Health Potential of Sorghum-Containing Pasta: *In Vitro* and Clinical Studies

by

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MSc (Hons)-Human Nutrition

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PUBLICATIONS

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Manuscript published

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CONFERENCE ABSTRACTS

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Khan, I., Yousif, A., Johnson, S. K., & Gamlath, S. Increasing the health potential of traditional grain foods using high antioxidant/high resistant starch sorghum. HDR Symposium: Research in Food and Nutrition (Show and Tell). Curtin University, Perth, Australia, June, 2011

Khan, I., Yousif, A., Johnson, S. K., & Gamlath, S. Effect of sorghum incorporation on functional components of pasta. Functional Foods Conference: Nutritional and Health Implications. University Putra, Malaysia, November, 2011.

ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] is a cereal crop that is grown in large quantities in Australia and is considered underutilised, as it is used mainly as animal feed (up to 60% of the crop). For human consumption sorghum grain is usually decorticated or milled to whole flour which contain substantial level of beneficial components, for example, slowly digestible starch, resistant starch and polyphenols. These components have been shown to be of benefit in modifying biomarkers for type 2 diabetes, obesity and cardiovascular disease (CVD). The presence of these beneficial components therefore make sorghum a promising candidate for the development of functional foods to reduce the risk of chronic diseases. Nevertheless, for such foods to deliver health benefits, they also need to be palatable so that they will be consumed repeatedly. Currently there is only scant data examining the role of whole grain sorghum flour in regards to its palatability and physiological functionality. Therefore the aim of this thesis was to evaluate the potential of whole grain sorghum flour as a palatable health beneficial ingredient through a series of product development, *in vitro* and clinical studies using pasta as a model food to examine the effect of sorghum on biomarkers for type 2 diabetes and obesity.

In **study 1 (chapter 3)**, pasta was prepared using different substitution levels (20, 30 and 40%) of either whole grain red sorghum flour (RSF) or whole grain white sorghum flour (WSF) for durum wheat semolina (DWS). These sorghum-containing pasta samples were compared to pasta made from 100% DWS (control) for resistant starch content, phenolic profile and antioxidant capacity. The addition of both RSF and WSF at all incorporation levels increased the resistant starch

content, polyphenolic content and antioxidant capacity compared to the control pasta in both uncooked and cooked forms.

In **study 2 (chapter 4)**, the same pasta samples used in study 1 were evaluated for *in vitro* starch digestibility, cooking quality and consumer acceptability using fifty untrained panellists. The addition of both RSF and WSF lowered the *in vitro* starch digestibility at all incorporation levels compared to the control pasta with no deleterious effects on most of the quality attributes (water absorption, swelling index, dry matter, adhesiveness, cohesiveness and springiness), except cooking loss, colour and hardness which were negatively affected. Consumer sensory results indicated that pasta samples containing 20% and 30% RSF or WSF had acceptable palatability based on meeting one or both of the pre-set acceptability criteria.

In **study 3 (chapter 5)**, pasta samples containing higher acceptable incorporation level (i.e., 30%) of both RSF and WSF, identified by a consumer panel in study 2, were used in a human study examining the effect of these pasta samples on post-meal glycaemia, satiety and energy intake. In a randomised cross-over design, 20 healthy subjects consumed four iso-caloric test meals of control pasta (CP), pasta containing 30% RSF (RSP), pasta containing 30% WSF (WSP) and white bread (WB) as a reference meal, following a 10-12 h fast. The RSP meal resulted in lower blood glucose response and glycaemic index (GI), improved satiety parameters and reduced subsequent energy intake from an *ad libitum* lunch compared to the CP meal. No effects of the WSP meal were observed on these parameters compared to the CP meal.

Study 4 (chapter 6), used the same cohort of subjects in study 3 to determine the effect of the sorghum-containing pasta on plasma total polyphenols, antioxidant capacity and oxidative stress biomarkers. In a randomised crossover design the subjects consumed three pasta meals (CP, RSP and WSP), as breakfast. The RSP meal resulted in higher level of plasma total polyphenols, antioxidant capacity, superoxide dismutase (SOD) activity and lower protein carbonyl, a marker of protein oxidation compared to the CP meal. However, WSP meal did not affect these parameters compared to the CP meal.

In conclusion, the RSF-containing pasta has shown potential as a palatable food product with postprandial blood glucose lowering, satiety enhancing, energy intake lowering and oxidant/antioxidant status enhancing effects. This suggest that this product and other made using RSF, may be of use in diets designed to reduce the risk of type 2 diabetes and obesity. No evidence was found to suggest that WSF-containing foods might provide these health beneficial effects. Future studies are now required to further substantiate the potential health benefits of diets incorporating RSF. These include investigations into (i) the *in vivo* mechanisms of the glycaemia lowering and satiety enhancing effects of the RSF (ii) the acute effect of RSF-containing foods on postprandial glycaemia and oxidative stress in individuals with type 2 diabetes (iii) the effect of RSF-containing foods on body weight and composition, lipid profile and oxidative stress in overweight/obese subjects in long term dietary intervention studies.

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LIST OF ABBREVIATIONS

8-iso-PGF$_{2\alpha}$	8-iso-prostaglandin F$_{2\alpha}$
ABS	Australian Bureau of Statistics
ABTS	2,2'–azinobis(3-ethylbenzothiazoline-6-sulfonic acid
BMI	Body mass index
CP	Control pasta
CVD	Cardiovascular disease
DNPH	2,4-dinitrophenylhydrazine
DU-HREC	Deakin University Human Research Ethics Committee
DWS	Durum wheat semolina
FDA	Food and drug administration
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
GI	Glycaemic index
GL	Glycaemic load
GOPOD	Glucose oxidase/peroxidase
GPx	Glutathione peroxidase
HPLC	High performance liquid chromatography
H$_2$O$_2$	Hydrogen peroxide
iAOC	Incremental area over the curve
iAUC	Incremental area under the curve
IDF	International Diabetes Federation
LH	Lipid hydroperoxide
MDA	Malondialdehyde
NSB	Non-specific binding

ORAC	Oxygen radical absorbance capacity
Ox-LDL	Oxidised-low density lipoprotein
PAC-bound	Phenolic acid content of bound extract
PAC-free	Phenolic acid content of free extract
PC	Protein carbonyl
pNpp	p-Nitrophenyl phosphate
RDS	Rapidly digestible starch
RONS	Reactive oxygen/nitrogen species
RS	Resistant starch
RSF	Red sorghum flour
RSP	Red sorghum pasta
SDS	Slowly digestible starch
SI	Satiety index
SOD	Superoxide dismutase
TA	Total activity
TAC	Total antioxidant capacity
TAS	Total antioxidant status
TBARS	Thiobarbituric acid reactive substances
TE	Trolox equivalent
TP	Total polyphenols
TRAP	Total radical-trapping antioxidant parameter
VAS	Visual analogue scale
WB	White bread
WSF	White sorghum flour
WSP	White sorghum pasta

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

The purpose of this introductory chapter is to examine the potential of whole grain sorghum flour in the formulation of high polyphenolic antioxidants and high resistant/slowly digestible starch containing foods. Sorghum flour will be examined for its potential as a palatable and physiologically effective food ingredient for the beneficial modification of biomarkers for type 2 diabetes and obesity. This chapter concludes by presenting the overall aims and hypotheses of this thesis.

1.1   INTRODUCTION

Sorghum is the world's fifth most important cereal crop which is consumed as a staple food in many countries of Africa and Asia (Taylor & Emmambux, 2010). It plays an important role in sustainable grain production, particularly in semi-arid regions of the world, due to its drought and high temperature tolerance and is therefore considered an important cereal crop for food security in these regions (Taylor, Schober, & Bean, 2006). In Australia, sorghum grain is used almost exclusively as an animal feed and is still underutilised as a human food source (Mahasukhonthachat, Sopade, & Gidley, 2010). Whole grain sorghum flour may be valuable as an ingredient in a number of food products due to the presence of health promoting constituents including slowly digestible starch, resistant starch and polyphenolic antioxidants (Yousif, Nhepera, & Johnson, 2012; Licata et al., 2013; Awika & Rooney, 2004; Dykes & Rooney, 2006). However, research studies conducted on sorghum grain so far have mainly focused on increasing its energy value and improving its starch and protein digestibility (Taylor et al., 2006) and little research has investigated using sorghum flour for the formulation of foods

with high levels of slowly digestible/resistant starch and polyphenolic antioxidants. Also information is lacking on the changes in starch digestibility and the stability of polyphenolic antioxidants in sorghum-containing foods during processing and cooking.

