Phenolic Profile and Carbohydrate Digestibility of Durum Spaghetti Enriched with Buckwheat Flour and Bran

By

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ABSTRACT

There is growing demand for functional foods and ingredients as a result of their health-promoting properties. In this work, the potential of common buckwheat flour (Supreme) and bran (Farinetta) in improving upon the phenolic and antioxidant properties of durum spaghetti was investigated. The effects of processing and cooking on these properties were also studied in addition to the cooking quality and carbohydrate digestibility of spaghetti products. The presence of buckwheat significantly ($p < 0.05$) elevated total phenolic content (TPC), total flavonoids content (TFC), rutin and phenolic acids levels, in flours, uncooked and cooked spaghetti samples. Significant increments were also recorded for antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) approaches. Among uncooked spaghetti samples, there were huge increments of between 114 and 522% for TPC, 50 and 242% for TFC, 359 and 1000% for DPPH antioxidant activity, and 101 and 197% for ORAC values of the experimental spaghetti samples over the control. Farinetta contributed more phenolic and antioxidant compounds than Supreme flour. Processing did not cause any significant losses in TPC, but losses ranging from 1.2 to 33.7% in TFC and 42.0 to 55.3% in DPPH antioxidant activity were incurred. Cooking generally resulted in significant losses ($p < 0.05$) of up to 39% in TPC, 40% in DPPH antioxidant activity, 22% in rutin content, and 55% in TFC among all buckwheat-containing products. Even after these losses, phenolic content and antioxidant properties of experimental samples especially those containing Farinetta were similar if not higher than those of commercial 100% buckwheat pasta (RefB). The introduction of buckwheat, particularly Farinetta, reduced cooking quality of spaghetti products. Cooking losses recorded for the experimental

samples were higher for Farinetta-substituted products, and ranged between 5.72 to 8.32%. These were generally higher than those of the control (6.33%). The introduction of buckwheat increased digestibility, although the amount of reducing sugars released after 120 min of hydrolysis was lower than that of the control. Readily digestible carbohydrate content of 212.8 mg/g to 339.6 mg/g was recorded for experimental samples while that of RefB was 362.5 mg/g. The results of this study show that the phenolic and antioxidant properties of durum spaghetti fortified with buckwheat milling fractions can compare favourably with those of 100% whole buckwheat pasta, and at the same time, maintain a higher cooking quality due to the presence of semolina.

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LIST OF ABBREVIATIONS

AACC	American Association of Cereal Chemists International
AAPH	2,2’-azobis (2-amidinopropane) dihydrochloride
AUC	Area under curve
BWPE	Buckwheat protein extract
DF	Dietary fibre
DNS	Dinitrosalycilate
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DW	Dry weight
GI	Glycemic index
GIm	Glycemic impact
GOPOD	Glucose oxidase/peroxidase
HPLC	High performance liquid chromatography
HT	High temperature (drying)
LT	Low temperature (drying)
ORAC	Oxygen radical absorbance capacity
RDC	Readily digestible carbohydrates
RS	Resistant starch
SDC	Slowly digestible carbohydrates
SI	Swelling index
SPI	Soy protein isolate
TDF	Total dietary fibre

TE	Trolox equivalent
TFC	Total flavonoids content
TPC	Total phenolic content
TROLOX	6-Hydroxyl-2,5,7,8-tetramethychromane-2-carboxylic acid
WA	Water absorption

CHAPTER 1: Literature review

1.1 Introduction

The awareness of eating healthy has become very evident as consumers are becoming more interested in their choices of foods. The food industry likewise is keen on meeting these demands by churning out foods and food ingredients that do not only sustain nutrition but also promote health. Functional foods, defined by Hasler (1998) as "processed foods containing ingredients that aid specific bodily functions in addition to being nutritious", are now the target of many who see the need to extend the benefits of their diets beyond basic nutrition, to the prevention and management of certain disease conditions such as diabetes, cardiovascular diseases and some cancers that plague modern society. This awareness has arisen due to mounting scientific evidence which supports the theory of functional foods containing physiologically-active components that enhance health.

In the manufacture of functional foods, cereals grains offer a wide range of alternatives as they can be exploited in many different fashions to meet specific needs (Charalampopoulos et al., 2002). Cereal based diets are a good source of energy and proteins (Li & Zhang, 2001; Pomeranz & Robbins, 1972), and many bioactive compounds, many of which possess antioxidant activity (Ötles & Cagindi, 2006; Liu, 2007; Sidhu et al., 2007). With the recent quest to eat healthier diets, other sources of natural food ingredients are being sought to complement traditional staples which have been relied upon as sources of energy for so many years.

The resurgence of non-traditional cereals or pseudo-cereals in our diets has paved the way for their use as functional food ingredients since they possess certain nutritional

and functional qualities which are absent or deficient in traditional cereal grains. Buckwheat is a pseudo-cereal crop with a lot of potential as a functional food ingredient. It is mainly cultivated for its seeds which are milled into flour and used for products such as bread, pancakes and soba noodles (Steadman et al., 2001a; Rayas-Duarte et al., 1998).

The prophylactic properties of buckwheat partly stem from its rich protein content and well-balanced amino acid profile (Pomeranz & Robbins, 1972). Protein extract from buckwheat has been proposed for the prevention and treatment of conditions such as hypertension and hypercholesterolemia (He et al., 1995; Kayashita et al., 1995). Buckwheat is also a rich source of phytochemicals such as polyphenols (Holasova et al., 2002; Oomah & Mazza, 1996; Przybylski et al., 1998) and fibre (Bonafaccia et al., 2003). In addition, buckwheat contains B vitamins (Bonafaccia et al., 2003), a high amount of unsaturated and polyunsaturated fatty acids (Steadman et al., 2001a) and minerals (Amarowicz & Fornal, 1987), making it an important functional food ingredient (Ötles & Cagindi 2006; Krkošková & Mrazova, 2005; Li & Zhang, 2001).

In this study, the potential of two commercial buckwheat milling products namely Supreme flour (comprising percentages of hull and endosperm) and bran Farinetta (mixture of aleurone layer of hulled seed and seed embryo), in improving the phenolic profile and antioxidant property of durum spaghetti was investigated. The effect of processing and cooking on these properties were also investigated. In addition, the effect of these buckwheat milling components on cooking quality and *in vitro* carbohydrate digestibility were also evaluated. While it is an excellent idea to fortify cereal grain products with pseudo-cereals such as buckwheat, the challenge still remains in manufacturing products which have similar, if not improved sensory attributes, in order

for them to compete on the consumer market. It is also essential to select optimal processing conditions that will preserve nutrients and bioactive compounds in fortified products.

1.2 Spaghetti

1.2.1 History

Spaghetti is probably the most common form of pasta, which is differentiated from other pasta products by its thin and long shape. Although the origin of pasta is not precisely known, it is believed to have been consumed in ancient China for many centuries before being introduced to ancient Rome (Antognelli, 1980). Today, spaghetti is manufactured and consumed all over the world. Its success and popularity has been ascribed to its ease of storage, handling, transportation and cooking (Tudorica et al., 2002). Over the years, manufacturers have sought to add value to spaghetti, which typically is rich in complex carbohydrates (Giese, 1992). Although spaghetti is usually eaten in combination with dishes which may be rich in other food groups, it is still pertinent to improve upon its nutritional and health promoting properties.

1.2.2 Processing and quality of spaghetti

Semolina from durum wheat (*Triticum turgidum* var. *durum*) possesses the best characteristics for the production of western-type spaghetti, which is popular across North America, Europe and beyond (Antognelli, 1980). It is a yellowish product of coarse granulation obtained from the endosperm of durum wheat during milling. The

quality of semolina is governed by environmental factors (climate, soil type and agronomic practices) and genotypic variations in durum wheat (Rharrabti et al., 2003). Durum wheat is milled in such a way so as to ensure that semolina with a uniform granulation and particle size distribution is obtained (Troccoli et al., 2000). A particle size range of 200-300 µm with less than 10% falling outside this range is ideal (Antognelli, 1980). Finer granulation may increase thermal stress during processing and affect dough properties, while larger particles result in inadequate absorption of water during dough hydration.

The protein content of semolina is a very important factor as it dictates the rheological property of the dough, and as such, semolina with high protein content is most ideal. The protein content of semolina could be as high as 15% and as low as 10% (Dexter et al., 1977). Gluten is the major protein found in semolina. It consists of two main types of proteins namely glutenins and gliadins.

Despite the fact that durum semolina is the raw material of choice for the production of spaghetti, different raw materials such as rice, corn and buckwheat have been used across different cultures (Trematerra, 2009), and in most cases, they have been used in conjunction with semolina. Other ingredients such as eggs and vegetables like spinach have also been added to boost processing, nutritional, and sensory qualities (Antognelli, 1980). Unlike western-type pasta, oriental noodles are manufactured with soft wheat flour. Also, the demand for gluten-free products has driven the need for more alternative sources of raw materials.

Spaghetti production involves hydration, mixing and forming, and a final drying step. In the first two steps, semolina is watered adequately and mixed uniformly to ensure

consistent unleavened dough of about 30% moisture level. At this stage, a dense protein network begins to form which physically entraps starch granules. This network is responsible for preventing considerable starch swelling and leaching during cooking (Bruneel et al., 2010). The mixing and forming step also results in some increment in damaged starch as a result of mechanical stress. Cultural differences are the main factors that influence the type of spaghetti processing. Depending on how the dough is formed or shaped after mixing, spaghetti may be manufactured in two basic ways: the sheeting and cutting method or the cold extrusion method (Serna-Saldivar, 2010). In the sheeting and cutting method, the dough is either mechanically or manually compressed to the desired thickness before being cut into thin strips. This method is mostly used in the oriental noodle industry. Technological advances in the industrial process have seen the shift from hitherto sheeting and cutting of the dough to the principle of cold extrusion. This method involves a push-through compressive forming process beginning with dough at room temperature. In an industrial set up, the preparation of dough and subsequent forming and cutting of products may all be achieved using a complex extruder.

Drying is the most crucial step of spaghetti processing (Troccoli et al., 2000). This step ensures that a final product of about 9 to 13% moisture content is achieved. Drying of spaghetti at the industrial scale is done in specially designed chambers under controlled conditions of temperature and humidity. High temperature (HT) drying programs have been developed to solve the problems associated with conventional low temperature (LT) drying (Baiano & Del Nobile, 2006; Destefanis & Sgrulletta, 1990; Guler et al., 2002; Zweifel et al., 2003). During drying, protein network formation continues (Bruneel et al., 2010), and the type of drying method used will dictate the

extent of this protein polymerization. LT drying uses temperatures of about 50 °C, while temperatures as high a 90 °C may be reached during HT drying. HT drying is believed to increase and preserve polymerization due to increased denaturation of proteins, which subsequently leads to superior quality products (Bruneel et al., 2010). There are also indications that protein-starch interactions are promoted by HT drying (Cunin et al., 1995; Vansteelandt & Delcour, 1998). In addition, HT drying inactivates lipoxygenase activity, which is responsible for the oxidative degradation of natural carotenoid pigments that provide the characteristic yellowish colour of spaghetti (Dexter et al., 1981). HT drying also has the added advantages of eliminating any threats of microbial contamination as well as shortening the drying stage, a factor which is crucial for maximizing profits. Although HT drying generally results in better quality products, the effect is barely noticeable when the quality of gluten in semolina is high to begin with (Cubadda et al., 2007). It is worthy of note however, that there are a few contradictory reports on the effect of different drying conditions on spaghetti quality. This may be due to differences in semolina properties such as protein content and gluten quality arising out of environmental variations and genetic differences in durum wheat.

Uncooked spaghetti quality is evaluated in terms of its colour, strength/flexibility, glassy nature and texture (Antognelli, 1980). A bright yellowish colour is of visual appeal to consumers. The quality of cooked spaghetti is evaluated in terms of its cooking time, water absorption, cooking loss and textural properties (firmness, stickiness and resilience) (Bruneel et al., 2010; Dexter, et al., 1985). The formation of a strong protein network which entraps starch granules during processing is necessary for the best quality of cooked spaghetti. Bruneel et al. (2010) demonstrated that some protein polymerization

also occurred during cooking, and that the balance of this protein network formation between the processing and cooking stages eventually determines the quality of the cooked product. Ideally, cooking quality is believed to be superior if the greater percentage of polymerization occurs during cooking.

1.3 Buckwheat

1.3.1 Introduction and history of crop

Buckwheat is a dicotyledonous crop of the family and genus Polygonaceae and *Fagopyrum* respectively (Ohnishi 1995). It has been a traditional crop in Asian for centuries. Records show that cultivation may have started in ancient China as early as the fifth century (Campbell, 1997). The crop was introduced into Europe during the middle ages, but by the turn of the nineteenth century, cultivation had plummeted due to a shift of attention to the more lucrative potato farming. After the resurgence of buckwheat as a result of interest in its highly nutritional and nutraceutical properties, successful breeding programs were implemented and its cultivation has now spread all over the world particularly in the northern hemisphere, as evidenced by its many local names across different cultures: *sarrasin* or *blé noir* (France), *ogal* (India), *fagopiro* (Italy) *soba* (Japan), *tatarka gryka* (Poland) and *buchweizen* or *heidekorn* (Germany) (Campbell, 1997).

Buckwheat is now regarded as an alternative crop along with many other ancient crops that have gained renewed interest such as quinoa and amaranth. Among the largest cultivators of buckwheat over the past four to five decades have been China, Russia,

Ukraine, United States, Canada, France, and Italy (Campbell, 1997). Together, these countries produce millions of tonnes of buckwheat seeds per year (Li & Zhang, 2001). In Canada, cultivation along the eastern prairies has existed for over four decades. The province of Manitoba contributes over 70% of Canadian buckwheat production with Ontario and Quebec contributing the remainder. AC Manisoba and Koban, as well as the Koto variety which is unique for its increased starch content have so far been developed (Agriculture and Agri-Food Canada, 2010). Currently, special frost resistant and self-pollinating varieties are being developed (Canadian Special Crops Association, 2012). Canada's contribution to the global buckwheat production which currently stands at a little over 2 million metric tonnes (FOASTAT, 2013) has been declining gradually over the years. Production stood at an average of just 5,000 metric tonnes within the past decade (Agriculture and Agri-Food Canada, 2007).

Many species of buckwheat have been identified in the wild, particularly in China (Ohnishi 1995). However, only about nine different species bear agricultural significance (Li & Zhang, 2001). Notable among these are the common buckwheat (*Fagopyrum esculentum* Moench) and the tartary buckwheat (*Fagopyrum tataricum*), sometimes referred to as sweet and bitter buckwheat, respectively. Common buckwheat, as its name implies, is the more widespread of the two. The success of the buckwheat crop has mainly hinged on its ability to adapt to diverse growing conditions. It is able to dwell under adverse conditions of insufficient water and soil nutrients, and can flourish without the help of fertilizers (Krkošková & Mrazova, 2005). In addition, it has a short growing season of 10-12 weeks (Eggum et al., 1980).

1.3.2 Structure and uses of the buckwheat grain

The buckwheat fruit, also referred to as the achene, is triangular in shape and measures about 4-9 mm long (Mazza & Oomah, 1993). The outer layer of the achene is a dark brown or black fibrous hull (pericarp). Dehulled achenes are called groats. Buckwheat groats bear structural resemblance to traditional cereal grains. The kernel is made up of a testa, an aleurone layer, an embryo, and a central endosperm (Wijngaard & Arendt, 2006) (Fig 1.1). After dehulling, whole groats may be cooked and consumed in the form of porridges. They may also be roller milled to yield flour of different seed fractions, each having its unique nutritional and functional properties.

Light flour, also referred to as 'fancy' flour is a grade of flour consisting mainly of the starchy endosperm fraction. This is the most common type of flour produced from buckwheat. Flour from buckwheat is used for products such as noodles, pancakes and baked goods like bread, cakes and biscuits (Campbell, 1997; Rayas-Duarte et al., 1998; Steadman et al., 2001a). Research has shown that the bran, which consists of the aleuronic and embryonic tissues, is by far the richest milling fraction in macronutrient and dietary fibre content (Steadman et al., 2001a).

