 °C for 16 h, during which time non-resistant starch was solubilised and hydrolysed to D-glucose by the combined action of the two enzymes. The reaction was terminated by the addition of pure ethanol and the RS was recovered as a pellet on centrifugation at 1500 x g for 10 min (Avanti^{TM} J-25 High Performance Centrifuge, California, USA). The residue was washed twice by suspension in aqueous ethanol 50% (v/v), followed by centrifugation. Free liquid was removed by decantation. RS in the pellet was dissolved in 2 M KOH by vigorously stirring in an ice water bath over a magnetic stirrer; this solution was neutralized with acetate buffer (pH 3.8) and the starch was quantitatively hydrolysed to glucose with AMG. D-Glucose was measured with glucose oxidase/peroxidase reagent (GOPOD) and this was the measure of the RS content. Absorbance at 510 nm was measured using a spectrophotometer (UV-2501PC, Shimadzu, Japan). RS content was calculated from absorbance value using an equation supplied from the kit.

6.2.9 Statistical analysis

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

6.3 Results and discussion

6.3.1 Mixing properties

The farinograph results of RS-enriched dough and the reference (without fibre) at constant water are reported in Fig. 6.1. Time required for dough development, or to reach the maximum consistency during mixing, was 12 min for the reference (Fig. 6.1A) and increased with the increase in RS content (15-18 min) due to a delay in hydration of the blend (Figs. 6.1B-D). Fibre addition did not induce changes in maximum dough consistency suggesting that water absorption was not influenced by RS addition. On the basis of these results, constant water content was used to produce pasta doughs with and without RS, and mixing times (before steam injection) were 25-50% higher for RS samples to assure proper hydration.

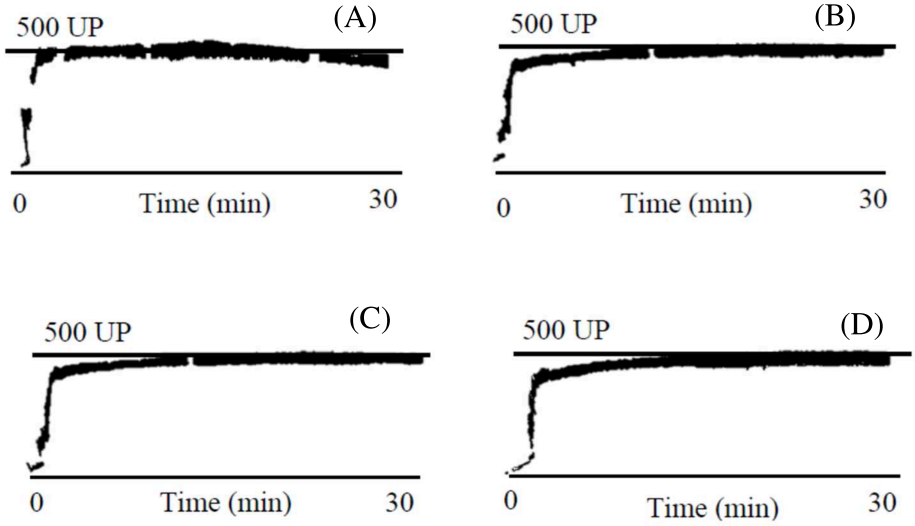

Fig. 6.1. Farinograph curves of rice flour blend (A) and flour blend enriched with RS at 10% (B), 15% (C) and 20% (D) at 59% water absorption.

6.3.2 Pasta cooking quality

Cooking quality of pasta samples with and without RS was established on the basis of OCT, CL, moisture content and textural characteristics of cooked pasta. The control showed lower OCT than pasta supplemented with RS and OCT slightly increased with the increase in fibre content (Table 6.1). This behaviour seemed to suggest that RS could acts as a texture modifier (Sajilata et al., 2006). Sozer et al. (2007b) demonstrated that substitution of durum wheat semolina with 10% of RS type 3 caused an increase in OCT compared to control pasta. On the contrary, other studies showed that replacing durum wheat semolina with RS type 2 and 3 caused a slight decrease in OCT values (Aravind et al. 2013; Vernaza et al., 2012). The disagreement between the studies could be due to the different pasta formulation (type and content of RS and flour) and the different process conditions for pasta production.