In order to deliver any health benefits, food products must be developed that are acceptable to consumers so that they will be consumed repeatedly. The incorporation of sorghum flour could potentially reduce product palatability and quality, through modifying flavour and texture of the products. These undesirable effects might arise due to the bitter and astringent flavour produced by sorghum phenolic compounds (Abdelghafor, Mustafa, Ibrahim, & Krishnan, 2011; Kobue-Lekalake, Taylor, & De Kock, 2007) and the absence of gluten protein in sorghum flour (Taylor & Emmambux, 2010). These potentially negative effects can be minimised by optimising the level of sorghum flour in food formulations using sensory evaluation and instrumental textural evaluation techniques to help guide new product development (Meilgaard, Civille, & Carr, 2007; Augustin, 2001).

The development of new products from whole grain sorghum flour is an important area of research since diet is considered an important determinant in the development of major chronic diseases such as type 2 diabetes, obesity and cardiovascular disease (CVD) (Nishida, Uauy, Kumanyika, & Shetty, 2004). Highly-refined cereal grains, which are low in slowly digestible starch, resistant starch and polyphenolic antioxidants but high in rapidly digestible starch, predominate in the Western diet and may contribute significantly to the development of such diseases (Aston, 2006). The use of whole grain sorghum flour,

containing slowly digestible starch, resistant starch and polyphenolic antioxidants, as an ingredient in staple foods such as pasta is a viable approach to develop new foods targeted at improving biomarkers for chronic diseases.

The purpose of this thesis is therefore to (i) identify formulations for maximum incorporation of whole grain sorghum flour into pasta, to increase its content of slowly digestible starch, resistant starch and polyphenolic antioxidants whilst maintaining cooking quality and consumer acceptability and to (ii) determine the effect of these newly developed sorghum-containing pastas on postprandial glycaemia, appetite and energy intake and oxidative stress biomarkers in healthy humans.

1.2   SORGHUM-THE CEREAL GRAIN

Cereal grains are the staple foods for a large proportion of the world population and are the major contributors of energy in the diet of people in both developed and developing countries (BNF, 2004). Sorghum is one of the cereal grains belong to the *Poaceae* family and is known by different names in different countries. The most common names are: milo or sorghum (USA), guinea corn (West Africa), Jowar (India), kaffir corn (South Africa) and sorghum (UK and Australia) (Dicko, Gruppen, Traore, Voragen, & van Berkel, 2006). In terms of production, sorghum is ranked fifth in the world and is the third most important cereal crop after wheat and barley in Australia (Mahasukhonthachat et al., 2010). In Australia sorghum production is about 1.4 million tonnes which is used almost exclusively as an animal feed (DAFF, 2012). Currently, grain sorghum varieties presented in **Table 1.1** are grown on approximately 600,000-700,000 hectare in north-eastern

Australia, with the major production areas being Queensland (approximately 60%) and New South Wales (approximately 38%) (DAFF, 2012).

Table 1.1. Grain sorghum varieties cultivated in Australia

Variety	Grain colour
Alpha	Red
Liberty	White
Dominator	Red
Venture	Bright red
MR-Pacer	Red
MR-Buster	Red
MR-Bounty	Red

Source: Adapted from DAFF (2012).

1.3 FOOD AND FEED USES OF SORGHUM

Sorghum is used as human food, animal feed and for industrial products. About 40% of the grain sorghum produced worldwide is used in the preparation of various traditional foods mainly in Africa and Asia (Pontieri et al., 2013). In these regions sorghum is milled as whole grain or decorticated (Dlamini, Taylor, & Rooney, 2007) and is then used in various food products such as tortilla, couscous, various types of porridges, cookies, snacks and fermented and unfermented breads (Carson, Setser, & Sun, 2000; Taylor et al., 2006). Furthermore sorghum is often recommended as a safe food for celiac patients, because it provides a good basis for gluten free breads and other baked products like cakes and biscuits (Kasarda, 2001). In developed countries like United States, UK and Australia sorghum is mainly grown for animal feed and is also used in the pet food industry (Taylor et al., 2006).

1.4   STRUCTURE AND NUTRIENT COMPOSITION OF SORGHUM GRAIN

Sorghum grain is a naked single-celled seed (caryopsis) with common colours of white, red, bronze, brown and black. It is composed of three major components: pericarp, germ (embryo) and endosperm. The pericarp is the outer layer and accounts for about 6% of the total grain weight and contains starch, fibre and various phytochemicals (Rooney & Serna-Saldivar, 2000). Some sorghum varieties contain a pigmented testa, located beneath the pericarp which contains condensed tannins (proanthocyanidins) (Dykes & Rooney, 2006). The endosperm (84% of grain weight) is the inner area and mainly consists of protein (primarily kafirin) and starch granules (Rooney & Serna-Saldivar, 2000). The embryo accounts for 10% of the grain weight and contains reserve nutrients, i.e., moderate amounts of oil, protein, enzymes and minerals (Rooney & Serna-Saldivar, 2000).

Nutritionally sorghum is comparable to other major cereals (Duodu, Taylor, Belton, & Hamaker, 2003). The levels of major nutrients are 55-75% carbohydrates, 7-12% protein, 1.4-4% fat, 0.5-2% ash and 6-10% total dietary fibre **(Table 1.2)**. However, sorghum is different from other cereals due to its low protein and starch digestibility (Rooney & Awika, 2005). Due to the low starch digestibility, sorghum may be advantageous for people with type 2 diabetes and obesity. Furthermore, sorghum grain contains substantial levels of a wide range of polyphenolic compounds which have health promoting properties, particularly antioxidant activity (Dykes & Rooney, 2006). The present review will therefore mainly focus on the starch digestibility and polyphenolic antioxidants of sorghum grain in relation to human health.

Table 1.2. Energy and nutrient values of major cereals and sorghum (/100g dry wt)

Cereal	Energy (kcal)	CHO (g)	Protein (g)	Fat (g)	Ash (g)	TDF (g)
Wheat	365	72.0	13.0	3.0	2.0	10.0
Rice	380	78	9.5	3.1	2.2	7.6
Maize	370	76	9.0	4.0	2.0	6.0
Sorghum	348	74	9.0	2.5	1.5	9.0

CHO: carbohydrates; TDF: total dietary fibre. Source: Adapted from FAO (1995).

1.5 SORGHUM STARCH AND ITS DIGESTIBILITY

Starch is the primary source of stored energy in cereal grains and generally compromises between 55 to 75% of the weight of the grain (Koehler & Wieser, 2013). In sorghum grain starch is deposited in the endosperm cells in the form of semicrystalline granules embedded in a continuous protein matrix and is composed of two types of polysaccharides: amylose and amylopectin (Taylor & Emmambux, 2010). Amylose is a linear polymer of α-1,4 linked glucose units. Amylopectin is a much larger extensively branched polymer of linear chains of α-1,4 linked glucose units, with α-1,6 branch points every 20 to 25 glucose residues. A normal sorghum starch granule contains 23-30% of amylose with the remainder being amylopectin (Taylor & Emmambux, 2010). Most cultivars of sorghum contain a hard corneous-type endosperm in which starch granules are tightly bound in a rigid protein matrix (Taylor & Emmambux, 2010). This physical matrix makes sorghum starch less susceptible to digestive enzyme attack and is one of the reasons for the low digestibility of sorghum starch.