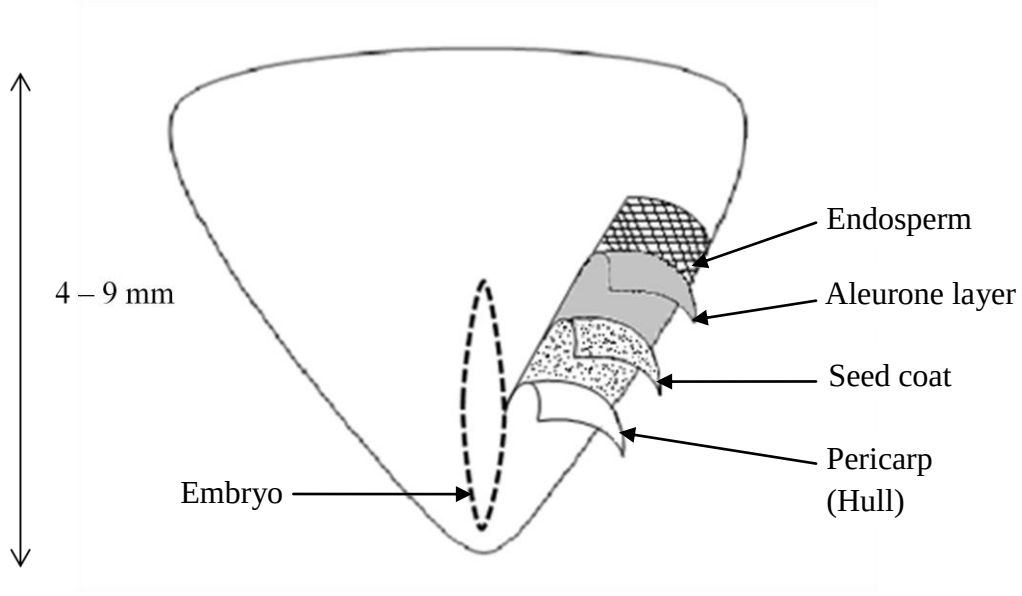

Figure 1.1. Buckwheat grain anatomy

1.3.3 Nutritional and functional properties of buckwheat grain and grain products

The macronutrient and chemical composition of buckwheat groats and milling fractions have been widely reported. Carbohydrates, proteins, lipids, minerals, and phytochemicals are all present in the achene (Campbell, 1997; Bonafaccia et al., 2003; Li & Zhang, 2001; Pomeranz & Robbins, 1972; Steadman et al., 2001a; Wijngaard & Arendt, 2006; Krkošková, & Mrazova, 2005). The levels of these components vary among cultivars, and environmental factors also play a part. For many decades, the nutritional superiority of the buckwheat grain over many traditional cereal grains has been discussed in terms of its protein quantity and well-balanced amino acid composition. Proteins are located in the aleurone and embryo of buckwheat grains. Unlike cereal grains which are limiting in the essential amino acid lysine, buckwheat is rich in lysine as well as arginine and aspartic acid (Pomeranz & Robbins, 1972). Buckwheat is, however, limiting in threonine and methionine, which are rich in cereals.

Bonafaccia (2003) reported levels of protein in grain, bran and flour of common buckwheat to be 11.7%, 21.6% and 10.6%, respectively, on a dry weight basis. Such is the excellent protein content that buckwheat flour is second only to oat flour in the cereal grain world (Li & Zhang, 2001). Eggum et al. (1980), however, found no significant difference in protein content between buckwheat and wheat grains. In buckwheat seeds, storage proteins consist mainly of globulins (64.5%) and albumins (12.5%), as well as a small amount of glutenin (8%) and prolamins (2.9%) (Ikeda et al., 1991). Buckwheat contains no gluten; therefore its products are well suited for the nutrition of celiac sufferers.

Groats contain about 67 to 70% carbohydrate, of which 55% is starch (Steadman et al., 2001a). This same study found levels of starch in fancy flour to be six times higher than in bran. Apart from starch, buckwheat groats also contain soluble carbohydrates mainly in the form of sucrose and fagopyritols (Steadman et al., 2000). These are quadrupled in the bran.

Buckwheat also contains minerals such as potassium, iron, magnesium, manganese, zinc, copper (Amarowicz & Fornal, 1987) and B vitamins (Bonafaccia et al., 2003). In the seed, about 80% of lipids are in the form of unsaturated fatty acids and over 50% of these constitute the polyunsaturated essential fatty acid linoleic acid (Steadman et al., 2001a). Other fatty acids present are oleic, palmitic, linolenic and stearic acids.

Buckwheat is known to have preventive and curative effects against certain chronic diseases such as diabetes (Kawa et al., 2003; Larner, 2002; Ortmeyer et al., 1995; Steadman et al., 2000), hypertension (Matsubara et al., 1985) and hypercholesterolemia (Sugiyama et al., 1985). It has also been recommended as a potential ingredient in the

formulation of low glycemic index foods (Skrabanja et al., 2001). These claims are the result of the numerous and in most cases, unique bioactive compounds present in the grain and other tissues of the plant.

The unique quality of buckwheat proteins, as mentioned earlier, has led to a lot of research into its health promoting properties. Following the work done by Pomeranz & Robbins (1972) on the amino acid characterization of buckwheat proteins, further studies have established that the sulphur-containing amino acids (cysteine and methionine) in buckwheat promote hypocholesterolemic activity (Sugiyama et al., 1985). Proteins with low lysine/arginine as well as methionine/glycine ratios are also believed to possess this cholesterol-lowering effect, although the mechanism is not fully understood (Li & Zhang, 2001). Through experiments with rats, this cholesterol-lowering effect was demonstrated using BWPE (Kayashita et al., 1995; Tomotake et al., 2000, 2001; Tomotake et al., 2006). The prophylactic properties of buckwheat protein extract (BWPE) have been compared with those of soy protein isolate (SPI) (Tomotake et al., 2002). Both have been shown to be effective in lowering blood cholesterol levels due to their uniquely low lysine/arginine and methionine/glycine ratios (Kritchevsky, 1979). Kayashita et al. (1995) later reported that BWPE had a better cholesterol-lowering effect than SPI because it has lower values for these ratios. Despite the excellent protein content of buckwheat, digestibility remains an issue (Ikeda & Kishida, 1993). Nevertheless, Kayashita et al. (1997), reports that this seemingly negative attribute could actually be a contributing factor to the cholesterol-lowering effect of BWPE.

Both D-chiro-inositol and its galactosyl derivatives, fagopyritols, have proven successful in lowering serum glucose levels in animal studies, and have therefore been

proposed for the treatment of diabetes (Kawa et al., 2003; Larner, 2002; Ortmeyer et al., 1995; Steadman et al., 2000).

One major phytochemical believed to be responsible for a wide array of health benefits is dietary fibre (DF) (Champ et al., 2003). Dietary fibre according to the American Association of Cereal Chemists International (AACC), is defined as the "*edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation*." Epidemiological evidence points to a link between the consumption of natural foods rich in dietary fibre (DF) and the reduced incidence of coronary heart disease (Liu et al., 1999; Wolk et al., 1999), as well as type-2 diabetes (Salmeron et al., 1997). Fibre content in buckwheat seeds, bran and flour was found to be approximately 27, 26 and 7%, respectively (Bonafaccia et al., 2003). Since fibre is mostly concentrated in the seed coat and hull, which comprise the outer tissues of the buckwheat grain, dehulled seeds could contain as low as 7% fibre and concentrations in fancy flour could be five to ten times lower than in bran (Steadman et al., 2001a). In all, buckwheat seeds contain about 70% more DF than wheat grains (Alvarez-Jubete et al., 2009). Skrabanja & Kreft (1998) found total starch content in autoclaved buckwheat groats to be 73.5% and resistant starch content to be 33.5% of this amount.

Buckwheat seeds and plant tissues are good sources of many phenolic compounds, particularly phenolic acids and flavonoids (Holasova et al., 2002; Oomah &

Mazza, 1996; Przybylski et al., 1998). These compounds are present in both free and bound forms and their levels are influenced by environmental factors and cultivar (Kitabayashi et al., 1995; Ohsawa & Tsutsumi, 1995; Oomah et al., 1996). Buckwheat is renowned for its high content of rutin, a flavonol glycoside (Figure 1.2). It is one of the few primary dietary sources of rutin. In groats, however, only low levels of between 0.02% and 0.03% on a dry weight basis have been found (Kreft et al., 2006; Steadman et al., 2001b). Higher levels of rutin can be attained in specific milling fractions as well as in leaves. Morishita et al. (2007) also found rutin content in tartary buckwheat seeds to be over a hundred times more than in common buckwheat. In Japan, Ohsawa & Tsutsumi, (1995) explored the possibility of increasing the rutin content of buckwheat through breeding programs owing to its importance in the functional food industry. Extensive research has established that rutin is beneficial in the treatment and prevention of rheological disorders of the blood such as capillary fragility which is associated with hypertension (Matsubara et al., 1985), as well as atherosclerosis (Wojcicki et al., 1995). It is also believed to be effective in reducing fasting blood glucose levels and increasing insulin levels (Hao et al., 2012; Yildizogluari et al., 1991). Catechins are also present in buckwheat seeds (Watanabe, 1998). Many of these phenolic compounds found in buckwheat including rutin possess antioxidant activity (Holasova et al., 2002; Sun & Ho, 2005; Watanabe, 1998; Watanabe et al., 1995).

Antioxidants are important for their protective effect against oxidative stress-related damage of cells which is associated with several degenerative diseases (Griendling & FitzGerald, 2003; Molavi & Mehta, 2004). In foods, they protect against rancidity leading to prolonged shelf life. The yield of extraction of phenolic compounds

depends on factors such as the chemical nature of the compounds, the type of solvent, as well as the time and temperature of extraction (Sun & Ho, 2005). The highest antioxidant activity in buckwheat grains was recorded in methanol extracts (Holasova et al., 2002; Przybylski et al., 1998; Sun & Ho, 2005).

Other unique functional components found in trace quantities within buckwheat seeds are summarized in Table 1.1. These include phytosterols, fagopyrins and thiamin-binding proteins, the latter being responsible for improving thiamine bioavailability (Mitsunaga et al., 1986). Phytosterols are known to inhibit cholesterol absorption (Lees et al., 1977; Mattson, Grundy, & Crouse, 1982), strengthen the immune system (Bouic, 2002), and possess anti-tumor properties (Awad & Fink, 2000). It has been established that on the whole, tartary buckwheat contains more nutrients and phytochemicals than common buckwheat (Bonafaccia et al., 2003).

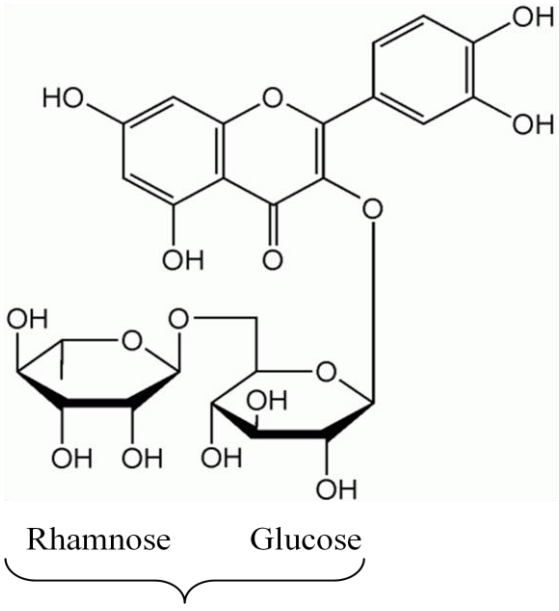

Figure 1.2. The chemical structure of rutin (quercetin 3-rutinoside)

Table 1.1. Physiological benefits of buckwheat

Bioactive components	Physiological benefit
Good quality proteins and balanced amino acid profile	Cholesterol-lowering effect (Kritchevsky, 1979)
Dietary fibre	Laxation, cholesterol & blood glucose attenuation (DeVries, 2003)
Fagopyrins	Attenuation of blood glucose (Kawa et al., 2003; Larner, 2002; Ortmeyer et al., 1995; Steadman et al., 2000)
Phytosterols	Inhibition of cholesterol absorption (Lees et al., 1977; Mattson, Grundy, & Crouse, 1982)
Thiamine-binding proteins	Improvement of thiamine bioavailability (Mitsunaga et al., 1986)
Rutin	Treatment and prevention of rheological disorders of the blood, eg. Hypertension (Matsubara et al., 1985; Wojcicki et al., 1995)
Other phenolic compounds	Anti-inflammatory and antioxidant properties (Holasova et al., 2002; Griendling & FitzGerald, 2003; Molavi & Mehta, 2004)

1.3.4 Dietary fibre and glycemic impact

Perhaps one of the most common global risk factors for ill health amongst both adults and children is obesity. Recent reports claim that the prevalence of obesity has reached epidemic proportions. Obesity and overweight are defined as body mass index (BMI) equal to or greater than 30 kg/m^2 and 25 kg/m^2, respectively (Guo et al., 2002). In 2010, more than half (52%) of the Canadian adult population aged 18 and above were regarded as either overweight or obese (Statistics Canada, 2012). The situation looks grim for children as well; between 2009 and 2011, close to a third of children aged between 5 and 17 years were classified as overweight or obese (Roberts et al., 2012). The increase in the consumption of energy-dense/high glycemic index (GI) foods coupled with the sedentary lifestyles of modernity, has put many at risk of developing overweight related illnesses such as diabetes (Pereira et al., 2005) and cardiovascular disease (Poirier & Eckel 2002; Van Gaal et al., 2006). Due to the expensive nature of healthcare delivery these days, a dietary solution is a more economical approach to tackling the obesity menace and its related illnesses compared to treatment (Brennan, 2005).

The AACC International defines Glycemic impact (GIm) as "*the weight of glucose that would induce a glycemic response equivalent to that induced by a given amount of food*" (Monro & Shaw, 2008). This concept was derived from the more familiar concept of GI, which is defined as "the total glycemic response in the 2 h immediately subsequent to the consumption of 50 g of carbohydrates" (Roberts, 2000). Foods ranked high on the GI scale are generally those with high carbohydrate content and high rates of digestibility (Roberts, 2000). Thus, the GIm of a food is a fair reflection of its GI, since the GI ranks foods based on their GIm. High GI foods release more glucose

during digestion and elevate postprandial blood glucose levels. The consumption of high GI foods has also been shown to increase hunger and cause overeating (Roberts, 2000). For people suffering from diabetes, reliance on diets with low glycemic responses is very crucial.

The digestibility of carbohydrates is based on their relative susceptibility to amylolytic activity. Rapidly digested carbohydrates are the quickest to be broken down into simple sugars, while slowly digestible carbohydrates, although eventually hydrolysed, are more resistant to enzyme activity. On the other hand, carbohydrate material that escapes digestion (dietary fibre) is now a topic of huge interest in the quest to influence the digestibility of our foods through processing. Increased DF consumption has been proposed as a solution to the negative effects of unhealthy eating (Jones, 2004). Different daily intakes of fibre have been recommended in different countries. In the United States and Canada, the Institute of Medicine has set 25 g and 38 g of fibre per day for women and men, respectively.

Dietary fibre has the ability to minimize the rate and extent of carbohydrate breakdown in the upper intestinal tract, leading to a gradual release of blood glucose after meals (Brennan, 2005). One mechanism proposed for this is that DF results in stomach distension which causes and maintains satiety for an extended period of time. In particular, soluble fibres delay gastric emptying by absorbing water and forming gels (Howarth et al., 2001). This causes a delay of movement of digesta through the gastrointestinal tract, giving a perception of satiety and thereby delaying hunger and minimizing the urge to eat. Gels also minimize access of digesta to digestive enzymes

and reduce glucose absorption. In addition, DF lowers the energy density of food by acting as a dilution factor (Howarth et al., 2001).