Table 6.1. Effect of resistant starch addition on cooking, mechanical and dough characteristics**.**

Sample		**RS0**	**RS10**	**RS15**	**RS20**
OCT (min)		13.0	13.5	14.0	14.5
CL (%)	*at OCT*	28.2^{a}	20.3^{b}	20.1^{b}	19.1^{b}
	at 10 min overcooking	33.4^{a}	26.2^{b}	24.6^{b}	24.8^{b}
Moisture content (%)		70.8^{a}	67.5^{b}	68.0^{b}	67.3^{b}
Firmness (N)	*at OCT*	1.3^{b}	1.7^{a}	1.6^{a}	1.7^{a}
	at 10 min overcooking	0.6^{c}	0.7^{b}	0.8^{a}	0.8^{a}
Stickiness (N)	*at OCT*	2.3^{a}	2.5^{a}	1.5^{b}	1.4^{b}
	at 10 min overcooking	2.5^{a}	2.3^{b}	1.3^{b}	1.2^{b}
G' (kPa)		139.0^{b}	-	163.0^{a}	155.0^{ab}

Values within rows with different superscuript letters are significantly different from each other.

Cooking loss of RS-enriched samples was 30% lower than reference suggesting the formation of a less soluble structure (Table 6.1). No significant differences in CL values were observed between samples containing RS ($p=0.05$).

Finally, moisture content (Table 6.1) was significant lower in the gluten-free pasta with fibre than the reference; this is probably due to low water-holding capacity (Fuentes-Zaragoza et al., 2010; Mudgil and Barak, 2013).

Textural properties, especially firmness and stickiness, are important for establishing pasta quality and consumer acceptability. Addition of RS significantly increased firmness of pasta cooked at optimum and overcooked ($p<0.05$) (Table 6.1). The positive effect of RS on pasta quality was confirmed by stickiness parameter (Table 6.1). Above 10% level of substitution, all gluten free pasta samples showed lower stickiness values than the reference (without RS). However, stickiness and CL values in the present study are still higher compared with pasta semolina (Aravind et al. 2013; Vernaza et al., 2012), ascribable to low protein contents of rice flour (Sissons, 2008). In fact, the protein matrix in conventional pasta is able to entrap starch granules during cooking and to limit the cooking loss and stickiness of pasta surface (Sissons, 2008).

6.3.3 Rheological properties of pasta doughs

Small deformation rheological tests give information on linear viscoelastic properties (LVP) of dough, which can help to understand macromolecular structures and how they change upon processing. Raw pasta samples were ground and used to produce doughs at constant water absorption for fundamental rheological tests. The main goal was to investigate pasta structure, which depends on formulation and processing. Fig. 6.2A shows the frequency sweep test at 25 °C for pasta dough containing 20% of RS and reference (RS0). Samples displayed weak gel behaviour because the magnitudes of G' were much higher than those of G" and viscoelastic moduli were frequency dependent. The addition of fibre considerably increased the storage (G') and loss (G") moduli of dough. The storage modulus at costant frequency (1 Hz) was used to compare samples (Table

6.1). Higher G' values for samples containing fibre indicate a higher number of elastically phisical interactions, which seem effective to improve characteristics of cooked pasta. This is consistent with the high correlation between indicators of pasta cooking quality and G' at 1 Hz (r = -0.82÷0.94 for CL vs. G' and firmness vs. G' and r = -0.90÷0.92 for stickiness).