Sorghum starch has a lower *in vitro* starch digestibility than other cereals in raw form and even after cooking has a lower starch digestibility compared to other

cereals (Barros, Awika, & Rooney, 2012). There is also strong evidence from animal studies that the energy value of sorghum grain is less than other cereals (Taylor & Emmambux, 2010). In *in vitro* studies sorghum and sorghum-based foods have shown low starch digestibility compared to other cereals. Zhang and Hamaker (1998) showed that the starch digestibility of cooked sorghum flour was 15-25% lower than that of cooked maize flour. Yousif et al. (2012) investigated the effect of whole grain sorghum flour incorporation on flat bread *in vitro* starch digestibility and showed that flat bread, containing sorghum flour at 40% incorporation exhibit reduced starch digestibility compared to the control flat bread made from wheat flour only. More recently Licata et al. (2013) showed that expanded snack-like products made from sorghum-maize composite flours using high-temperature high-pressure extrusion exhibit lower *in vitro* starch digestibility compared to expanded snack-like products made from maize flour only.

Several intrinsic and extrinsic factors have been shown to be responsible for the reduced digestibility of sorghum starch. Among the intrinsic factors amylose to amylopectin ratio play a role in the digestibility of sorghum starch. A higher ratio of amylose in the starch is associated with lower starch digestibility. *In vitro* and animal studies have consistently shown that waxy sorghum (having no or little amylose) is more digestible than normal sorghum (24-33% amylose) (reviewed by Rooney & Pflugfelder, 1986). Compared to other cereals some varieties of sorghum have high amylose content and hence exhibit low starch digestibility (Akerberg, Liljeberg, Granfeldt, Drews, & Bjorck, 1998).

The interaction between sorghum endosperm proteins (kafirins) and starch granules has been studied extensively as a main extrinsic factor that influences sorghum

starch digestibility (Ezeogu, Duodu, Emmambux, Taylor, 2008; Taylor & Emmambux, 2010). This interaction of starch with endosperm proteins seems to reduce the susceptibility of both native and processed starch to hydrolytic enzymes and consequently decrease its digestibility (Rooney & Pflugfelder, 1986). Kafirins in sorghum are mainly formed by intermolecular disulphide cross-linking (Chandrashekar & Mazhar, 1999). Ezeogu, Duodu, and Taylor (2005) found that cooking sorghum flour in the presence of a reducing agent (2-mercaptoethanol) improved its starch digestibility by preventing or reducing disulphide-bonded polymerization of the kafirins that can occur during moist heating, suggesting that sorghum proteins may act as barrier to starch digestion.

The interaction of sorghum polyphenols with starch is another extrinsic factor contributing to the low digestibility of sorghum starch. Barros et al. (2012) investigated the interaction of sorghum tannins (proanthocyanidins) with starch molecules and the resulting effect on *in vitro* starch digestibility. They reported that sorghum tannins interact strongly with starch and that this contributes to decreased starch digestibility.

The inhibitory effect of sorghum polyphenols on digestive enzymes has also been extensively studied as a potential factor contributing to the low digestibility of sorghum starch. Kim, Hyun, and Kim (2011) investigated the *in vitro* inhibitory effect of ethanolic extract from sorghum, foxtail millet and proso millet on α-glucosidase and α-amylase activities. They demonstrated that the ethanolic extract of sorghum inhibited both α-glucosidase and α-amylase activities while those of the millets had no detectible effect on the activity of these enzymes. Hargrove,

Greenspan, Hartle, and Dowd (2011) compared the effect of bran extracts from tannin-containing sumac sorghum and tannin-free (but flavonoids rich) black sorghum on *in vitro* α-amylase activity. They reported that both flavonoids and tannins from sorghum inhibited α-amylase activity. More recently Mkandawire et al. (2013) determined the effect of extracts from tannin-containing sorghum on α-amylase activity and *in vitro* starch digestibility. Their results indicated that sorghum extracts reduced α-amylase activity in a dose dependent manner. In addition the extracts decreased the slowly digestible starch and increased the resistant starch levels in maize starch.

1.6   LINK BETWEEN STARCH DIGESTIBILITY AND HUMAN HEALTH

Starch can be classified into three types, namely rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) based on the action of digestive enzymes to release glucose from the starch (Englyst, Kingman, & Cummings, 1992). The starch fraction digested within 20 min of incubation is classified as RDS while the starch fraction digested between 20 and 120 min is classified as SDS and the starch fraction which is not digested even after 120 min of incubation is termed as RS (Englyst et al., 1992). During digestion when the food enters into the small intestine, the RDS present in the food is quickly hydrolysed by the amylolytic enzymes (pancreatic α-amylase and the intestinal brush border enzymes) (Nichols et al., 2003) into glucose units which results in an immediate increase in blood glucose. Like RDS, SDS is also completely digested in the small intestine, however SDS requires a longer digestion time and the glucose units from SDS release at a slower pace and hence the effect of SDS on blood glucose is not immediate (Miao, Jiang, Cui, Zhang, & Jin, 2013). RS escapes digestion in the

small intestine and is fermented into short chain fatty acids by the colonic microflora. Since RS is not digested to glucose units it has less impact on blood glucose concentration than digestible starch (Rahman et al., 2007). Food sources and digestion rate of RDS, SDS and RS are given in **Table 1.3**.

Table 1.3. Classification of starch (based on Englyst et al., 1992)

Starch fraction	Example	Digestion time and rate in small intestine
RDS	Freshly cooked starchy foods, white bread	Rapid and complete (20 min)
SDS	Most cereals such as wheat, maize, sorghum	Slow but complete (20-120 min)
RS		
1. Physically inaccessible (RSI)	Partially milled/whole grains	Resistant to digestion
2. RS granule (RSII)	Banana, raw potato, beans	Resistant to digestion
3. Retrograded (RSIII)	Cooled cooked potato	Resistant to digestion
4. Chemically modified (RS IV)	Many types	Resistant to digestion

RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch.

Frequent and immediate increase in blood glucose concentration has been shown to be associated with insulin resistance, oxidative stress and inflammation (Del Prato et al., 1994; Ceriello, 2000). Elevated blood glucose and insulin levels have also been associated with increased hunger and decreased satiety (reviewed by Sloth & Astrup, 2006). These conditions in turn are associated with the progression of diet related chronic diseases such as type 2 diabetes, obesity and CVD (Willett, Manson, & Liu, 2002; Maritim, Sanders, & Watkins, 2003; Bagatini et al., 2011; Liu et al., 2000; Brand-Miller, Holt, Pawlak, & McMillan, 2002). The long term consumption

of foods containing SDS and RS compared with those containing RDS may therefore decrease the risk of type 2 diabetes, obesity and CVD. A number of randomised controlled trials have investigated the effect of foods containing high levels of SDS and RS compared to those containing high level of RDS on post-meal blood glucose, insulin, appetite and energy intake. The results of these studies are inconsistent but the majority have shown beneficial effects of foods high in SDS and RS on these parameters **(Table 1.4)**. Nilsson, Johansson, Ekstrom, and Bjorck (2013) in a randomised crossover design, evaluated the effect of brown beans (providing 6.5 RS) in comparison to a white wheat bread on blood glucose, insulin and subjective appetite. The foods were provided as evening meals to 16 healthy subjects. The results showed that brown beans meal reduced postprandial blood glucose, insulin and sensation of hunger compared to the white wheat bread meal within a time frame of 11-14 h. Eelderink et al. (2012) in a randomised crossover study in healthy male, compared the effect of ^{13}C-enriched pasta (as a source of SDS) with ^{13}C-enriched bread (as a source of RDS) on blood glucose and insulin for a period of 6 h. The results showed that pasta meal reduced postprandial insulin without being able to reduce postprandial glycaemic response compared to the bread meal. These authors concluded that, a similar glycaemic response after pasta and bread, despite high levels of SDS in pasta, can be explained by slower uptake of glucose into tissues. Klosterbuer, Thomas, and Slavin (2012) documented postprandial reduction in blood glucose and insulin following consumption of muffins containing RS or RS and pullulan compared with a low-fibre control muffin. However, appetite and energy intake were not affected following consumption of these muffins. The other studies (van Can, van Loon, Brouns, & Blaak, 2012; Anderson et al., 2010; Willis, Eldridge, Beiseigel, Thomas, & Slavin,

2009; Sands, Leidy, Hamaker, Maguire, & Campbell, 2009; Ells, Seal, Kettlitz, Bal, & Mathers, 2005; Harbis et al., 2004; Seal et al., 2003) presented in **Table 1.4** have shown beneficial effects of foods containing SDS and RS on blood glucose, insulin, appetite or energy intake.

