UNIVERSITY OF MOLISE

EFFECT OF PASTA PROCESSING ON PHYSICOCHEMICAL PROPERTIES OF BARLEY BETA-GLUCAN AND PHENOLIC ACIDS

PHD THESIS

in

FOOD BIOTECHNOLOGY (BIOTECNOLOGIA DEGLI ALIMENTI)

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SUMMARY

The increasing interest in the relationship between health and nutrition has steered the scientific research towards studying the role of specific food components/ingredients (phytochemicals), which are naturally included in many foods, in the treatment and/or prevention of ailments.

Cereals are generally suitable vehicles of bioactive substances (phytochemicals) such as β-glucan and phenolic compounds. Indeed, available literature report that β-glucan and phenolic compounds have a beneficial role in diet and health. However, the literature seems to show that processing of grains, such as pasta processing, may affect bioactive compounds, increasing or decreasing the health-enhancing properties of cereals products.

Based on these remarks, the current PhD research work aims at developing barley pasta rich in β-glucan as a functional food with the use of waxy barley as a rich source of soluble dietary fiber, such as β-glucan, and phenolic acids. The research work includes three studies:

- in the 1st study chemical, molecular and rheological properties of waxy barley individually and in blend with semolina was investigated;
- in the 2nd study impact of processing (extrusion, drying and cooking) on the concentration and physicochemical properties of the flours and blends, and molecular characterization of β-glucan, as well as viscosity of the developed pasta products was evaluated;
- in the 3th part of the research antioxidant properties and phenolic acid composition of the developed pasta products was studied.

The obtained results show that pasta processing may affect β-glucan and phenolic acids.

This research would make a great contribution to the barley research and assist barley industry in the development of innovative functional foods.

RIASSUNTO

Il crescente interesse nella correlazione tra alimentazione e salute ha indotto la comunità scientifica ad un maggior impegno nel campo della ricerca di alcune sostanze dalle riconosciute proprietà salutistiche (phytochemicals), naturalmente presenti in una vasta gamma di prodotti di interesse agro-alimentare.

I cereali in genere, nonché i prodotti da essi derivati, sono tra gli alimenti più idonei a veicolare composti bioattivi (phytochemicals) come ad esempio β-glucani e composti fenolici. Evidenze scientifiche hanno dimostrato, infatti, che β-glucani e acidi fenolici possono avere importanti effetti fisiologici. Tuttavia, diversi studi riportano che il processo produttivo può influenzare positivamente o negativamente la componente bioattiva, determinando pertanto cambiamenti nelle proprietà fisiologiche del prodotto finito.

Alla luce di queste considerazioni, l'attività di ricerca di dottorato ha avuto come principale finalità la produzione di pasta funzionale a base d'orzo ricca in β-glucani ed è stata sviluppata in tre sezioni:

- valutazione delle proprietà chimiche, molecolari e reologiche di sfarinati di orzo waxy in miscela e non con semola di frumento duro;
- studio dell'impatto dei processi tecnologici sulla concentrazione e caratteristiche molecolari e di solubilità dei β-glucani in paste funzionali;
- valutazione della capacità antiossidante e degli acidi fenolici delle paste a base d'orzo waxy prodotte nell'ambito della sperimentazione.

I risultati ottenuti consentono di affermare che il processo di pastificazione può influenzare la componente bioattiva, in particolare β-glucani e acidi fenolici, presente nel prodotto finito. La presente attività di dottorato vuole fornire un contributo al mondo della

ricerca e dell'industria, nonché fornire informazioni per la realizzazione di cibi funzionali a base d'orzo.

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CHAPTER I

Introduction

Introduction

Whole cereal grains, including barley, and their products are important foods in human diet mainly because they are rich sources of energy, dietary fiber, vitamins and minerals. Their consumption has been linked to the prevention of chronic diseases.

Numerous laboratory and clinical studies have demonstrated that consumption of barley-based products can lower serum cholesterol levels, reduce glucose uptake, decrease plasma insulin response, control weight through prolonged satiety (Yokoyama et al., 1997; Cavallero et al., 2002; Wood, 2007) and encourage growth of beneficial gut microflora (Brennan and Cleary, 2005). These physiological effects of barley are primarily attributable to the increasing of viscosity in the gastrointestinal tract (Wood, 2007), caused mainly by β-glucan. Previous studies have demonstrated that the concentration, molecular weight (MW), and structural features of β -glucan influence its physical properties (viscosity and solubility) (Wood et al., 1994). The physicochemical characteristics of β-glucan are influenced by many factors such as genotype, environment, agronomic input, and the interactions of these factors and food processing methods.

In addition to β-glucan, barley contains many other health-promoting components, which include phenolic compounds (Hernanz et al., 2001; Adom and Liu, 2002). Phenolic acids are the dominant group present in free and bound form primarily in the outer layers of the cereal kernels. Phenolic compounds found in cereals are known for their beneficial antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties and overall for the promotion of human health (Kahkonen et al., 1999). They may act as scavengers of free radicals (Ragaee et al., 2006), chelating agents of pro-oxidant metals, quenchers of singlet oxygen (Larson, 1988), and inhibitors of human LDL cholesterol oxidation (Abdel-Aal and Gamel, 2008).

Viewing the importance of barley as vehicle minor components with functional properties, in the last years, food researchers and the food industry has been investigating the possibility of incorporating barley flours and by-products into various food products (bread, muffins, pasta, noodles, bars, cookies, etc) (Izydorczyk et al., 2008; Vitaglione et al., 2008; Verardo et al., 2011). Although significant numbers of studies illustrate the benefit of consuming barley-rich products, few studies document the changes to the bioactive compounds of barley-based products that the production process brings about. In particular, the available literature reveals that food processing operations can have a negative or positive impact on the molecular weight and solubility of β-glucan (Tiwari and Cummins, 2009) and levels of phenolic compounds in foods (Ragaee et al., 2014). This has implications for their bioactive properties and potential health benefits they can offer. Therefore, cereal recipe and processing should be carefully chosen to preserve the health properties of foods after processing.

1. Literature review

1.1 The history of the use of barley

The beginning of agriculture is dated back to 10,000 years ago in the Pre-Pottery Neolithic Near East. Barley (*Hordeum vulgare* L.) is one of the cereal founder crops and it is believed that first plant domestication took place within the Fertile Crescent (Lev-Yadun et al., 2000).

Hordeum vulgare L. subsp. *spontaneum* (wild barley) is said to be the ancestor of today's barley. The spread of barley most likely started in present-day Israel, northern Syria, southern Turkey, eastern Iraq and western Iran. With the movement of civilizations accompanied by the establishment of trade routes the use and cultivation of barley reached Europe. Barley was a popular food in ancient Greece and Italy and used as an ingredient for preparing porridge or unleavened bread. Greeks and Romans such as Hippocrates or Pliney the Elder, respectively, considered barley as a healthy and nourishing food and barley gained as well recognition for medical treatments. In the ancient Rome, gladiators believed that barley could increase strength and stamina and thus preferred it to other cereals. Barley reached Spain around 5,000 BC and spread then over today's Germany and France. Indications of domestication of barley on the British Isles date back until 3,000 BC and one millennium later, barley was introduced to Northern Europe. Probably due to the nourishing properties and the ruggedness of the crop, barley became a major food especially for poor people throughout history (Newman & Newman, 2005).

In 2009, barley was the twelfth most important agricultural commodity of the world in terms of production. After maize, wheat and paddy rice it was the fourth most important cereal crop (FAO, 2011). The annual production was about 152 million tons, of which the Russian Federation produced around 17.9 million tons followed by France,

Germany and Ukraine with 12.9, 12.3 and 11.8 million tons, respectively. In the European Union, barley still attains distinction as the second cereal crop after wheat.

1.2 Barley grain

Barley belongs to the family Poaceae and the genus Hordeum. The most common form of barley is *Hordeum vulgare*. It can be classified as spring or winter types, two-row or six-row (in two-row barley only the central spikelet is fertile, while in six-row barley has fertile lateral spikelets also), hulled or hull-less (which relates to the presence or absence of an outer husk attached to the grain) and malting or feed (which relates to its end-use). The composition of the grain can be classified as normal, waxy or high amylose starch types, high lysine, high β-glucan or proanthocyanidin-free. Barleys of different classes often differ widely in both their physical and compositional characteristics, and as a result they have different processing properties and end-uses.

Generally wholegrain barley consists of approximately 70% starch, 10-20% protein, 5-10% β-glucan, 2-3% free lipids and approximately 2.5% minerals (Czuchajowska et al., 1998; Izydorczyk et al., 2000; Quinde et al., 2004) with total dietary fibre and soluble dietary being reported to range from 11 to 34% and 3-20% respectively (Fastnaught, 2001) (Table 1.1). Barley composition is influenced by genetics, environmental factors and can also be influenced by the interaction between the two factors (Aman and Newman, 1986).

Barley is rich in prolamin storage proteins (hordeins). Barley proteins have a moderate nutritional quality, and recent studies have shown that there may be a link between barley proteins and grain hardness (Fox et al., 2007). Barley has been reported to contain a number of essential amino acids, including threonine, valine, lysine and arginine. Essential amino acids are amino acids that cannot be made by the body, or cannot be produced fast enough to meet the body's demand for them. These amino acids

therefore must be supplied by the diet as they play important roles in metabolic pathways. For example, arginine is involved in the synthesis of urea in the liver, while lysine is involved in the production of carnitine, which is a substance that transports fatty acids within cells (Sullivan et al., 2010).

Table 1.1 Composition of hulled and hulless barleys in nonwaxy and waxy genotypes (Newman and Newman, 2005)

	Nonwaxy		**Waxy**	
Property	**Hulled**	**Hulless**	**Hulled**	**Hulless**
Protein, %	15.6	16.3	16.1	16.9
Ether extract, %	2.0	2.1	2.3	2.6
Ash, %	2.7	2.1	2.8	2.1
Starch, %	55.9	61.3	51.5	58.5
Soluble carbohydrates, %	2.3	2.9	5.0	5.5
Fiber, %				
Total dietary fiber	17.0	13.2	19.6	13.8
Soluble dietary fiber	4.4	4.9	5.9	6.3
Arabinoxylans	6.2	4.4	6.7	4.6
Cellulose	3.8	2.1	4.4	1.9
Klason lignin	2.0	0.9	1.8	0.9
Total β-glucan	4.4	4.7	5.3	6.3
Soluble β-glucan	2.6	2.6	3.2	3.4
Viscosity, cP	2.8	3.1	3.3	4.9

Generally, starch is most abundant in the endosperm fraction of the barley kernel (fig. 1.1), as starch is the major component of flour. Smaller levels of starch are present in the aleurone, subaleurone and germ tissues (Flores et al., 2005; Izydorczyk et al., 2003). Studies have been undertaken on the pasting properties of barley starches. Ragaee and Abdel-Aal (2006) found that wholegrain barley had peak and final viscosities that were significantly higher than those of hard wheat, but lower than those of soft wheat. The authors studied the addition of 15% barley wholegrain to both hard and soft wheat, finding that the addition lead to increases in the peak and final viscosities of the hard

wheat formulations, while the addition of barley lead to a decrease in the pasting attributes of the soft wheat formulations. This suggests that the addition of barley to hard wheat would be more likely to produce an end-product with increased rates of starch retrogradation and would therefore have to be taken into account when formulating a barley-containing product. The authors suggested that barley had the potential to be included in the development of a healthy food product.

Barley starches generally have a high amylose-to-amylopectin ratio, meaning that there is a significantly higher proportion of amylose in the starch than amylopectin. Amylose molecules, because of their linearity, line up more readily than amylopectin molecules and hence have a more extensive network of hydrogen bonds. More energy will be required to break these bonds and gelatinise the starch. As a result of this, barley starches retrograde more readily than wheat starches (van Amelswoort and Westrate, 1992). The amylose content of barley starch can vary however. Waxy barleys generally have an amylose content of approximately 5%, the most common barley cultivars have an amylose content of 20-30% whereas high amylose barley can have an amylose content of up to 45% (Sullivan et al., 2013).

The main non-polar lipid fraction in barley is triacylglycerol, with the other lipids being made up of steryl esters, diacylglycerol, monoacylglycerol and free fatty acids (Morrison, 1993) whereas polar lipids consist primarily of phospholipids. Linoleic acid (50-60%), palmitic (20-30%), oleic (10-15%) and linolenic acid (4-9%) make up the non-starch lipids found in the barley kernel.

The ash content of barley is usually in the range of 2-3%, with the main minerals present being phosphorous, potassium and calcium, while chlorine, magnesium sulphur and sodium are found in smaller amounts (Owen et al., 1977). Minerals were found to be in their highest concentrations in the embryo, with the lowest concentrations found in

the endosperm (Liu et al., 1974). Bhatty (1997) found phosphorous and potassium to be the minerals with the highest abundance in barley (0.21 and 0.25% respectively). Calcium (0.02%), sulphur (0.12%), magnesium (0.08%), iron (49.9 mg/kg), zinc (24.4 mg/kg), manganese (13.9 mg/kg) and copper (12 mg/kg) were also detected in the study.

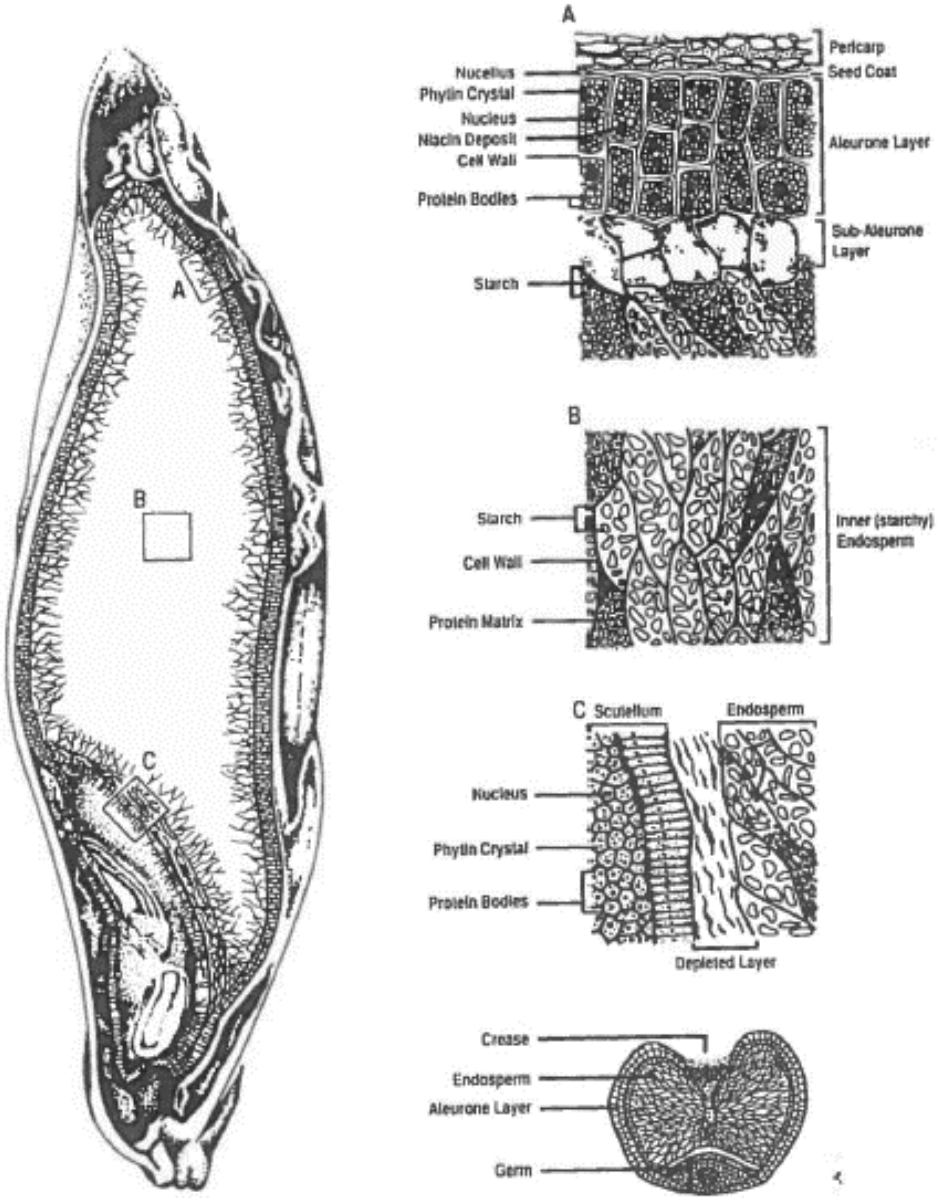

Figure 1.1 Barley grain with enlarge cross sections (Sullivan et al., 2013)

Fibre plays an important role in the digestibility of foods. Several authors have shown barley to have fibre levels ranging from 11 to 20% (Marconi et al., 2000; Fastnaught, 2001; Virkki et al., 2004). Fibre can be classified as either soluble or insoluble. Soluble fibre can be defined as "the edible parts of plants or similar carbohydrates resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine" whereas insoluble fibre passes through digestion without being absorbed and aid bowel function (AACC, 2001). The main constituent of soluble fibre is β-glucan (MacGregor and Fincher, 1993; Panfili et al., 2008).

Barley was found to be an excellent source of phenolic compounds with antioxidant properties, such as phenolic acids (Goupy et al., 1999; Ragaee et al., 2006).

1.3 Barley beta-glucan

1.3.1 Occurrence and molecular-structural characteristics of β-glucan

β-glucan is the more common name for (1-3, 1-4)- β-D-glucan. β-glucan consists of homopolymers of D-glucopyranosyl residues, usually linked by two (cellotriose) or three (cellotetraose) β-(1-4) linkages (~70%) separated by one β-(1-3) linkage (~30%) (Cui et al., 2000). Barley grain is notable for a high content of β-glucan that ranges from 2.5% to 11.3% (Izydorczyk and Dexter, 2008). In barley endosperm cell walls, β-glucan account for 75% of the total cell wall polysaccharides, the rest of which are made up of arabinoxylans, cellulose, glucamannans and proteins. The content of β-glucan in barley is influenced by both genetic and environmental factors and the interactions between the two (Andersson et al., 1999). Numerous studies showed that barley genotypes with anomalous starch composition (waxy or high amylose) have higher content of β-glucan than those with normal starch. It appears that the waxy starch character and high levels of β-glucan are usually inherited simultaneously, although the precise nature of the association between the waxy starch and high levels of β-glucan has not yet been revealed. The total content of β-glucan in the grain generally increases when barley is grown in hot and dry conditions (Swanston, 1997) although, the content of β-glucan may be reduced in response to short periods of very high temperature (Savin et al., 1997).