One component of dietary fibre that has huge implications on carbohydrate digestibility of processed foods is resistant starch (RS). Englyst et al. (1992) defines RS as the fraction of starch, which escapes digestion in the small intestine, and may be digested in the large intestine. Resistant starch can be put into three categories, depending on the factors responsible for their resistance to amylolytic degradation: physically inaccessible starch (RS_1), ungelatinized starch (RS_2) and retrograded starch (RS_3). There is also a fourth group of chemically modified starches (RS_4). Resistant starch that is of interest when studying cereal grain and grain products are RS_1 and RS_3, respectively (Englyst et al., 1992). Physically inaccessible starch is typical of whole or partly milled grains as a result of starch granules being entrapped within the food matrix (Liu, 2007). For example, starch granules entrapped within a dense network of proteins which results in limited or no exposure to starch degrading enzymes. In the formation of retrograded starch, the chemical structure and composition of starch plays a very important role. RS_3 is formed through an initial gelatinization of starch during hydrothermal treatment and a subsequent cooling. This results in the re-association of starch polymers, particularly amylose, into tightly packed helical structures that are stabilized by hydrogen bonding and are heat-stable (Haralampu, 2000). Almost all cooking processes of starch and starch products involve some level of hydration, this, and for the fact that cereal grain starches contain between 20 and 30% of amylose (Jane et al., 1999; Tester & Morrison, 1990), makes retrograded starch the most important group of resistant starches in cereal grain products. Unlike amylose, amylopectin chains do not favour retrogradation because they

have a lower degree of polymerization, between 20 and 40, compared to about 100 for amylose. In addition, the branches of amylopectin interfere with the re-association process during cooling (Haralampu, 2000).

The influence of fibre on the carbohydrate digestibility and postprandial blood glucose levels has been brought to light in many *in vitro* studies (Brennan et al., 2004; Brennan & Samyue, 2004; Brennan et al., 2012; Brennan et al., 2008) and human studies (Cummings et al., 1996; Raben et al., 1994; Reader et al., 1997; Wisker & Feldheim, 1990; Wisker et al., 1992).

Buckwheat and cereal grains are among the best sources of natural fibres and will be useful ingredients in manufacturing food products that can help minimize glucose release during digestion. Skrabanja et al. (2001) reported that a 50% substitution with buckwheat flour was able to retard the carbohydrate digestibility of white wheat bread and lower the glycemic index by more than 30%. Buckwheat was also able to increase satiety compared to the control in the same study.

CHAPTER 2: Phenolic Profile and Antioxidant Properties of Durum Spaghetti Enriched with Commercial Buckwheat Flour and Bran

ABSTRACT

Spaghetti is a popular dish the world over which is particularly known for its high carbohydrate content. It is, however, deficient in other nutrients and protective substances, and therefore is a suitable product to which value can be added. This study investigated the effect of common buckwheat Supreme flour and bran Farinetta supplementation of semolina on the phenolic and antioxidant properties of spaghetti, as well as the effect of processing and cooking on these properties. Up to 40% substitution was achieved. Seven typologies of spaghetti were manufactured in addition to 100% semolina spaghetti as control. One hundred percent semolina spaghetti (RefA) and whole buckwheat soba noodles (RefB) were commercially obtained and used as references. Total phenolic content (TPC), total flavonoids content (TFC), antioxidant capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC), as well as HPLC analysis of rutin and ten other phenolic compounds were carried out. The effects of processing and cooking were also investigated. There were huge increments of between 114 and 522% for TPC, 50 and 242% for TFC, 359 and 1000% for DPPH antioxidant activity, and 101 and 197% for ORAC values in uncooked experimental spaghetti samples RefA. Samples containing the largest amounts of Farinetta had the highest scores. In addition to rutin, all ten phenolic compounds analysed were present in Supreme flour and Farinetta. The presence of buckwheat in samples saw

the introduction of five out of the ten phenolic compounds, among these was rutin. With the exception of ferulic acid, the concentration of all other phenolic compounds increased with the addition of buckwheat. Processing did not cause any significant losses in TPC, but losses ranging from 1.2 to 33.7% in TFC and 42.0 to 55.3% in DPPH antioxidant activity were incurred. Cooking generally resulted in significant losses ($p < 0.05$) of up to 39% in TPC, 40% in DPPH antioxidant activity, 22% in rutin concentration, and 55% in TFC among all buckwheat-containing products. Results show that low levels of substitution with buckwheat flours and bran can be successfully used in the manufacture of spaghetti with enhanced phenolic and antioxidant properties even after cooking.

2.1 Introduction

Spaghetti is manufactured and consumed all over the world. Its success and popularity has been ascribed to its ease of storage, handling, transportation and cooking (Tudorica et al., 2002). The presence of semolina as the sole raw material makes the product less nutritious since it is only rich in complex carbohydrates (Giese, 1992). Over the years, manufacturers have sought to add value by including other ingredients to enhance the nutritional and bioactive profile. Research has proposed the use of ingredients such as bean flour (Rivas et al., 2012; Gallegos-Infante et al., 2010), pseudo cereals (Schoenlechneret al., 2010); Caperuto et al., 2001) and fibre (Edwards et al., 1995; Gelencser et al., 2008; Tudorica et al., 2002) just to name a few.

The necessity to improve upon the nutraceutical quality of foods has been spurred by growing awareness of the benefits of eating healthy. Consumers are now keen on choosing foods and food ingredients that do not only sustain nutrition but also promote

health. Attention has recently been drawn to the protective benefits of physiologically-active plant metabolites such as phenolic and antioxidant compounds which have been strongly associated with the reduced risk of chronic diseases such as heart disease, some cancers (Block et al., 1992; Shahidi, 2004) and diabetes mellitus (Ford & Mokdad, 2001).

Common buckwheat (*Fagopyrum esculentum* Moench) is a dicotyledonous crop which has similar structural properties and usage to cereal grains. It has been a traditional crop in Asia for centuries, but is now cultivated globally, especially in the northern hemisphere. Buckwheat is mainly cultivated for its seeds which are milled into flour and used in products such as bread, biscuits, pancakes, ready-to-eat breakfast cereals and soba noodles (Steadman et al., 2001a; Rayas-Duarte et al., 1998).

The health-promoting properties of buckwheat stem from a number of factors: it has excellent protein content and a well balanced amino acid composition (Pomeranz & Robbins, 1972). Buckwheat protein extract has been compared to soy protein isolate and found to be more potent in lowering blood cholesterol levels due to its uniquely low lysine/arginine and methionine/glycine ratios (Kritchevsky, 1979). It has therefore been proposed for the prevention and treatment of conditions such as hypertension and hypercholesterolemia (He et al., 1995; Kayashita et al., 1995). Buckwheat is also a rich source of phytochemicals.

Phenolic compounds have been extracted from the leaves, seeds and hulls of buckwheat. Buckwheat is one the most important dietary sources of rutin (Kreft et al., 2006). Depending on the variety, buckwheat hulls contain more than three times the flavonoid concentration in seeds, and about one and-a-half times more rutin, with levels

averaging 77 and 47 mg/10 0g respectively (Oomah & Mazza, 1996). Several other phenolics present in the seeds and hulls include quercetin and phenolic acids (Watanabe et al., 1997), flavanols such as catechins (Watanabe, 1998) and proanthocyanidins (Quettier-Deleu et al., 2000). Many of these compounds have also been reported in buckwheat flour and have been shown to possess antioxidant activity in vivo (Holasova et al., 2002; Watanabe et al., 1997). Antioxidants are important for their protective effect against oxidative stress-related damage of cells which is associated with several degenerative diseases (Griendling & FitzGerald, 2003; Molavi & Mehta, 2004). In addition, buckwheat is rich in B-vitamins, lipids and minerals, making it an important functional food ingredient (Ötles & Cagindi, 2006; Krkošková & Mrazova, 2005; Bonafaccia et al., 2003; Li & Zhang, 2001; Steadman et al., 2001a; Wijngaard & Arendt, 2006).

Despite the fact that cereal grains are important sources of antioxidant compounds, alternate crops such as buckwheat can be more nutritious and offer even more elevated levels of these bioactive compounds, and as such, can be exploited in complementing cereals grains in the production of functional foods.

Although there are studies which have investigated the phenolic profile of buckwheat flours (Inglett et al., 2011; Van Hung & Morita, 2008) as well as the effect of buckwheat flours on the quality of spaghetti (Manthey et al., 2004; Schoenlechner et al., 2010), there seems to be no information on the phenolic and antioxidant properties of spaghetti enriched with buckwheat flour and bran. In this study, the potential of two commercial buckwheat milling products obtained from the endosperm, hull, embryo and

aleurone, for improving the phenolic profile and antioxidant properties of durum spaghetti was investigated. The effects of cooking were also examined.

2.2 Materials and methods

2.2.1 Raw materials

Durum semolina flour was obtained from the local market (Winnipeg, MB, Canada). Common buckwheat (*Fagopyrum esculentum)* Supreme flour (comprising percentages of hull and endosperm) and bran Farinetta (mixture of aleurone layer of hulled seed and seed embryo) were purchased from Minn-Dak Growers, Ltd. (Grand Forks, ND, USA).

2.2.2 Chemicals

HPLC grade methanol, ethyl acetate, acetic acid, acetonitrile and phosphoric acid, as well as sodium hydroxide, sodium nitrite, aluminium chloride, sodium bicarbonate, hydrochloric acid and anhydrous sodium sulfate were all purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Phenolic standards, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) and Folin-Ciocalteu were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Fluorescein and 6-hydroxyl-2,5,7,8-tetramethychromane-2-carboxylic acid (Trolox) were purchased from Fisher Acros Organics (Morris Plains, NJ, USA).

2.2.3 Spaghetti processing

Seven different typologies of spaghetti were manufactured by substituting semolina with different amounts of buckwheat Supreme flour, Farinetta or both. Between 10 and 40% w/w of substitutions were reached (Table 2.1). Products substituted with Supreme flour alone were denoted by the code 'nS', where 'n' represents the percentage substituted and 'S' stands for Supreme flour. Similarly, 'nF' was used for samples substituted with only Farinetta. Samples containing both components were denoted by the code 'nSnF'. Processing was achieved with a twin screw extruder (APV Baker Ltd, Peterborough, England, UK) fitted with a 2-mm die. One hundred percent semolina spaghetti was also manufactured to serve as the control. The substitution amounts were predetermined and based on achieving a compromise between nutritional/nutraceutical enhancement and spaghetti quality. The flours were first mixed manually before being loaded into the hopper. Water was pumped at a rate between 0.614 to 0.614 L/h depending on the absorptive capacity of the dough mixtures in order to achieve a desirable moisture content of approximately 30%. A summary of the processing parameters of extrusion are presented in Table 2.2. Extruded products were dried for 18 h in a Moffat® convection oven (Model ECO-3, DeltaRex Canada Inc. Toronto, ON, Canada) preheated to a temperature of 80 ºC and allowed to cool to room temperature. The dried spaghetti samples were broken into lengths of 10 cm, kept in zip-lock bags and stored at -18 ºC.

Two commercial products were used as reference samples and analysed alongside the experimental samples. The former included RefA- 100% durum spaghetti (ARRIGHI®) purchased from Canada Safeway Limited (Winnipeg, MB, Canada), and

RefB- 100% whole buckwheat soba noodles (EDEN FOODS®) purchased from Well Canada online store.

Table 2.1. Flour formulations, cooked and uncooked spaghetti samples

Number	Sample Code	% Durum semolina	% Supreme	% Farinetta
1	Control[a]	100	0	0
2	20S[a]	80	20	0
3	10S-10F[a]	80	10	10
4	20F[a]	80	0	20
5	30S[a]	70	30	0
6	15S-15F[a]	70	15	15
7	30F[a]	70	0	30
8	20S-20F[a]	60	20	20
9	RefA[b]	na	na	na
10	RefB[b]	na	na	na
11	100S[c]	0	100	0
12	100F[c]	0	0	100

[a] Code represents flour formulations, uncooked spaghetti and cooked spaghetti
[b] Code represents both uncooked and cooked commercial reference spaghetti samples
[c] Code represents only flour samples
Abbreviations: F, Farinetta; S, Supreme flour; RefA, commercial 100% semolina spaghetti; RefB, commercial 100% whole buckwheat soba noodles; na, not applicable

Table 2.2. Processing parameters of extrusion

Spaghetti code	Product temperature (°C)	Die pressure (bar)	Torque (%)	Screw speed (rpm)	Feed rate (kg/h)	Water injection (L/h)
Control	41	21	31	30	2.0	0.748
20S	45	33	52	30	2.0	0.631
10S-10F	45	22	32	30	2.0	0.696
20F	45	36	51	30	2.0	0.614
30S	45	25	39	30	2.0	0.696
15S-15F	45	19	31	30	2.0	0.696
30F	45	17	28	30	2.0	0.614
20S-20F	44	18	22	30	2.0	0.696

2.2.4 Proximate analyses of raw materials

Moisture content was determined using the Gravimetric Method 44-15 A, AACC (2000). Ash content was determined according to Method 08-12 of the AACC (2000). Crude protein analysis was performed using the Kjeldahl procedure outlined by Serna-Saldívar (2012). Samples were digested with concentrated sulphuric acid and then distilled with excess alkali. Liberated ammonia was trapped in boric acid and titrated against standard HCl solution. A conversion factor of 5.7 was used in computing % protein.

2.2.5 Preparation of cooked samples for analyses

Cooking of spaghetti followed the procedure outlined by Manthey et al. (2004). About 10 g of spaghetti was broken into lengths of about 5 cm and added to 300 mL of boiling distilled water in a beaker. Optimum cooking was achieved at 12 min after the disappearance of the inner white core as set out in the Approved Method 66-50 (AACC 2000). The cooked spaghetti was then drained with a Buchner funnel, frozen at -18 °C and then freeze-dried. Freeze-dried samples were then milled with a multi-use blade grinder, model PCC770 (Loblaws Inc., Brampton, ON, Canada) and stored at -18 °C for further analysis.

2.2.6 Extraction of antioxidants and phenolics

2.2.6.1 Extraction of antioxidant compounds

The extraction of antioxidant compounds from flour blends, uncooked and cooked spaghetti were done as follows: to 2 g of sample, 20 mL of acidified methanol (HCl : methanol : water, 1:80:20) was added in 50 mL centrifuge tubes and shaken at room temperature for 2 h using a Wrist Action Shaker, model 75 (Burrell Scientific, Pittsburgh, PA, USA). The mixture was then centrifuged at 5 °C for 15 min at 7,800 x g (Sorvall RC-6 Plus Centrifuge, Thermo Fisher Scientific Inc., Asheville, NC, USA) and the supernatant collected and used for the analysis of DPPH radical scavenging activity, ORAC, and total phenolics assays. Extractions were done in triplicate.

2.2.6.2 Extraction of phenolic acids

Phenolic acids for HPLC analysis were extracted following the method described by Hirawan et al. (2010). Two grams of each group of samples was hydrolysed with 60 mL of 4 M NaOH for 4 h. Oxidation was minimized by infusing nitrogen gas every hour for 5 min. Using 6 M HCl, the pH of the mixture was lowered to between 1.5 to 2 units before centrifuging at 5 °C for 20 min at 7,800 x g (Sorvall RC-6 Plus Centrifuge, Thermo Fisher Scientific Inc., Asheville, NC, USA). The supernatant was collected and extracted three times with ethyl acetate using a total volume of 70 mL. The extracts were pooled together and dehydrated with anhydrous sodium sulphate before being evaporated to dryness using a rotary vacuum evaporator (IKA RV10, IKA® Works Inc., Wilmington,

NC, USA). Reconstitution was done with 5 mL of 50% methanol and then filtered through a 0.45 µm PTFE filter. Extractions were done in duplicate.

2.2.6.3 Extraction of rutin and flavonoids

Extractions were done in accordance with Vogrincic et al. (2010). To 1 g of each group of samples, 25 mL of 80% methanol was added and shaken at room temperature for 8 hours with a Wrist Action Shaker, model 75 (Burrell Scientific, Pittsburgh, PA, USA). Extracts were filtered through a Whatman^{TM} No. 4 filter paper. For HPLC analysis of rutin, extracts were further filtered through a 0.45 membrane filter. Extractions were done in duplicate.

2.2.7 Analysis of antioxidant activity and phenolic compounds

2.2.7.1 Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity using the DPPH method was employed from Beta et al. (2005). To 0.1 mL of extract as prepared in section 2.2.6.1, 3.9 mL of DPPH working solution (60 µmol/L) was added and the absorbance read at 515 nm at 0 and 30 min with an Ultraspec 1100 pro, UV/Visible spectrophotometer (Biomicron Ltd. Cambridge, England, UK). Methanol was used as blank. DPPH radical scavenging activity (%) was calculated according to the equation: $(1 - [A_{sample}/A_{control,t=0}] \times 100)$. A standard curve of different Trolox concentrations (0, 125, 250, 375, 500 and 1000 µM) versus activity (%)

was constructed and results were expressed as µmol equivalent of Trolox/g. All tests were done in triplicate.