Table 1.4. Randomised controlled studies of foods containing SDS/RS on glycaemia, insulinaemia, subjective appetite and energy intake

Reference	Design and subjects	Treatment	Outcome measures	Major results
Nilsson et al. (2013)	Single meal CO; $n = 16$ Hlty, Nrmwt M/F, 23* y	White bread (C) vs brown beans (RS)	Glucose, insulin, appetite	Brown beans meal ↓ blood glucose, insulin and hunger compared to white bread meal
Eelderink et al. (2012)	Single meal CO; $n = 10$ Hlty, Nrmwt M, 21* y	^{13}C-enriched bread (C) vs ^{13}C-enriched pasta (SDS)	Glucose, insulin	Pasta ↓ insulin but did not affect blood glucose compared to bread
van Can et al. (2012)	Two meals CO; $n = 10$ Hlty, Ovwt M/F, 56* y	Glucose or sucrose (C) vs trehalose or isomaltulose (SDS)	Glucose, insulin	Trehalose and isomaltulose ↓ plasma glucose and insulin compared to both glucose and sucrose
Klosterbuer et al. (2012)	Single meal CO; $n = 20$ Hlty, Nrmwt M/F, 18-60 y	Low-fibre muffin (C) vs soluble corn fibre, RS, soluble corn fibre + pullulan or RS + pullulan containing muffins	Glucose, insulin, appetite, energy intake	RS and RS + pullulan muffins ↓ blood glucose and insulin but did not affect appetite and energy intake compared to low-fibre muffin
Anderson et al. (2010)	Single meal CO; $n = 17$ Hlty, Nrmwt M, 20-30 y	Tomato soup (C) or maltodextrin (RDS) vs whole grain (Hi-maize), high amylose (Hi-maize 260), regular corn starch (SDS, RS)	Glucose, appetite, energy intake	Hi-maize, Hi-maize 260 and regular corn starch ↓ blood glucose, appetite and energy intake compared to maltodextrin

(Continued)

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Table 1.4. (Continued)

Reference	Design and subjects	Treatment	Outcome measures	Major results
Willis et al. (2009)	Single meal CO; $n = 20$ Hlty, Nrmwt M/F, 18-65 y	Low-fibre muffin (C) vs polydextrose, barley-oat, RS or corn bran muffins (RS)	Satiety	Resistant starch and corn bran muffins ↑ satiety compared to low-fibre muffin
Sands et al. (2009)	Single meal CO; $n = 12$ Hlty, Nrmwt M/F, 23* y	White bread (C) vs waxy maize starch (SDS) vs maltodextrin (RDS)	Glucose, insulin, appetite	Waxy maize starch ↓ blood glucose and insulin but appetite was not affected compared to maltodextrin
Ells et al. (2005)	2 week CO; $n = 10$ Hlty, Nrmwt F, 20-37 y	Thinned waxy maize starch (RDS) vs native waxy maize starch (SDS)	Glucose, insulin	Native waxy starch ↓ blood glucose and insulin compared to thinned waxy starch
Harbis et al. (2004)	Single meal CO; $n = 9$ Hlty, Ob M/F, 25-45 y	Wheat biscuit (SDS) vs wheat flakes (RSD)	Glucose, insulin	Biscuit meal ↓ blood glucose and insulin compared to flakes meal
Seal et al. (2003)	Single meal CO; $n = 8$ Hlty, Nrmwt M/F, 44* y and $n = 13$ Type 2 diabetic, Ovwt and Ob M/F, 52* y	Starch (SDS) vs starch (RDS)	Glucose, insulin	Starch (SDS) ↓ blood glucose and insulin compared to starch (RDS) in both healthy and diabetic subjects

*mean age; CO: crossover; Hlty: healthy; Nrmwt: normal-weight; Ovwt: over-weight; Ob: obese; M: male; F: female; C: control; RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch; ↑: increase; ↓: decrease.

1.7 SORGHUM POLYPHENOLS

Phenolic compounds, of which more than 8000 are known, represent a wide range of plant secondary metabolites (Pietta, 2000). Research studies have shown that cereal grains, fruits, vegetables and legumes contain phenolic compounds that may provide health benefits beyond that of basic nutrients (Hanhineva et al., 2010). In all cereal grains, phenolic compounds are mainly concentrated in the outer bran layer and a substantial amount is lost when the bran is removed during decortication (Ragaee, Seetharaman, & Abdel-Aal, 2012).

Among cereal grains, some varieties of sorghum have the higher contents of phenolic compounds (Awika & Rooney, 2004) ranging from 0.5-22.5 mg GAE/g **(Table 1.5)**. Phenolic compounds in sorghum can be classified into three main categories, namely phenolic acids, flavonoids and tannins **(Table 1.6)**. Phenolic acids are found in almost all sorghum varieties and include derivatives of benzoic acid (hydroxybenzoic acids) or cinnamic acid (hydroxycinnamic acids) (Dykes & Rooney, 2006). These compounds are present either in free form (in pericarp, testa and aleurone layers) or in bound form (esterified to cell wall). It has been shown that phenolic acids of sorghum are mainly present in the bound form (85%), with ferulic acid being the major bound phenolic acid (47%) (Dykes & Rooney, 2006).

Flavonoids constitute the largest group of phenolic compounds in the plant kingdom and are present in nature as glycosides (Cook & Samman, 1996). Several classes of flavonoids have been reported in sorghum grain such as anthocyanins, flavanols, flavanones, flavones and flavan-4-ols (Awika & Rooney, 2004; Awika, Rooney, &

Waniska, 2004a). Among flavonoids, anthocyanins are the major phenolics studied in sorghum. The most common anthocyanins in sorghum are 3- deoxyanthocyanidins (e.g. apigeninidin and luteolinidin, which confer yellow and red colours, respectively). These anthocyanins are distinct because they do not contain the hydroxyl group in the 3-position of the C-ring (Pale, Kouda-Bonafos, Nacro, Vanhaelen-Fastre, & Ottinger, 1997). Luteolinidin and apigeninidin represent 36 to 50% of the total anthocyanin contents in black and tannin sorghum and 19% in red sorghum (Awika, Rooney, & Waniska, 2004b). The common flavones in sorghum are apigenin and luteolin and are predominant in tan varieties (Dykes & Rooney, 2006).

Some sorghum varieties with a pigmented testa contain condensed tannins (proanthocyanidins) that are often called polyflavans (Dykes & Rooney, 2006). Condensed tannins are polymeric flavonoids and consist of flavan-3-ols (proanthocyanidins) and/or flavan 3, 4-diols (pro-3-deoxyanthocyanidins) units linked by C-C interflavan bonds, called B-type proanthocyanidins (Krueger, Vestling, & Reed, 2003; Awika & Rooney, 2004). The most abundant proanthocyanidins in sorghum are homopolymers of catechin/epicatechin, with uniform B-type interflavan bonds (Krueger et al., 2003). On the basis of tannin content sorghum varieties are classified as type I (without tannins), type II (tannins present in pigmented testa), or type III (tannins present in pigmented testa and pericarp) (Dykes & Rooney, 2006).