The molecular structure of β-glucan was derived by analysing the oligomers obtained from the digestion of β-glucan using specific enzymes that break the linkages between the D-glucopyranosyl units. Approximately 90-95% of the oligosaccharides produced during digestion are trisaccharides (3-O-β-D-cellobiosyl-D-glucose, DP3) and tetrasaccharides (3-O-β-D-cellotriosyl-D-glucose, DP4), with the remaining 5-10% made of longer oligosaccharides (≥DP5). The DP3/DP4 ratio is considered to be a

fingerprint of the structure of cereal β-glucan. In barley β-glucan, the molar ratio of DP3/DP4 may range from 2.3-3.4 (Lazaridou et al., 2007).

1.3.2 Physicochemical properties

It is generally accepted that β-glucan can lower plasma cholesterol, improve lipid metabolism, and reduce glycaemic index. The physiological activity of β-glucan has been attributed to its physicochemical properties, mostly the increased viscosity in the upper digestive tract (Izydorczyk and Dexter, 2008).

Viscosity is defined as the proportional relationship between the flow of a fluid and force directed on the fluid, and calculated as the shear stress (the force applied tangential to the fluid plane) divided by shear rate (the velocity gradient in a particular fluid due to applied shear stress) (Dikeman and Fahey, 2006) . The viscosity unit is millipascal second (mPa.s) or centipoise (cP), where 1 mPa.s is equal to 1 cP. Apparent viscosity, the most common term used for dietary fiber, is defined as the viscosity of a non-Newtonian fluid expressed as if it were a Newtonian fluid (Bourne, 2002). It is a coefficient calculated from empirical data as if the fluid being measured exhibited Newtonian flow and obeyed Newton's Law, allowing for a single viscosity reading at one shear rate (McDonald and others 2001).

The Rapid Visco Analyser (RVA) is used to measure the apparent viscosity of β-glucan solutions or oat-flour slurries as a function of temperature, time, and stirring speed. It is a simple, rapid, and reliable measurement, which needs only a small amount of sample and provides a graphical record of viscosity change with time. The measured viscosity is reported in centipoise (cP) or Rapid Visco Units (RVU).

In a freshly prepared solution, β-glucan behave as random-coil polysaccharides and exhibit high-flow viscosity at relatively low concentrations (Wood, 2002). At low

concentration (<0.2%), the β-glucan solution behaves as a Newtonian solution, whose viscosity does not be affected by an increasing shear rate. When the concentration of β-glucan above 0.2%, the high molecular weight β-glucan molecules start to entangle and form viscous and pseudoplastic solutions; whereas the low molecular weight β-glucan can form soft gels at higher concentrations. Wood (2007) reported that the viscosity is an exponential function of the concentration of dissolved β-glucan and of its molecular weight.

The molecular weight (MW) values of β-glucan ranged from 0.57×10^6 to 5.9×10^6 (Beer et al., 1997a; Knuckles et al., 1997). The discrepancies in β-glucan MW estimate might come from genetic and environmental factors as well as from the method used for extraction (solvent and temperature affect the solubilisation) and purification, aggregation phenomena (dependent on the structural features and solvent quality) and depolymerisation events (by endogenous or microbial β-glucanases from contaminating microorganisms) occurring during the extraction step (Lazaridou and Biliarderis, 2007).

1.3.3 Health benefits of β-glucan

Numerous laboratory and clinical studies have demonstrated that consumption of barley β-glucan can lower serum cholesterol levels, reduce glucose uptake, decrease insulin response, and control weight through prolonged satiety in humans (Brennan and Cleary, 2005).

The water-soluble β–glucan can effectively reduce the serum cholesterol levels associated with a lower risk of coronary health disease and the hypoglycemic effects (Davidson et al., 1991; Cavallero et al., 2002).

The hypocholesterolemic effect of β-glucan was related to the increasing of intestinal viscosity caused by β-glucan (Bourdon et al., 1999). The increased viscosity in the

gastrointestinal tract may lower the re-absorption of bile acid (BA), leading to an increased excretion of BA in the feces (Lia et al., 1997). The BAs, as part of the mixed micelles in the small intestine, are necessary for lipid digestion. Normally, the BAs are almost completely re-absorbed and transported to the liver through the enterohepatic circulation by active and passive mechanisms (Hofman, 1994). Increased excretion of BA stimulates the hepatic synthesis of BA from cholesterol, which removes the cholesterol from the circulation and eventually lowers the serum cholesterol level (LaRusso, 1983). The hypoglycemic effects of β-glucan are also attributed to the viscosity of β-glucan (Wood et al., 1994).

The increasing of viscosity caused by β-glucan slows intestinal transit, and delays gastric emptying and intestinal absorption of nutrients, such as digestible carbohydrates, thereby reducing postprandial hyperglycemia and insulin secretion.

These actions, in turn, increase satiety and promote weight loss (Mälkki and Virtanen, 2001; Lazaridou and Biliaderis, 2007).

1.3.4 Processing impact on β-glucan

There has been much speculation and insufficient investigation over the years concerning the effects of processing on the physicochemical characteristics of β-glucan incorporated into food products. This speculation has frequently supposed that processing would be detrimental to bioactivity. Processing seems to affect the molecular (chemical structure and degree of polymerisation), structural (molecular interactions) and functional properties (viscosity, water binding capacity and solubility), depending upon processing methods and conditions.

Table 1.2 summarizes the effect of some of the food processing operations on molecular weight, solubility and level of β-glucan in processed foods.

Wet milling, dry milling, and air classification are processing methods used for obtaining β-glucan-rich flour. Wikstrom et al. (1994) observed the variation in the intrinsic viscosity of different milling fractions of oats. Thus, milling can influence the molecular weight of oats and barley. In a study by Andersson et al. (2004), the structure of β-glucan was not affected by milling. However, a lower molecular weight for white flour and lower bran fraction for barley and higher molecular weight for shorts and whole meal was observed.

Table 1.2 Effect on molecular weight, solubility and level of β-glucan during food processing operations[a] (Tiwari and Cummins, 2009)

Food Processing Operations	Molecular Weight	Solubility/Extractability	β-Glucan Levels	Reference
Milling	(↓)(↑)	(↓)(↑)	(↓)(↑)	Andersson et al (2004)
Storage	(↓)	(↓)		Beer et al (1997)
Germination	(↓)	(↑)	(↓)	Wilhelmson et al (2001)
Malting	(↓)		(↓)	Wang et al (2004)
Fermentation			(↓)	Lambo et al (2005)
		(↑)		Degutyte-Fomins et al (2002)
Thermal processing		(↑)		Jaskari et al (1995)
Radiation		(↑)		Bhatty and MacGregor (1988)
Baking	(↓)			Andersson et al (2004); Trogh et al (2004)
Freezing	(~)	(↓)		Suortti et al (2000)
Cooking (glucans incorporated pasta)			(~)	Cavallero et al (2000)
Cooking of porridge	(~)	(~)		Kerkhoffs et al (2003)
Cooking		(↑)		Buckeridge et al (2004)
Pressure cooking (barley bulgur)			(↑)	Köeksel et al (1999)
Autoclaving (barley meal)	(↓)			Huth et al (2000)

[a] Increased (↑); decreased (↓); no change (~).

Changes in the properties of β-glucan may also arise from shearing damage due to mechanical processing (Wood et al., 1989), or by excessive heat treatment of food products. Unfavorable structural changes may also occur during commercial purification such as the depolymerization of the linear structure (Würsh and Pi-Sunyer, 1997), resulting in decreased molecular weight and reduced viscosity. A survey of molecular weights in processed foods (Åman et al., 2004) showed a partial depolymerisation of β-glucan in common oat foods, as macaroni and fresh pasta. In the foods where the partial depolymerisation occurred, the molecular weight distributions were frequently wide and bimodal indicating a nonrandom enzymatic hydrolysis.

Regand et al. (2009) also found an extensive degradation of β-glucan in foods such as oat pasta and bread. Degutyte-Fomins et al. (2002) showed that fermentation of oat bran using rye sourdough starter increased the solubility and the degradation of β-glucan during mixing and fermentation, probably due to the activity of β-glucanase. However, the molecular weight of β- glucan may remain unaffected (Lambo et al., 2005). The controversial results might be due to the different acidity levels obtained (pH 4.0 for oat bran concentrate and pH 5.2 for oat bran) and the differences in the chemical composition and enzyme activity of the preferment. Beer et al. (1997b) showed that baking of muffins increased the extractability but decreased the molecular weight of β-glucan. In their study, frozen storage decreased the extractability but did not change the molecular weight of β-glucan. However, Lan-Pidhainy et al. (2007) reported that repeated freezing and thawing reduced solubility of β-glucan, which in turn affected the glycemic response. Åman et al. (2004) observed that yeast-leavened bread, muffins, and other products like porridge and breakfast cereals retained higher molecular weight when compared with its raw material. Kerckhoffs et al. (2003) also observed depolymerization during bread production decreased the molecular weight of β-glucan. Where there was prolonged treatment at lower temperatures and presence of wheat flour, the β-glucan depolymerization is partially due to the β-glucanases activity of wheat flour (Andersson et al., 2004; Andersson et al., 2008).

Earlier research has indicated that extrusion cooking also causes fragmentation of food molecules such as starch and β-glucan. Extrusion-induced fragmentation may influence the functionality and nutritional quality of these cell wall polysaccharides. Research has been extended to investigate the effect of extrusion-induced fragmentation on the molecular characteristics (size and chain length) and functionality of barley β-glucan. The effect of extrusion cooking on fiber content and composition and on its

physicochemical characteristics depends on both the process parameters used (temperature, pressure, shear forces, screw design) and on the composition of the ingredients (Tiwari and Cummins, 2009).

However, food processing is also reported to increase the physiological activity of β-glucan by increasing solubility (cooking, extrusion), although the molecular size of the polymer may be partly reduced (due to enzymatic hydrolysis, milling, and pumping) (Robertson et al., 1997; Izydorczyk et al., 2000). Similarly, Johansson et al. (2007) reported an increasing of extractability in cooked porridges, while Beer et al. (1997b) found that cooking of porridge did not affect the extractability or the molecular weight of β-glucan.

1.4 Phenolic acids

1.4.1 Occurrence and molecular-structural characteristics of phenolic acids

Phenolic acids are the most common phenolic coumpounds that naturally occur in whole-grain cereals, in addition to flavonoids (Gani et al., 2012). Phenolic compounds may be defined as substances that possess an aromatic ring bearing one or more hydroxyl (OH) substituents (Duodu et al., 2011). Phenolic acids are derivatives of benzoic and cinnamic acids. Hydroxybenzoic acid derivatives include *p*-hydroxybenzoic, protocatechuic, vanillic, syringic and gallic acids. Hydroxyl cinnamic acid derivatives include *p*-coumaric, caffeic, ferulic and sinapic acids. A significant proportion of these phenolic acids is known to be linked to lignans and arabinoxylans (Nordkvist et al., 1984). Ferulic acid is highly concentrated in the cell walls of aleurone layer that is rich in arabinoxylan (Maillard and Berset, 1999).

The phenolic acids reported in cereals occur in both free and insoluble-bound form. Free phenolic acids are found in outer layer of the pericarp and are extracted using

organic solvents. Bound phenolic acids are esterified to cell wall polysaccharides; acid or base hydrolysis is required to release these bound compounds from the cell matrix (Dykes and Rooney, 2007). Phenolic acids can be esterified with small molecules such as alcohols, other phenolic acids, phenols, and alkaloids, among others. Non-starch polysaccharides such as xylose, and arabinose units may easily esterify with phenolic acids. Phenolic acids with carboxyl and hydroxyl groups can bind either starch or other polysaccharides through hydrogen bonds, chelation, or covalent bonds (Yu et al., 2001). Ferulic and *p*-coumaric are the major acids found in many cereals, including barley (Madhujith and Shahidi, 2009). Phenolic acid levels vary among cereals; their barns concentrate these compounds threefold (Table 1.3).

Table 1.3 Phenolic acid content in cereal grains (Dykes and Rooney, 2007)

Sample	**Amount (µg/g)**
Whole grains:	
Barley	450-1346
Finger millet	612
Foxtail millet	3907
Maize	601
Oat	472
Pearl millet	1478
Rice	197-376
Rye	1362-1366
Sorghum	385-746
Wheat	1342
Brans:	
Oat	651
Rye	4190
Wheat	4527

1.4.2 Health benefits of phenolic acids

Phenolic compounds, including phenolic acids, in our diet provide health benefits associated with reduced risk chronic diseases. Phenolic compounds have antioxidant properties and protect against degenerative diseases like hearth diseases and cancer in which reactive oxygen species i.e., superoxide anion, hydroxyl radicals and peroxyl radicals are involved (Rhodes and Price, 1997). It is emerging that polyphenols may have far more important effect *in vivo* such as enhancing endothelial function, cellular signaling and anti-inflammatory properties (Sies et al., 2005; Ramos, 2008). Emerging research has also suggested that undigested polyphenols associated with dietary fibre may provide important protection at the intestinal environment level (Vitaglione et al., 2008). However, whether the protective effect of polyphenols on health is via antioxidant or other mechanisms, research strongly supports a positive relationship between polyphenol intake and decreased risk of certain chronic diseases (Weichselbaum and Buttriss, 2010).

Ferulic and p-coumaric acids, the most common phenolic acids in cereal grains, are suggested to have antioxidant effect scavenging free radicals (Gani et al., 2012). They are also able to chelate ions of transition metal thereby reducing the metals' capacity to generate free radicals in Fenton reaction (Leibovitz and Mueller, 1993).

Ferulic acid was shown as a chemoprotectant, which was thought to deter the process of carcinogenesis by inhibiting the formation of N-nitroso-compounds (Kuenzig et al., 1984). This compound is also able to prevent peroxynitrite-mediated nitration of tyrosine residues in collagen thus implicating a chemoprotectant role for this antioxidant in the prevention of tissue injury (Kato et al.,1997).

Coumaric acid also has been suggested to have antitumor activity against human malignant tumors. Coumaric acid induces cytostasis and inhibits the malignant

properties of human tumor cells in vitro. A 50% reduction in the level of cell proliferation was achieved by concentration ranging from 1-4.5 mmol/L. *p*-coumaric acids have also potentially protective effect against heart diseases because of its ability to decrease the resistance of low density lipoproteins (LDL), cholesterol oxidation, lipid peroxidation and of apo-protein B100 (Garrait et al., 2006).

1.4.3 Processing impact on phenolic acids

A wide range of food processing methods are used to process cereal grains into a wide variety of food products. Processing of cereals may enhance or reduce levels of phenolic compounds in foods and this has implications for their bioactive properties and potential health benefits they can offer.

In cereal grains phenolic compounds are concentrated in their outer layers (pericarp, testa or seed coat) (Duodu, 2011). Therefore the decortication and milling process reduce phenolic content and subsequently, antioxidant activity of the flours produced. In general, the extent of reduction in phenolic content increases in direct proportion to the extent of removal of the outer layers of the grain during decortication and refining process of milling (Duodu, 2011; Ragaee et al., 2014).

Processing of food using heat is the most common method of food processing and preservation. Apart from the preservation effect, thermal processing can influence other quality aspects such as sensory, nutritive and phytochemical quality. Heat processing can take up various forms including cooking, roasting, microwave heating and extrusion cooking, to mention a few. The severity and mode of the thermal process also has a bearing on the quality of the food. The literature seems to show that thermal processing of grains may increase or decrease phenolic content and antioxidant activity. Cheng et al. (2006) reported increases in phenolic acids such as ferulic, syringic, vanillic and *p*-

coumaric acids in wheat flour upon thermal treatment. It could be that thermal processing of grains could bring about release of bound phenolics from the breakdown of cellular components and cell walls. In this regard, increase in antioxidant activity of thermally-processed sweet corn has been attributed to increase in solubilized ferulic acid esters and release of bound phenolics from the cell matrix (Dewanto et al., 2002). Fares et al. (2010) determined the effect of processing semolina into pasta and cooking on phenolic acids profile and antioxidant properties of pasta samples enriched with wheat debranning fractions and found both decreases and increases in phenolic content due to processing. Specifically, there was a decrease of free phenolic acids in control and wheat bran-enriched samples on processing semolina into pasta, mainly due to a decrease in *p*-hydroxybenzoic acid. There was however no change in the bound phenolic acid fraction. The results contradict those of Khan et al. (2013) who did not report any decrease in the free phenolic acids during extrusion of pasta made from durum wheat and sorghum flour.

However, Fares et al. (2010) found that cooking the pasta increased the levels of bound phenolic acids with generally no variation in free phenolic acid levels. This is somewhat in contrast with Zielinski et al. (2001) who reported increases in free and bound phenolic acids after severe hydrothermal processing of some cereal grains. Conversely, Verardo et al. (2011) reported a phenolic acid degradation in barley spaghetti after cooking.

According to Fares et al. (2010), during pasta processing, oxygen, water and heat treatment induce oxidative degradation of antioxidants including phenolics as suggested by other authors (Borrelli et al., 1999). This may account for the observed decrease in free phenolics on pasta processing which are considered to be more reactive than bound phenolics in counteracting the effects of oxygen and heat. On the other hand, during

cooking, boiling water can enhance the release of bound phenolics from the food matrix such as ferulic acid ester-linked to cell walls. This could increase bound phenolics content and antioxidant activity.