2.2.7.2 Determination of oxygen radical absorbance capacity (ORAC)

The oxygen radical absorbance capacity assay was conducted according to the method of (Li et al., 2007), which had previously been described by Huang et al. (2002). A reaction mixture consisting of 20 µL each of diluted sample extracts as prepared in section 2.2.6.1, buffer solution (blank) and Trolox standard (0, 6.25, 12.5, 25 and 50 µM), as well as 120 µL of fluorescein and 60 µL of 2,2'- azobis (2 - amidinopropane) dihydrochloride (AAPH) serving as a generator of peroxyl radical were all transferred into designated wells of a 96-well flat bottom polystyrene microplate (Corning Incorporated, Corning, NY, USA) in a systematically controlled reagent transfer program delivered by a Precision 2000 automated microplate pipetting system (Bio-Tek Instruments, Inc., Winooski, VT, USA). Fluorescence generated was read every minute for 50 min at 37 °C by an Flx800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) controlled by KC4 3.0 software (version 29). Reaction mixtures and fluorescence measurements were done in triplicate. A regression equation was obtained from a plot of Trolox concentrations versus net area under the fluorescence decay curve (AUC). This was used to calculate ORAC values which were expressed as Trolox equivalents. The AUC was achieved based of the following equation:

$$AUC = 0.5 + \frac{f1}{f0} + \ \ldots + \frac{fi}{f0} + \ \ldots + \frac{f49}{f0} + \ 0.5\frac{f50}{f0}$$

Where f_0 = initial fluorescence reading at 0 min and f_i = fluorescence reading at time I min. Net AUC = AUC (blank) – AUC (sample). Calculated ORAC results were expressed as μmol equivalent of Trolox/g.

2.2.7.3 Determination of total phenolic content

The Folin-Ciocalteau method (Singleton & Rossi, 1965) adapted by Beta et al. (2005) was used in the determination of total phenolic content in all three sample types (flour composites, cooked and uncooked spaghetti). A portion of extract (0.2 mL), as prepared in section 2.2.6.1, was added to 1.5 mL of a 10-fold freshly diluted Folin-Ciocalteau's reagent. After vortexing, the mixture was allowed to equilibrate for 5 min, after which 1.5 mL of sodium carbonate solution (60 g/L) was added with further vortexing. The reaction was allowed to progress at room temperature and away from light for 90 min. Absorbance was read at 725 nm with an Ultraspec 1100 pro, UV/Visible spectrophotometer (Biomicron Ltd. Cambridge, England, UK). Acidified methanol was used as blank. A standard curve of concentration (0, 50, 100, 200, 250, 300 and 400 μg/mL) versus absorbance was constructed using ferulic acid standard. All tests were done in triplicate and results expressed as mg equivalent of ferulic acid/100 g.

2.2.7.4 Determination of total flavonoids content

The total flavonoid content was colorimetrically determined as described by Liu et al. (2002). Extracts (0.25 mL), as prepared in section 2.2.6.3, were diluted with 1.25 mL of distilled water, after which 75 μL of 5% sodium nitrite was added. After 6 min,

150 µL of a 10% aluminium chloride solution was added and the mixture allowed to stand for 5 min. Next 0.5 mL of 1 M NaOH was added and the volume topped to 2.5 mL with distilled water. After mixing the solution, the absorbance was read immediately at 510 nm against a prepared blank using an Ultraspec 1100 pro, UV/Visible spectrophotometer (Biomicron Ltd. Cambridge, England, UK). Measurements were done in duplicate and compared with a rutin standard curve with concentrations: (0, 150, 225, 300, 450 and 600 µg/mL). Results were expressed as mg equivalent of rutin/100 g.

2.2.7.5 HPLC analysis of phenolic compounds

Reverse-phase chromatography was performed using a Waters Alliance 2695 HPLC instrument (Waters, Mississauga, ON, Canada) equipped with a Waters 2996 photodiode array detector. Separation was achieved using a Gemini 5µ C 18 110A guarded column (150 mm × 4.6 mm) (Phenomenex®, Torrance, CA, USA) held at 35 °C. Components were separated with a gradient made up of 0.1% acetic acid in water (solvent A) and 0.1% acetic acid in methanol (solvent B) for 70 min at a flow rate of 0.9 mL/min. The initial compositions of the gradient were set at 91% A and 9% B. Sample volumes of 10 µL each, as prepared in section 2.2.6.2 were injected and detection was set at 280 nm. Phenolic acids were identified by comparing retention times with those of their respective standards: gallic acid, protocatechuic acid, p-hydroxybenzoic acid, (+)catechin, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid and sinapic acid. Quantification was done in duplicate for all samples.

2.2.7.6 HPLC analysis of rutin

Rutin was analysed with a with a Waters Alliance 2695 HPLC instrument (Waters, Mississauga, ON, Canada) equipped with Waters 2996 photodiode array detector. A Gemini 5µ C 18 110A guarded column (150 mm × 4.6 mm) (Phenomenex®, Torrance, CA, USA) was used for separation. A linear gradient of solvent A consisting of acetonitrile and methanol (1:2, vol/vol) and solvent B consisting of 0.75% aqueous phosphoric acid was used to run the samples. From an initial composition of 0% A and 100% B, it run to 60% A and 40% B in 17 min, and then to 100% A and 0% B in a further 3 min before being reverted to 100% B in 2.5 min. An additional 2.5 min period of equilibration was set after this, taking the entire runtime to 25 min. Sample volumes of 10 µL each, as prepared in section 2.2.6.3, were injected. Flow rate was set at 1 mL/min and the detector at 380 nm. Rutin was identified by comparing the retention time with that of a rutin standard. Quantification was done in duplicate for all samples.

2.2.8 Statistical analysis

Data were expressed on a dry weight basis and reported as means ± standard deviations. Using SAS statistical software, version 9.2 (SAS Institute Inc., Cary, NC, USA), a one- way analysis of variance (ANOVA) and means testing (Tukey's range test) were performed with level of significance set at $p < 0.05$.

2.3 Results and discussion

2.3.1 Total phenolic content and antioxidant activity of spaghetti products

The TPC of flour composites, uncooked and cooked spaghetti samples are presented in Table 2.3. It was observed that the addition of buckwheat to semolina significantly ($p < 0.05$) increased TPC in all test samples. The effect was more pronounced with the presence Farinetta. Among the composites, TPC values ranged from 217.1 mg FE/100 g for 20S to 571.7 mg FE/100 g for 20S-20F, while the control was 82.0 mg FE/100 g. A 20% substitution with Farinetta was enough to quadruple TPC of all three controls. At a 40% level of substitution (20S-20F), TPC increased by more than six times compared to the controls. Overall, Farinetta contributed two-fold more TPC than Supreme flour. The acquisition of Farinetta from the aleuronic and embryonic tissues of the buckwheat grain makes it highly concentrated in bioactive compounds. Inglett et al. (2011) also found Farinetta to contain the highest TPC among flours which also included Supreme, whole and fancy flour. In another study, Van Hung et al. (2008) recorded a 30-fold increase in TPC for bran over the endosperm fraction of buckwheat. This indication of a higher TPC in the outer layers of the buckwheat grain was also confirmed by Sedej et al. (2011). In this study, Supreme and Farinetta flours had TPC's of 851.4 and 1,429.3 mg FE/100 g DW respectively. These are strikingly higher than the 726 mg/100 g recorded for buckwheat seeds by Velioglu et al. (1998). Supreme flour constitutes the endosperm of buckwheat enriched by the presence of certain percentages of the hull. It is therefore more phenolic-dense than ordinary buckwheat endosperm flour and can be as potent as whole buckwheat flour.

Statistically, processing did not alter TPC in spaghetti. Contrasting reports were, however, reported by Verardo et al. (2011), who reported a 45.9% decrease in TPC of whole buckwheat spaghetti after processing. Total phenolic contents of 98.2 and 77.1 mg FE/100 g were recorded for the control and the 100% semolina reference spaghetti (RefA), respectively. These values are similar to those obtained for five regular commercial spaghetti samples which averaged 86.5 mg/100 g (Hirawan et al. 2010). The highest TPC was recorded for 30F (611.1 mg/100 g), and this was slightly but statistically lower than the 693.6 mg/100 g recorded for the commercial 100% whole buckwheat pasta (RefB). The fact that spaghetti containing 30% of Farinetta was able to compare favourably with 100% whole buckwheat spaghetti underscores the rich phenolic content of the aleurone layer and embryo of buckwheat.

Cooking significantly ($p < 0.05$) reduced TPC in all spaghetti samples. Although the addition of buckwheat flours resulted in significantly higher TPC in all spaghetti typologies, the average percentage losses in TPC due to cooking were higher in buckwheat-containing spaghetti samples. These ranged from 33.0 to 39.5%. The percentage loss recorded for the control was 31.3%, while that recorded for the whole buckwheat reference pasta (RefB) was 35.2%. These losses incurred are lower than the 53.5% reported by Verardo et al. (2011) after cooking whole buckwheat spaghetti. The reputation of semolina as the best raw material for spaghetti production is largely contributed by its gluten proteins. Gluten provides the foundation of a strong protein network which helps minimize leaching during cooking (Bruneel et al., 2010). Since buckwheat contains no gluten proteins, their substitution for semolina weakened this

network, and led to a greater losses of phenolic compounds through leaching during cooking.

A similar trend in the effect of buckwheat components was observed in antioxidant activity measurements of test samples (Table 2.4.). The higher the substitution with buckwheat, the higher the DPPH radical scavenging activity recorded. Among the composites, 20S scored the lowest DPPH value of 1.34 µmol TE/g while 20S-20F recorded the highest value of 3.33 µmol TE/g. Both were significantly higher than that of the control, with a value of 0.24 µmol TE/g. Similarly, amongst spaghetti products, samples containing the highest amounts of Farinetta recorded the largest DPPH scores. These were 20S-20F and 30F, with scores of 1.87 and 1.77 µmol TE/g respectively. There were no significant differences in DPPH values between these samples and RefB (1.84 µmol TE/g). The average DPPH activity in 20S-20F and 30F was more than ten times that of the control, which scored 0.17 µmol TE/g. This clearly shows the dominance of Farinetta in its level of antioxidant compounds. Processing led to a significant ($p < 0.05$) reduction in DPPH radical scavenging activity ranging from 42.1 to 55.4% among test samples. The control, however, recorded only a decrease of 27.0%. These reductions were observed to increase with increasing levels of buckwheat addition, particularly with Farinetta. This could be the outcome of degradation of antioxidant components during the high temperature drying process. The presence of buckwheat also mitigated losses in DPPH activity incurred after cooking. This is in contrast to what was observed for TPC, and might be explained by the fact that cooking may have eliminated the majority of phenolic compounds in buckwheat which do not necessarily possess antioxidant activity. In other words, the phenolic compounds which possess antioxidant

activity in buckwheat may be more heat-stable than those other compounds which possess little or no antioxidant activity. Also, while cooking may have generally resulted in the loss of antioxidant activity, there may have been a separate regeneration of antioxidant activity during the heating process. There are reports of TPC and antioxidant activity being enhanced after cooking. Turkmen et al. (2005) reported an increase, though not significant, in the TPC of boiled spinach. They also found significant increases in the antioxidant activity in three other vegetables. It is therefore safe to assume that in the case of buckwheat, any increment in TPC due to boiling occurred with a corresponding increase in antioxidant activity, and this helped to mitigate the effect of cooking on the degradation of antioxidant compounds. Cooking resulted in a 22.4% to 40.5% reduction in the DPPH antioxidant activity among buckwheat containing products, while the control and RefB recorded losses of 54.0% and 40.9% respectively. This is understandable, considering the fact that the DPPH antioxidant activity in the control was much lower than those of the test samples even before the cooking process. The antioxidant capacity determined by ORAC showed increments in all experimental samples over the control. These ranged from 100% to 214%. There were no significant differences in ORAC values of spaghetti samples that contained Farinetta (Table2.4.). Yet again, the best performing products were 30F and 20S-20F with ORAC values of 17.04 and 16.11 µmol TE/g, respectively. These were not found to be significantly different from that of RefB (17.51 µmol TE/g). No significant difference was also found between the control and RefA which scored 5.42 and 4.66 µmol TE/g respectively, and are consistent with ORAC values reported by Hirawan et al. (2010) for commercial 100% semolina spaghetti which ranged from 5.6 to 15.9 µmol TE/g.

Table 2.3. Total phenolic content (TPC) of flour composites, uncooked and cooked spaghetti (mg equivalent of ferulic acid/100 g DW)

Sample code	Flour	Uncooked spaghetti	Cooked spaghetti
Control	81.98 ± 4.8^{e}	98.23 ± 1.0^{g}	67.51 ± 3.8^{f}
20S	217.08 ±15.7^{d}	209.86 ± 27.2^{f}	136.60 ± 9.5^{e}
10S-10F	355.73 ± 6.1^{c}	361.08 ± 10.6^{d}	218.62 ± 9.0^{d}
20F	477.66 ±19.8^{b}	474.71 ± 15.9^{c}	301.10 ± 9.8^{c}
30S	289.51 ± 2.7^{c,d}	271.89 ± 17.5^{e}	171.77 ± 6.5^{d,e}
15S-15F	473.91 ± 8.3^{b}	458.57 ± 9.2^{c}	301.10 ± 22.5^{c}
30F	650.55 ±17.0^{a}	611.11 ± 15.7^{b}	409.63 ± 25.5^{a,b}
20S-20F	571.73 ± 80.3^{a}	591.70 ± 29.3^{b}	363.96 ± 30.0^{b}
RefA	na	77.06 ± 3.1^{g}	44.23 ± 1.6^{f}
RefB	na	693.62 ± 5.6^{a}	449.19 ± 49.2^{a}
100S	851.42± 45.6	na	na
100F	1429.34± 23.4	na	na

Values are mean ± standard deviation (n=3). Values with different letters in each column are statistically different at $p < 0.05$ (Tukey's range test)
na, not applicable; DW, dry weight

Table 2.4. DPPH radical scavenging activity and oxygen radical absorbance capacity (ORAC) of flour composites, uncooked and cooked spaghetti samples

Sample code	DPPH (μmol equivalent of Trolox/g DW)			ORAC [y] (μmol equivalent of Trolox/g DW)
	Flour	Uncooked spaghetti	Cooked spaghetti	
Control	0.24 ± 0.02^{g}	0.17 ± 0.02^{d}	0.08 ± 0.01^{f}	5.42 ± 1.1^{c,d}
20S	1.34 ± 0.11^{f}	0.78 ± 0.08^{c}	0.46 ± 0.03^{e}	10.91 ± 1.9^{b,c}
10S-10F	1.85 ± 0.08^{e}	0.86 ± 0.04^{c}	0.65 ± 0.05^{d,e}	13.29 ± 2.5^{a,b}
20F	2.30 ± 0.08^{d}	1.16 ± 0.02^{b}	0.83 ± 0.05^{c,d}	14.64 ± 2.5^{a,b}
30S	1.85 ± 0.05^{e}	0.83 ± 0.01^{c}	0.64 ± 0.07^{d,e}	13.45 ± 1.0^{a,b}
15S-15F	2.48 ± 0.23^{d}	1.26 ± 0.02^{b}	0.92 ± 0.13^{b,c}	13.48 ± 1.4^{a,b}
30F	3.12 ± 0.10^{c}	1.77 ± 0.07^{a}	1.18 ± 0.15^{a}	17.04 ± 2.3^{a}
20S-20F	3.33 ± 0.10^{c}	1.87 ± 0.14^{a}	1.16 ± 0.09^{a}	16.11 ± 1.8^{a,b}
RefA	na	0.20 ± 0.01^{d}	0.07 ± 0.01^{f}	4.66 ± 0.6^{d}
RefB	na	1.84 ± 0.06^{a}	1.08 ± 0.09^{a,b}	17.51 ± 3.2^{a}
100S	5.46 ± 0.20^{b}	na	na	21.67 ± 2.5
100F	7.31 ± 0.11^{a}	na	na	29.88 ± 3.1

Values are mean ± standard deviation (n=3). Values with different letters in each column are statistically different at $p < 0.05$ (Tukey's range test)
[y] ORAC results represent uncooked spaghetti, Supreme and Farinetta flour.
na, not applicable; DW, dry weight

2.3.2 Rutin and total flavonoids concentration in spaghetti products

The rutin concentration of Farinetta and Supreme flours was 264.0 µg/g and 188.9 µg/g, respectively (Table 2.5). In an earlier study by Steadman et al. (2001a) rutin concentrations of 465.0 µg/g and 193.0 µg/g, respectively, for Farinetta and Supreme were reported. Sedej et al. (2010) and Kreft et al. (2006) also found rutin in whole buckwheat flour to be 179.2 µg/g and 218.5 µg/g, respectively. It is worthy of note that differences in bioactive components in similar samples may be the result of environmental and genotypic variations (Mpofu et al., 2006). There was no rutin found in semolina. The highest rutin contents were recorded for samples containing the largest amounts of Farinetta. In spaghetti, 20S-20F and 30F had concentrations of 84.4 µg/g and 71.8 µg/g, respectively. Rutin is highly concentrated in the hull of buckwheat, therefore, the presence of hull in Supreme flour makes it richer in rutin compared to fancy flour, which consists of only the endosperm fraction of the grain. This also explains why the rutin concentration in 20S-20F was higher than that in 30F. Farinetta nonetheless contributed about 50% more rutin than did Supreme flour on an equal weight basis. After processing, rutin content significantly reduced by a range of 6.7 to 14.9% in experimental samples. Reports have been made of the presence of rutin degrading enzymes in buckwheat seeds (Yasuda and Nakagawa, 1994) which are activated upon milling and are responsible for heavy losses of rutin in flour in the presence of water during processing. This was confirmed by Kreft et al. (2006) who reported a decrease from 218 to 78 µg/g after manufacturing noodles from dark buckwheat flour. Vogrincic et al. (2010) also reported an 85% reduction in rutin in tartary buckwheat flour after mixing with water. The rutin content of RefB was significantly higher than all experimental samples with

132.7 µg/g. Significant losses in rutin were observed after cooking. The higher the substitution with buckwheat, the higher the rutin losses incurred. These ranged from 5.9% in 20S to 22.5% in both 30F and 20S-20F.