Table 1.5. Total phenolic content of different sorghum grain types

Sorghum type	Total phenolic (mg GAE/g)	Reference
(A) Tannin-free		
White	2.7	Dlamini et al. (2007)
	0.5	Dykes et al. (2005)
	0.8	Awika et al. (2005)
	0.524-0.763	Kamath et al. (2004)
Red	5.3	Dlamini et al. (2007)
	1.9-5.0	Dykes et al (2005)
	4.8	Awika et al (2004b)
Black	5.3-19.8	Dykes et al. (2013)
	7.6-9.8	Awika et al. (2005)
(B) Tannin-containing		
Red	19.7-24.5	Dlamini et al. (2007)
	7-8.8.8	Dykes et al (2005)
Black	8.9	Dykes et al (2005)
Brown	11.7-22.5	Awika et al. (2004b)

GAE: gallic acid equivalent (Folin Ciocalteu method).

Table 1.6. Major Phenolic acids, flavonoids and tannins in sorghum grain

Major class	Subclass class	Compound
Phenolic acids	Hydroxybenzoic acids	Gallic, protocatechuic, salicylic, gentisic, *p*- hydroxybenzoic, vanillic, syringic
	Hydroxycinnamic acids	Ferulic, caffeic, *p*-coumaric, sinapic, cinnamic
Flavonoids	Anthocyanins	Apigeninidin and derivatives, luteolinidin and derivatives
	Flavones	Apigenin, luteolin
	Flavanones	Eriodictyol and derivatives
	Flavonols	Kaempferol
	Flavan-4-ols	Apiforol, luteoforol
	Dihydroflavonols	Taxifolin and derivatives
Condensed tannins	Proantocyanidin	Catechin, procyanidin B-1

Source: Adapted from Taylor, Belton, Beta, & Duodu (2013).

1.8 ANTIOXIDANT ACTIVITY AND BIOAVAILABILITY OF SORGHUM POLYPHENOLS

Foods rich in phenolic compounds such as cereal grains have been shown to possess antioxidant activity (Ragaee, Abdel-Aal, & Noaman, 2006). A number of studies have reported the antioxidant activity of different sorghum grain types, ranging from 10-427 μmol TE/g (ABTS method) and 3-310 μmol TE/g (DPPH method) **(Table 1.7)**. Antioxidant capacity of sorghum grain extracts is strongly correlated with its total phenolic content indicating that phenolic compounds are primarily responsible for the antioxidant activity of this grain (Dykes, Rooney, Waniska, & Rooney, 2005).

All of the major classes of sorghum phenolics including phenolic acids, flavonoids and condensed tannins have been shown to have antioxidant activity (Dykes & Rooney, 2006). The antioxidant activity of phenolic acids depends on the number of hydroxyl groups. Between the two main types of phenolic acids, hydroxycinnamates have greater antioxidant activity than hydroxybenzoates due to the presence of more hydroxyl groups, which ensure greater hydrogen donating ability and radical stabilisation (Rice-Evans, Miller, & Paganga, 1996). Due to the small molecular size phenolic acids are more readily absorbed from the intestinal tract compared to high molecular weight polyphenols (flavonoids and tannins) (Scalbert, Manach, Morand, Remesy, & Jimenez, 2005). The majority of phenolic acids in sorghum and other cereals are tightly bound to cell wall and can only be extracted by alkaline hydrolysis. However, microbial fermentation of cereal grains has been shown to increase the bioavailability of bound phenolic acids. Andreasen, Kroon, Williamson, and Garcia-Conesa (2001) reported that esterases present in human colon are capable of releasing esterified diferulates and other hydroxycinnamic acids from cereal grains. This implies that the bound phenolic acids of sorghum may also be potentially bioavailable and will contribute to the *in vivo* antioxidant activity.

The antioxidant activity of flavonoids depends on the configuration of hydroxyl group on the B ring (Burda & Oleszek, 2001). Hydroxyl group on the B ring denote hydrogen and electron to the hydroxyl and peroxyl radicals, as a result these radicals are stabilised and give rise to stable flavonoid radicals, which in turn reacts with free radicals and terminate the radical chain reaction (Cook & Samman, 1996). Awika and Rooney

(2004) reported that the antioxidant capacity of sorghum is well correlated with its flavonoid (anthocyanin) contents. The bioavailability of sorghum anthocyanins and other flavonoids has not yet been reported. However, the bioavailability of flavonoids reported from other food sources (Mulleder, Murkovic, & Pfannhauser, 2002; Cao, Muccitelli, Sanchez-Moreno, & Prior, 2001; Manach, Williamson, Morand, Scalbert, & Remesy, 2005), implies that the flavonoids from sorghum and sorghum-containing foods may also be absorbed from the human intestinal tract.

The antioxidant activity of sorghum tannins (proanthocyanidins) depends on the *ortho*-phenolic hydroxyl group located in the B ring (Schofield, Mbugua, & Pell, 2001). High molecular weight tannins have the greatest antioxidant activity among phenolic compounds due to the presence of many hydroxyl groups in their structures (Bors, Michel, & Stettmaier, 2000). Like other phenolics the bioavailability of sorghum tannins has not yet been reported. In previous studies bioavailability of proanthocyanidins from other food sources is reported to be poor as polymeric proanthocyanidins are not absorbed as such and only dimers and monomers of these compounds are absorbed in low quantities (reviewed by Manach et al. 2005).

Table 1.7. Antioxidant activity of different sorghum grain types

Reference	Sorghum grain type	Antioxidant activity	
		ABTS (µmol TE/g)	DPPH (µmol TE/g)
Dykes et al. (2013)	Black	80-334.2	32.4-177.2
Dlamini et al. (2007)	White	22	3
	Red	52	12
	Red (tannin)	359- 427	175- 310
Awika et al. (2005)	Brown (tannin)	114-240	103-202
	Black	89-104	49-52.6
	White	9.8	6.2
Dykes et al. (2005)	White	10	5
	Red	30-85	12-30
	Red (tannin)	145-150	55-70
	Black (tannin)	175	85
Awika et al. (2004b)	Brown (tannin)	103-226	97-202

ABTS: 2,2-azinobis (3-ethyl-benzothiazoline-6-sulphonic acid); DPPH: 1,1-diphenyl-2-picrylhydrazyl; TE: trolox equivalent.

1.9   LINK BETWEEN POLYPHENOLS AND HUMAN HEALTH

Polyphenols in foods have attracted great interest in recent years due to their potential for beneficial effects on human health. In epidemiological studies, an inverse association has been shown between the consumption of polyphenol-rich foods and the risk of chronic diseases such as diabetes, CVD and cancer (Scalbert et al., 2005; Arts & Hollman, 2005; Wang, Chun, & Song, 2013). Recent literature suggests that polyphenols may reduce the risk of chronic diseases by reducing oxidative stress through their antioxidant properties (Santhakumar, Bulmer, & Singh, 2014).

Oxidative stress is an imbalance between the generation of free radicals in the body, such as reactive oxygen/nitrogen species (RONS) and the antioxidant defence system (Perez-Matute, Zulet, & Martinez, 2009). The primary antioxidant defence system consists of endogenous antioxidants enzymes such as glutathione peroxidase (GPx), catalase and superoxide dismutase (SOD) and external antioxidants obtained by dietary intake such as polyphenols, α-tocopherol, β- carotene, ascorbic acid, zinc, selenium, and copper (Scalbert & Williamson, 2000). Free radicals include the hydroxyl radical (OH), superoxide radical (O^-_2), nitric oxide radical (NO) and non-radicals such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) (Lamb & Goldstein, 2008). The presence of a small number of these free radicals is essential for the normal functions of the body as they are involved in signal transduction (Circu & Aw, 2010). However, in certain conditions (**Figure 1.1**), the production of RONS exceeds that of antioxidant defence system. In such conditions free radicals predominate, which then attack biological molecules such as lipids, protein, carbohydrates and DNA, resulting in membrane and tissue damage and enzyme inactivation. These condition in turns disturb the normal body functions leading to the development of chronic diseases such as type 2 diabetes, CVD and cancer (Ruskovska & Bernlohr, 2013). Examples of biological molecules damaged by the action of RONS include oxidised lipids (oxidised low-density lipoproteins, 8-isoprostanes, thiobarbituric acid reactive substances, malondialdehyde), oxidised proteins (protein carbonyls, protein thiols, 3-nitrotyrosine), oxidised carbohydrates (glycated products) and oxidised DNA bases (8-hydroxydeoxyguanosine, 5-hydroxymethyldeoxyuridine). These oxidised molecules are used as biomarkers of oxidative stress in biological studies (Perez-Matute et al.,