Zielinski et al. (2001) reported the behavior of phenolic compounds present in selected cereals (wheat, barley, rye, and oat) during extrusion cooking at different temperatures (120, 160, 200◦C). They found that significant increases in phenolic acids content and free and bound phenolic acids except for sinapic and caffeic acids were not detected in the extruded grains. The highest content of free and bound phenolic acids was reported in rye and oat. The changes in free phenolic acids were more pronounced when compared to the bound ones. The liberated phenolic acids may contribute to the high antioxidant potential of extrudates when they are considered as a dietary antioxidant. Ferulic acid was found as a predominant compound in raw wholegrain as well as in extruded grain.

Baking could result in an increase in the concentration of phenolic compounds of wholegrain bread regardless of baking time (10, 20, or 35 minutes). Some studies reported negligible changes in total phenolics caused by baking (Ragaee et al., 2014). The effect of baking appeared to be dependent on type of baked product, type of phenolic acids, recipe and baking conditions (Abdel-Aal and Rabalski, 2013). Moore et al. (2009) reported enhanced antioxidant properties in whole wheat pizza crust increasing baking temperature and time, and dough fermentation time. During fermentation, the metabolic activities of microorganisms which also involves a variety of enzyme activities have a significant effect on the chemical constituents of the food including phenolic compounds and their bioactive properties. Such effects may include binding of phenolos to other plant constituents such as proteins which makes these phenolos unextractable or degradation of phenolos by microbial enzymes (Duodu,

2011). On the other hand, fermentation with Lactobacillus rhamnosus A71 and Saccharomyces cerevisiae both increased total phenolic content and antioxidant activity in barley grains (Đorđević et al., 2010).

Aims of PhD research activity

This PhD study aimed 1) to develop barley spaghetti rich in β-glucan as a functional food with the use of waxy barley as a rich source of β-glucan, dietary fiber and antioxidants; 2) to investigate chemical, molecular and rheological properties of waxy barley individually and in blends with semolina; 3) to evaluate impact of processing (extrusion, drying and cooking) on the concentration and physicochemical characteristics (molecular weight and solubility) of β-glucan, as well as viscosity of the developed pasta products; 4) to look into antioxidant properties and phenolic acid composition of the developed pasta products.

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CHAPTER II

Effect of pasta processing on beta-glucan physicochemical properties of barley and semolina pastas

Abstract

Nutritional studies into beta-glucan have shown a link between its regular consumption and a number of health benefits, including a decrease in the risk of chronic heart disease by lowering blood cholesterol and an increased insulin response in diabetics. Functional and nutritional properties of β-glucan are correlated with their concentration, molecular weight, and structure. Various food processing techniques can have a negative or positive impact on the level and molecular weight of β-glucan in the end product. Viewing the importance of β-glucan, waxy barley flour rich in β-glucan (10%, d.b) was used to produce barley functional spaghetti, which was compared to semolina spaghetti. The impact of processing (extrusion, drying and cooking) on physicochemical properties of barley blends, and pastas as well as molecular characterization of β-glucan, were investigated. The barley spaghetti reached the Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) requirements of β-glucan content (0.75-1.0g/serving), which could allow these pastas to warrant the health claims "may reduce the risk of heart disease" and "contributes to the maintenance of normal blood cholesterol levels", respectively. Pasta processing did not significantly affect the amount of β-glucan, but it impacted the β-glucan physicochemical properties in the end products. In all pastas, extrusion and drying were detrimental to the β-glucan properties, while cooking significantly increased the extractability and molecular weight of β-glucan, and in turn the viscosity, which is associated with physiological effectiveness. Cooked pastas containing 30% of barley flour (w/w) showed the lowest value of β-glucan content, but the highest value of viscosity between barley pastas, indicating that viscosity cannot be explained by β-glucan content only. In general, replacing wheat semo-

lina with barley flour rich in β-glucan (even if moderate amounts), it will be able to produce barley pastas which have enhanced β-glucan properties and related health benefits.

2.1 Introduction

Whole cereal grains, including barley, are an excellent source of bioactive substances (phytochemicals) such as healthy dietary fiber, particularly β-glucan. Consequently, they are gaining renewed interest as an ingredient for the production of functional foods, such as pasta, bakery products, flakes, snacks, etc., which are common components of human diet.

The mixed linkage (1-3, 1-4)-β-D-glucan, commonly referred to as β-glucan, is classified as a soluble dietary fiber (AACC, 2001) and is a major component of the cell walls of barley and oat endosperm. The β-glucan content of cereals ranges mostly from about 5-11% in barley and 3-7% in oats (Skendi et al., 2003), to 2% in rye (Ragaee et al., 2001) and <0.5% in wheat grains (Beresford and Stone, 1983). Although consumption of rye has beneficial effects, barley and oats are the primary sources of β-glucan in the human diet, but their levels can vary dramatically between varieties.

β-glucan has been found to be effective in attenuating postprandial blood glucose and insulin, and in lowering blood lipids, especially serum total and LDL-cholesterol (Wood, 2002; Cavallero et al., 2002; Yokoyama et al., 1998). The documented relationship between consumption of foods rich in soluble fiber, especially β-glucan, and reducing the risk of heart disease led to the first health claim for a specific food by the Food and Drug Administration (FDA, 1997). In particular, the physiological role of β-glucan in reducing glycemic responses has been mostly associated with its capacity to increase viscosity in solution at low concentration in the upper digestive tract, a property of soluble high molecular weight polysaccharides (Wood et al., 1994; Dikeman and Fahey, 2006). The viscosity of β-glucan depends on its concentration and molecular weight, which in turn are dependent on its extractability and solubility (Tosh et al., 2008). Molecular weight and solubility of β-glucan

are affected by genotype, environment, agronomic input, and the interactions of these factors and food processing methods (Tiwari and Cummins, 2009). Available literature (Buckeridge et al., 2004; Tiwari and Cummins, 2009) reveals that the level of β-glucan in a finished product (e.g. bread, cake, muffins) depends upon several factors in the production chain, whereas food processing operations are mainly affecting molecular weight and solubility of β-glucan (Wood et al., 1989; Izydorczyk et al., 2000; Regand et al., 2009).

There has been much speculation and insufficient investigation over the years concerning the effects of processing on the physicochemical characteristics of β-glucan incorporated into food products. This speculation has frequently supposed that processing would be detrimental to bioactivity. Thus, processing may affect the molecular (chemical structure and degree of polymerisation), structural (molecular interactions) and functional properties (viscosity, water binding capacity and solubility) which, in turn, could affect the sensory, physiological and ultimately the health benefits of β-glucan. Changes in the properties of β-glucan may arise from shearing damage due to mechanical processing (Wood et al., 1989), or by excessive heat treatment of food products.

The current study aimed at developing barley pasta rich in β-glucan able to satisfy the FDA (0.75 g of β-glucan per serving, 2006) and EFSA (≥ 1g of β-glucan per quantified portion, 2011) health claim requirements. Pastas were made from waxy barley as a rich source of soluble dietary fiber, individually and in blends with semolina. A preliminary study of the pasting properties of barley and semolina flour blends was carried out in order to understand the contribution of β-glucan to viscosity of raw materials and end products. The health benefits are controlled by the solubility and molecular weight of β-glucan, that in turn affect the final viscosity of the product in the gut (Wood et al. 1994). Thus, the impact

of processing (extrusion, drying and cooking) on the concentration and physicochemical properties of the flours and blends, and molecular characterization of β-glucan, as well as viscosity of the developed pasta products was evaluated.

2.2 Materials and Methods

2.2.1 Materials

The two-rowed, hulless, waxy barley cultivar (CDC Fibar) was selected based on its high β-glucan content (Gray et al., 2009) and obtained from the University of Saskatchewan (Saskatoon, Saskatchewan, Canada). The 6 six-rowed, hulled, normal barley (Celebrity) was provided by a Canadian supplier.

The samples for investigation were provided in 25 kg and a 6 kg representative sample of each cultivar was obtained from the original seed bags. Commercial durum wheat semolina, xanthan gum (El Peto Products), annato food colour (Calico), sea salt (Life stream) were bought from a local store in Guelph (ON, Canada).

CDC Fibar grain was ground using a cyclone Sample Mill (UDY Corp., Fort Collins, CO,USA) equipped with a 0.5 mm screen. The barley flour and semolina were refrigerated until pasta preparation.

Microbial α-amylase thermostable (for TDF and Starch Assay; 100ml; 3.000 U/ml; 45 U/mg) and pancreatin from porcine pancreas was purchased (P7545, activity equiv. 8× USP) from Megazyme International (Ireland) and Sigma-Aldrich (Canada), respectively.

2.2.2 Pasta preparation

Barley flour (CDC Fibar) was blended with different amounts of semolina (100, 50, 30 g barley flour/100g of semolina) subsequently referred to as 100% barley flour, 30% barley flour, 50% barley flour. Durum wheat semolina pasta (100% semolina) was made as a control. Salt, xanthan gum and annatto solution were added to flours to improve flavor, texture

and color of pastas (Aldughpassi et al., 2012). The recipes for making pastas were chosen based on the aforementioned study, to meet the FDA and EFSA health claim requirements. Moisture content of pastas was adjusted during processing on the basis of water absorption of barley blends. The formulations of pastas are described in Table 2.1. The dry ingredients were mixed in a pasta maker (PastaMatic MX700, SIMAC VETRELLA) for 3 minutes. After that, water was added and all ingredients were mixed for 6 minutes, then the dough was extruded. The optimum cooking time of pastas (the time necessary to obtain complete gelatinization of starch shown by the disappearance of the white central core of the spaghetti strand) was determined according the Approved Method 66-50 (AACC, 2005).

Table 2.1 Pasta formulations

	Semolina (g)	**Barley flour (g)**	**Salt (g)**	**Xanthan gum (g)**	**Annato solution 2.8%(v/v) (uL)**	**Water (mL)**
100% barley flour		100.0	1.0	1.0	85.0	75.0
50% barley flour	50.0	50.0	1.0	0.5	42.5	59.5
30% barley flour	70.0	30.0	1.0	0.3	25.5	53.3
100% semolina	100.0	-	1.0	-	-	42.0

After the extrusion, half of each batch was freeze-dried (e.g. fresh pastas), half was dried at 80°C in an air oven (Baking Center Duke, Model E101-EV) for 4 hours (e.g. dried pastas) (fig. 2.1). Part of fresh and dried pastas was cooked (e.g. fresh cooked, and dried cooked pastas). Cooked pasta were freeze-dried for chemical assays (Virtis Genesis 25 EL Laboratory - Pilot Freeze Dryer). All samples were ground using cyclone Sample Mill equipped with a 0.5 mm screen and refrigerated for future analysis.

Figure 2.1 Developed pasta products

2.2.3 Chemical analyses

Moisture content of raw ingredients and pastas was determined according the standard methods as outlined in the AACCI Method 44-15 (AACCI 2010). Total β-glucan content of samples was determined using the AACCI Method 32-23 (AACCI 2010). For this method, β-glucan is hydrolyzed to D-glucose with lichenase and β-glucosidase.

2.2.4 Physicochemical properties of raw materials and pastas

2.2.4.1 Pasting properties of flours

Pasting properties of barley flour, semolina and their blends were measured on a Rapid Visco Analyser (RVA-4) (AACC, 2005), using the RVA General Pasting Method (Newport Scientific Pty. Ltd., Warriewood, Australia). A sample of 3.5 g of flour (14% moisture basis) was transferred into a canister and approximately 25 ± 0.1 ml distilled water was added (corrected based on 14% moisture basis). The slurry was heated to 50°C and stirred at 160 ppm for 10 s for thorough dispersion. The slurry was held at 50°C for up to 1 min, and then heated to 95°C over 7.3 min and held at 95°C for 5 min, and finally cooled to 50°C over 7.7 min. The pasting temperature (the temperature where viscosity first increases by at least 25 cP over a 20 s period), peak time (the time at which peak viscosity occurred), peak viscosity (the maximum hot paste viscosity, PV), holding strength or trough viscosity (the trough at the minimum hot paste viscosity, TV), final viscosity (the viscosity at the end of test after cooling to 50°C and holding at this temperature, FV), breakdown (peak viscosity-holding strength or trough viscosity, BD) and setback (final viscosity-holding strength, SB) were calculated from the pasting curve, using Thermocline version 2.2 software Newport Scientific Pty. Ltd. (Warriewood, Australia). All RVA experiments were run in duplicate and the coefficient of variation of viscosity properties was less than 10% at any value.

2.2.4.2 RVA Method for Viscosity Measurement of β-glucan in pasta

To produce slurries, an amount of milled sample containing 1.0% β-glucan amount was weighed into an RVA canister. A volume of 20 mM sodium phosphate buffer (pH 6.9) containing 10 mM NaCl, equal to 25 mL minus the moisture present in the sample, was added

to the RVA canister. All the digestive enzymes were added to the canister at the beginning of the run in the following amounts: 100 μL of thermostable microbial amylase and 600 μL of pancreatin (0.5 mg/mL in sodium phosphate buffer, pH 6.9), as used in the in vitro digestion protocol (Gamel et al., 2012). The RVA (RVA-4, Newport Scientific, Warriewood, Australia) equipped with Thermocline software version 2.2 for Windows was held constant at 37°C, and mixing speed was set at 480 rpm for 10 sec followed by 2 hr at 160 rpm. Viscosity was recorded every 8 sec, and the final viscosity was noted at the end of 2 hr, when a plateau of the final segment of viscosity curve was achieved. Viscosity of samples was measured at 37°C for 2 h to mimic human body temperature.

2.2.4.3 Viscosity Measurement with a Controlled-Strain Rheometer

The viscosity of pasta sample extracts (after centrifugation at 8,000 × g for 10 min) was measured with a controlled-strain rheometer (ARES, TA Instruments, New Castle, DE). Up and down shear ramps were conducted over a shear rate range of 0.1–400 sec–1 at 37°C with a cone-and-plate geometry (angle of 0.04 radians, diameter of 50 mm). Apparent viscosity was recorded at the shear rate of 30 sec^{-1} on the down cycle, primarily for consistency with earlier publications.

Gamel et al. (2012) reported highly significant Pearson correlation between the RVA method and a specific in vitro extraction protocol for barley, especially at a 1% β-glucan level.

2.2.4.4 Characterization of β-Glucan Solubilized at 37°C

The peak molecular weight (M_p) of β-glucan was determined using high-performance size-exclusion chromatography (HPSEC) with postcolumn calcofluor addition as described by

Wood et al. (1991) but using two columns (300 x 7.5 mm) in series of Shodex OHpak KB806 M (J. M. Science Inc., Grand Island, NY) and Waters Ultrahydrogel (Waters, Milford, MA). The columns were maintained at 40°C and eluted with 0.1M tris buffer at 1mL/min using a Shimadzu 10ATVP pump. A Perkin- Elmer ISS 100 autosampler and injector were used with an injection volume of 100 µL. Postcolumn, the eluant was mixed 1:1 with 20 mg/L of calcofluor in 0.1 M tris buffer (pH 8.0) using a Waters model 590 Pump. The fluorescence was measured using an RF-10AXL fluorescence detector (excitation, 360 nm; emission, 540 nm). β-Glucan molecular weight (MW) of the standards was determined essentially as described also by Wang et al. (2003).

To determine the solubility of the β-glucan at 37°C, the concentration in the extract (c_e) was determined using flow injection analysis (FIALab Instruments, Bellevue, WA) essentially as described by Jørgensen (1988). A standard curve was prepared by solubilizing pure β-glucan (Megazyme International) in water (1 mg/mL) at 90°C for 2 h and diluting to concentrations from10 to 100 µg/mL. The extracts were diluted with water to give β-glucan concentrations in the same range. The standards and extracts were mixed 1:1 with 50 mg/L Calcofluor solution (in 0.1M Tris-HCl, pH 8.0) in the FIA, and the fluorescence intensity was measured (excitation, 360 nm; emission, 450 nm). The percent extractable (solubilized) β-glucan was calculated from the two values (% soluble β-glucan = soluble β-glucan/total β-glucan x 100). Triplicate measurements were made on each of the supernatants.

2.2.5 Statistical analysis

All analyses were carried out in duplicate or triplicate, and the data were reported as means ± standard deviation (SD). The data were subjected to least significant difference (LSD) tests and principal component analysis (PCA) with PASW 18 (SPSS, Chicago, IL) to identify relationships between barley pastas and processing. Differences were considered to be significant when $P < 0.05$.

2.3 Results and discussion

2.3.1 Pasting properties of raw ingredients

The contribution of β-glucan to pasting was demonstrated by the significant decrease of apparent viscosity after enzymatic degradation of β-glucan with lichenase (Wood, 2007). Based on these remarks, a preliminary study of the pasting properties as measured by a Rapid Visco Analyzer (RVA) was performed to investigate the flours individually and in blends with potential higher health benefits. Moreover, the contribution of xanthan gum and gluten to pasting properties was evaluated.

Table 2.2 and fig. 2.2 show the RVA pasting properties of normal and waxy barley flours, and semolina individually and in blends, with and without addition of xanthan gum and gluten. The shape of the pasting curve differed depending on type of cereal flours and formulations. In particular, the cereal samples tested showed different behaviour during heating, holding and cooling cycles in excess of water. Semolina, waxy and normal barley flour had only one peak viscosity (PV) at different pasting temperatures and peak times, while waxy barley flour in blend with semolina had double peak viscosities. The PV provides an indication of the viscous load. Waxy and normal barley had higher peak viscosities (3579 cP and 3585 cP, respectively) compared to that of semolina (1493cP). This might be due to the lower quantity of free water during the heating, partially induced by β-glucan. The important effect of β-glucan component on the pasting properties of barley has been reported (Glennie-Holmes, 1995), because it would greatly compete for water during pasting. Besides β-glucan, other molecules (such as starch, proteins, lipids, and arabinoxylans) and their interactions are responsible for changes of peak viscosity. The amylose content appears to play a critical role in determining pasting properties using RVA (Gupta et al.,

2009). At lower amylose content, the structure of starch gel is easily disrupted by heating. Amylose suppresses swelling and maintains the integrity of swollen starch granules (Hermansson and Svergmark, 1996). Since starch swelling is mainly a property of amylopectin (Tester and Morrison, 1990), waxy starch swells rapidly and swollen granules degrade at lower temperature, indicating that waxy starch rapidly develops viscosity. This could explain the lower pasting temperature and shorter peak time of whole waxy barley (55°C and 5.7 min) than normal barley (76°C and 9.4 min) and semolina (85°C and 8.8 min), which had a higher amylose content. When 1% of xanthan gum was added to waxy barley flour, the highest PV was obtained (more than 5% of gluten was added).