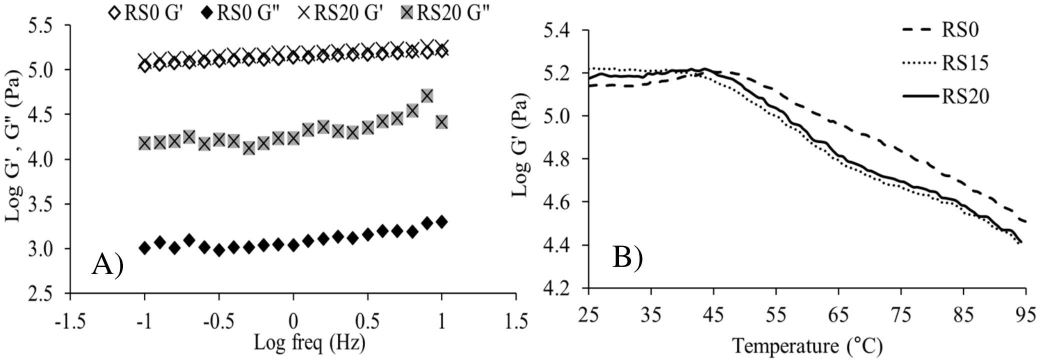

Fig. 6.2. A) Storage (G') and loss moduli (G") vs frequency at 25 °C for dough. Flour blend dough enriched with RS at levels of 0% and 20% and 59% water absorption. B) Storage modulus (G') vs. temperature at 1 Hz for dough obtained from raw pasta. Flour blend dough enriched with RS at levels of 0, 15% and 20% and 59% water absorption.

In order to evaluate dough rheological properties during pasta cooking, a dynamic temperature ramp test from 25 °C to 95°C was carried out (Figure 6.2B). Changes in G' profile as a function of temperature reflects structural transitions associated with heating of the system above the starch gelatinization temperature. Swelling of starch granules due to gelatinization is clearly highlight by a G'$_{peak}$ (Peressini, Foschia, Tubaro and Sensidoni, 2015; Peressini, Pin and Sensidoni, 2011; Rolee and LeMeste, 1999). Fig. 6.2B shows G' curves for pasta doughs with and without RS. Elastic properties decreased with the increase in temperature above 45 °C indicating the absence of native granules of rice starch. As expected, processing conditions promoted a complete gelatinization of rice starch granules. Below 45 °C, RS0 showed a lower G' curve than RS-enriched pasta doughs according to frequency sweep results (Fig.6.2A, Table 6.1). An opposite behavior was observed above 45 °C indicating that the plasticizing effect of temperature was higher for RS samples Besides, RS samples exhibited a linear decrease in G' between 55-70 °C and a deviation from linearity above 70 °C, which could identify changes in RS granules. Polarized light microscopy images of heat treated RS confirmed this hypothesis (Fig. 6.3) An increase in starch granules size (swelling) with theincrease in temperature and a partial loss of birefrangence are associated with gelatinisation phenomenon (Fig. 6.3 A-D). In fact, from 25 °C to 95 °C a gradual loss of birefringence at the centre of the granules, but not at their periphery, was apparent. These results were in agreement with Li et al. (2011) who observed a decrease in birefringence more pronounced at the center of mung bean starch as the processing temperature increased. The thermal energy seemed to promote the mobility of the molecules, which reduces their radial orientation and cause a loss of birefringence of resistant starch (Zhang et al., 2014). In Fig. 6.3D (90 °C) this loss was more evident and suggest that pasta cooking could determine a decrease in fibre content. Cai et al. (2014) identified a gelatinization temperature of high-amylose between 72-98 °C. Indeed, it was reported that RS type 2 added in semolina pasta decreased about 50-60% compared with the initial amount (Gelencsér et al., 2010; Vernaza et al., 2012).