2009). Under the above conditions, an exogenous source of antioxidants may be required in order to prevent oxidative stress-related damage of membranes and tissues. The polyphenols, possess structural characteristics favourable for radical scavenging, which enable them to act as effective antioxidants against RONS (Visioli et al., 2011).
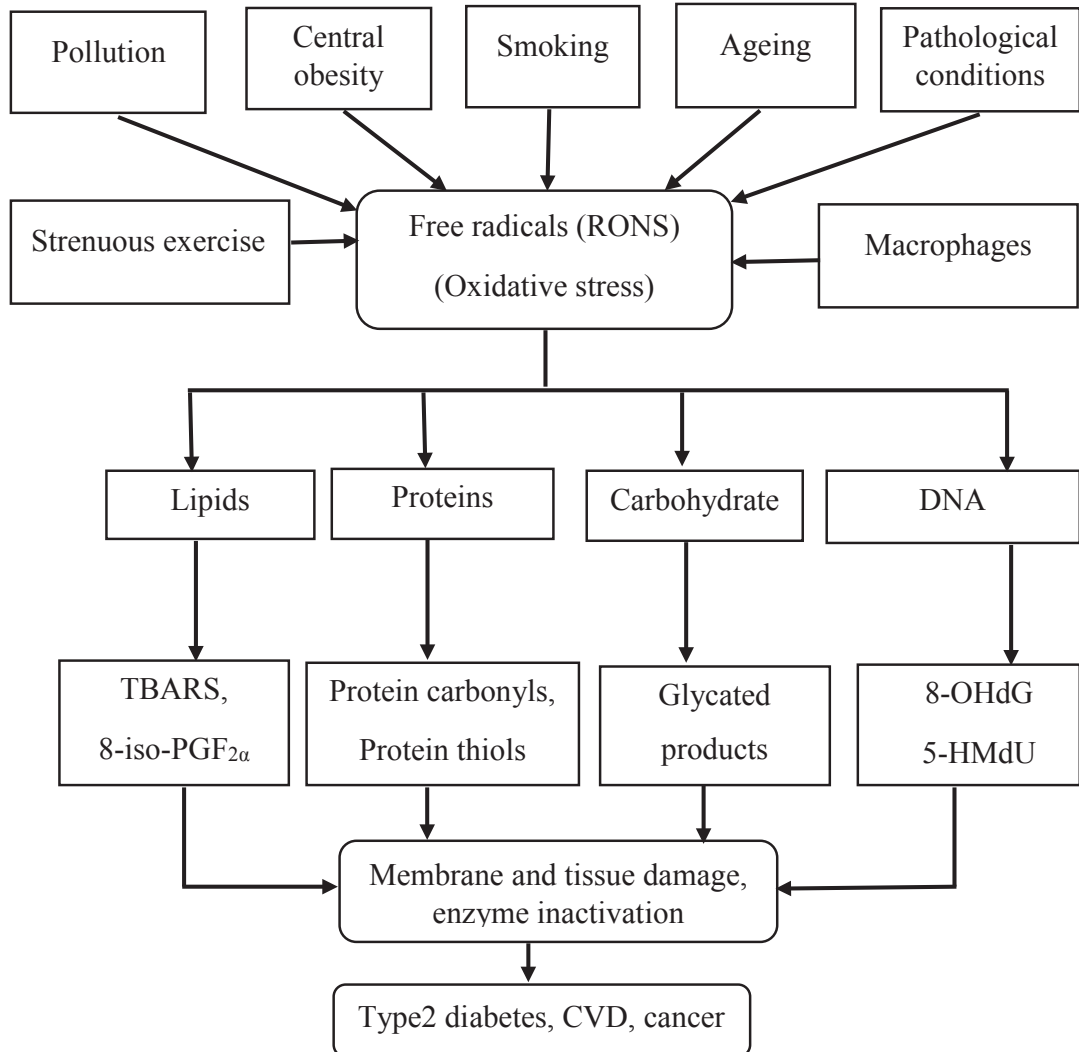
Figure 1.1. Schematic representation of the role of oxidative stress in the development of chronic diseases. RONS: reactive oxygen/nitrogen species; TBARS: thiobarbituric acid reactive substances; 8-iso-PGF$_{2\alpha}$: 8-iso-prostaglandin F$_{2\alpha}$; 8-OHdG: 8-hydroxydeoxyguanosine; 5-HMdU: 5-hydroxymethyldeoxyuridine. (Source: Adopted from Santhakumar et al., 2014; Perez-Matute et al., 2009).

The effect of polyphenol-rich foods on antioxidant status and oxidative stress biomarkers has been investigated in a number of randomised controlled studies **(Table 1.8)**. The results of these studies are inconsistent, but the majority have shown beneficial effects of polyphenol-rich foods on some or all of the oxidant/antioxidant biomarkers in both healthy and diabetic individuals. Among the range of foods studied, nuts were the most commonly investigated. Haddad, Gaban-Chong, Oda, and Sabate (2014) investigated the effect of a meal containing walnut (providing 1400 mg total polyphenols) in comparison to a control meal containing white bread (providing 11.8 mg total polyphenols) on plasma total phenols, antioxidant capacity (ferric reducing antioxidant power and oxygen radical absorbance capacity) and oxidative stress markers (malondialdehyde and oxidised-low density lipoprotein). The meals were provided as breakfast to 16 healthy subjects. The results indicated that the walnut meal increased oxygen radical absorbance capacity (ORAC) and decreased malondialdehyde (MDA) levels within a postprandial time frame of 5 h. Liu, Liu, Chen, Chang, and Chen (2013) in a randomised crossover design in type 2 diabetic subjects, compared the effect of an almond-containing meal with a control meal on total polyphenols, ferric reducing antioxidant power (FRAP) and oxidative stress markers (MDA, oxidised-low density lipoprotein and protein carbonyl) for 4 weeks. The results showed that the almond-containing meal reduced protein carbonyl and oxidised-low density lipoprotein (ox-LDL). Price et al. (2012) investigated the effect of aleurone-enriched foods (bread and extruded cereal products) with control foods (bread and extruded cereal products without aleurone) on FRAP antioxidant capacity and superoxide dismutase (SOD) activity in a parallel controlled study (duration of 4

weeks) and found no effect of aleurone-enriched foods on these parameters. The other studies (Bogdanski et al., 2012; Vieira et al., 2012: Villano, Lettieri, Guadagni, Schmid, & Serafini, 2012; Hudthagosol et al., 2010; Torabian, Haddad, Rajaram, Banta, & Sabate, 2009; Li, Jia, et al., 2007; Nemzer et al. 2012) presented in **Table 1.8** have shown beneficial effects of polyphenol-rich foods on most of the oxidant/antioxidant markers.