The analysis of total flavonoids content (TFC) showed that Farinetta contained more than twice the amount in Supreme flour (Table 2.6.). Samples with higher Farinetta amounts therefore had higher TFC. The highest concentration was found in 30F with 590.1 mg/100 g, which was higher than the control (172.7 RE mg/100 g) and RefB (395.7 RE mg/100 g). Statistical analysis showed that TFC in spaghetti were significantly ($p < 0.05$) lower than their corresponding flours. This could possibly be contributed by degradation from elevated temperatures during drying. High temperature drying however is essential for achieving better quality spaghetti products as it contributes to the formation of a stronger starch-protein network (Bruneel et al., 2010) and the production of a more brightly coloured and attractive finished product (Dexter et al., 1981). Analysis of hydrolyzed extracts of buckwheat flours confirmed that the majority (72%) of bound phenolics present in buckwheat flours were flavonoids, while the free phenolic fraction consisted of 60% flavonoids (Verardo et al., 2011). Cooking also significantly reduced the TFC by as much as 72.4% in the control. In buckwheat-containing products, losses ranging from 20.2% for 15S-15F to 54.8% in 20S were recorded after cooking. It appears that the presence of Farinetta mitigated these losses. Six different flavonoids were reported in buckwheat grains by Dietrych-Szostak & Oleszek (1999): rutin, orientin, vitexin, quercetin, isovitexin, and isoorientin. Out of these, only rutin and isovitexin were found present in dehulled grains. Reports on TFC in buckwheat grains have been very contradictory. Dietrych-Szostak and Oleszek (1999) reported TFC in dehulled seeds and

hulls to be 18.8 mg/100 g and 74.0 mg/100 g respectively. On the other hand, Oomah and Mazza, (1996) found TFC of four different common buckwheat cultivars to range between 37.2 mg/100 g and 40.8 mg/100 g in seeds and 121.3 mg/100 g and 146.4 mg/100 g in hulls. They attributed the variations to differences in growing seasons and location. Surprisingly, Inglett et al. (2011) found no significant difference in TFC between Farinetta (106.1 mg/100 g), Supreme flour (105.6 mg/100 g) and whole flour (103.1 mg/100 g), using 50% ethanol as solvent. These results are much lower than those obtained in this study. Farinetta which is made up of fractions from the outer layers of the kernel is expected to be far richer in phenolic content than the hull-containing Supreme flour. The type of solvent used for extraction affects the yield of phenolic compounds. Polar solvents have been shown to be more effective in the extraction of antioxidant and phenolic compounds than non polar ones. In this study, extraction was achieved by using 80% methanol (v/v). Methanol was found to be the most potent solvent of extraction of flavonoids by Przybylski et al (1998) who found over a 400% increase in TFC of buckwheat seeds extracted using methanol when compared with acetone. When compared with water, 80% methanol extracted over 60 times the total phenolics in whole grain buckwheat (Zielinski & Kozlowska, 2000).

Table 2.5. Concentration of rutin in flour composites, uncooked and cooked spaghetti samples (µg/g DW)

Sample code	Flour	Uncooked spaghetti	Cooked spaghetti
Control	nd	nd	nd
20S	35.63 ± 1.33^{e}	30.60 ± 0.93^{g}	26.07 ± 1.20^{e}
10S-10F	44.62 ± 3.38^{e}	37.95 ± 2.38^{f,g}	35.72 ± 3.94^{d}
20F	53.83 ± 3.03^{d}	47.44 ± 2.24^{e,f}	37.69 ± 1.54^{d}
30S	53.93 ± 1.40^{d}	50.10± 0.32^{d,e}	39.67 ± 1.44^{d}
15S-15F	66.15 ± 1.28^{c}	61.13 ± 2.73^{c,d}	49.36 ± 0.10^{c}
30F	79.68 ± 0.19^{b}	71.75 ± 3.43^{c}	55.61 ± 0.14^{c}
20S-20F	90.54 ± 3.91^{a}	84.44 ± 7.54^{b}	65.44 ± 2.05^{b}
RefA	na	nd	nd
RefB	na	132.74 ± 0.23^{a}	119.91 ± 1.73^{a}
100S	188.90 ± 3.99	na	na
100F	264.05 ± 3.80	na	na

Values are mean ± standard deviation (n=2). Values with different letters in each column are statistically different at $p < 0.05$ (Tukey's range test).
nd, not detected, na, not applicable; DW, dry weight.

Table 2.6. Total flavonoids content (TFC) of flour composites, uncooked and cooked spaghetti (mg equivalent of rutin /100 g DW)

Sample code	Flour	Uncooked spaghetti	Cooked spaghetti
Control	220.53 ± 0.31^{c}	172.73 ± 20.09^{d,e}	47.70 ± 24.22^{d,e}
20S	259.61 ± 2.82^{c}	260.37 ± 27.77^{c,d,e}	117.64 ± 2.02^{c,d,e}
10S-10F	377.24 ± 28.52^{b,c}	305.66 ± 29.32^{c,d,e}	222.98 ± 2.02^{b,c,d}
20F	514.01 ± 46.55^{a,b}	421.56 ± 87.87^{a,b,c}	331.39 ± 59.03^{a,b}
30S	358.02 ± 17.18^{b,c}	237.29 ± 49.24^{c,d,e}	144.71 ±20.20^{b,c,d,e}
15S-15F	484.63 ± 114.58^{a,b}	349.38 ± 60.70^{b,c,d}	278.81 ± 40.36^{a,b,c}
30F	642.62 ± 82.68^{a}	590.08 ± 20.92^{a}	442.43 ± 50.70^{a}
20S-20F	548.81 ± 14.17^{a,b}	542.21 ± 40.45^{a,b}	315.66 ± 80.22^{a,b}
RefA	na	125.07 ± 2.78^{e}	21.96 ± 40.30^{e}
RefB	na	395.72 ± 80.08^{a,b,c}	312.71 ± 93.22^{a,b}
100S	761.06 ± 189.78	na	na
100F	1824.38 ± 372.77	na	na

Values are mean ± standard deviation (n=2). Values with different letters in each column are statistically different at $p < 0.05$ (Tukey's range test).
na, not applicable; DW, dry weight.

2.3.3 Phenolic compounds from HPLC analysis

Table 2.7 shows ten major phenolic compounds other than rutin that were identified and quantified by reversed-phase high performance liquid chromatography with the aid of retention times of their respective standards. The phenolic compounds detected in chronological order are as follows: *1* gallic acid (GA), *2* protocatechuic acid (PCA), *3* p-hydroxybenzoic acid (p-OH-BA), *4* (+)catechin (CAT), *5* vanillic acid (VNA), *6* caffeic acid (CFA), *7* syringic acid (SYA), *8* p-coumaric acid (p-COA), *9* ferulic acid (FA), *10* sinapic acid (SIA). Out of these, five (*1, 2, 3, 5 and 7*) are hydroxybenozic acids, while four (*6, 8, 9 and 10*) are hydroxycinnamic acids. CAT is a flavanol. All ten phenolic compounds were present in Farinetta and Supreme flours. Four compounds: PCA, CFA, SYA and SIA, however, were not detected in the control. From Table 2.8, it can be seen that the trend with the control was very similar to RefA, with the only difference being the presence of SYA in the reference. With the exception of FA, the concentration of all other phenolic compounds increased with the addition of buckwheat. In the best performing products (30F, 20S-20F and 20F), there were huge increments in concentrations of p-OH-BA (13-16 times), CAT (6-9 times) and VNA (6-8 times) over the control (Figures 2.1 & 2.2). The observed increments are a result of the elevated levels of these compounds in both Supreme flour and Farinetta, as seen in Figure 2.3. Hirawan et al. (2010) found the average FA concentration of regular commercial spaghetti to be about 50 µg/g. This is slightly lower than what was obtained for the control (73.9 µg/g). As expected, cooking reduced the concentration of phenolic compounds in spaghetti (Table 2.9). In the control, the sum of all measured phenolic compounds decreased from 99.4 to 77.2 µg/g after cooking. Among test samples, the sum

of measured phenolic compounds ranged from 121.2 to 290.8 µg/g, these were reduced to a range of 86.3 to193.5 µg/g after cooking.

Table 2.7. Concentrations of phenolic acids and (+)catechin in raw materials (µg/g DW)

Sample code	GA	PCA	p-OH-BA	(+)CAT	VNA	CFA	SYA	p-COA	FA	SIA	Total
Control	1.09^{b}±0.09	nd	1.85^{c}±0.29	4.47^{c}±0.17	3.12^{c}±0.14	nd	nd	2.5c±0.05	62.31^{a}±2.38	nd	75.35
100S	7.68^{b}±6.35	9.21^{b}±0.73	55.39^{a}±4.22	117.30^{b}±8.96	33.29^{b}±1.98	6.97^{b}±1.34	7.79^{b}± 0.99	14.13^{b}±0.92	18.64^{b}±0.32	14.39^{b}±0.53	284.78
100F	50.95^{a}±2.64	21.35^{a}±0.44	188.72^{b}±4.665	400.12^{a}±9.86	103.78^{a}±3.69	20.99^{a}±0.15	22.95^{a}±1.71	32.12^{a}±2.30	27.02^{b}±4.71	40.29^{a}±7.76	908.29

Data represent means of duplicate determinations

GA, gallic acid; PCA, protocatechuic acid; p-OH-BA, p-hydroxybenzoic acid; (+)CAT, (+)catechin; VNA, vanillic acid; CFA, caffeic acid; SYA, syringic acid; p-COA, p-coumaric acid; FA, ferulic acid; SIA, sinapic acid

DW, dry weight; nd, not detected

Table 2.8. Concentrations of phenolic acids and (+)catechin in uncooked spaghetti (µg/g DW)

Sample code	GA	PCA	p-OH-BA	(+)CAT	VNA	CFA	SYA	p-COA	FA	SIA	Total
Control	4.97^{bc}±0.42	nd	2.8^{f}±0.89	11.21^{f}±2.97	3.27^{f}±0.03	nd	nd	3.2^{f}±0.31	73.87^{a}±1.43	nd	99.32
20S	7.93^{b}±0.62	2.8^{e}±0.06	13.59^{e}±0.71	12.26^{f}±0.07	7.5^{e}±0.53	nd	2.06^{bc}±0.68	4.66^{e}±0.27	66.16^{a}±3.29	6.04^{abc}±0.01	123.00
10S-10F	2.85^{c}±0.01	3.92^{cde}±0.08	25.14^{cd}±0.49	53.15d^{e}±1.03	12.61^{d}±0.09	nd	1.97^{bc}±0.74	6.31^{d}±0.13	73.81^{a}±2.66	2.94^{bc}±0.19	182.70
20F	3.02^{c}±0.36	4.51^{cd}±0.10	35.67^{b}±0.82	75.48^{bc}±1.74	18.53^{c}±1.29	5.37^{a}±1.27	5.7^{a}±0.32	7.82^{c}±0.20	77.47^{a}±1.06	9.96^{ab}±5.03	243.53
30S	5.4b^{c}±0.07	3.62^{de}±0.12	17.18^{de}±0.65	36.26^{e}±1.37	9.88^{de}±0.76	nd	1.55^{bc}±0.12	5.71^{d}±0.17	62.22^{a}±3.77	4.42^{bc}±0.00	146.24
15S-15F	3.27^{c}±0.68	4.94^{bc}±0.54	32.27^{bc}±3.26	68.26^{cd}±6.91	17.77^{c}±1.64	6.32^{a}±0.65	3.87^{ab}±2.42	8.48^{c}±0.15	73.4^{a}±0.87	11.15^{ab}±4.56	229.73
30F	3.51^{c}±0.40	5.86^{b}±0.07	45.8^{a}±4.25	96.97^{a}±9.02	25.27^{b}±1.34	7.21^{a}±0.04	6.74^{a}±0.70	10.22^{b}±0.18	70.6^{a}±3.54	14.60^{a}±0.05	286.78
20S-20F	4.68^{bc}±1.14	5.71^{b}±0.73	45.69^{a}±3.19	96.72^{a}±6.76	26.45^{b}±1.53	7.33^{a}±0.62	6.71^{a}±0.31	10.30^{b}±0.06	73.57^{a}±3.37	13.72^{a}±1.88	290.88
RefA	3.86^{c}±0.79	nd	2.62^{f}±0.72	6.20^{f}±0.02	2.73^{f}±0.20	nd	1.58^{bc}±0.01	2.61^{f}±0.02	38.21^{b}±16.12	nd	57.81
RefB	11.81^{a}±2.38	8.46^{a}±0.22	44.62^{a}±0.68	94.46^{ab}±1.44	30.46^{a}±0.36	7.16^{a}±1.12	nd	12.26^{a}±0.60	11.66^{c}±1.14	10.29^{ab}±0.02	231.18

Data represents means of duplicate determinations.

GA, gallic acid; PCA, protocatechuic acid; p-OH-BA, p-hydroxybenzoic acid; (+)CAT, (+)catechin; VNA, vanillic acid; CFA, caffeic acid; SYA, syringic acid; p-COA, p-coumaric acid; FA, ferulic acid; SIA, sinapic acid.