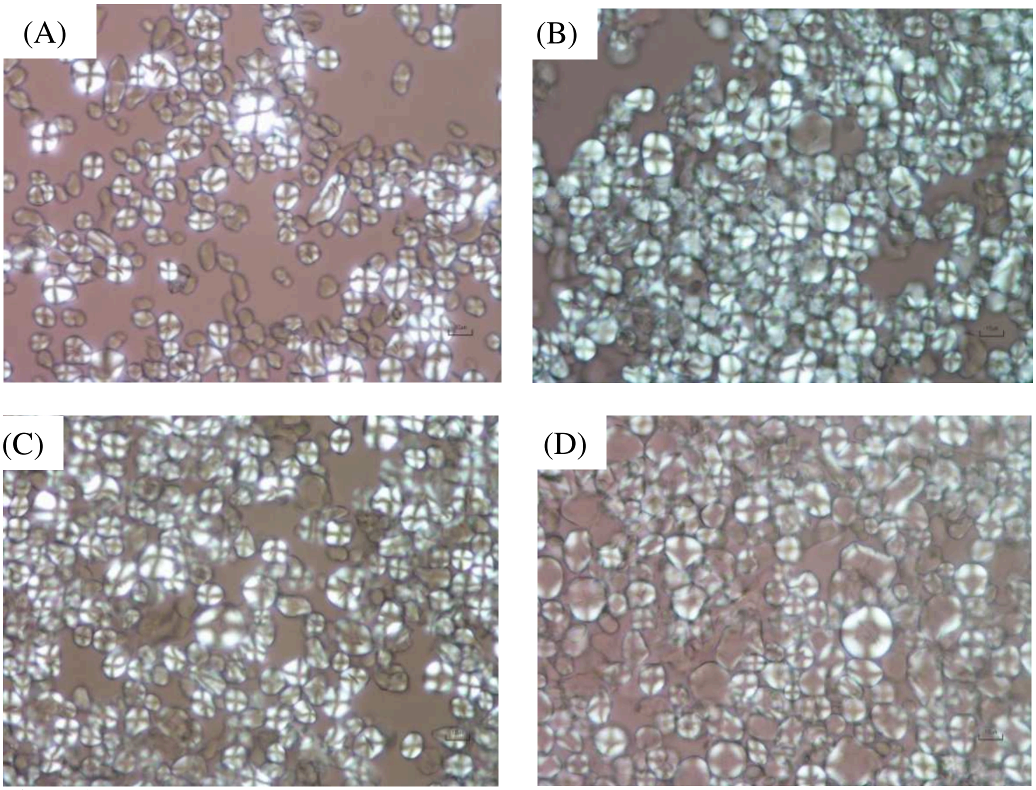

Fig. 6.3. Polarized light microscopy images of resistant starch treated for 5 min at room temperature (A), 60 °C (B), 70 °C (C) and 90 °C (D) (400X).

6.3.4 RS degradation

The present work analysed the resistant starch content in the pure ingredient and raw gluten free pasta samples (Table 6.2). In particular, the aim was to better understand how processing conditions could affect RS degradation during gluten free pasta production.

Table 6.2. Resistant starch degradation of all raw gluten-free pasta samples.

Samples	RS content (%)	RS loss (%)
Hi-maize 260	46.87 ± 5.03	
RS0	1.87 ± 0.49	
RS10	4.06 ± 0.42	36.62
RS15	6.05 ± 0.10	30.35
RS20	7.94 ± 1.26	27.48

Hi-maize 260 resulted to contain 46.87% of RS. The process conditions affected the RS loss that ranged between 36.6% and 27.5% (Table 6.2). Based on what reported in literature, it can be hypothesized that extrusion process for pasta does not affect considerably the initial amount of resistant starch added in the formulation (Gelencsér et al., 2010; Sajilata et al., 2006). This suggested that RS loss in our product was probable ascribable to the steam injection during the kneading step (thermal treatment of the hydrated dough). A possible solution could be to use, in gluten free pasta formulation, ingredients as an alternative to the heat treatment of the dough during pasta production. These ingredients, such as gelatinized starch, biopolymers or reticulation agents, should allow for the network formation of the product.

6.4. Conclusions

In this study, the effect of substitution of rice flour with RS (10, 15 and 20%) on quality characteristics and fibre degradation in gluten free-pasta were evaluated. The results indicated that RS was able to improve pasta quality thanks to its ability to decrease CL and stickiness and increase firmness at OCT. This trend was registered also during overcooking for all parameters analyzed. In conclusion, the critical points for RS stability resulted to be processing conditions and pasta cooking. The present research work showed a RS loss during the production of about 31%, while the literature reported a loss during pasta cooking of about 50-60%. Taking in account this information, only RS20 can be considered as a food product source of DF, since it contains 3 g of fibre per 100 g of pasta (Vernaza et al., 2012).

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Chapter 7 - Conclusions and future prespectives

The results of the present PhD research project showed that inclusion of dietary fibre significantly modified dough rheological properties and quality characteristics of the end product. In general, these changes depend on the level and type of fibre in terms of degree of polymerisation, presence of low molecular sugars and water absorption properties.