The double-peak viscosities of all flour blends showed up at pasting time and temperature of amylopectin (first peak) and amylose (second peak). Our results were consistent with those of literature (Gupta et al., 2009; Hatcher et al., 2005). The association between amylose and amylopectin molecules in mixed flour was different from that of individual starches, which induced specific chain interactions (between molecules, starch granules, swollen granules, and granules fragments) during heating, and each starch gelatinized independently of the other, thus providing double-peak viscosities (Gupta et al., 2009).

During the holding period of the viscosity test, the material slurries are subjected to high temperature and mechanical share stress with further disrupt in starch granules in the grains, resulting in amylose leaching out and alignment (Ragaee and Abdel-Aal, 2006). This period is commonly associated with a breakdown (BD) in viscosity. High values of breakdown are associated with high peak viscosities, which in turn, are related to the degree of swelling the starch granules during heating. More starch granules with a high swelling capacity result in a higher peak viscosity, such as in the waxy barley flour, which had a

higher peak viscosity and breakdown than normal barley and semolina. The BD is related to the ability of starches to withstand heating at high temperatures.

During the cooling, re-association between starch molecules, especially amylose, will result in the formation of a gel structure and, therefore, viscosity will increase to a final viscosity (FV). This phase is commonly described as the setback region and is related to retrogradation and reordering of starch molecules. The low setback values indicate low rate of starch retrogradation and syneresis. For semolina, waxy and non-waxy barley flours final viscosity was 2460 cP, 2114 cP, and 3852 cP, while setback values were 1397 cP, 1028 cP, and 2271 cP, respectively. This suggests that starch with lower amylose content (waxy barley) decreased the amount of leached out amylose, suppressing viscosity during cooling. The PV, BD and FV values of semolina control were similar to those found by Güler et al. (2002), showing the consistence of our results.

The results of RVA pasting properties indicated that the addition of xanthan gum (1%) to waxy barley flour increases the peak viscosity and, thus, the potential human health benefits. Additionally, pasting properties could help to determine the replacement level of semolina with waxy barley flour in order to obtain the desired pasting properties of end products.

Table 2.2 Average RVA pasting properties of semolina, waxy and non-waxy whole barley flours, and their formulations with and without xanthan gum (1%) and gluten (5%)

Samples	Pasting temperature (°C)	Peak visc. 1 (cP)	Peak 1 time (min)	Peak visc. 2 (cP)	Peak 2 time (min)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final Visc. (cP)	Setback viscosity (cP)
Semolina	85			1493	8.8	1063	430	2461	1398
Celebrity barley (non-waxy)	76	-	-	3585	9.4	1581	2004	3853	2272
CDC Fibar barley (waxy)	55	3580	5.7	-	-	1086	2494	2114	1028
Celebrity+Semolina (50/50)	83	-	-	2520	9.3	1303	1218	3091	1789
CDC Fibar+Semolina (50/50)	63	1296	5.4	1627	8.9	1112	515	1849	737
CDC Fibar+Semolina (30/70)	66	550	5.4	1492	8.9	770	722	1727	957
CDC Fibar+Semolina (20/80)	68	306	5.4	1499	9.1	891	608	2007	1117
CDC Fibar+Semolina (10/90)	85	172	5.3	1560	9.1	1002	558	2264	1267
CDC Fibar + gluten (5%)	58	3314	5.7	-	-	1014	2300	1937	923
CDC Fibar + Xanthan gum (1%)	50	3932	5.7	-	-	1136	2796	2252	1116

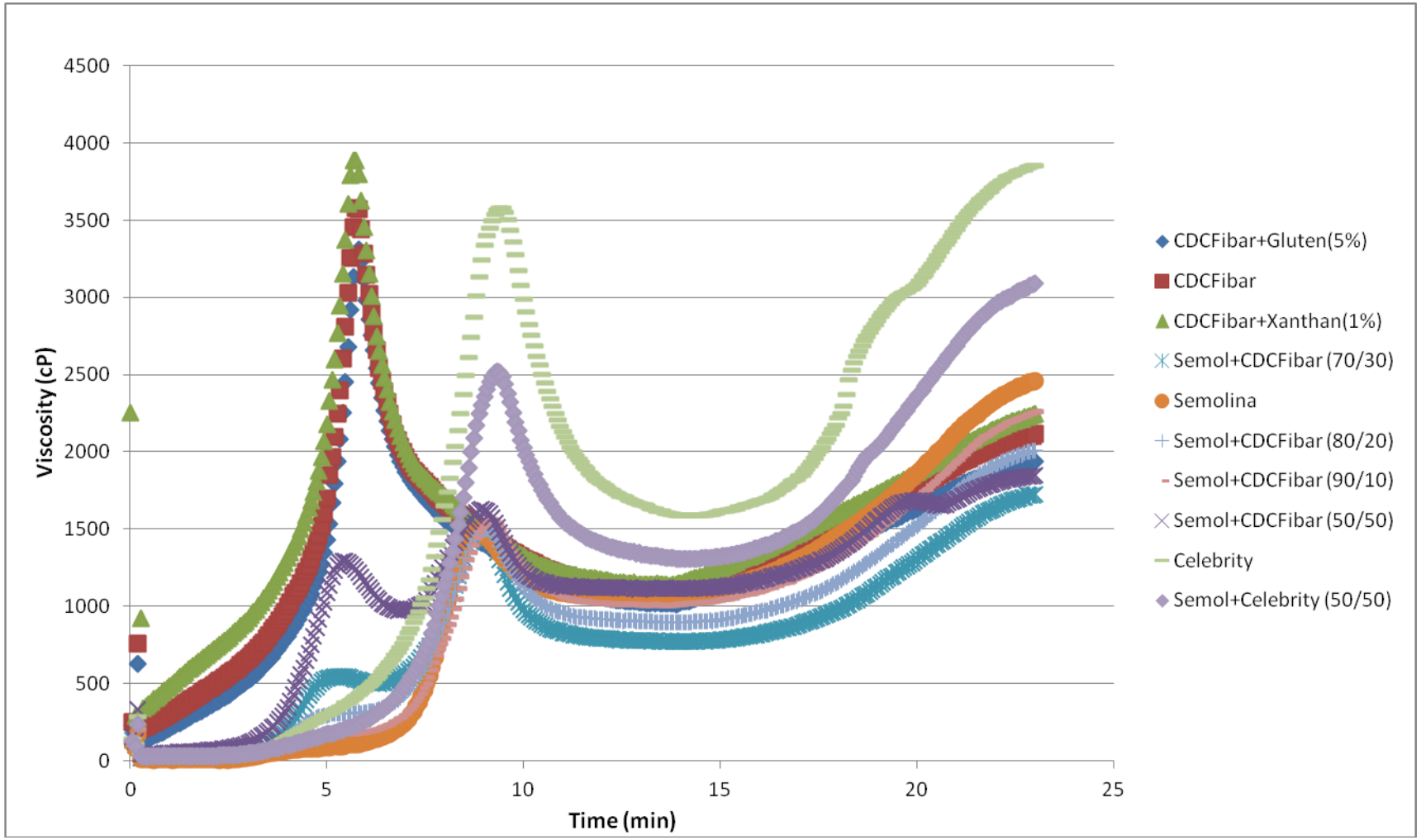


Figure 2.2 RVA pasting curves of semolina, waxy and non-waxy whole barley flours, and their formulations with and without xanthan gum (1%) and Gluten (5%)

2.3.2 Physicochemical properties of β-glucan in raw materials and pastas

The concentrations of β-glucan in the raw materials (barley flour and semolina, and their blends) and corresponding pastas are givens in Table 2.3. Barley flour individually accounted for much higher β-glucan content (9.9% d.b.) than both barley flour/semolina blends and semolina, being in good accordance with data of literature (Izydorczyk and Dexter, 2008; Beresford and Stone, 1983). It has been reported that hulless barley contains much higher β-glucan than either wheat or hulled barley (Bhatty, 1999). Moreover, waxy hulless barley has been described to contain approximately 7 or 8% β-glucan, whereas regular hulless barley comprises significantly less (4.6%) (Gao et al., 2009) which are in good accordance with data obtained for barley flours.

Analysis of variance (ANOVA), used to determine effects of pasta processing (mixing, extrusion and drying) and cooking on total β-glucan content in pasta samples, showed that β-glucan concentrations did not change during processing ($P<0.05$) (Table 2.3), in accordance with Marconi et al. (2000). Although high total β-glucan is indicative of high dietary fiber content in barley, it is important to recognize the soluble component of β-glucan that is responsible for the beneficial ability of β-glucan to lower serum cholesterol and blood glucose levels (Wood et al., 1994; Yokoyama et al., 1997). In fact, the physiological functions of cereal β-glucan are associated with viscosity, which in turn corresponds to the amount and molecular weight of β-glucan solubilized in the intestine (Wood et al., 2000; Tosh et al., 2010). Based on this remark, β-glucan viscosity was measured by RVA (pasta slurry viscosity) and controlled-strain rheometer (extract viscosity), of samples digested into RVA canister (Table 2.3). Solubility and molecular weight of β-glucan extracted from raw materials and pastas are also presented in Table 2.3.

Barley flour individually had the highest value of β-glucan viscosity as measured by RVA and rheometer, and peak molecular weight (M_p). Conversely, semolina showed the lowest values of all physicochemical properties investigated. The obtained value of β-glucan solubility in barley flour alone was similar to those found by Izydorczyk et al. (2000) in whole flour of hulless waxy barley cultivars.

Extrusion and drying processing significantly influenced viscosity, solubility and M_p of β-glucan, which were lower in all uncooked pastas than the original flours (Table 2.3 and fig. 2.3). Reduction in M_p and solubility of β-glucan could be attributed to the extrusion processing and the presence of β-glucanase in the wheat flour used to make pasta.

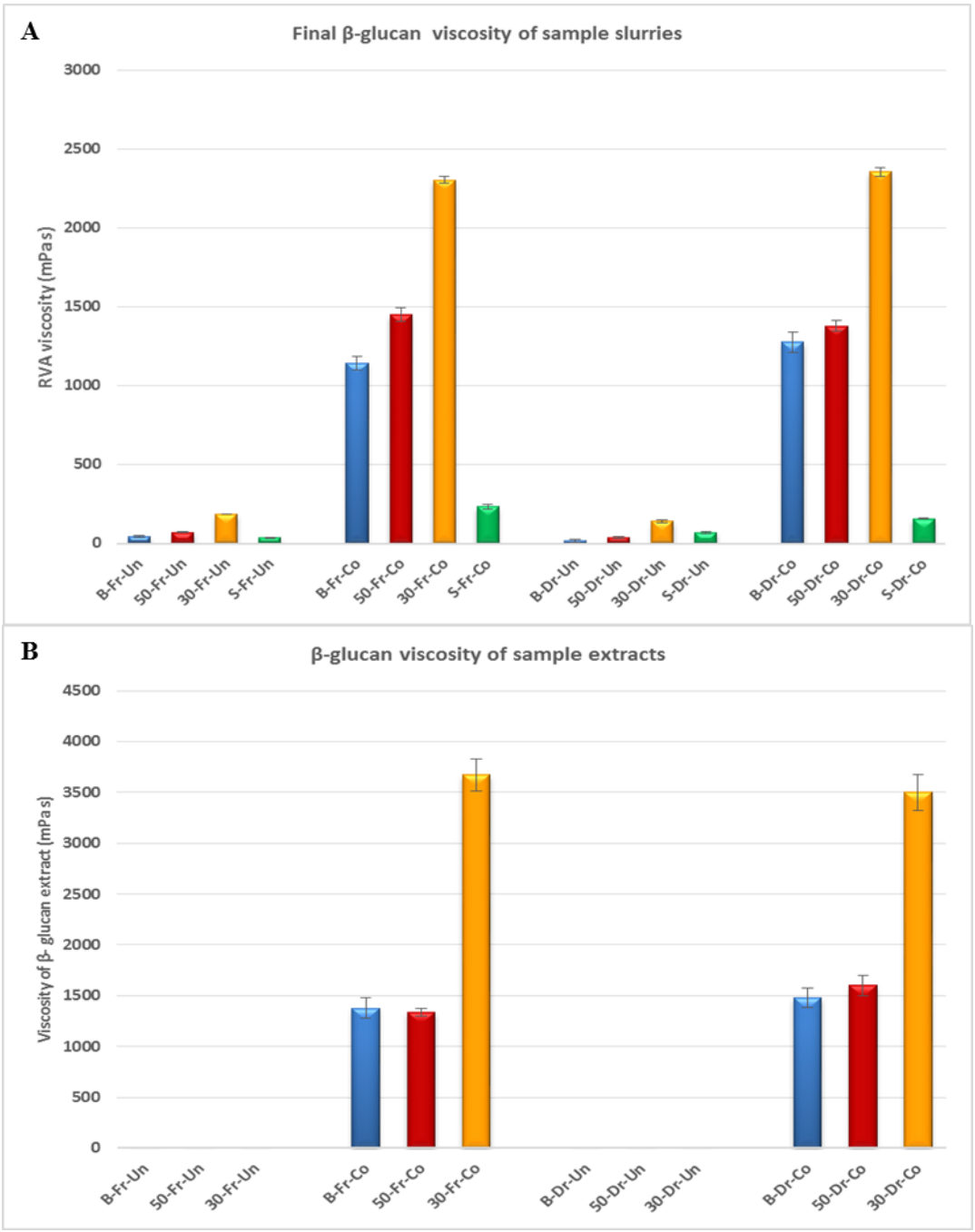
Table 2.3 β-glucan physicochemical properties in raw materials and pastas

	Total β-glucan content (%,db)	Soluble β-glucan/ total β-glucan (%, db)	$M_W \times 10^3$ (g/mol)	Viscosity by RVA (cP)	Viscosity by Rheometer (mPa/s at 30 s)
100% barley flour					
Raw materials	9.87 (0.08) a	57.73 (2.68) *a*	504 (13) *a*	294 (32) *a*	223 (63) *a*
Fresh-Uncooked	9.96 (0.04) *a*	18.71 (1.14) *b*	313 (11) *b*	43 (5) *b*	4 (<0.1) *b*
Fresh-Cooked	9.86 (0.06) *a*	83.09 (1.50) *c*	1074 (0.7) *c*	1144 (43) *c*	1377 (101) *c*
Dried-Uncooked	9.72 (0.21) *a*	9.60 (0.13) *d*	142 (3) *d*	17 (9) *b*	3 (<0.1) *b*
Dried-Cooked	9,74 (0.06) *a*	67.31 (2.97) *e*	1163 (113) *c*	1277 (65) *d*	1480 (92) *d*
50% barley flour					
Raw materials	5.21 (0.01) *a*	60.19 (4.52) *a*	159 (14) *a*	152 (17) *a*	172 (3) *a*
Fresh-Uncooked	5.19 (0.11) *a*	11.12 (0.29) *b*	102 (5) *a*	71 (1) *b*	4 (<0.1) *a*
Fresh-Cooked	5.10 (0.06) *a*	73.08 (3.53) *c*	1013 (44) *b*	1450 (41) *c*	1337 (35) *b*
Dried-Uncooked	5.00 (0.24) *a*	11.69 (0.22) *b*	136 (11) *a*	36 (7) *b*	3 (<0.1) *a*
Dried-Cooked	5.18 (0.17) *a*	55.42 (2.53) *d*	865 (24) *c*	1379 (32) *d*	1599 (103) *c*
30% barley flour					
Raw materials	3.34 (0.05) *a*	64.22 (2.42) *a*	89 (7) *a*	159 (2) *a,b*	12 (11) *a*
Fresh-Uncooked	3.04 (0.11) *b,c*	25.78 (0.31) *b*	64 (8) *a*	184 (1) *a*	6 (0.1) *a*
Fresh-Cooked	3.13 (0.03) *b,d*	62.74 (1.14) *a*	1158 (34) *b*	2303 (23) *c*	3674 (157) *b*
Dried-Uncooked	2.93 (0.06) *c*	17.32 (0.62) *c*	50 (4) *a*	140 (10) *b*	4 (<0.1) *a*
Dried-Cooked	3.22 (0.002) *a,d*	51.71 (4.45) *d*	1220 (9) *b*	2355 (27) *d*	3499 (174) *c*
100% semolina					
Raw materials	0.31 (0.01) *a*	6.01 (0.08) *a*	30 (0.3) *a*	36 (10) *a*	2 (<0.1) *a, b*
Fresh-Uncooked	0.35 (0.06) *b,c*	4.25 (0.12) *b*	31 (3) *a*	35 (2) *a*	2 (<0.1) *a*
Fresh-Cooked	0.30 (0.01) *b,d*	6.93 (0.13) *a*	109 (2) *b*	232 (14) *b*	4 (<0.1) *c*
Dried-Uncooked	0.29 (0.01) *c*	5.31 (0.10) *b*	42 (1) *c*	70 (5) *c*	2 (<0.1) *b*
Dried-Cooked	0.28 (0.01) *a,d*	7.21 (0.43) *c*	92 (4) *d*	156 (2) *d*	3 (<0.2) *c*

Values are means ± SD. Values in a formula with different letters are significantly different ($P < 0.05$, LDS test)

It seems that prolonged treatment at ambient temperature, such as during the kneading and extrusion processes in pasta at which enzymes remain active, may result in extensive degradation. During processing, the action of enzyme causes depolymerisation and release polysaccharides from the cell wall structure, thereby increasing their solubility (Regand et al., 2009), similar to processes in germination or malting. Subsequently,

however, the increased mobility might lead to self-association and formation of insoluble β-glucan aggregates, lowering solubility values (Tosh et al., 2008).