DW, dry weight; nd, not detected

Table 2.9. Concentrations of phenolic acids and (+)catechin in cooked spaghetti (µg/g DW)

Sample code	GA	PCA	p-OH-BA	(+)CAT	VNA	CFA	SYA	p-COA	FA	SIA	Total
Control	7.66^{a}±0.62	nd	2.14^{b}±0.05	4.58^{e}±0.48	1.77^{ef}±0.17	nd	0.73^{c}±0.44	1.97^{f}±0.22	58.34^{ab}±1.93	nd	77.19
20S	1.83^{b}±0.26	1.74^{cd}±0.32	9.59^{ab}±1.64	12.83^{de}±2.66	4.09^{def}±0.34	nd	0.74^{c}±0.02	3.03^{def}±0.54	52.65^{ab}±9.16	nd	86.50
10S-10F	0.82^{b}±0.24	2.36^{b} ±0.42	16.44^{ab}±0.53	28.05^{cd}±1.53	6.58^{cd}±0.18	1.86^{abc}±0.08	0.81^{c}±0.06	4.42^{cd}±0.07	60.49^{ab}±0.57	1.8b^{c}±0.29	123.63
20F	1.11^{b}±0.02	2.71^{bc}±0.15	22.87^{a}±0.26	45.48^{abc}±3.38	9.81^{c}±0.15	2.56^{ab}±0.09	0.85^{c}±0.05	5.66^{bc}±0.28	67.61^{a}±0.17	5.82^{a}±0.67	164.48
30S	1.00^{b}±0.37	2.08^{bc}±0.01	12.58^{a}b±0.67	16.74^{de}±0.01	4.88d^{e}±0.14	1.51^{bc}±0.00	0.63^{c}±0.03	3.69^{cde}±0.17	50.11^{b}±7.99	1.05^{b}±0.01	94.27
15S-15F	2.36^{b}±0.32	3.22^{bc}±0.02	20.27^{ab}±2.08	30.04^{bcd}±1.92	9.09^{c}±1.28	2.05^{ab}±0.02	0.88^{c}±0.01	5.49^{bc}±0.55	56.93^{ab}±5.03	1.52^{b}±0.10	131.85
30F	7.62^{a}±0.24	3.62^{b}±1.12	28.95^{a}±1.87	56.92^{a}±8.77	14.41^{ab}±2.18	3.31^{ab}±0.51	1.13^{c}±0.04	6.62^{ab}±1.43	61.38^{ab}±2.60	9.56^{a}±3.32	193.52
20S-20F	7.76^{a}±1.58	6.07^{a}±0.60	27.02^{a}±15.36	47.82^{ab}±11.47	13.40^{b}±0.50	3.43^{a}±0.24	8.17^{a}±0.39	6.89^{ab}±3.94	56.73^{ab}±0.97	8.14^{a}±0.76	185.43
RefA	2.29^{b}±0.19	nd	1.28^{b}±0.09	4.34^{e}±0.11	1.49^{f}±0.07	nd	0.66^{c}±0.04	1.46^{f}±0.00	31.32^{c}±4.46	nd	42.84
RefB	11.71^{a}±2.89	7.63^{a}±0.42	26.35^{a}±0.55	59.96^{a}±0.66	17.69^{a}±0.22	3.06^{ab}±1.39	7.11^{b}±0.32	8.70^{a}±0.20	8.11^{d}±1.88	6.95^{a}±2.10	157.27

Data represents means of duplicate determinations.

GA, gallic acid; PCA, protocatechuic acid; p-OH-BA, p-hydroxybenzoic acid; (+)CAT, (+)catechin; VNA, vanillic acid; CFA, caffeic acid; SYA, syringic acid; p-COA, p-coumaric acid; FA, ferulic acid; SIA, sinapic acid.

DW, dry weight; nd, not detected

2.4 Conclusions

The introduction of buckwheat flours in the spaghetti dough resulted in elevating the total phenolic content and antioxidant activity, as well as introducing phenolic components which were absent in the control. The effect of Farinetta in improving the phenolic profile of spaghetti samples was greater than that of Supreme flour. Over all, the products with the highest phenolic and antioxidant contents were those containing higher amounts of Farinetta, particularly 30F and 20S-20F. The average TPC and TFC of these two products were six and three times higher than the control, respectively. Also, the DPPH and ORAC values recorded for these products were three and eleven times higher than those of the control respectively. These products were also potent enough to compete with the phenolic and antioxidant properties of the commercial whole buckwheat reference product (RefB). Cooking resulted in significant losses ($p < 0.05$) of up to 39% in TPC, 40% in DPPH antioxidant activity, 22% in rutin content, and 55% in TFC among all buckwheat-containing products. Processing, however, did not affect TPC of the experimental products. Based on the results of this study, it is possible to improve upon the phenolic and antioxidant profile of spaghetti through fortification with buckwheat milling fractions. However, to further explain the phenolic and antioxidant losses incurred during processing and cooking, the structural and technological properties of buckwheat substituted spaghetti need to be properly investigated.

CHAPTER 3: Cooking properties and carbohydrate digestibility of buckwheat enriched spaghetti

ABSTRACT

Spaghetti is a popular commodity known for its low glycemic index. Nutritionally, however, it remains unbalanced, and paves the way for fortification, which may alter the technological properties and quality of the end product. The purpose of this study was to investigate the effect of buckwheat Supreme flour and bran Farinetta on the cooking quality and *in vitro* carbohydrate digestibility of durum spaghetti. Seven different types of spaghetti were manufactured by substituting durum semolina with up to 40% of buckwheat. In addition, 100% semolina spaghetti was manufactured to serve as control. Comparison was also made with commercially available 100% semolina spaghetti (RefA) and whole buckwheat soba noodles (RefB). The swelling index, cooking loss, water absorption and dry matter of the samples were investigated to evaluate their cooking quality. Carbohydrate digestibility was investigated by monitoring the release of reducing sugars over 120 min of hydrolysis. Cooking losses recorded for the experimental samples were higher for Farinetta-substituted products, and ranged between 5.72 to 8.32%. These were generally higher than that of the control (6.33%). Cooking losses recorded for Farinetta-containing samples were also higher than those recorded for Supreme flour-containing ones by as much as 12% at a 30% level of substitution (30S & 30F). The presence of Farinetta also resulted in lower water absorption scores compared to Supreme flour by 26%. Over the course of 120 min, buckwheat-containing samples had a higher rate of carbohydrate breakdown compared to the control. After the end of 120 min,

however, the extent of digestion was lower compared to the control. This was manifested by the fact that the control had the highest concentration of reducing sugars (744.08 mg/g) while samples substituted with only Farinetta (30F and 20F) had the lowest concentrations with 604.02 mg/g and 615.53 mg/g, respectively. This represents an average reduction of 18% in the extent of carbohydrate breakdown compared to the control. Results showed that it is possible to fortify semolina with buckwheat flour and bran in the manufacture of spaghetti while still maintaining a high standard of cooking quality as well as a low glycemic index characteristic of traditional spaghetti products.

3.1 Introduction

Perhaps one of the most common risk factors for ill-health amongst both adults and children is obesity. Current global statistics of this epidemic reveal that over a billion people are overweight, out of which about a third are regarded obese (Arroyo & Herron, 2013). The past few decades have seen a sharp increase in the consumption of energy-rich foods and this coupled with the sedentary lifestyles of modernity, has put many lives at risk of developing weight related illnesses such as diabetes (Pereira et al., 2005), cardiovascular diseases (Van Gaal et al., 2006; Poirier & Eckel, 2002) as well as some cancers (Calle & Kaaks, 2004). There is a high level of awareness of the implications of unhealthy eating, and consumers will opt for healthier foods if given the necessary incentive (Brennan, 2005; Evans et al., 2005). It has now been established that a dietary mediation is a more economical approach to tackling the obesity menace and its concomitant illnesses compared to treatment through medication (Brennan, 2005). A dietary mediation partly involves the consumption of foods with low rates and extents of

carbohydrate digestibility (glycemic impact), leading to low postprandial blood glucose levels. The Glycemic impact (GIm) of a food is defined as "the weight of glucose that would induce a glycemic response equivalent to that induced by a given amount of food" (Monro & Shaw, 2008). This concept is derived from the more familiar concept of glycemic index (GI), which is defined as "the total glycemic response in the 2 h immediately subsequent to the consumption of 50 g of carbohydrates" (Roberts, 2000). Foods ranked high on the GI scale are generally those with high carbohydrate content and high rates of digestibility. Thus, the GIm of a food is a fair reflection of its GI. Apart from high GI foods increasing postprandial glucose levels, they also have been shown to increase hunger and cause overeating (Roberts, 2000). For people with glucose related intolerances, reliance on diets with low glycemic responses is very crucial.

Spaghetti is known for its low GI (Jenkins et al., 1988; Jenkins et al., 1983). It is known that the relatively slow breakdown of carbohydrates from spaghetti is as a result of its compact nature, resulting from the extrusion process and also from the formation of a tight gluten network which entraps starch granules (Fardet et al., 1998). Spaghetti, however, is nutritionally unbalanced, being only rich in complex carbohydrates (Giese, 1992). Effort has therefore been made to boost the nutritional and nutraceutical properties of this popular commodity through fortification.

The re-emergence of buckwheat as an alternative crop has led to the production of many functional foods such as noodles, pancakes and baked goods like bread, cakes and biscuits (Campbell, 1997; Rayas-Duarte et al., 1998). Buckwheat (*Fagopyrum esculentum* Moench) is a dicotyledonous pseudo-cereal crop cultivated widely but more prominently in the northern hemisphere. Countries such as Russia, China, Ukraine,

Canada, the United States and Italy are among the leading producers of buckwheat (Campbell, 1997). After many years of intensive research, buckwheat is now well renowned for its healing properties. Its high protein content and complete amino acid profile are known to be the basis for its cholesterol-lowering property (Kayashita et al., 1995; Kritchevsky, 1979; He et al., 1995; Wojcicki et al., 1995). It is also rich in phenolic acids and flavonoids, many of which possess antioxidant activity (Holasova et al., 2002; Kreft et al., 2006; Oomah & Mazza, 1996; Przybylski et al., 1998). Buckwheat is regarded as one of the best sources of rutin, a flavonol glycoside which has been shown to be effective in the treatment and prevention of rheological disorders of the blood such as hypertension (Matsubara et al., 1985), and atherosclerosis (Wojcicki et al., 1995). In addition to being a healthy source of B-vitamins (Bonafaccia et al., 2003), polyunsaturated fatty acids (Steadman et al., 2001a), and minerals (Amarowicz & Fornal, 1987), buckwheat is also a rich source of dietary fibre (Bonafaccia et al., 2003). Dietary fibre (DF) generally has the ability to minimize the rate and extent of carbohydrate breakdown in the upper intestinal tract, leading to a gradual release of blood glucose after meals (Brennan, 2005). DF is said to cause stomach distension which causes and maintains satiety for an extended period of time. Soluble fibres particularly have the potential to delay gastric emptying by absorbing water and forming gels (Howarth et al., 2001). These gels delay the movement of digesta through the gastrointestinal tract, thereby giving a perception of satiety, delaying hunger and minimizing the urge to eat. Gels also minimize the access of digestive enzyme to digesta, resulting in reduced glucose production and absorption. In addition, DF lowers the energy density of food by acting as a dilution factor (Howarth et al., 2001). Studies have also shown that

Fagopyrins, a class of soluble carbohydrates present in buckwheat have proven successful in lowering serum glucose levels in animal studies, and have therefore been proposed for the treatment of diabetes (Kawa et al., 2003; Larner, 2002; Ortmeyer et al., 1995; Steadman et al., 2000). Furthermore, rutin is believed to be effective in reducing fasting blood glucose levels and increasing insulin levels (Hao et al., 2012; Yildizogluari et al., 1991).

Despite the fact that several studies have reported on the carbohydrate digestibility of fortified spaghetti products, there are very few studies, if any at all, that have reported on the effect of buckwheat flour and bran. In an effort to enhance the nutritional and nutraceutical properties of spaghetti, it is apparent to do so in a manner that will preserve the cooking quality and low GI characteristic of traditional spaghetti products. This study therefore sought to investigate the effect of partial substitution of semolina with buckwheat flour and bran on the cooking quality and *in vitro* carbohydrate digestibility of spaghetti products.

3.2 Materials and methods

3.2.1 Raw materials

Durum semolina flour was obtained from the local market, (Winnipeg, MB, Canada). Common buckwheat (*Fagopyrum esculentum)* Supreme flour (comprising percentages of hull and endosperm) and bran Farinetta (mixture of aleurone layer of hulled seed and seed embryo) were purchased from Minn-Dak Growers, Ltd. (Grand Forks, ND, USA).

3.2.2 Chemicals

For the determination of slowly and readily digestible carbohydrates as well as resistant starch, the following enzymes and chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA): amyloglucosidase, malic acid, glucose standard, phenol, pancreatin and dinitrosalycilate (DNS). The following were purchased from Fisher Scientific (Fair Lawn, NJ, USA): potassium sodium tartrate, sodium bicarbonate ($NaHCO_{3)}$, hydrochloric acid (HCl), pepsin, potassium hydroxide (KOH), sodium sulfite ($Na_2SO_{3)}$, sodium phosphate dibasic ($Na_2HPO_{4)}$, sodium phosphate monobasic ($NaH_2PO_{4)}$, calcium chloride ($CaCl_2$), sodium hydroxide (NaOH). Glucose oxidase/peroxidase (GOPOD) assay kit was purchased from Megazyme Int. (Bray, Wicklow, Ireland)

For fibre analysis, acetone was purchased from Fisher Scientific (Fair Lawn, NJ, USA), while acid-washed celite, heat-stable α-amylase and protease were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

3.2.3 Spaghetti processing

Seven different typologies of spaghetti were manufactured by substituting semolina with different amounts of common buckwheat Supreme flour, Farinetta or both. Between 10 and 40% w/w of substitutions were reached (Table 3.1.). Products substituted with Supreme flour alone were denoted by the code ‘nS’, where ‘n’ represents the percentage substituted and ‘S’ stands for Supreme flour. Similarly, ‘nF’ was used for samples substituted with only Farinetta. Samples containing both components were

denoted by the code ‘nSnF’. Processing was achieved with a twin screw extruder (APV Baker Ltd, Peterborough, England) fitted with a 2-mm die. One hundred percent semolina spaghetti was also manufactured to serve as the control. The substitution amounts were predetermined and based on achieving a compromise between nutritional/nutraceutical enhancement and spaghetti quality. The flours were first mixed manually before being loaded into the hopper. Water was pumped at a rate between 0.614 to 0.614 L/h depending on the absorptive capacity of the dough mixtures in order to achieve a desirable moisture content of approximately 30%. A summary of the processing parameters of extrusion are presented in Table 3.2. Extruded products were dried for 18 h in a Moffat® convection oven (Model ECO-3, DeltaRex Canada Inc. Toronto, ON, Canada) preheated to a temperature of 80 ºC and allowed to cool to room temperature. The dried spaghetti samples were broken into lengths of 10 cm, kept in zip-lock bags and stored at -18 ºC.

Two commercial products were used as reference samples and analysed alongside the experimental samples, these were RefA- 100% durum spaghetti (ARRIGHI®) purchased from Canada Safeway Limited (Winnipeg, MB, Canada), and RefB- 100% whole buckwheat soba noodles (EDEN FOODS®) purchased from Well Canada online store.

Table 3.1. Flour formulations, cooked and uncooked spaghetti samples

Number	Sample Code	% Durum semolina	% Supreme	% Farinetta
1	Control[a]	100	0	0
2	20S[a]	80	20	0
3	10S-10F[a]	80	10	10
4	20F[a]	80	0	20
5	30S[a]	70	30	0
6	15S-15F[a]	70	15	15
7	30F[a]	70	0	30
8	20S-20Fa	60	20	20
9	RefA[b]	-	-	-
10	RefB[b]	-	-	-
11	100S[c]	0	100	0
12	100F[c]	0	0	100

[a] Code represents flour formulations, uncooked spaghetti and cooked spaghetti
[b] Code represents both uncooked and cooked commercial reference spaghetti samples
[c] Code represents only flour samples
Abbreviations: F, Farinetta, S, Supreme flour, RefA, commercial 100% semolina spaghetti, RefB, commercial 100% whole buckwheat soba noodles.

Table 3.2. Processing parameters of extrusion

Spaghetti code	Product temperature (°C)	Die pressure (bar)	Torque (%)	Screw speed (rpm)	Feed rate (kg/h)	Water injection (L/h)
Control	41	21	31	30	2.0	0.748
20S	45	33	52	30	2.0	0.631
10S-10F	45	22	32	30	2.0	0.696
20F	45	36	51	30	2.0	0.614
30S	45	25	39	30	2.0	0.696
15S-15F	45	19	31	30	2.0	0.696
30F	45	17	28	30	2.0	0.614
20S-20F	44	18	22	30	2.0	0.696

3.2.4 Cooking of spaghetti for *in vitro* carbohydrate digestion and fibre analysis

Cooking of spaghetti followed the procedure outlined by Manthey et al. (2004). About 10 g of spaghetti was broken into lengths of about 5 cm and added to 300 mL of boiling distilled water in a beaker. Optimum cooking was achieved at 12 min after the disappearance of the inner white core as outlined in the Approved Method 66-50 (AACC 2000). The cooked spaghetti was drained with a Buchner funnel. For fibre analysis, cooked samples were frozen at -18 °C and then freeze-dried. Freeze-dried samples were then milled with a multi-user blade grinder, model PCC770 (Loblaws Inc., Brampton, ON, Canada) in order to pass through a screen size of 0.42 mm.