Table 1.8. Randomised controlled human studies of polyphenol-rich foods on antioxidant status and oxidative stress biomarkers

Reference	Design and subjects	Treatment	Outcome measures	Major results
Haddad et al. (2014)	Single meal CO; $n = 16$ Hlty, Nrmwt M/F, 26* y	White bread (C) vs walnut	TP, ORAC, FRAP, MDA, Ox-LDL	Walnut meal ↑ ORAC and ↓ MDA but did not affect TP, FRAP and ox-LDL compared to white bread meal
Liu et al. (2013)	12 week CO; $n = 20$ Type 2 diabetic, Ovwt and Ob M/F, 58* y	Control meal (C) vs Almond-containing meal	TP, FRAP, MDA, Ox-LDL, PC	Almond meal ↓ PC and ox-LDL but did not affect TP, FRAP and MDA compared to control meal
Villano et al. (2012)	Single meals CO; $n = 21$ Hlty, Nrmwt M/F, 28* y	Placebo beverage (C) vs oolong tea	TRAP, FRAP	Oolong tea ↑ TRAP and FRAP compared to placebo beverage
Bogdanski et al. (2012)	12 week Parallel; $n = 28$/gp hypertensive, Ob M/F, 49* y	Cellulose capsule (C) vs green tea capsule	TAS	Green tea capsule ↑ TAS compared to placebo capsule
Price et al. (2012)	4 week Parallel; $n = 40$/gp Hlty, Nrmwt M/F, 45-65 y	White bread + extrude cereal (C) vs aleurone-enriched bread extruded cereal	FRAP, SOD	Aleurone-enriched bread and extruded cereals did not affect FRAP and SOD compared to control diet
Vieira et al. (2012)	Single meal CO; $n = 9$ Hlty, Nrmwt F, 21-27 y	Water (C) vs apple juice	ABTS, FRAP, LH, TBARS	Apple juice ↑ ABTS and FRAP and ↓ LH and TBARS compared to water

(Continued)

Table 1.8. (Continued)

Reference	Design and subjects	Treatment	Outcome measures	Major results
Hudthagosol et al. (2011)	Single meal CO; $n = 16$ Hlty, Nrmwt M/F, 23-44 y	White bread (C) vs pecan	TP, ORAC, FRAP, MDA, Ox-LDL	Pecan meal ↑ TP, ORAC and ↓ ox-LDL but did not affect FRAP and MDA compared to white bread meal
Torabian et al. (2009)	Single meal CO; $n = 14$ Hlty, Nrmwt M/F, 19-65 y	Control meal (C) vs walnut meal vs almond meal	TP, FRAP, ORAC, TBARS	Both walnut and almond meal ↑ TP, FRAP and ORAC ↓ TBARS compared to control meal
Li, Jia, et al. (2007)	4 week CO; $n = 60$ Hlty, Nrmwt M, 22^* y	Pork meal (C) vs almond meal	SOD, GPx, catalase, MDA, 8-OHdG	Almond meal ↑ SOD and GPX and ↓ MDA and 8-OHdG but did not affect catalase activity
Nemzer et al. (2012)	Single meal parallel; $n = 16$/gp Hlty, Nrmwt M/F, 45-55 y	Pear juice (C) vs poylphenoic-rich juice	8-iso-PGF$_{2\alpha}$, AOPP	Polyphenolic rich juice ↓ 8-iso-PGF$_{2\alpha}$ and AOPP compared to pear juice

* mean age; CO: crossover; Hlty: healthy; Nrmwt: normal-weight; Ovwt: over-weight; Ob: obese; M: male; F: female; gp: group; C: control; TP: total polyphenols; FRAP: ferric reducing antioxidant power; ORAC: oxygen radical absorbance capacity; MDA: malondialdehyde; Ox-LDL: oxidised-low density lipoprotein; PC: protein carbonyl; TRAP: total radical-trapping antioxidant parameter; TAS: total antioxidant status; SOD: superoxide dismutase; GPx: glutathione peroxidase; ABTS: 2,2′–azinobis(3-ethylbenzothiazoline-6 sulfonic acid; LH: lipid hydroperoxides; TBARS: thiobarbituric acid reactive substances; 8-OHdG: 8-hydroxydeoxyguanosine; 8-iso-PGF$_{2\alpha}$: 8-iso-prostaglandin F$_{2\alpha}$; AOPP: advanced oxidation protein products; ↑: increase; ↓: decrease.

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1.10 EFFECT OF SORGHUM ON RISK FACTORS FOR TYPE 2 DIABETES AND OBESITY

Diet-related chronic diseases including type 2 diabetes and obesity are highly prevalent in both developed and developing countries. Type 2 diabetes is a metabolic disorder which is associated with a decreased uptake of glucose into muscle and adipose tissue, resulting in macro and micro-vascular complications such as atherosclerosis, cataract formation and retinopathy (Henriksen, Diamond-Stanic, & Marchionne, 2011; Nathan, 1993). According to the International Diabetes Federation (IDF) report, about 382 million people are diabetic worldwide (IDF, 2013). In Australia, about 1 million people (4% of total population) are suffering from diabetes (AIHW, 2013).

Overweight and obesity are leading risks for deaths worldwide and at least 3.4 million people die each year as a result of being overweight or obese (WHO, 2013). According to the Australian Bureau of Statistics (ABS) report, about 61% of Australians were either overweight or obese in 2007-2008 (ABS, 2011).

Persistent high levels of blood glucose and excessive energy intake have been identified as the major risk factors responsible for the development of type 2 diabetes and obesity (Willett et al., 2002; Williams, Grafenauer, & O'Shea, 2008). Other factors such as oxidative stress, play a role in the onset of type 2 diabetes (Perez-Matute et al., 2009).

Sorghum and sorghum-containing foods have been shown to decrease blood glucose, oxidative stress and energy intake in animals. Kim and Park (2012) determined the effect of a sorghum grain extract on the hepatic gluconeogenesis and the glucose uptake of muscle tissues in diabetic male Wistar rats. The results indicated that the sorghum extract reduced the concentration of blood glucose which was attributed to the inhibition of hepatic gluconeogenesis by the sorghum extract. In a similar study Chung et al. (2011) showed that phenolic extracts from sorghum exert hypoglycaemic effects in diabetic rats by decreasing serum glucose level. Sorghum flour has also been shown to beneficially affect markers of oxidative stress in animals. In a rat study Moraes et al. (2012) showed that a diet containing sorghum flour significantly decreased thiobarbituric acid reactive substances (TBARS) (a marker of lipid peroxidation) in the liver. The authors suggested that the higher phenolic content and antioxidant capacity of sorghum flour may be responsible for the observed effect.

Only a few studies have been reported that investigated the effect of sorghum on energy intake/weight reduction in non-human mammals (Al-Mamary, Molham, Abdulwali, & Al-Obeidi, 2001; Muriu, Njoka-Njiru, Tuitoek, & Nanua, 2002). Appleton, Rand, Priest, Sunvold, and Vickers (2004) evaluated the effect of sorghum-maize composite diet in comparison to a rice diet on energy intake and weight gain in overweight cats. Compared to the rice diet, cats fed on sorghum-maize composite diet consumed less energy and gained less weight.

Despite the beneficial effects of sorghum and sorghum-containing foods on blood glucose and energy intake in non-human mammals, the literature is limited to only two studies on the effect of sorghum-containing foods on post meal blood glucose in humans. Abdelgadir et al. (2005) studied the effect of six traditional Sudanese foods made from sorghum, maize, millet and wheat on blood glucose and insulin responses in type 2 diabetic subjects. Their results showed that blood glucose values for the 2 h duration were lowest for millet products and highest for maize products while sorghum products gave intermediate values. In a similar study, conducted on type 2 diabetic individuals, Lakshmi and Vimala (1996) showed that whole grain sorghum products gave lower plasma glucose response than rice and wheat products.

1.11   CONCLUSIONS

Sorghum is a cereal grain that is underutilised for human consumption in the developed world. The literature on sorghum grain shows it to be a good source of health beneficial components including slowly digestible starch, resistant starch and polyphenolic antioxidants. These components have the potential to assist good health when incorporated into foods through lowering postprandial glycaemia, appetite and energy intake and oxidative stress biomarkers. The presence of these beneficial health components in sorghum gives this grain great potential as an ingredient in functional foods. Product development studies are now required to develop palatable sorghum foods acceptable to consumers in developed countries which maintain high levels of slowly digestible starch, resistant starch and polyphenolic antioxidants. Substantiation studies of the beneficial effect of these newly developed sorghum products on

biomarkers of chronic diseases in human participants are required, commencing with postprandial studies, to confirm their effects on glycaemia, appetite and energy intake and oxidative stress biomarkers.