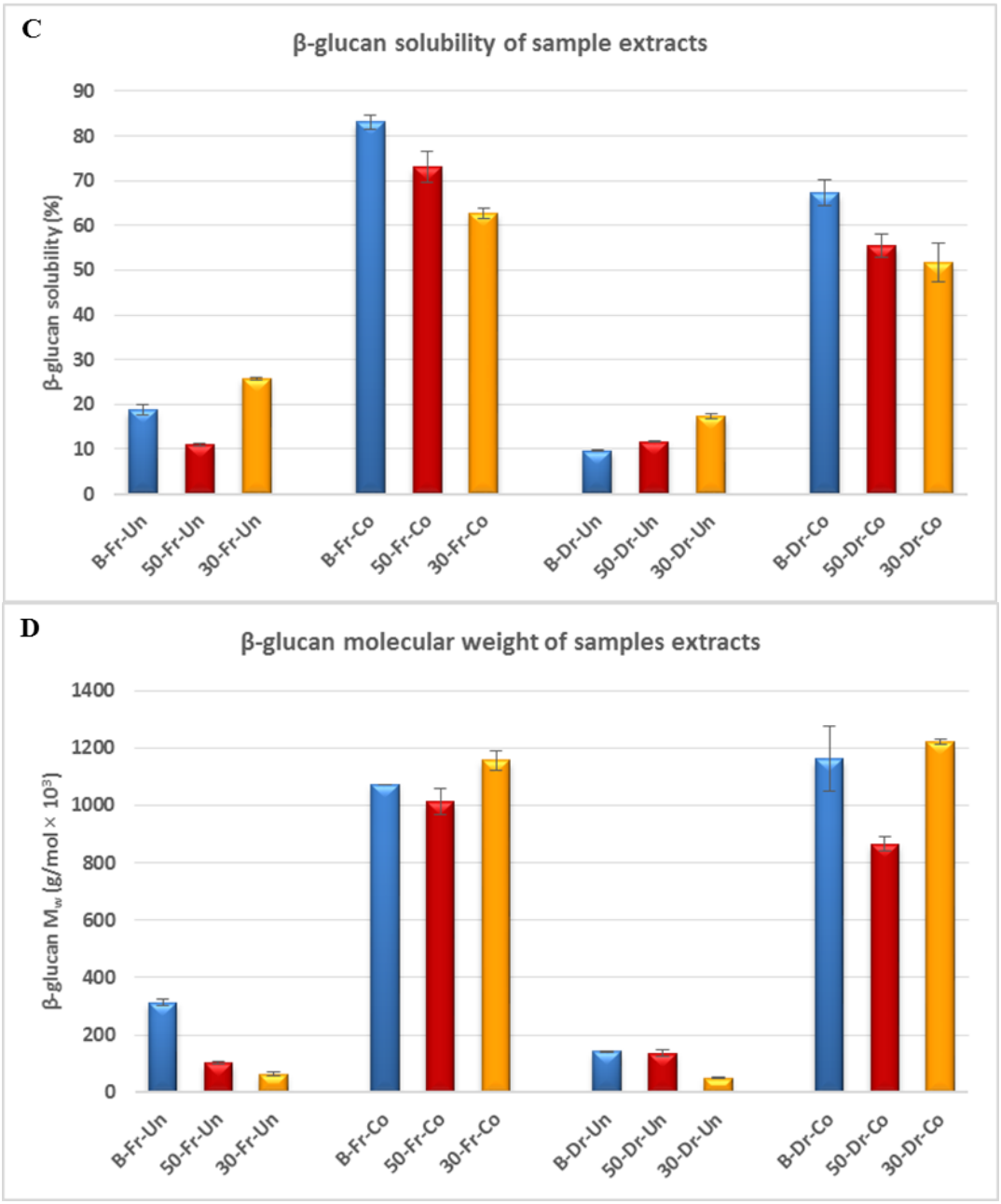


Figure 2.3 β-glucan viscosity as measured by RVA (A) and controlled-strain rheometer (B), solubility (C) and molecular weight (D) of fresh and dried, uncooked and cooked, pastas made from 100 barley flour (blue colour), 50/50 barley flour/semolina blend (red colour), 30/70 barley flour/semolina blend (orange colour), and 100 semolina (green colour)

The decrease of the amount of soluble β-glucan after drying process was in accord to what reported by Johansson et al. (2007). Moreover, Beer et al. (1997) reported that during drying process water is repelled from the polysaccharide structure, decreasing

the amount of soluble β-glucan. It possibly reflected changes in molecular weight and crystallinity.

On the other hand, at least where the cooking is concerned, processing significantly increased the extractability and M_p of β-glucan, and in turn the viscosity, which is associated with physiological effectiveness (Table 2.3 and fig. 2.3). Thus, cooked barley-containing pastas could be expected to increase the viscosity in the upper gut and reduce serum cholesterol levels to a greater extent as compared to semolina pasta.

The increase in extractability could be attributed to the dismantling of β-glucan from the other cellular components, as the β-glucan is found in the cell wall of grains with cellulose and other noncellulosic polysaccharides and heating releases it from the matrix.

Similarly, assessing the susceptibility of β-glucan in barley to disruption, and their release as soluble β-glucan during cooking, Robertson et al. (1997) found that the cooking increased the extractability of polysaccharides from barley and the susceptibility of β-glucan to solubilisation by proteolysis because of the close association between β-glucan and protein in the cell wall. The denaturation of protein during cooking increased the availability of protein to proteases. Johansson et al. (2007) also reported a significant increase in β-glucan solubility upon cooking, probably due to enzyme activity in wheat flour towards the β-glucan polysaccharide.

The high M_p values of β-glucan in cooked samples suggested the disruption of the covalent and non-covalent bonds between β-glucan and other cell wall components, releasing and solubilizing the initially insoluble β-glucan (Izydorczyk et al., 2000).

Since the viscosity is a function of molecular weight and concentration of β-glucan, the highest viscosity of cooked pastas could be explained by the highest values of solubility and molecular weight of these samples. Above all, it is interesting to note that cooked pastas made from 30% barley flour had very high values of viscosity, while they had the

lowest β-glucan content. As Izydorczyk et al. (2000) found, viscosity development profiles cannot be explained by β-glucan content only, but extractability and molecular weight had a significant effect on viscosity.

Conversely, semolina pastas had very low viscosity because of the low solubility (from 4.3% to 7.2%) and low molecular weight (from 16,000 to 60,000 g/mol) of β-glucan. Regand et al. (2009) found similar results of solubility (6.7%) and molecular weight (33,000 g/mol) in dried wheat pasta.

2.3.3 Relationship between physicochemical properties of β-glucan in pasta samples

Principal component analysis (PCA) was applied to pooled data of barley-containing pastas in order to detect the most causes of variability. The PCA of pasta samples results in two principal components that account for 99% of the total variability. In the figure 2.4, the first principal component explains 78% of the total variability, and could be considered as representative of type of processing because cooked and uncooked pastas have been placed on the positive and negative side of the horizontal axis, respectively. Viscosities as measured by RVA and controlled-strain rheometer, solubility and molecular weight are placed close together on the positive side of the horizontal axis, indicating that they are positively correlated with each other.

The second principal component explains 21% of the total variability and could be considered as representative of the type of formulation. In fact, pastas made from barley flour alone are clustered on the positive side of PC2, pastas made from 50/50 barley flour/semolina blend are placed in the middle and pastas made from 30/70 barley flour/semolina blend are located on the negative side of PC2. Moreover, total β-glucan content is placed on the positive side of PC2, indicating a positive correlation between type of formulation and total β-glucan content.

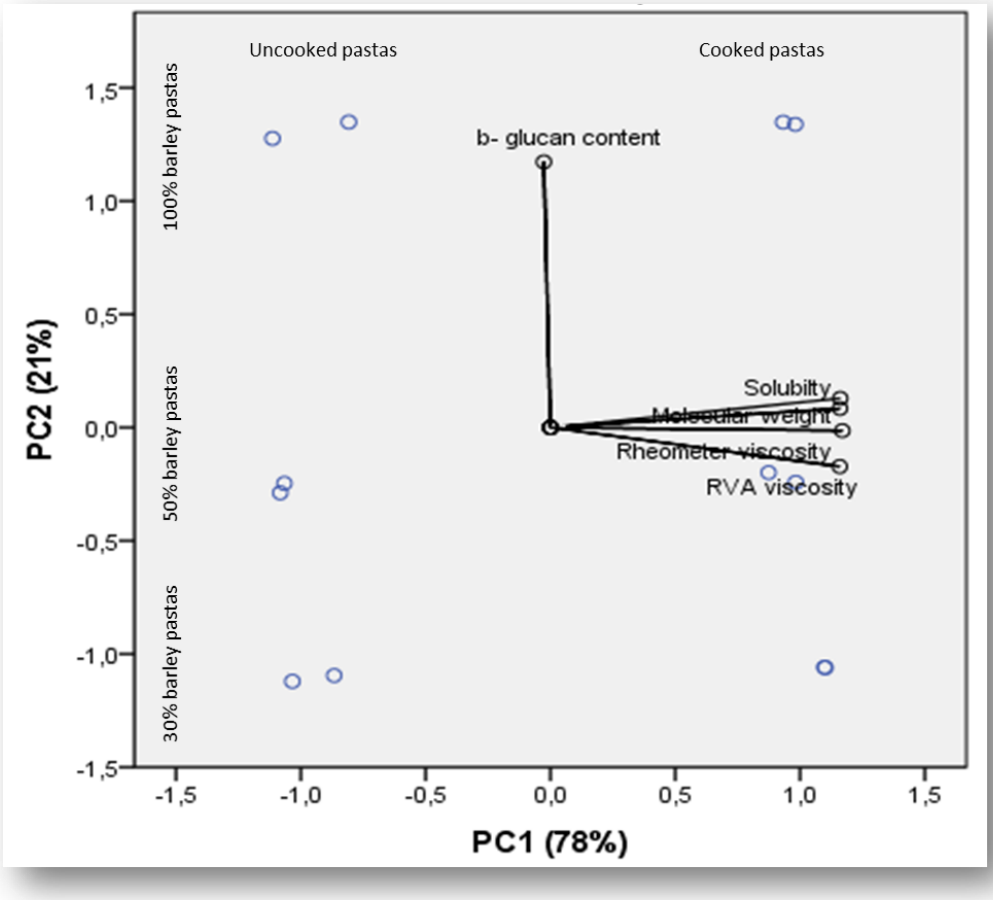


Figure 2.4 Principal component analysis. Loading plot of barley-containing pastas as function of the first two principal components

2.4 Conclusions

The barley spaghetti showed an increase of β-glucan in the range of 2.8-9.9%, proportionally at the barley flour replacement. This content meets the FDA and EFSA requirements, which could allow these pastas to deserve the health claims "may reduce the risk of heart disease" and "contributes to the maintenance of normal blood cholesterol levels", respectively.

Processing (extrusion, drying and cooking) differently impacted β-glucan physicochemical properties of the flours and blends, as well as the developed pasta products. The total β-glucan content did not vary, while viscosity, solubility and molecular weight were affected by processing,

Indeed, extrusion and drying affect β-glucan physicochemical properties in all formula. Conversely, cooking significantly increased the extractability and M_p of β-glucan, and in turn the viscosity, which is associated with physiological effectiveness. Cooked pastas containing only 30% of barley flour showed the lowest value of β-glucan content, but the highest value of viscosity between barley pastas, indicating that viscosity cannot be explained by β-glucan content only.

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CHAPTER III

Effect of pasta processing on phenolic acid composition and radical scavenging capacity of barley and semolina pastas

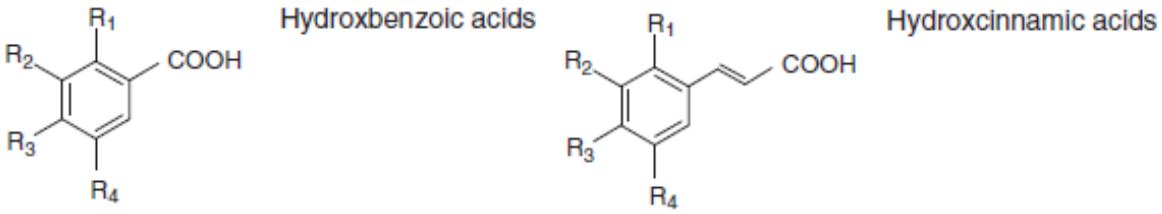
Abstract

Whole grains, including barley, are rich in a wide range of compounds with known health benefits. Barley grain is an excellent source of healthy dietary fiber, particularly β-glucan, and other bioactive constituents such as tocols, B complex vitamins, minerals, and phenolic compounds, and consequently, it is gaining renewed interest as an ingredient for the production of functional foods. Phenolic acids are the main phenolic compounds in barley that could contribute to health benefits of barley foods. In the current study waxy barley flour rich in β-glucan was used to produce 3 barley spaghetti functional products to study impact of pasta-making and drying process on phenolic acid composition and scavenging capacity of free radicals. The content of individual phenolic acids, total phenols and radical scavenging capacity in raw ingredients, fresh and dried spaghetti, and in uncooked and cooked spaghetti were evaluated and compared with semolina spaghetti. The addition of barley flour into pasta at incorporation levels of 30, 50 and 100% effectively increased phenolic acid and total phenols. Pasta processing did not significantly affect the total phenols content and free radical scavenging capacity, but a significant reduction in total phenolic acids content measured by HPLC was found. Drying resulted in an increase in phenolic acids, compared to corresponding fresh-uncooked pasta. Cooking did not greatly affect total phenolic acids, more leading to conserving free and bound phenolic compounds.

3.1 Introduction

Demand for functional foods that offer therapeutic and disease-prevention is globally increasing. In this regard, barley represents an opportunity because it contains high levels of β-glucan, dietary fiber (Gray et al., 2009) and phenolic compounds (Madhujith and Shahidi, 2009). Phenolic compounds are naturally occurring phytonutrients that possess one or more aromatic rings with one or more hydroxyl groups. In barley, there are several classes of compounds that have a phenolic structure, such as benzoic and cinnamic acid derivatives, proanthocyanidins, quinones, flavonols, chalcones, flavones, flavanones and amino phenolic compounds (Goupy et al., 1999; Hernanz et al., 2001; Adom and Liu, 2002; Abdel-Aal et al., 2006).

Currently interest in phenolic and other secondary metabolites is rising because of their potential protective roles in human health. They are known for their ability to inhibit oxidation of methyl linoleate (Kahkonen et al., 1999), to scavenge free radicals (Abdel-Aal et al., 2012), to inhibit oxidation of human LDL cholesterol (Abdel-Aal and Gamel, 2008) and to impede singlet oxygen or chelate pro-oxidant metals (Larson, 1988). Phenolic acids are the main phenolic compounds in cereal grains, such as barley and wheat. They are present in free and bound form primarily in the outer layers of the cereal kernels. Phenolic acids are derivatives of benzoic acid (*p*-hydroxybenzoic, protocatechuic, vanillic, syringic and gallic acids) and cinnamic acid (*p*-coumaric, caffeic, and ferulic acids) (fig. 3.1). Ferulic acid and its dehydrodimer derivatives are the major phenolic compounds in cereals present mainly in bound form, which is ester-linked to the cell wall in the outer layers of the grain kernels (Hernanz et al., 2001; Manach et al., 2004). Polyphenols, including phenolic acids, could play significant roles in human nutrition and health due to their anti-inflammatory antioxidant, and anticarcinogenic effects (Sies et al., 2005; Kahkonen et al., 1999).



Group	Position	Name
	Derivatives of hydroxybenzoic acid	
OH	2	Salicylic acid
OH	3	3-Hydroxybenzoic acid
OH	4	*p*-Hydroxybenzoic acid
OH	3,4	Protocatechuic acid
OH	2,5	Gentisic acid
OH	3,4,5	Gallic acid
OCH_3	3,5	Syringic acid
OH	4	
OCH_3	3	Vanillic acid
OH	4	
	Derivatives of hydroxycinnamic acid	
OH	2	*P*-Coumaric acid
OH	3	*m*-Coumaric acid
OH	3,4	Caffeic acid
OCH_3	3,5	Sinapic acid
OH	4	
OH	3,4	Chlorogenic acid (ester of caffeic acid and quinic acid)
OH	4	Ferulic acid
OCH_3	3	

Figure 3.1 Chemical structures of common phenolic acids in cereals (Ragaee et al., 2014)

Content and bioavailability of phenolic acids could be altered during processing. Processing of cereals may positively or negatively affect the content of phenolic compounds which possibly impacts their bioactive properties and health benefits (Duodu, 2011). In particular, the literature seems to show that pasta production and cooking may increase or decrease phenolic acids content and its antioxidant activity (Fares et al., 2010; Verardo et al., 2011). The occurrence of phenolic acids in free or bound form would affect their behaviour during processing and eventually their bioavailability for absorption and subsequent physiological effects. In this regard, to achieve the maximum benefits from a food it is critical to understand its com-

position of nutrient and bioactive components as well as the effects of food formula, food processing and cooking on the composition and bioavailability of these beneficial components.

Based on these remarks, this study was designed to investigate effects of formulation, pasta-making (e.g. extrusion and drying), and cooking on individual phenolic acids in fresh, dried and cooked barley spaghetti. Barley spaghetti was made from high β-glucan barley alone or in blends with semolina and compared with 100% semolina spaghetti.

3.2 Materials and methods

3.2.1 Materials

Description of grains, ingredients and end products was provided in chapter I. Barley grain samples were milled with a UDY cyclone sample mill equipped with a 0.5 mm screen (UDY, Fort Collins, CO). The Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the HPLC-grade phenolic acids, including gallic, protocatechuic, *p*-hydroxybenzoic, gentisic, 3-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, sinapinic, and *o*-coumaric acids were purchased from Sigma-Aldrich Canada (Oakville, ON).

3.2.2 Extraction of free and bound phenolics

The free phenolic acids were extracted from 0.5 g of the whole grain flour twice on an IKA shaker VXB (IKA Works, Wilmington, NC) with 80% methanol. The extraction was carried out under nitrogen for 30 min. The tube contents were centrifuged at 10,000 × g for 20 min. The extracts were pooled together and evaporated under vacuum at 40°C using a rotary evaporator to remove methanol and concentrated to approximately 2 mL; then, they were diluted to 4 ml with water and acidified to pH 2 with 2M HCl. The free acidified extract was centrifuged at 10,000 × g for 15 min and transferred into a clean separatory funnel. The free acidic supernatant was extracted three times with 10 mL of ethyl ether and ethyl acetate in a 1:1 ratio (v/v) on an IKA shaker for 10 min each and then centrifuged. The organic phase was collected, passed through anhydrous sodium sulfate, and dried under a stream of nitrogen. The residue was redissolved in 1.5 mL of Nanopure water. Prior to HPLC analysis, the free phenolic extracts were filtered through a 0.45 μm Acrodisc syringe filter (PN4484, Pall, Port Washington, NY).