3.2.5 Determination of cooking properties of spaghetti

The following parameters were determined as described by Tudorica et al. (2002) after cooking 40 g of pasta in 300 mL of distilled water.

Swelling index (SI) was evaluated as grams of water per gram of oven dried spaghetti using the following equation: $\frac{(\text{weight of cooked spaghetti} - \text{weight after drying})}{\text{weight after drying}}$

Cooking loss was determined by weighing the residue left after evaporating the drained cooking water to dryness. Cooking loss was calculated as follows: $\left(\frac{\text{weight of residue}}{\text{weight of uncooked spaghetti}}\right)$ X 100

Water absorption was determined by weighing cooked spaghetti and comparing it to uncooked spaghetti using the formula:

$$\left(\frac{\text{weight of cooked spaghetti} - \text{weight of uncooked spaghetti}}{\text{weight of uncooked spaghetti}}\right) \text{X } 100$$

Dry mater was determined according to the Gravimetric Method 44-15 A, AACC (2000).

3.2.6 Determination of readily and slowly digestible carbohydrates

3.2.6.1 *In vitro* carbohydrate digestion

The method employed by Brennan et al. (2008) in determining *in vitro* carbohydrate digestibility was used. Two grams of previously cooked and minced spaghetti were mixed with 30 mL of distilled water and 0.8 mL of 1 M HCl to achieve a pH of 2.5. After incubating in a water bath set at 37 °C for 10 min, pepsin (1 mL of 10% solution in 0.05 M aqueous HCl) was added and the samples kept for a further 30 min at 37 °C. The pH was then adjusted to 6 using 2 mL of 1 M $NaHCO_3$ after which 0.1 mL of amyloglucosidase and 5 mL of pancreatin (2.5% solution in sodium maleate buffer, pH 6) were added. The total volume was adjust to 53 mL with buffer and incubated at 37 °C with slow constant mixing. Duplicate 1 mL aliquots were withdrawn at 0, 20, 60 and 120 min and placed into tubes containing 4 mL of absolute ethanol and mixed. These were stored at 4 °C for subsequent analysis of reducing sugars using the dinitrosalycilate (DNS) method.

Readily digestible carbohydrates (RDC) and slowly digestible carbohydrates (SDC) were determined as grams of reducing sugars (maltose equivalent) released between 0 and 20 min, and 20 and 120 min, respectively.

3.2.6.2 Measurement of reducing sugars with DNS method

The quantification of reducing sugars using the DNS method involves the oxidation of the aldehyde group of reducing sugars with the subsequent reduction of 3,5-dinitrosalicylic acid to 3-amino,5-nitrosalicylic acid under alkaline conditions and with the production of a dark red colouration which is measured colorimetrically. Following the method outlined by Serna-Saldívar (2012), the ethanol-containing samples obtained after the *in vitro* hydrolyses were centrifuged, after which they were evaporated using a water bath set at 80 °C. Then, 10 mL of distilled water was added to dissolve the sugars. Extracts (0.5 mL) were transferred in duplicates to test tubes and the volumes topped to 3 mL with distilled water. Then, 3 mL of previously prepared DNS solution (10 g of DNS, 2 g of crystalline phenol and 0.5 g of sodium sulfite in 1 L of 1% NaOH) was added to each tube. The tubes were covered with aluminium foil and heated in a water bath set at 100 °C for 5 min. While the contents of the tubes were still warm, 1 mL of 40% potassium sodium tartrate solution was added. After cooling, the absorbance was read at 510 nm with an Ultraspec 1100 pro, UV/Visible spectrophotometer (Biomicron Ltd. Cambridge, England, UK). Reducing sugar concentrations were calculated with the aid of a maltose standard curve using the following concentrations: 0.0, 1.0, 2.0, 3.0 & 3.5 mg/ mL. Results were reported as maltose equivalent mg/g of sample.

3.2.7 Determination of resistant starch

Resistant starch was determined using the official AACC 2000, Method 32-44 procedure. By this method, glucose from non-resistant starch was released through the hydrolysis of samples using pancreatic alpha amylase and amyloglucosidase for 16 h at 37 °C. The reaction was stopped by the addition of absolute ethanol and resistant starch recovered as pellet after centrifugation. This portion was again hydrolysed using amyloglucosidase and the resulting glucose measured using the glucose oxidase-peroxidase method. In this method, glucose was oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. Through side reactions involving hydrogen peroxide, a pink colour developed which was measured at 540 nm against a reagent blank. Glucose was used as standard.

3.2.8 Determination of total dietary fibre

Total dietary fibre was determined according to the standard enzymatic-gravimetric method AOAC 2005, Method 985.29. This method is based on the principle of enzymatic treatment for starch and protein removal with subsequent weighing of residue as fibre. Into a 400 mL beaker, 1 g of previously cook and freeze-dried sample was incubated with 50 mL of phosphate buffer (pH 6) and 0.1 mL heat-resistant α-amylase at 95 °C to 100 °C for 30 min. The solution was then cooled to room temperature and the pH adjusted to 7.5 using 0.275 N NaOH. Next 0.1 mL of a 50 mg protease in 1 mL phosphate buffer solution was added and incubated at 60 °C for 30 min. Finally, the solution was cooled again and the pH adjusted to 4 to 4.6 with 0.325 M HCL

before the addition of 0.3 mL amyloglucosidase for further incubation at 60 °C for 30 min. To each beaker, 280 mL of warm 95% ethanol was added and allowed to stand for 1 h to enhance precipitation. Contents were filtered through celite while washing three times with 20 mL of 78% ethanol, twice with 10 mL of 95% ethanol and twice with 10 mL acetone. The residues were dried overnight at 105 °C and weighed. Analyses were done in duplicate. Contents of one filter were analysed for protein using the Kjeldahl method, while the other portion was analysed for ash. These were used to correct dietary fibre values. A blank was also run together with the samples.

Total dietary fibre was calculated using the formula:

$$\% \text{ TDF} = \frac{(\text{Rsample} - \text{Psample} - \text{Asample} - \text{B})}{\text{SW}} \text{ X } 100$$

Where TDF = total dietary fibre, R= average residue weight, P= average protein weight, A= average ash weight, B = blank, SW = Average sample weight

3.2.9 Statistical analysis

Data were expressed on a dry weight basis and reported as means ± standard deviations. Using SAS statistical software, version 9.2 (SAS Institute Inc., Cary, NC, USA), a one- way analysis of variance (ANOVA) and means testing (Tukey's range test) were performed with level of significance set at $p < 0.05$.

3.3 Results and discussion

3.3.1 Cooking quality of spaghetti products

The quality of cooked spaghetti is of utmost importance to spaghetti consumers (Cubadda et al., 2007). Uncooked spaghetti quality is dependent on the quantity and quality of gluten proteins as well as on the gelatinization of starch granules (Bruneel et al., 2010).

The cooking quality parameters of spaghetti samples are summarised in Table 3.3. The addition of buckwheat to semolina did not produce a clear pattern in swelling index (SI), although compared to the 100% semolina reference sample (RefA), all but 30S had significantly lower ($p < 0.05$) SI values. Comparison with RefA in this instance is ideal because it is a prototype of regular commercial spaghetti with good cooking properties. Results showed that the lower the buckwheat substitution, the higher the SI, and that Supreme flour was better at producing spaghetti with a higher SI than Farinetta. The swelling index of spaghetti is important because it determines if the product will have the characteristic textural properties such as firmness, resilience and being less sticky (Dexter et al., 1985). During processing of spaghetti, a gluten protein network begins to develop which entraps starch granules (Bruneel et al., 2010). There is a further polymerisation of this network during cooking. This network provides the framework upon which the desired characteristic textural properties such as firmness and resilience are formed during cooking. If there is insufficient swelling of starch granules due to a poorly formed protein network, the texture of the product will be poor. On the other hand if swelling is too high, there will be interruption of the formed protein network and this will lead to leaching out of starch granules into the water, causing stickiness. Substituting

semolina with buckwheat therefore resulted in products with reduced swelling capacities due to the absence of gluten in buckwheat. The lower SI for Farinetta-containing samples compared to Supreme flour can also be explained in terms of the higher fibre content of Farinetta (Table 3.4). Fibre has the ability to compete for water in the product which ultimately inhibits the swelling of starch granules (Bruneel et al., 2010). This assertion was also confirmed by Tudorica et al. (2002) after studying the effect of pea fibre, inulin and guar on cooking quality of pasta.

The cooking loss contained in the drained cooking water also defines the quality of spaghetti. The best quality spaghetti has lower cooking losses; however, the introduction of other raw materials and ingredients other than semolina increases cooking losses. Results indicate that cooking loss increased upon the addition of buckwheat. The cooking loss for 30F (8.32%) was significantly higher ($p < 0.05$) than that for 30S (7.31%). Similarly, 20F had a higher cooking loss than 20S, suggesting that the addition of Farinetta led to a bigger disruption of the protein network which is responsible for preventing leaching. Manthey & Schorno (2002) and Aravind et al. (2012) also recorded increases in cooking loss when whole-wheat flour and semolina blended with fibre respectively were used in manufacturing spaghetti. Although cooking loss is vital in defining the cooking quality of spaghetti, this may not be of too much relevance from a nutritional point of view if the dish is prepared and consumed in a soup-like fashion, as most of the nutrients retained in the cooking water will still be available.

The water absorption (WA) of a product is a measure of its content of water-soluble solids (Rooney, 2007). The absorption of water during cooking decreased with the introduction of buckwheat. WA scores for test samples ranged from 129.7 to 168.2%.

Farinetta caused the highest reductions. For samples 30F, 20S-20F and 20F which had the highest levels of Farinetta, reductions of between 23% and 34% in WA compared to the control were recorded. WA of the control and RefA were 174.2 and 155.6%, respectively, while that of RefB was 95.9%. Supreme flour predominantly consists of buckwheat endosperm which is rich in starch (about 78%) (Bonafaccia et al., 2003). This property enhances its ability to absorb more water than Farinetta which consists of a mixture of the aleurone layer and embryo. A well formed protein network within spaghetti will have the ability to entrap swollen starch granules and maintain a high WA, which is what was observed with the control by virtue of its 100% semolina base.

Table 3.3 Cooking quality of spaghetti products

Sample code	Swelling index	Cooking loss (%)	Water absorption (%)	Dry matter (%)
Control	$1.65 \pm 0.00^{d,e}$	6.33 ± 0.09^{f}	174.24 ± 0.46^{a}	37.74 ± 0.02^{b}
20S	1.61 ± 0.02^{e}	5.72 ± 0.32^{g}	152.20 ± 0.78^{d}	38.36 ± 0.25^{b}
10S-10F	1.92 ± 0.04^{b}	$6.83 \pm 0.01^{d,e,f}$	150.87 ± 0.14^{d}	34.25 ± 0.48^{d}
20F	1.69 ± 0.01^{d}	7.62 ± 0.03^{c}	141.15 ± 0.32^{e}	37.17 ± 0.09^{c}
30S	2.07 ± 0.01^{a}	$7.31 \pm 0.02^{c,d}$	168.21 ± 0.39^{b}	32.57 ± 0.09^{e}
15S-15F	1.77 ± 0.00^{c}	$6.96 \pm 0.09^{d,e}$	141.82 ± 0.20^{e}	36.05 ± 0.04^{c}
30F	$1.66 \pm 0.01^{d,e}$	8.32 ± 0.00^{b}	129.77 ± 0.05^{g}	37.56 ± 0.09^{b}
20S-20F	1.31 ± 0.01^{f}	$7.88 \pm o.17^{b,c}$	137.90 ± 0.65^{f}	43.27 ± 0.17^{a}
RefA	2.02 ± 0.02^{a}	$6.48 \pm 0.03^{e,f}$	155.58 ± 0.71^{c}	$33.08 \pm 0.26^{d,e}$
RefB	1.33 ± 0.04^{f}	26.06 ± 0.28^{a}	95.85 ± 1.14^{h}	42.89 ± 0.72^{a}

Values are means of duplicate determinations. Values with different letters in each column are statistically different at $p < 0.05$ (Tukey's range test).

Table 3.4. Total dietary fibre (TDF) and resistant starch (RS) content of spaghetti products (g/100 g of sample DW)

Sample code	TDF	RS
Control	3.49 ± 0.09[d]	1.54 ± 0.01[a]
20S	4.23 ± 0.11[d]	1.51 ± 0.00[a]
10S-10F	4.23 ± 0.03[d]	1.51 ± 0.02[a]
20F	4.36 ± 0.05[d]	1.50 ± 0.00[a]
30S	5.20 ± 0.01[c]	1.51 ± 0.01[a]
15S-15F	5.53 ± 0.23[c]	1.44 ± 0.09[a]
30F	6.98 ± 0.08[b]	1.49 ± 0.01[a]
20S-20F	5.58 ± 0.11[c]	1.45 ± 0.06[a]
RefA	3.02 ± 0.03[d]	1.50 ± 0.03[a]
RefB	12.77 ± 0.24[a]	1.48 ± 0.01[a]

Values are mean ± standard deviation (n=2). Values with different letters in each column are statistically different at $p < 0.05$ (Tukey's range test).

3.3.2 *In vitro* carbohydrate digestion

Table 3.5 summarises the results of *in vitro* carbohydrate digestibility of spaghetti samples determined by monitoring the release of reducing sugars over 120 min of hydrolysis. Results point to an increase in digestibility upon the introduction of buckwheat. At a 20% level of substitution, the Farinetta-containing sample (20F) had the lowest amount of reducing sugars in dialysate at all stages of analysis. Differences of 15.7%, 17.6% and 17.3%, at 20 min, 60 min and 120 min, respectively, compared to the control were recorded for 20F. Similarly, at a 30% level of substitution, the lowest amount of reducing sugars liberated was recorded for 30F, with differences of 14.7%, 21.1% and 18.8% at 20 min, 60 min and 120 min, respectively, compared to the control. The initial reducing sugar concentration at time 0 min was highest for the control and this reduced significantly upon introduction of buckwheat, with the greatest reductions occurring for samples substituted with only Farinetta (20F and 30F). As expected, there

was a steady increase in the reducing sugar content released over the 120 min duration of hydrolysis for all samples. At the end of 120 min of *in vitro* digestion, the control had the highest concentration of reducing sugars (744.08 mg/g) while 30F and 20F had the lowest concentrations with 604.02 mg/g and 615.53 mg/g, respectively. These represent an 18% reduction in the extent of carbohydrate breakdown. In order to fully perceive the effect of buckwheat on the carbohydrate digestibility of the products, a mathematical correction was performed to eliminate the effect of any free reducing sugars that were present before the start of the digestive process. After this correction, all buckwheat-containing samples with the exception of 20S-20F, showed a higher liberation of reducing sugars compared to the control (Figure 3.1). Despite this, the total reducing sugars present at the end of 120 min of digestion, with initial free sugars included, was still lower than the control. This may suggest that apart from 20S-20F, all other buckwheat-containing samples had a higher rate of carbohydrate digestion compared to the control although the extent of digestion was lower in the end. Figure 3.2 clearly shows that slowly digestible carbohydrates decreased upon the introduction of buckwheat. It can therefore be inferred that the effect of buckwheat on the carbohydrate digestibility of spaghetti is based on the dilution of available carbohydrates (starch) rather than on a mechanism which slows down their breakdown. Spaghetti is known for its low GI due to its compactness resulting from the extrusion process as well as the presence of a tight protein network which entraps starch granules (Fardet et al., 1998). The higher rate of carbohydrate breakdown observed for the buckwheat samples can therefore be traced to the initial quality of spaghetti produced.