The main effects are summarised for product type below:

- ✓ Extruded snacks: inulin enrichment lowered dough consistency and caused different kinetics of starch gelatinization. Inulin GR (short-chain) had a greater effect than HPX (long-chain). Inulin GR increased product expansion and hardness compared with the reference, while no significant differences in volume and mechanical properties were observed between reference and inulin HPX enriched samples up to 5%.
- ✓ Pasta: the addition of DF led to an increase of OCT, CL, WA and SI and to deterioration in textural characteristics. Moreover, raw pasta enriched with DF showed a darker colour than semolina pasta control. In the cooked pasta this trend changed; in fact, all inulin enriched pasta (except for GR-P samples) showed higher L* value than semolina pasta. Our data suggests an improvement in cooking characteristics when oat bran flour is added in combination with another DF; whereas a negative effect is observed when psyllium fibre was added in combination with DFs.
- ✓ Gluten-free pasta: RS was able to improve pasta quality thanks to its ability to decrease CL and stickiness and increase firmness at OCT compared with the control. This trend was registered also during overcooking for all parameters analyzed.

Based on these results, it might be hypothesised that DF 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. In fresh pasta case study, DF seems to compete with starch and protein for water during dough formation and would hydrate quicker than other components of the semolina, thus leading to starch and protein fractions of pasta more discrete and less incorporated into the matrix. However, it should have taken in account that in fresh unsubstituted pasta the gluten matrix is not as strong as in a dried pasta sample. This consideration suggests that the relation between the amount of DF added and starch digestibility is more complex in dried pasta because the gluten–fibre matrix may be strengthened by elevated temperature drying. Thus, it can be concluded that the effect of DF on pasta structure and subsequently its nutritional characteristics depends not only on the type and amount of DF added but also on the manufacturing process conditions.

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, GR, HPX, GG, P and O showed significant lower AUC values compared to control pasta sample. Many factors have been suggested to explain the slow digestion in DF enriched pasta. The reduction in the digestibility of pasta added with soluble fibre can be explained by the changes in the microstructure of cereal based products and the limitation of water availability for starch gelatinisation due to the hydration of soluble non-starch polysaccharides. The functional properties of β-glucans have been ascribed to their ability to increase lumen viscosity. It has been suggested that cereal β -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.

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.

The present study demonstrated that process conditions affected dietary fibre stability. In particular, the evaluation of inulin content in extruded snacks showed that the loss ranged between 12.4% and 29.5% compared with the initial amount added to the dough. This degradation occurred partially during extrusion-cooking leading to low molecular weights products, which probably determined a further reduction in dough consistency and elasticity. On the basis of these results dough was subjected to heating and shearing conditions not too severe for the stability of inulin. Extruded snacks enriched with 5% of inulin can be considered as a functional food. On the other hand, the critical points for RS stability resulted to be processing conditions and pasta cooking. Indeed, the loss of RS in raw gluten-free pasta ranged between 36.6% and 27.5%. It can be hypothesized that extrusion process for pasta does not affect considerably the initial amount of resistant starch added in the formulation. This suggested that RS loss in our product was probable ascribable to the steam injection during the kneading step (thermal treatment of the hydrated dough). A possible solution could be to use, in gluten free pasta formulation, ingredients as an alternative to the heat treatment of the dough during pasta production. These ingredients, such as gelatinized starch, biopolymers or reticulation agents, should allow for the network formation of the product. Moreover, based on what is reported in literature, pasta cooking could cause a RS loss of about 50-60%. Taking in account this information, only RS20 can be considered as a food product source of DF, since it contains 3 g of fibre per 100 g of pasta.

Food fortification is the costeffective way of achieving a particular nutritional goal. Indeed, dietary fibre ingredients utilised in this research project could be purchased at low price without causing equipment modifications.

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. Additional research would be necessary in order to evaluate the acceptance of DF enriched pasta from consumers.

Dietary fibers can be used in processed food products not only to improve the dietary fiber content but also to improve the viscosity, texture, sensory characteristics and shelf-life of food products. As mentioned previously, dietary fibre exhibits several physicochemical properties such as water binding, gelling and structure building in creating novel food structures. It is also possible to utilise fibre-rich by-products from primary or secondary food production streams as ingredients in food products as inexpensive, non-caloric bulking agents for partial replacement of flour, fat or sugar, as enhancers of water and oil retention and to improve emulsion or oxidative stabilities. DFs are generally classified as soluble or insoluble, based on whether they form a solution when mixed with water (soluble) or not (insoluble). Compared with insoluble dietary fibre, in food processing the soluble fraction demonstrates greater capacity to provide viscosity, ability to form gels and/or act as emulsifiers, has neither bad texture nor bad taste and is easier to incorporate into processed food and drink. Moreover, DF concentrates could be used as functional ingredients in food to avoid syneresis, to stabilise products with a high percentage of fat and emulsion and to modify the texture and the viscosity of formulated products by virtue of their high WHC and OHC and their rheological properties.