The residual pellet obtained after extraction of free phenolic acids was immediately processed for the analysis of bound phenolic acids. First the pellet was washed with 15 mL of hexane,

and then it was centrifuged at 10,000 × g for 10 min. The supernatant was discarded. Five milliliters of 2M NaOH was added to the pellet, and then the contents were purged with nitrogen and mixed on an IKA stirring plate for 1 hr at 70°C. After hot extraction, the mixture was cooled, acidified to pH 2 with 2M HCl, and centrifuged at 10,000 × g for 15 min. The acidic supernatant was transferred into a clean separatory funnel. The pellet was washed with 10 mL of Nanopure water and then centrifuged at 10,000 × g for 15 min. The water supernatant was combined with the acidic supernatant, and the mixture was extracted three times with 10 mL of ethyl ether and ethyl acetate in a 1:1 ratio (v/v) on an IKA shaker for 10 min each and then centrifuged. The organic phase was collected, passed through anhydrous sodium sulfate, and dried under a stream of nitrogen. The residue was redissolved in 5 mL of Nanopure water and then filtered through a 0.45 μm Acrodisc syringe filter (PN4484, Pall) and stored in a freezer prior to HPLC analysis.

3.2.3 Analysis of Free and Bound Phenolic Acids by HPLC (High Performance Liquid Chromatography)

Phenolic acids in barley extracts were separated and quantified by HPLC equipped with a G1311A quaternary pump, G1329A temperature-controlled injector, G1316A temperature-controlled column thermostat, G1322A degasser, G1315B photodiode array detector (DAD), and ChemStation Rev.B.02.01-SR2 data acquisition system with the capability of conducting isoabsorbance plot and 3D graphic analyses (Agilent Technologies Canada, Mississauga, ON). A 25 cm × 4.6 mm i.d., 5 μm Supelcosil column LC-18 (58298, Supelco, Bellefonte, PA) was employed for the separation of phenolic compounds. A mixture of 12 authentic phenolic acid standards including gallic, protocatechuic, *p*-hydroxybenzoic, gentisic, 3-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, sinapinic, and *o*-coumaric acids was used for calibration, identification, and quantification. For separation of phenolic ac-

ids, a gradient system was applied starting with 100% of 6% formic acid and 0% of acidified acetonitrile. The gradient was gradually changed over 35 min to 82% of 6% formic acid and 18% acidified acetonitrile and then held for next 5 min. After that, 2 min was allowed to return to the starting conditions. The total run time was 42 min. Separated phenolic acids were monitored at five different wavelengths (260, 275, 300, 380, and 330 nm) to enhance accuracy of phenolic acid quantification. In other words, protocatechuic, *p*-hydroxybenzoic, and vanillic acids were quantified at 260 nm; syringic acid at 275 nm; *p*-coumaric acid at 300 nm; and caffeic, ferulic, and sinapinic acids at 380 nm. The identity of phenolic acids was confirmed by the isoabsorbance plot analysis. Typical HPLC chromatogram depicting separation of phenolic acids from standard mixture is presented in figure 3.2. The chromatogram was obtained at 275 nm, at which all 12 phenolic acids showed reasonable response except for gentisic acid. Gentisic acid appeared clearly at 330 nm, but other phenolic acids had low responses. In the current study, each phenolic acid was quantified at its maximum absorption wavelength to enhance the method's accuracy (Abdel-Aal et al., 2012).

3.2.4 Analysis of Total Phenols Content (TPC) in Free and Bound Extracts

Total phenols content in free and bound phenol extracts was measured based on the Folin–Ciocalteu method described by Kaluza et al. (1980), with ferulic acid, the main phenolic in barley, used as a standard for calibration and quantification. The reaction mixture contained 250 μL of sample extract, 250 μL of diluted Folin–Ciocalteu reagent, and 500 μL of saturated sodium carbonate solution. The mixture was brought up to a volume of 5 mL with distilled water. The contents were mixed and kept in darkness for 30 min and then centrifuged at 10,000 × g for 10 min, and then the absorbance was read at 725 nm. A series of ferulic acid standard solutions were prepared at 0–347 μg/mL and read at 725 nm against a reagent blank.

The concentrations showed a linear relationship against absorbance, having a determination coefficient (R^2) of 0.999 and generating the following regression equation:

$$y = 54.968x + 0.0297$$

where x is the concentration of ferulic acid (μg/mL) and y is the absorbance at 725 nm.

The results of total phenols content are expressed as milligrams of ferulic acid equivalents per kilogram of sample. In this study total phenols content in free and bound extracts of barley are referred to as total free phenols and total bound phenols.

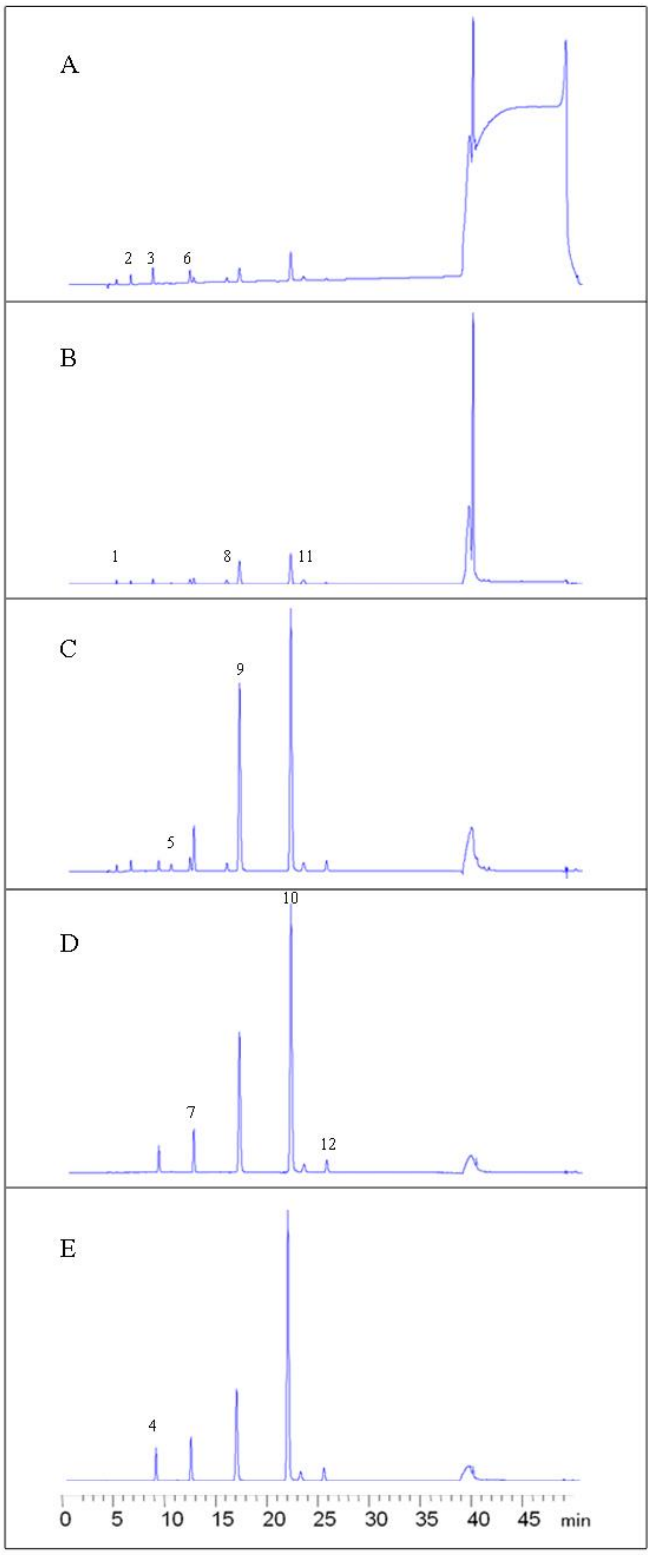


Figure 3.2 HPLC chromatograms showing separation of phenolic acids in standard mixture at 260 nm (A), at 275 nm (B), at 300 nm (C), at 380 nm (D), and at 330 nm (E). 1 = gallic acid; 2 = protocatechuic acid; 3 = benzoic acid; 4 = gentisic acid; 5 = 3-hydroxybenzoic acid; 6 = vanillic acid; 7 = caffeic acid; 8 = syringic acid; 9 = *p*-coumaric acid; 10 = ferulic acid; 11 = *o*-coumaric acid; and 12 = sinapinic acid

3.2.5 DPPH Radical Scavenging Capacity

The free radical scavenging capacity of free and bound barley extracts was determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl), as outlined earlier (Abdel-Aal et al., 2008). The antioxidant reaction was initiated by transferring 1 mL of sample extracts into a test tube containing 4 mL of 80% methanol and 1 mL of freshly prepared 1mM DPPH solution. The final concentration of DPPH in the reaction mixture was 167μM. The reaction was monitored by reading absorbance at 517 nm for 20 min at 1 min intervals. A blank reagent was used to study stability of the DPPH radical over the test time. The absorbance measured at 10 min was used for the calculation of micromolar units of DPPH radical scavenged by barley extracts. The kinetics of the antioxidant reaction in the presence of phenolic extracts were also determined over a 20 min period and compared with butylated hydroxytoluene (BHT) as an antioxidant reference. The antioxidant capacity of the samples was reported as percentage relative depletion (RD) of DPPH using the following equation:

$$\%\ \text{RD of DPPH} = \frac{\text{C of control}_{\text{t = 0 min}} - \text{C of sample}_{\text{t = 10 min}}}{\text{C of control}_{\text{t = 0 min}}} \times 100$$

where C is the concentration of DPPH (μM).

3.2.6 Statistical Analysis

All analyses were carried out in triplicate, and the data were reported as means ± standard deviation (SD). Analysis of variance and least significant difference (LSD) tests were conducted with PASW 20 (SPSS, Chicago, IL) in order to identify differences among means, while a Pearson correlation test was conducted to determine the correlations among means. Statistical significance was declared at $P < 0.05$.

3.3 Results and Discussion

3.3.1 Effect of processing and cooking on phenolic acids

Phenolic acids are the main phenolic compounds in barley and wheat (Abdel-Aal et al., 2012; Zielìnski et al., 2011; Adom and Liu, 2002) and they occur primarily in ester form linked to insoluble polysaccharides. Free and bound phenolic acids were assessed in barley and semolina flours, uncooked and cooked pastas to determine if loss of specific polyphenols or change in their profile occurred during pasta processing (extrusion, drying) and cooking. Tables 3.1 and 3.2 show average concentrations of the major phenolic acids found in the free and bound extracts of raw materials and pastas. According to several reports, the widespread phenolic acids in barley and wheat are gallic, protocatechuic, *p*-hydroxybenzoic, gentisic, chlorogenic, vanillic, caffeic, syringic, p-coumaric, ferulic, sinapinic and *trans*-cinnamic acids (Abdel-Aal et al. 2012; Wu et al., 1999; Irakli et al., 2012).

In the current study, ferulic acid was the major phenolic acid found in the free and bound phenolic extracts in all the investigated pasta samples. Ferulic acid could be a health-promoting component as a chemoprotectant, which was thought to deter the process of carcinogenesis by inhibiting the formation of N-nitroso-compounds (Kuenzig et al., 1984). This compound is also able to prevent peroxynitrite-mediated nitration of tyrosine residues in collagen thus implicating a chemoprotectant role for this antioxidant in the prevention of tissue injury (Kato et al., 1997). Other phenolic acids were found in the raw ingredients and end products at measurable amounts where *p*-hydroxybenzoic, vanillic, caffeic and *p*-coumaric acids were present in the free extracts (Table 3.1) and protocatechiuc, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric and sinapinic acids were found in the bound extracts (Table 3.2). A typical chromatogram of free and bound phenolic acids of tested samples is presented in figure 3.3. Except for *p*-hydroxybenzoic and vanillic acids, which were mainly in free form in semolina and pasta made from it, the other phenolic acids were prevalent in bound form.

The amount and type of free and bound phenolic acids were in a fair agreement with those reported by Mattila et al. (2005) in durum wheat semolina and by Abdel-Aal et al. (2012) in yellow whole barley flour.

Inclusion of whole barley flour into pasta formulations resulted in an increase of the phenolic content. The highest content of free and bound forms of phenolic acids was in barley flour (13.9 µg/g and 654.0 µg/g, respectively). Semolina showed comparable free phenolic acid content to that in the 30% barley flour in blend with semolina, mainly due to the recovery of *p*-hydroxybenzoic acid. However, semolina possessed the lowest bound phenolic acid content (202.2 µg/g) among all the flour samples. Since phenolic acids are concentrated in the outer layers of wheat (Lilyana-Pathirana and Shahidi, 2007), the low total phenolic acids content is expected in semolina, a product derived from the endosperm.

Compared to the other pasta formulations, pastas made from 100% barley flour had the highest levels of free and bound phenolic acids, a finding that is not unexpected given the higher levels of free and bound phenolic acid content of the corresponding raw material. Likewise the free phenolic acids in semolina and 30% barley flour blend were close, semolina pasta showed comparable free phenolic acid content to pasta containing 30% barley flour. As expected the order of bound phenolic acids was pasta made from 100% barley flour > 50% barley flour >30% barley flour > semolina.

The pasta-making process (mixing and extrusion) decreased the free phenolic acids in all fresh and uncooked pasta formulations. The observed variation in the free phenolic acid content was mainly due to a decrease in ferulic, caffeic and slightly *p*-hydroxybenzoic acid (Table 3.3). Free vanillic and *p*-coumaric acid were not significantly affected by processing. Conversely, the bound fraction did not change, except for pasta control (Table 3.3). The results from the present study were in agreement with those of Fares et al. (2010) who reported a decrease in the free phenolic acids during pasta processing, attributed to a reduction in *p*-

hydroxybenzoic acid. It is well documented that during pasta processing the presence of oxygen, water and heat treatment induces the oxidative degradation of antioxidants (carotenoids pigments, polyphenols) (Borrelli et al., 1999; Fares et al., 2008).

Table 3.1. Composition of phenolic acids and total phenolic acids content (TPAC, sum) in free extracts of raw materials and uncooked and cooked spaghetti (mg/kg, dry basis, means ±SD)

	***p*-HBA**	**VA**	**CA**	***p*-CA**	**FA**	**TPAC**
100% barley flour						
Raw materials	n.d	3.1 (0.16) *a*	1.9 (0.15) *a*	1.9 (0.07) *a,c*	6.5 (0.08) *a*	13.9 (0.46) *a*
Fresh-Uncooked	n.d.	3.2 (0.29) *a*	0.9 (0.02) *b*	1.6 (0.02) *b*	5.3 (0,14) *b*	11.5 (0.47) *b*
Fresh-Cooked	n.d	3.7 (0.27) *a*	n.d	2.0 (0.15) *a,c*	4.4 (0.15) *c*	10.7 (0.57) *b*
Dried-Uncooked	n.d	3.7 (0.27) *a*	0.9 (0.01) *b*	1.8 (0.03) *b,c*	6.0 (0.37) *d*	13.1 (0.68) *a*
Dried-Cooked	n.d	3.6 (0.20) *a*	n.d	1.7 (0.08) *b,c*	5.3 (0.03) *b*	10.9 (0.31) *b*
50% barley flour						
Raw materials	n.d	2.7 (0.05) *a,b,c*	1.3 (0.09) *a*	1.3 (0.01) *a*	5.4 (0.19) *a*	10.7 (0.34) *a*
Fresh-Uncooked	n.d.	2.8 (0.07) *b*	0.8 (0.01) *b*	1.3 (0.01) *a*	4.2 (0.06) *b*	9.1 (0.15) *b*
Fresh-Cooked	n.d	2.8 (0.07) *b*	n.d	1.3 (0.02) *a*	4.2 (0.03) *b*	8.3 (0.12) *c*
Dried-Uncooked	n.d	2.5 (0.01) *c*	0.8 (0.01) *b*	1.4 (0.04) *a*	4.9 (0.04) *c*	9.6 (0.10) *d*
Dried-Cooked	n.d	2.7 (0.14) *b*	n.d	1.3 (0.04) *a*	4.9 (0.02) *c*	8.9 (0.20) *b,c*
30% barley flour						
Raw materials	n.d	2.4 (0.04) *a,c*	n.d.	1.1 (0.01) *a,b*	4.9 (0.10) *a*	8.4 (0.15) *a*
Fresh-Uncooked	n.d.	2.4 (0.17) *a,b*	n.d	1.2 (0.09) *b*	4.2 (0.05) *b*	7.8 (0.31) *b*
Fresh-Cooked	n.d	2.4 (0.17) *a,b*	n.d	1.0 (0.05) *c*	4.1 (0.03) *c*	7.5 (0.25) *c*
Dried-Uncooked	n.d	2.2 (0.10) *b*	n.d	1.3 (0.03) *b*	4.5 (0.05) *d*	8.0 (0.18) *b*
Dried-Cooked	n.d	2.5 (0.06) *c*	n.d	1.0 (0.01) *a,c*	4.3 (0.03) *b*	7.8 (0.10) *b*
100% semolina						
Raw materials	1.6 (0.04) *a*	2.0 (0.10) *a*	n.d	1.0 (0.07) *a*	3.8 (0.03) *a*	8.4 (0.24) *a*
Fresh-Uncooked	1.5 (0.1) *b*	2.0 (0.09) *a*	n.d	0.8 (0.04) *a*	3.6 (0.09) *b*	7.9 (0.32) *b*
Fresh-Cooked	1.5 (0.03) *b*	2.0 (0.09) *a*	n.d	0.8 (0.03) *a*	3.5 (0.01) *c*	7.8 (0.16) *b*
Dried-Uncooked	2.9 (0.01) *c*	1.9 (0.16) *a*	n.d	0.9 (0.05) *a*	4.0 (0.01) *a*	9.7 (0.23) *c*
Dried-Cooked	2.6 (0.11) *d*	2.1 (0.04) *a*	n.d	0.8 (0.05) *a*	3.8 (0.01) *a*	9.3 (0.21) *c*

Values in a formula with different letters are significantly different ($p<0.05$, LDS test). *p*-HBA = *p*-hydroxybenzoic acid; VA = vanillic acid; CA = caffeic acid; *p*-CA = *p*-coumaric acid; FA = ferulic acid; TPAC = total phenolic acid content.