As expected the presence of buckwheat significantly increased TDF content in all samples (Table 3.4). TDF content in experimental products ranged from 4.23% in 20S and 10S-10F to 6.98% in 30F. While the control, RefA and RefB had TDF content of 3.49, 3.02 and 12.77%, respectively. Farinetta-containing samples showed higher fibre contents than Supreme flour-containing ones. At 30% substitution with Farinetta, the TDF was double that of the control. It has been established that the introduction of non-traditional raw materials such as buckwheat leads to the formation of weakened protein networks which are incapable of keeping starch granules intact (Edwards et al., 1995). As a result, gelatinized starch granules within the weakened gluten network are left exposed and susceptible to the swift action of digestive enzymes. The presence of high amounts of TDF can lead to such a disintegration of this gluten-starch network. Similar reports on the increase in the amount of liberated sugars during *in vitro* digestion of fibre-enriched spaghetti have been made (Aravind et al., 2012; Tudorica et al., 2002). Statistical analysis however showed no differences between samples in RS content (Table 3.4.), suggesting that RS concentration played no role in the differences observed in carbohydrate digestibility of the samples. The ability of buckwheat to reduce the glycemic impact of spaghetti has huge nutritional implications as it affects postprandial blood glucose and insulin levels.

Table 3.5. Concentration of reducing sugars in dialysates (mg/g sample DW)

Sample code	Time (min)			
	0	20	60	120
Control	$394.48 \pm 0.8^{a,b}$	604.35 ± 0.7^{c}	709.10 ± 3.6^{c}	744.08 ± 1.5^{b}
20S	$351.72 \pm 1.3^{a,b,c}$	608.69 ± 3.1^{c}	732.61 ± 1.5^{b}	738.94 ± 2.1^{b}
10S-10F	$280.40 \pm 0.0^{b,c}$	563.55 ± 4.8^{d}	652.84 ± 0.6^{e}	655.26 ± 0.7^{d}
20F	169.91 ± 0.5^{c}	509.47 ± 11.3^{e}	584.27 ± 1.8^{h}	615.53 ± 0.7^{g}
30S	$319.29 \pm 2.4^{b,c}$	652.32 ± 13.5^{b}	670.74 ± 1.0^{d}	728.23 ± 1.9^{c}
15S-15F	$239.14 \pm 1.6^{b,c}$	565.13 ± 4.2^{d}	607.93 ± 1.5^{g}	651.66 ± 0.3^{d}
30F	$197.46 \pm 1.0^{b,c}$	515.27 ± 1.6^{e}	559.55 ± 1.6^{i}	604.02 ± 1.3^{h}
20S-20F	$350.74 \pm 16.4^{a,b,c}$	563.57 ± 0.6^{d}	554.09 ± 1.9^{i}	624.60 ± 1.7^{f}
RefB	$254.22 \pm 16.4^{b,c}$	616.69 ± 0.7^{c}	617.99 ± 0.1^{f}	637.16 ± 2.8^{e}

Values are mean ± standard deviation (n=2). Values with different letters in each column are statistically different at $p < 0.05$ (Tukey's range test).

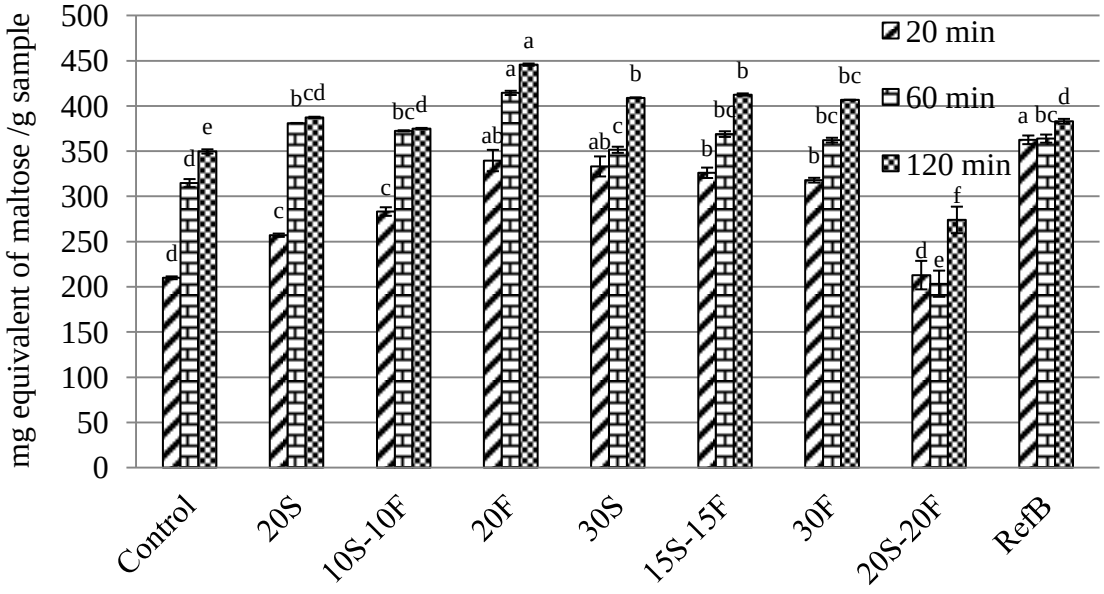

Figure 3.1. Concentration of reducing sugars released during *in vitro* digestion. Values represent means of duplicate determinations. Bars of the same time point marked by different letters are significantly different from each other ($p < 0.05$, Tukey's range test).

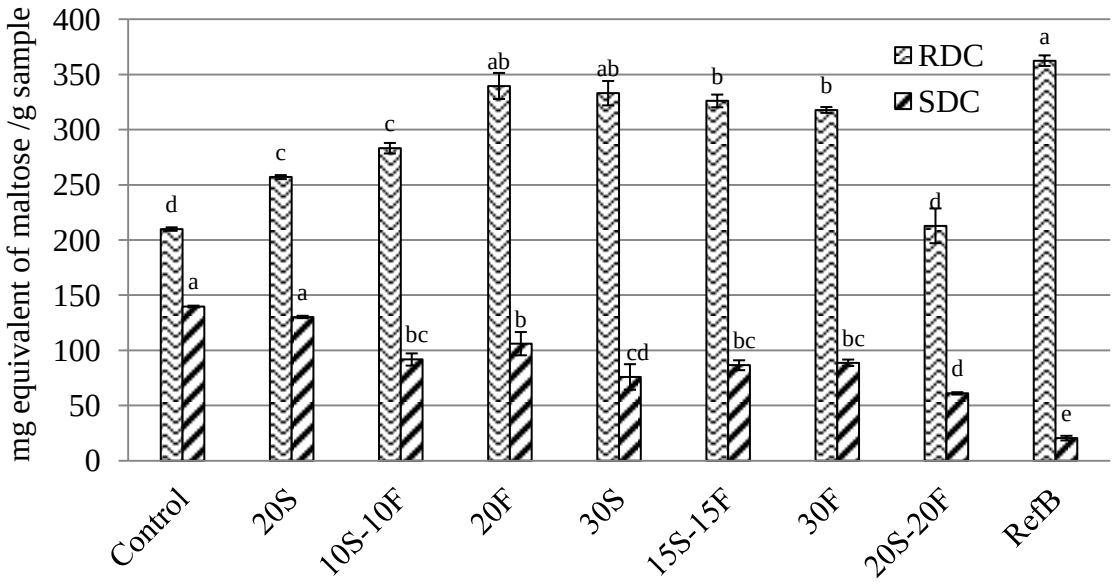

Figure 3.2. Readily digestible carbohydrates (RDC) and slowly digestible carbohydrates (SDC) of spaghetti products. Values represent means of duplicate determinations. Bars of the same time point marked by different letters are significantly different from each other ($p < 0.05$, Tukey's range test).

3.4 Conclusion

The overall quality of cooked spaghetti was reduced by the introduction of buckwheat. Cooking losses recorded for Farinetta-containing samples were higher than those recorded for Supreme flour-containing samples by as much as 12% at a 30% level of substitution (30S & 30F). At this same level of substitution, the presence of Farinetta resulted in lower water absorption scores compared to Supreme flour by 26%. The cooking losses recorded for the experimental products, however, were about six times lower than those of the 100% whole buckwheat commercial pasta (RefB). Although buckwheat increased digestibility of products, it was able to reduce the glycemic impact by lowering the total amount of reducing sugars in dialysate after 120 min of *in vitro*

digestion. Products substituted with only Farinetta (30F & 20F) had the biggest impact on carbohydrate digestibility by lowering the total amount of reducing sugars released by as much as 18 and 17%, respectively, compared to the control. These results indicate that buckwheat-fortified spaghetti have better cooking quality compared to 100% whole buckwheat noodles and elicit a lower glycemic response upon digestion compared to 100% durum spaghetti.

GENERAL CONCLUSION

It is essential that the fortification of spaghetti be done in a manner which preserves the physical and technological properties that define the product. Fortification of spaghetti with common buckwheat Supreme flour and bran Farinetta resulted in elevated levels of phenolic compounds and antioxidant properties. The effect was greatest with Farinetta. The presence of buckwheat also resulted in a decrease in the amount of available carbohydrate, which ultimately has a bearing on the postprandial blood glucose levels and insulin response. Despite this, the addition of buckwheat resulted in an increase in *in vitro* carbohydrate digestibility of products.

The fortification of durum spaghetti with buckwheat milling fractions which are concentrated in bioactive components is feasible and is a better alternative to 100% buckwheat spaghetti considering the fact that the latter has very poor cooking qualities.

RECOMMENDATIONS FOR FURTHER STUDIES

Further input is required in order to improve upon the cooking quality of buckwheat enriched spaghetti products, and also to investigate their acceptability through sensory analysis.

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APPENDIX

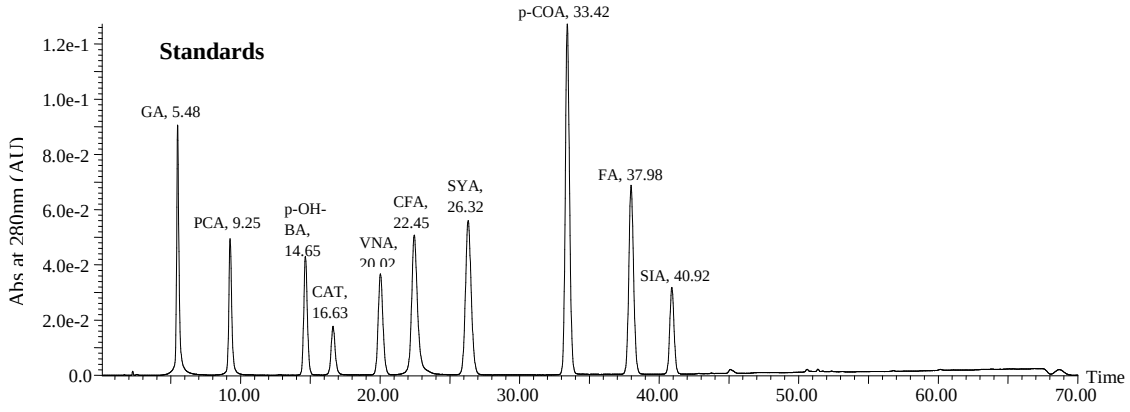
A.1. Moisture, ash and protein content of spaghetti raw materials

	% Moisture	% Ash	% Protein
Control (Semolina)	11.59	0.7	13.29
100S	10.76	2.27	16.43
100F	9.81	5.87	37.83

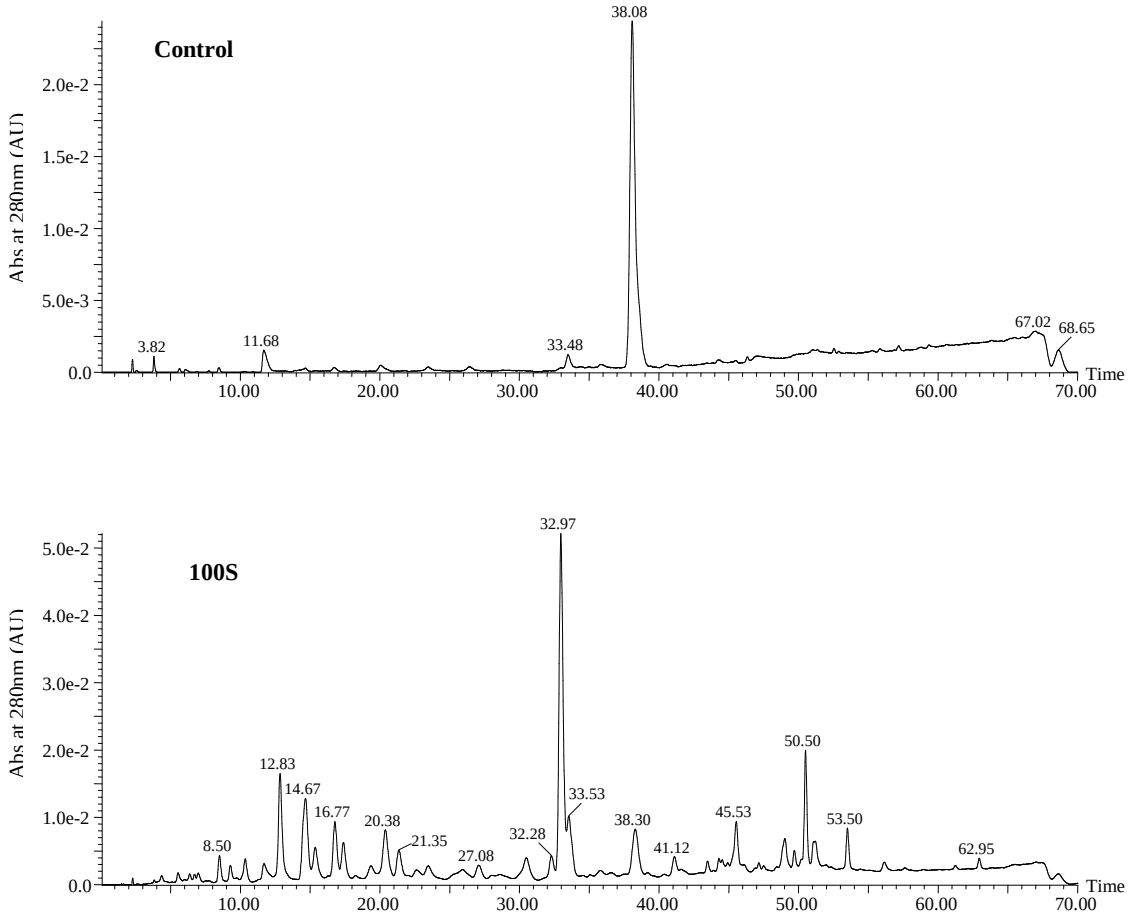
Values are means of duplicate analysis and expressed on a dry weight basis.

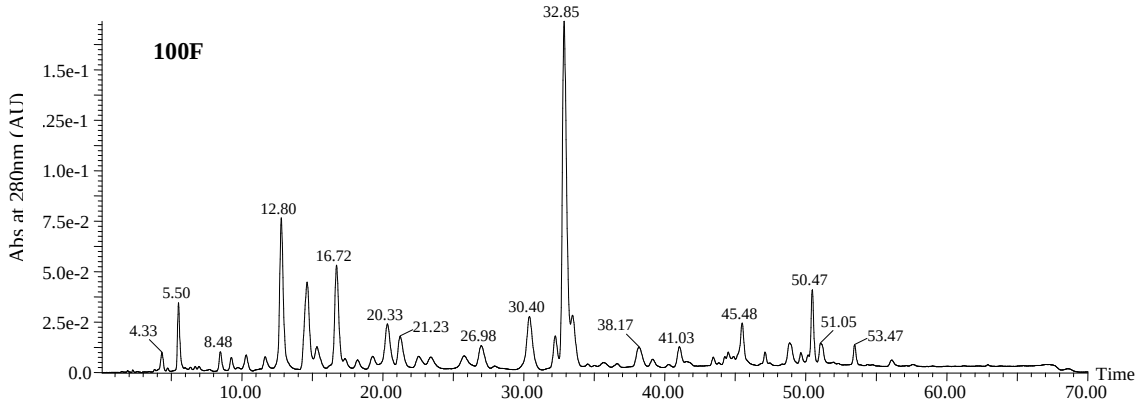
A.2. HPLC chromatograms

A.2.1. HPLC chromatogram of phenolic standards. GA, gallic acid; PCA, protocatechuic acid; p-OH-BA, p-hydroxybenzoic acid; (+)CAT, (+)catechin; VNA, vanillic acid; CFA, caffeic acid; SYA, syringic acid; p-COA, p-coumaric acid; FA, ferulic acid; SIA, sinapic acid.



A.2.2. HPLC chromatograms of phenolic compounds in control, Supreme flour (100S), and Farinetta (100F)





A.2.3. HPLC chromatograms of rutin in Supreme flour (100S) and Farinetta (100F)