Cereals and cereal co-products such as wheat, oat, barley and rice represent common dietary fibre sources. However, novel sources of fibre such as apple, grape, lemon, mango, orange, peach, carrot, cauliflower, onion, pea, potato, tomato could be utilised. At present, up to one third of fruit and vegetables in the form of peels, pips and skins can be discarded during preparation and processing, therefore creating a ‘waste’. Utilization of fibres from exotic fruit by-products would not only open new businesses and profits, but would also contribute to give alternate uses to the huge quantities of the by-products wasted in the food industry. Currently these by-products are dispatched to animal feed, landfill or incineration, thus potentially creating negative effects on the environment. Extensive research has shown fruit and vegetable by-products to be a high source of dietary fibre. Also, their use can impart such functional benefits as gelling, thickening and water binding. These properties are advantageous and may be utilized in many fields such as bakery products. Finally, utilization of exotic fruits and their by-product could also increase the nutraceutical property of the end product by increasing its antioxidant activity.

List of publications relevant to this PhD research activity

Foschia M., Peressini D., Sensidoni A., Brennan M. A., Brennan C. S. (2015). How combinations of dietary fibres can affect physicochemical characteristics of pasta. *LWT - Food Science and Technology, 61*, 41-46.

Foschia M., Peressini D., Sensidoni A., Brennan M. A., Brennan C. S. (2015). Synergistic effect of different dietary fibres in pasta on *in vitro* starch digestion*? Food Chemistry, 172*, 245-250.

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Foschia M., Peressini D., Sensidoni A., Brennan M. A., Brennan C. S. (2014). Mastication or maceration: does the preparation of sample affect the predictive *in vitro* glycaemic response of pasta? *Starch*, *66*, 1096-1102.

Foschia M., Peressini D., Sensidoni A., Brennan C. S. (2013). The effects of dietary fibre addition on the quality of common cereal products. *Journal of Cereal Science, 58*, 216-227.

Additional publications

Anese M., Manzocco L., Panozzo A., Beraldo P., Foschia M., Nicoli M.C. (2012). Effect of radiofrequency assisted freezing on meat microstructure and quality. *Food Research International*, *46*, 50-54.

Calligaris S., Foschia M., Bartolomeoli I., Maifreni M., Manzocco L. (2012). Study on the applicability of high pressure homogenization for the production of banana juice. *LWT- Food Science and Technology*, *45*, 117-121.

Manzocco L., Foschia M., Tomasi N., Maifreni M., Dalla Costa L., Marino M., Cortella G., Cesco S. (2011). Influence of hydroponic and soil cultivation on quality and shelf life of ready-to-eat lamb's lettuce (*Valerianella locusta* L. Laterr). *Journal of the Science of Food and Agriculture*, *91*, 1373-1380.

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Vita

Martina Foschia was born on June 18th 1979 in San Vito al Tagliamento (Pordenone, Italy). After obtaining the scientific diploma (High School Ettore Majorana, San Vito al Tagliamento) she went on to study Food Science and Technology at the University of Udine (Italy), completing her Bachelor's of Science, which then led her to pursue a Master's degree in Food Science. During her MSc studies, she was involved in research collaborations with the following topics:

- Study of different strategies aimed to remove/reduce the suspected carcinogen acrylamide in biscuits, potato chips, French fries and breakfast cereals;
- Identification and evaluation of quality parameters for frozen ready-to-eat foods during different storage conditions;
- Application of non-conventional technologies (High-pressure homogenization, UV-C light, pulsed light) on vegetable products in order to obtain safe products by destroying spoilage microorganisms and inactivating enzymes.

She wrote her MSc thesis on the "Study and development of a radiofrequency assisted freezing system".

Winner of a FSE (European Social Fund) grant for a PhD course in Food Science at the University of Udine, she focused on the "Study and development of new functional foods containing cereals". Her work was performed at the Food Science (University of Udine, Italy) and Food, Wine and Molecular Biosciences (Lincoln University, New Zealand) Departments.

She is fist author and coauthor of several scientific articles.

She is currently at the University College Cork (Ireland) as a postdoctoral researcher in the Cereal and Beverage Science research group.