Table 3.2. Composition of phenolic acids and total phenolic acids content (TPAC, sum) in bound extracts of raw materials and uncooked and cooked spaghetti (mg/kg, dry basis, means ±SD)

	PRCA	*p*-HBA	VA	CA	SRA	*p*-CA	FA	SA	TPCA
100% barley flour									
Raw materials	4.9 (0.41) *a*	18.7 (1.58) *a*	27.5 (4.23) *a,c*	14.6 (1.21) *a*	22.6 (0.14) *a*	61.7 (1.58) *a*	520.7 (49,29) *a,b*	23.3 (1.99) *a*	694.0 (60.43) *a*
Fresh-Uncooked	4.8 (0.17) *a*	18.8 (0.54) *a*	27.9 (0.74) *a*	14.8 (0.94) *a*	28.1 (1.66) *b,c*	61.5 (1.65) *a*	537.8 (45.40) *a*	25.9 (2.50) *a,b*	719.6 (53.71) *a*
Fresh-Cooked	4.8 (0.10) *a*	19.4 (0.89) *a*	26.9 (1.62) *a*	11.1 (1.09) *a,b*	24.7 (1.24) *a,b*	60.7 (1.52) *a*	445.2 (49.81) *a,b*	27.6 (2.72) *b*	620.4 (58.99) *a,b*
Dried-Uncooked	9,1 (0.48) *b*	23.9 (1.46) *b*	30.9 (1.64) *b*	9.9 (0.46) *b,c*	30.8 (0.94) *c*	61.0 (2.84) *a*	467.1 (52.27) *a,b*	29.8 (2.45) *c*	662.5 (62.54) *a,b*
Dried-Cooked	7,5 (0.17) *c*	20.5 (1.61) *a*	24.8 (0.89) *c*	9.3 (0.36) *c*	25.4 (2.11) *a,b*	58.4 (1.47) *a*	404.3 (43.46) *b*	20.8 (0.61) *d*	571.0 (50.68) *b*
50% barley flour									
Raw materials	2.5 (0.06) *a*	16.1 (0,13) *a*	21.0 (0.40) *a,b*	9.6 (0.15) *a*	21.6 (0.79) *a*	33.6 (0.71) *a,b*	315.9 (41.75) *a,b*	18.5 (0.44) *a*	438.8 (44.43) *a,b*
Fresh-Uncooked	2.6 (0.19) *a*	16.1 (0.45) *a*	20.1 (0.73) *a*	10.0 (1.04) *a*	21.3 (1.33) *a*	34.8 (1.36) *a*	259.4 (5.17) *a*	18.7 (2.74) *a*	383.0 (13.01) *a*
Fresh-Cooked	2.8 (0.24) *a*	16.4 (0.32) *a*	20.3 (1.37) *a*	n.d.	20.0 (0.90) *a*	32.4 (0.04) *b*	306.4 (12.34) *a*	18.2 (0.74) *a*	416.5 (15.95) *a*
Dried-Uncooked	5.0 (0.11) *b*	18.7 (1.94) *b*	23.3 (2.97) *b*	9.8 (0.05) *a*	23.3 (2.01) *a*	33.7 (0.29) *a,b*	295.4 (13.01) *a*	18.8 (0.17) *a*	428.0 (20.55) *a,b*
Dried-Cooked	4.6 (0.34) *b*	17.9 (0.13) *b*	21.4 (0.73) *a,b*	n.d.	21.6 (1.03) *a*	33.4 (1.28) *a,b*	369.3 (18.74) *b*	19.0 (1.08) *a*	487.2 (23.33) *b*
30% barley flour									
Raw materials	n.d.	15.3 (0,13) *a*	19.8 (0,02) *a*	n.d.	21.2 (0.71) *a,b*	22.8 (0.72) *a*	252.2 (4.29) *a,b*	17.0 (0.49) *a*	348.3 (6.36) *a,d*
Fresh-Uncooked	n.d.	14.8 (0.85) *a*	19.6 (1.94) *a*	n.d.	20.8 (1.24) *b*	24.4 (1.40) *a*	242.3 (5.88) *a*	17.9 (1,35) *a,c*	339.8 (12.66) *a*
Fresh-Cooked	n.d.	n.d	n.d	n.d.	n.d	23.5 (1.87) *a*	257.3 (9.98) *a,c*	16.2 (0.85) *b*	327.0 (12.70) *a*
Dried-Uncooked	n.d.	17.5 (0.33) *b*	20.9 (0.05) *b*	n.d.	21.7 (0.14) *b*	23.4 (0.13) *a*	287.8 (24.39) *b,c*	18.6 (1.74) *c*	389.9 (26.78) *c*
Dried-Cooked	n.d.	16.0 (1.26) *a*	20.5 (0.30) *b*	n.d.	19.2 (0.21) *c*	22.5 (0.81) *a*	289.5 (18.74) *c*	18.1 (1.43) *c*	385.8 (22.75) *c,d*
100% semolina									
Raw materials	n.d.	n.d.	n.d.	n.d.	n.d.	10.9 (0.70) *a*	174.2 (4.06) *a,b,c*	17.1 (0.48) *a*	202.2 (5.24) *a*
Fresh-Uncooked	n.d.	n.d.	n.d.	n.d.	n.d.	9.6 (0.41) *b*	161.1 (5.11) *a*	n.d.	170.7 (5.52) *b*
Fresh-Cooked	n.d.	n.d.	n.d.	n.d.	n.d.	12.1 (0.37) *c*	168.9 (3.43) *a,b*	n.d.	181.0 (3.80) *b*
Dried-Uncooked	n.d.	n.d.	n.d.	n.d.	n.d.	11.1 (0.09) *a,c*	178.4 (1.08) *b,c*	15.3 (0.27) *b*	204.8 (1.44) *a*
Dried-Cooked	n.d.	n.d.	n.d.	n.d.	n.d.	10.6 (0.36) *a,b*	185.9 (9.38) *c*	14.7 (1.09) *b*	211.2 (10.83) *a*

Means in a formula with different letters are significantly different ($p<0.05$, LDS test). PRCA = protocatechuic acid; *p*-HBA = *p*-hydroxybenzoic acid; VA = vanillic acid; CA = caffeic acid; SRA = syringic acid; *p*-CA = *p*-coumaric acid; FA = ferulic acid; SA = sinapinic acid; TPAC = bound total phenolic acid content.

Table 3.3 Changes in free and bound phenolic acids during the pasta making process in uncooked and cooked spaghetti compared to corresponding raw flours (+ = % increase and − = % decrease)

	100% barley flour				**50% barley flour**				**30% barley flour**				**100% semolina**			
	Fr-Un	**Fr-Co**	**Dr-Un**	**Dr-Co**	**Fr-Un**	**Fr-Co**	**Dr-Un**	**Dr-Co**	**Fr-Un**	**Fr-Co**	**Dr-Un**	**Dr-Co**	**Fr-Un**	**Fr-Co**	**Dr-Un**	**Dr-Co**
Free Phenolic acids																
p-HBA													-6.3	-6.3	81.3	62.5
CA	-52.6		-52.6		-38.5		-38.5									
FA	-18.5	-32.3	-7.7	-18.5	-22.2	-22.2	-10.3	-16.8	-14.3	-16.3	-8.2	-12.2	-5.3	-7.9	5.3	0.0
TPAC	-17.3	-23.0	-5.8	-21.6	-15.0	-22.4	-10.3	-16.8	-7.1	-10.7	-4.8	-7.1	-6.0	-7.1	15.5	10.7
Bound Phenolic acids																
PRCA	-2.0	-2.0	85.7	53.1	4.0	12.0	100.0	84.0								
p-HBA	0.5	3.7	27.8	9.6		1.9	16.1	11.2	-3.3		14.4	4.6				
VA	1.5	-2.2	12.4	-9.8					-1.0		5.6	2.4				
SRA									-1.9		2.4	-9.4				
SA	11.2	18.5	27.9	-10.7											-10.5	-14.0
TPAC													-15.6	-10.5	1.3	4.5

Fr-Un = fresh and uncooked; Fr-Co = fresh and cooked; Dr-Un = dried and uncooked; Dr-Co = dried and cooked; PRCA = protocatechuic acid; *p*-HBA = *p*-hydroxybenzoic acid; VA = vanillic acid; CA = caffeic acid; SRA = syringic acid; FA = ferulic acid; SA = sinapinic acid; TPAC = total phenolic acid content.

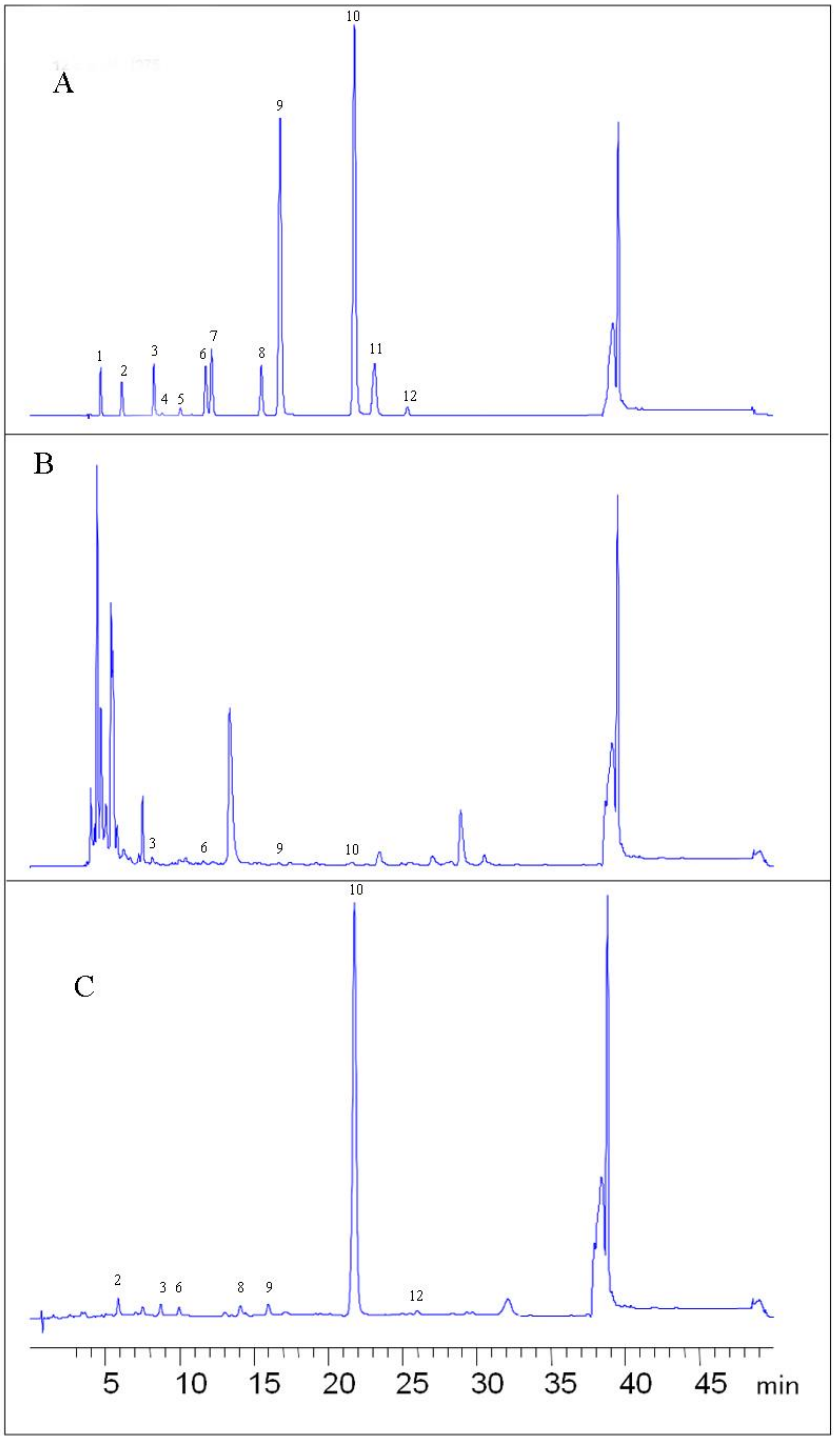


Figure 3.3. Typical HPLC chromatograms (at 275nm) showing separation of phenolic acids in standard mixture (A), in free (B) and bound (C) extracts. 1 = gallic acid; 2 = protocatechuic acid; 3 = *p*-hydroxybenzoic acid; 4 = gentisic acid; 5 = 3-hydroxybenzoic acid; 6 = vanillic acid; 7 = caffeic acid; 8 = syringic acid; 9 = *p*-coumaric acid; 10 = ferulic acid; 11 = *o*-coumaric acid; and 12 = sinapinic acid.

The observed reduction in the content of phenolic acids during pasta processing may be due to the combined impact of the addition of water and oxygen, and the temperature rise during extrusion and kneading. In our study the loss was observed mainly in the free fraction of phenolic acids, which is more reactive in counterbalancing the action of oxygen and heat.

Drying process differently affected individual phenolic compounds in the free and bound fractions, and thus, the total phenolic acid content. In our study, the composition and content of phenolic acids in dried and uncooked semolina pasta were of the same magnitude of those reported by Mattila et al. (2005). Free vanillic, caffeic and *p*-coumaric acids did not significantly change, while *p*-hydroxybenzoic and ferulic acids of the free extracts showed higher values compared to the corresponding fresh-uncooked pasta. Regarding the bound fraction, drying process did not significantly affect some of the bound phenolic acids detected, such as caffeic, syringic, *p*-coumaric, ferulic and sinapinic acids, but it increased protocatechiuc, *p*-hydroxybenzoic and vanillic content in the bound extracts of dried-uncooked 100%, 50% and 30% barley flour-containing pastas. In general, it is interesting to note that where the increase was concerned, the value of total phenolic acids in free and bound extracts of dried-uncooked pasta increased, with respect to the corresponding uncooked pasta (Table 3.3). It seems that the effect of drying on free and bound phenolic acids could be due to the nature of phenolic compounds. The bound phenolic compounds ester-linked to cell walls (not measurable before drying) could be released by pasta matrix during drying, increasing both bound and free phenolic fractions. Therefore, drying temperature could enhance the extraction of phenolic compounds from the food matrix and thereby increase the amount of them during the subsequent chemical extraction.

Conflicted results have been reported with regard to the effect of pasta-making process (mixing, extrusion, and drying) on free and bound phenolic acids in pasta. Verardo et al. (2011) found that the main effect of pasta processing was a decrease of free and bound phenolic acids. Similarly, Fares et al. (2010) observed a decrease in free phenolic content of durum wheat pasta enriched with bran fractions of wheat, but the bound phenolics did not change. On the other hand, the results from the study of Khan et al. (2013) contradict those of the two mentioned studies; the pasta processing did not change the free and bound phenolic acid content of fettuccine made by replacing durum wheat semolina with red or white sorghum flour. The discrepancy between the mentioned studies could be explained by different conditions applied by the authors. Similarly, the process applied in the current research could produce different effects from the literature. The severity and mode of thermal process could also have a bearing on the phenols in the food (Duodu, 2011). Moreover, the effect of each processing step of flour into pasta should be taken into account to understand effects of pasta-making and drying on phenolic compounds.

Cooking led to slight changes in free and bound phenolic acids compared to the equivalent uncooked pasta. These data confirmed the results of Mattila et al. (2005), who demonstrated that, on dry basis, the contents of phenolic acids in wheat flours and corresponding products made from these flours (bread and pasta) were found to be similar. On the other hand, Verardo et al. (2011) reported a strong decrease of free and bound phenolic acids of pasta after cooking. This effect could be laid to the solubility of phenolic compounds in the cooking water. Fares et al. (2010) and Khan et al. (2013) also found decrease in the free phenolic acid content, but increase in the bound fraction of nonconventional pasta. They suggested that boiling can enhance the extractability of bound phenolic acids from the food matrix during cooking and hence can increase their

recovery. Unlike bound phenolic acids, free phenolic acids are not physically trapped in protein network (Naczk et al., 2011; Prigent et al., 2009), therefore the cooking process could have resulted in leaching of these compounds into the cooking water.

3.3.2 Total Free and Bound Phenols Contents

Table 3.4 reports on the total free and bound phenols contents of the flours and pastas as determined by the Folin–Ciocalteu assay. This assay determines the total amount of reducing substances by measuring the change in color when metal oxides are reduced by phenolic antioxidants and other reducing substances may present in the extract. This method would also serve as an indication of antioxidant capacity for free or bound substances present in the extracts. Although its low accuracy is well known, the Folin-Ciocalteu assay has been widely applied for the analysis of phenolic compounds in grains, so it could be useful to compare data on the current samples with those present in the literature. The disagreeing results of the literature could be explained by the difference of extraction method, solvent type, and grain variety. In the present study, the total phenols content was expressed as ferulic acid equivalent, since it is the predominant phenolic acid in barley flour and semolina.

Formulation containing 100% barley flour had higher free and bound phenolics content (235.9 mg/kg and 1590.2 mg/kg, respectively) than the other formulations. Total free and bound phenolics content broadly ranged among samples. The total free phenolic content ranged from 235.9 mg/kg in 100% barley flour to 19.9 mg/kg in semolina. As expected, total bound phenolic content contributed the highest portion of total phenolic compounds in the all flours, ranging from 1590.2 mg/kg in 100% barley flour to 342.4 mg/kg in semolina. The values are in a fair agreement with those reported by Irakli et al.

(2012) and Fares et al. (2010), concerning whole barley flour and semolina, respectively.

Compared to the control pasta, all barley pastas had higher free and bound total phenolic content. The total phenolic content of the uncooked and equivalent cooked pastas were very close to the that in the raw materials, denoting that pasta-processing (extrusion, drying and cooking) did not significantly affect the total phenolic content of free and bound extracts.

In barley flour individually and in blend with semolina, and in all barley pastas, the free and bound phenolic contents as assessed by Folin-Ciocalteu method were nearly 20 times and 2 times, respectively, higher than those measured by HPLC-DAD analysis. A major reason for this difference is the lack of specificity of the Folin-Ciocalteu reagent to phenolic acids that did not represent the profile of the total phenolic compounds. Indeed, the literature reports that other phenolic and reducing compounds found in barley might contribute to the total content of phenols (Bonoli et al., 2004; Verardo et al., 2011). On the other hand, HPLC-DAD values of semolina and control pastas were close to those obtained with the Folin-Ciocalteu method, denoting that phenolic acids represent the majority of phenolic compounds.

Table 3.4 Total phenols content of free and bound extracts of raw materials and corresponding pasta

	TPCf (mg/kg on d.b)	TPCb (mg/kg on d.b)
100% barley flour		
Raw materials	235.9 (21.30) *a*	1590.2 (50.09) *a*
Fresh-Uncooked	205.7 (12.21) *a,b*	1436.31 (59.45) *b*
Fresh-Cooked	180.8 (0.82) *b,c*	1436.69 (15.64) *b*
Dried-Uncooked	197.2 (17.90) *b,c*	1533.54 (20.75) *a,b*
Dried-Cooked	166.1 (1.21) *c*	1637.45 (44.53) *a*
50% barley flour		
Raw materials	113.6 (3.71) *a*	1031.7 (54.99) *a*
Fresh-Uncooked	113.9 (10.44) *a*	909.1 (54.92) *b,c*
Fresh-Cooked	97.7 (2.24) *a*	995.1 (14.54) *a,b*
Dried-Uncooked	107.7 (0.24) *a*	882.4 (11.02) *c*
Dried-Cooked	104.8 (7.15) *a*	958.4 (34.78) *a,b,c*
30% barley flour		
Raw materials	88.8 (8.31) *a,b*	808.0 (26.45) *a*
Fresh-Uncooked	74.1 (5.90) *b*	735.9 (21.59) *a*
Fresh-Cooked	75.8 (6.55) *b*	758.3 (55.11) *a*
Dried-Uncooked	91.1 (5.47) *a,b*	811.7 (25.50) *a*
Dried-Cooked	101.1 (9.23) *a*	789.0 (62.59) *a*
100% semolina		
Raw materials	19.9 (1.80) *a*	342.4 (16.95) *a*
Fresh-Uncooked	17.9 (1.20) *a,b*	370.1(4.43) *a,b*
Fresh-Cooked	13.2 (0.44) *c*	397.4 (22.60) *b*
Dried-Uncooked	15.6 (0.97) *b,c*	442.9 (7.97) *b,c*
Dried-Cooked	13.5 (0.25) *c*	462.4 (32.8) *c*

Values are means ±SD. Means in a formula with different letters are significantly different ($p<0.05$, LDS test).
TPCf = total phenolic content of free extract. TPCb = total phenolic content of bound extract.

3.3.3 DPPH Radical Scavenging Capacity

The DPPH free radical scavenging capacity of the flours and, uncooked and cooked pastas is presented in Table 3.5. The DPPH assay measures the ability of an antioxidant to reduce the stable deep-purple DPPH radical, and the reduction in color is monitored over a given time. The color intensity of DPPH radicals with no antioxidant or sample extracts was stable over the test time with an average absorbance of 1.763 (fig. 3.3). The antioxidant extracts from samples tested in this study exhibited different reaction kinetics curves compared with the BHT antioxidant standard (75ppm). The free and bound extracts of barley flour individually showed a sharp drop in DPPH colour intensity, indicating high antioxidant activity in quenching DPPH radicals during the first 10 minutes, followed by a gradual logarithmic decline. In the presence of BHT, however, the color intensity of DPPH gradually reduced over time following a logarithmic decline. The different kinetics curve of the free and bound extracts of semolina indicated their low radical scavenging capacity.

The free and bound extracts of barley flour individually had the highest percentage of depleted DPPH radical (70.0 and 60.4%, respectively) (Table 3.5). On the other hand, free and bound phenolic extracts of semolina showed the lowest DPPH depletion percentage (10.5% and 26.3 %, respectively). Similar results were found by Abdel-Aal et al. (2012) in bound extracts of yellow whole barley samples. In general, free and bound phenolic extracts of barley flour individually and in blend with semolina had higher antioxidant capacity as determined by DPPH assay than those of semolina. Similar to total phenolic content, pasta processing (extrusion, drying and cooking) did not significantly affect the antioxidant capacity of both free and bound phenolic extracts of all uncooked and cooked pastas, independently from the formulation.

Table 3.5 Scavenging capacity of DPPH free radical (% relative depletion) of raw materials and corresponding pasta

	Free Phenolic Extract	Bound Phenolic Extract
100% barley flour		
Raw materials	60.4 (0.28) *a,b*	70.0 (1.28) *a*
Fresh-Uncooked	55.1(1,92) *a*	65.8 (1.93) *a*
Fresh-Cooked	55.7(0.94) *a*	65.5 (6.14) *a*
Dried-Uncooked	62.9 (5.63) *b*	75.4 (3.48) *a*
Dried-Cooked	61.4 (3.30) *b*	67.5 (5.49) *a*
50% barley flour		
Raw materials	38.4 (1.98) *a*	49.1 (1.63) *a*
Fresh-Uncooked	37.9 (0.04) *a*	46.7 (1.21) *a*
Fresh-Cooked	36.7 (2.04) *a*	50.7 (3.82) *c*
Dried-Uncooked	36.0 (0.25) *a*	63.8 (1.24) *b*
Dried-Cooked	37.8 (1.61) *a*	58.5 (5.47) *b,c*
30% barley flour		
Raw materials	26.2 (1.40) *a*	37.2 (0.96) *a*
Fresh-Uncooked	22.2 (2.26) *a*	39.1 (0.06) *a,c*
Fresh-Cooked	25.6 (0.77) *a*	48.5 (1.24) *b*
Dried-Uncooked	24.9 (2.30) *a*	43.3 (0.34) *b,c*
Dried-Cooked	26.0 (2.59) *a*	45.8 (4.36) *b*
100% semolina		
Raw materials	10.5 (3.03) *a,b*	26.3 (0.44) *a*
Fresh-Uncooked	12.2 (1.71) *a,b*	27.9 (2.50) *a*
Fresh-Cooked	7.5 (1.53) *b*	26.8 (1.29) *a*
Dried-Uncooked	11.3 (3.42) *a,b*	29.8 (0.40) *a*
Dried-Cooked	16.2 (5.14) *a*	28.0 (2.76) *a*

Means in a formula with the same letter within a formulation are not significantly different at $P < 0.05$. DPPH = 2,2-diphenyl-1-picrylhydrazyl

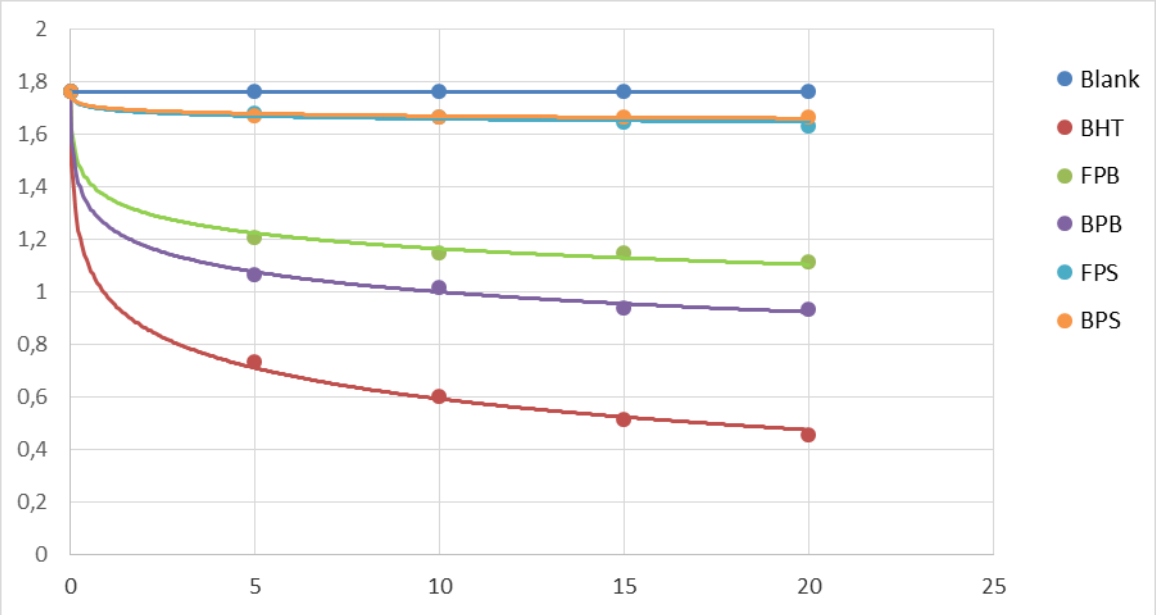


Figure 3.3 Kinetics of 2,2-diphenyl-1-picrylhydrazyl radical with free (FP) and bound phenolic (BP) extracts of barley flour (B) and semolina (S), and butylated hydroxytoluene (BHT).

3.3.4 Contribution of Phenolics to Antioxidant Capacity

All data on total free phenols, total bound phenols, free phenolic acids, bound phenolic acids, and DPPH scavenging capacity were subjected to Pearson correlation analysis to determine the relationship between phenolic compounds and their contribution to antioxidant capacity of the investigated samples (Tables 3.6 and 3.7). In all extracts, ferulic acid was the predominant phenolic acid and exhibited the significantly strongest correlation with total phenolic acids content (TPAC) and total phenols content (TPC).

Total phenols content (Folin-Ciocalteu method) gave strong positive correlations with total phenolic acids content (HPLC-DAD measurement) for both free and bound extracts, suggesting that phenolic acids made considerable contributions to phenolic compounds content in the tested samples under the experimental conditions.

Except for *p*-hydroxybenzoic acid, all phenolic acids were positively correlated with total phenolic acids content and total phenols content, indicating that they are the main

phenolic compounds in the free extracts. In addition, negative correlations between free *p*-hydroxybenzoic and all other free phenolic compounds were found in this study.

In the bound fraction, all phenolic acids were significantly correlated, particularly ferulic and *p*-coumaric acids showed the strongest correlations and were prevailing in the bound phenolic acids. Overall, *p*-coumaric and ferulic acids were found to have a high correlation with the total bound phenols content and the total amount of phenolic acids determined by HPLC. This strong correlation confirmed the prevalence of ferulic and *p*-coumaric acids in the bound extracts.

The antioxidant capacity, measured as the DPPH radical scavenging capacity, showed significant correlations with total phenolic content (r = 0.965) suggesting that phenolic compounds in the samples examined may be able to fight free radicals formed in the human body. In addition, significant correlations were also found between the phenolic acids (except for free *p*-hydroxybenzoic acid) in free and bound extracts, and DPPH radical scavenging capacity, indicating the role of examined phenolic acids in inhibiting free radicals.

Table 3.6 Correlation coefficients between free phenolic acids, total phenolic content, total phenolic acids content and DPPH scavenging capacity

	TPC	*p*-HBA	VA	CA	*p*-CA	FA	TPAC	DPPH
TPC		-0.711**	0.900**	0.641**	0,936**	0,864**	0,787**	0,965**
***p*-HBA**			-0.624**	-0.328	-0.630**	-0.564**	-0.192	-0.665**
VA				0.402	0.916**	0.697**	0.715**	0.962**
CA					0.534**	0.762**	0.767**	0.569**
***p*-CA**						0.779**	0.779**	0.945**
FA							0.844**	0.836**
TPAC								0,797**

**indicates at $P = 0.05$; n=20. TPC = total phenols content (determined by Folin-Ciocalteu assay); *p*-HBA= *p*-hydroxybenzoic acid; VA = vanillic acid; CA = caffeic acid; *p*-CA = *p*-coumaric acid; FA = ferulic acid; TPCA = total phenolic acids content (determined by HPLC-DAD method); DPPH = % relative depletion of 2,2-diphenyl-1-picrylhydrazyl.

Table 3.7 Correlation coefficients between bound phenolic acids, total phenolic content, total phenolic acids content and DPPH scavenging capacity

	TPC	PA	*p-HBA*	VA	CA	SRA	*p-CA*	FA	SA	TPCA	DPPH
TPC		0,863**	0,827**	0,858**	0,802**	0,824**	0,923**	0,948**	0,775**	0,967**	0,938**
PA			0,723**	0,727**	0,754**	0,708**	0,784**	0,610**	0,657**	0,833**	0,902**
p-HBA				0,992**	0,612**	0,993**	0,693**	0,781**	0,739**	0,842**	0,830**
VA					0,671**	0,993**	0,754**	0,831**	0,763**	0,884**	0,848**
CA						0,634**	0,842**	0,794**	0,608**	0,808**	0,778**
SRA							0,711**	0,786**	0,746**	0,847**	0,815**
p-CA								0,935**	0,748**	0,935**	0,859**
FA									0,781**	0,992**	0,923**
SA										0,817**	0,772**
TPCA											0,948**

**indicates at $P = 0.05$; n=20. TPC = total phenols content (determined by Folin-Ciocalteu assay); PA = protocatechuic acid; *p*-HBA= *p*-hydroxybenzoic acid; VA = vanillic acid; CA = caffeic acid; SRA = syringic acid; *p*-CA = *p*-coumaric acid; FA = ferulic acid; SA = sinapinic acid; TPCA = total phenolic acids content (determined by HPLC-DAD method); DPPH = % relative depletion of 2,2-diphenyl-1-picrylhydrazyl.

3.4 Conclusions

In general, total and individual phenolic acids strongly correlated and contributed to the free radical scavenging capacity of pastas. The addition of barley flour into pasta at all incorporation levels effectively increased the phenolic acid content and total phenols content, showing antioxidant potential and increasing the possible benefits in diet to help prevention of chronic diseases related to oxidative stress.

Pasta processing did not significantly affect the total phenols content and free radical scavenging capacity, but it led to vary phenolic acids content of pastas. The significant reduction in total phenolic content after extrusion might be due to oxidizing reactions trigged by water, oxygen and heat. Interesting drying of pasta resulted in improved phenolic acids, compared to corresponding fresh-uncooked pasta. Cooking did not greatly affect the total phenolic acids, more leading to conserving free and bound phenolic compounds. Based on this, different processing technologies have been found to produce various effects, and thus the choice of cereal formula and technological process is crucial in preserving phenolic acids and their anticipated health-enhancing properties.

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CHAPTER IV

General Conclusions

4.1 Overall Conclusions

The demonstrated link between the intake of whole grains and various health benefits has heightened consumer awareness of good nutrition and increased interest in functional foods. Although whole cereal grains contain many health-promoting components (phytochemicals or phytonutrients) such as β-glucan and phenolic compounds, food processing could increase or reduce them. This has implications for their bioactive properties and potential health benefits they can offer.

Therefore, cereal recipe and processing should be carefully chosen to preserve the health properties of foods after processing.

The aim of this study was to develop functional pasta (spaghetti), a common food in the world. Spaghetti were produced from waxy barley as a rich source of healthy dietary fiber, in particular β-glucan, and other bioactive constituents, such as phenolic acids. Most of phenolic substances are mainly concentrated in the outer layer of cereal grains, and thus whole barley was considered as the best solution to reduce the loss of bioactive compounds and to increase the potential health benefits of spaghetti. Then we evaluated the impact of processing on some health-promoting components of spaghetti.

For the developed barley spaghetti the health claims “may reduce the risk of heart disease” (FDA, 2006) and “contributes to the maintenance of normal blood cholesterol levels” (EFSA, 2011) could be adopted, based on the required β-glucan content (chapter 2). The total content of β-glucan did not change in barley pasta compared to the corresponding raw materials, indicating that pasta processing did not have any influence on β-glucan levels. Conversely, pasta processing affected the physicochemical properties of β-glucan that are associated with physiological effectiveness. In particular, extrusion and drying negatively affect solubility and molecular weight, and in turn viscosity of β-glucan in all pasta formula, while cooking significantly increased them.

Thus, cooked pasta made from barley seems to be healthier than that made from semolina alone. The viscosity of barley spaghetti was not related to β-glucan content as the highest viscosity was found in barley spaghetti containing the lowest β-glucan. This illustrates the importance of evaluating the extractability and molecular-structural characteristics of β-glucan, and not just their concentration for potential health benefits in foods.

In addition to β-glucan, whole barley is considered a good source of phenolic acids, known for their beneficial antioxidant properties and overall for the promotion of human health. So in the next stage of research (Chapter 3), the contribution of total and individual phenolic acid to antioxidant properties of barley spaghetti was evaluated. In general, total and individual phenolic acids strongly correlated and contributed to the free radical scavenging capacity of pastas. The addition of barley flour into regular pasta at all incorporation levels effectively increased the phenolic acid content and total phenols content, showing antioxidant potential and increasing the possible benefits in diet to help prevention of chronic diseases related to oxidative stress.

Pasta processing did not significantly affect total phenols content (measured by Folin-Ciocalteu method) and free radical scavenging capacity, but it caused changes in the content of individual phenolic acids content (measured by HPLC method) of pastas. After extrusion total phenolic acids content decreased, while drying of pasta resulted in improved phenolic acids, compared to corresponding fresh-uncooked pasta. Cooking did not greatly affect the total phenolic acids, more leading to conserving free and bound phenolic compounds.

These results would be useful in the development of barley-based functional pastas with improved health benefits. In the development of barley lines with high level of β-glucan special care should be given to its molecular structure and impact of processing such as

mixing, extrusion, cooking, etc. Therefore, food manufactures should pay attention not only to the potential physiological properties of raw barleys, through evaluating the initial phytochemicals content such as β-glucan and phenolic acids, but also evaluate the possible changes of these bioactive compounds during processing.

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