1.12 AIMS

The overall aim of this thesis is to evaluate the potential of whole grain sorghum flour as a palatable health beneficial food ingredient. This will be investigated by using pasta as a model food in a series of product development, *in vitro* and postprandial clinical studies. These studies will provide new knowledge on the potential for the sorghum pasta to provide postprandial effects on glycaemia, appetite and energy intake and oxidative stress biomarkers that may be of benefit for the prevention and control of type 2 diabetes and obesity.

The specific aims of the thesis are:

1. To evaluate the effect of substituting durum wheat semolina with whole grain red or white sorghum flour on resistant starch content, phenolic profile and antioxidant capacity in both uncooked and cooked pasta.

2. To elucidate the effect of whole grain red or white sorghum flour addition into durum wheat semolina pasta on its *in vitro* starch digestibility, cooking quality and consumer acceptability.

3. To investigate the effect of pasta containing whole grain red or white sorghum flour on postprandial glycaemia, appetite and energy intake in healthy humans.

4.   To examine the effect of pasta containing whole grain red or white sorghum flour on postprandial plasma total polyphenols, antioxidant capacity and markers of oxidative stress in healthy human subjects.

1.13   HYPOTHESES

It is hypothesised that:

1. The addition of whole grain red or white sorghum flour to durum wheat pasta will increase its resistant starch content, polyphenolic content and antioxidant capacity in both uncooked and cooked forms.

2. The addition of whole grain red or white sorghum flour to durum wheat pasta will decrease its starch digestibility without affecting the cooking quality and palatability.

3. Pasta containing whole grain red or white sorghum flour will induce lower postprandial glycaemia, appetite and energy intake compared to control pasta made from durum wheat semolina only.

4. Pasta containing whole grain red or white sorghum flour will increase postprandial plasma total polyphenol, antioxidant capacity and decrease markers of oxidative stress to a greater extent than control pasta made from durum wheat semolina only.

CHAPTER TWO

GENERAL METHODS

This chapter describes the general methods used to complete the four studies conducted in this thesis. The specific methods used within an individual study will be reported in the respective study chapter.

2.1   RAW MATERIALS

Durum wheat semolina (DWS) (the endosperm of selected Australian durum wheat milled according to manufacturer's specifications to volume weighted mean particle size of 356.4 μm) was purchased from Manildra Group of Companies (Tamworth, NSW, Australia). Red sorghum grain (var. Alpha), a tannin-free variety, grown in the summer of 2009-2010, was obtained from Lochabar Enterprises Pty Ltd. (Tara, QLD, Australia). White sorghum grain (var. Liberty), a commercial hybrid, grown in the summer of 2010-2011 at Hermitage Research Station (Warwick, Queensland, Australia), was supplied by the Queensland Department of Agriculture Fisheries and Forestry (Cooper's Plains, QLD, Australia). The red and white sorghum whole grains were milled to flours using a rotor Mill (ZM 200, Retsch GmbH, Haan, Germany) fitted with a 500 micron screen at the Department of Agriculture and Food, Western Australia. The particle size distribution of the flours **(Table 2.1)** was determined in triplicate with laser light scattering by air dispersion using a Mastersizer 2000 (Malvern instruments Ltd, Malvern, UK). Data was calculated by the instrument software as d(0.1), d(0.5) and d(0.9) which represents the maximum diameter of 10%, 50% and 90% of the particles, respectively. In addition the volume weighted mean particle size,

D[4,3], was also calculated by the software. All flours were vacuum packed and stored at 15 °C in the dark prior to use.

Table 2.1. Particle size distribution of sorghum flours[*]

Sample	d(0.1)	d(0.5)	d(0.9)	D [4, 3]
	μm	μm	μm	
RSF	48.4 ± 0.6	243.4 ± 0.3	514.2 ± 3.9	267.5 ± 1.2
WSF	32.9 ± 0.6	228.1 ± 0.9	503.5 ± 3.0	213.6 ± 3.4

[*]Means of triplicate analyses ± SD. d(0.1), d(0.5), d(0.9), are maximum diameters of 10%, 50% and 90% (of total volume) of particles; D[4,3] is the volume weighted mean particle size. RSF: red sorghum flour; WSF: white sorghum flour.

2.2 PASTA PREPARATION

Formulations consisting of 100% DWS (control) or by replacing DWS with whole grain red sorghum flour (RSF) or whole grain white sorghum flour (WSF) at 20%, 30% and 40% (w/w), were prepared for fettuccine-type pasta processing **(Table 2.2)**. The maximum inclusion level of both sorghum flours i.e. 40% was identified by measuring dough strength in preliminary experiments. For each formulation, dry ingredients were added into a Hobart mixer (model N-50, Hobart, Australia) and mixed at low speed for 5 min. Water at 35-40 ml per 100 g of flour depending on formulation, (based on preliminary experiments) was added to give a uniform, smooth and non-sticky dough. The dough was kneaded by hand for 10 min and then allowed to rest at room temperature for a further 10 min. The dough was folded and sheeted four times through a pasta machine (Atlas, model 150, Padova, Italy) with a 4 mm gap. The sheet was cut into 25 cm long and 0.6 cm wide ribbons and dried in open air at ambient temperature

(21-25 ºC) for 30 h to a final moisture level of ≤ 10%. Formulations were prepared in duplicate. Dried pasta was sealed in doubled moisture proof plastic bags and stored in the dark at 4 ºC.

Table 2.2. Formulations for pasta preparation

Ingredients	Control	20% RSF	30% RSF	40% RSF	20% WSF	30% WSF	40%WSF
DWS (g)	100	80	70	60	80	70	60
RSF (g)	-	20	30	40	-	-	-
WSF (g)	-	-	-	-	20	30	40
Water (ml)	35	37	38	40	36	38	39

DWS: durum wheat semolina; RSF: red sorghum flour; WSF: white sorghum flour.

2.3 PASTA COOKING

The optimum cooking time for each type of pasta was determined using AACC method 66-50 (AACC, 2000). Briefly, 10 g of pasta was cooked in 300 ml of boiling distilled water. Optimum cooking time was when the white core in the pasta was still present but disappeared after squeezing between two plexiglass plates.

2.4 FREEZE DRYING OF COOKED PASTA AND MILLING

After cooking for the optimal time, pasta was drained and immediately cooled with distilled water at 20 ºC. The cooked pasta was frozen in liquid nitrogen and dried in a laboratory freeze-drier (Flexi-Dry™ model FD-3-55D-MP, FTS Systems, Stone Ridge, New York, USA). A sample mill (Black and Decker, Hunter Valley, MD, USA) was used to mill both the uncooked and freeze-dried cooked pasta to pass 100% through a

0.5 mm screen. The milled samples were stored at 4 ºC in sealed plastic containers in the dark.

2.5 PROXIMATE AND DIETARY FIBRE ANALYSIS OF FLOURS AND PASTA

Proximate and dietary fibre analyses were performed on raw materials (flour samples) and freeze-dried cooked pasta. Moisture content was determined by oven drying at 100 ºC for 16 h (AOAC, 1997). Total protein content was determined using the Kjeldahl digestion distillation procedure with a nitrogen-to-protein conversion factor of 5.7 and 6.25 for durum wheat semolina and sorghum flours, respectively (AACC, 2000). Ash and fat contents were measured according to AOAC methods 923.03 and 920.85, respectively (AOAC, 1997). Total dietary fibre was determined by an enzymatic-gravimetric method according to AOAC method 985.29 (AOAC, 1997), using the Sigma-Aldrich total dietary fibre assay kit (TDF-100A, Sigma-Aldrich, St. Louis, MO, USA).

CHAPTER THREE

STUDY 1 - EFFECT OF WHOLE GRAIN SORGHUM FLOUR ADDITION ON RESISTANT STARCH CONTENT, PHENOLIC PROFILE AND ANTIOXIDANT CAPACITY OF DURUM WHEAT PASTA

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3.1   ABSTRACT

Foods containing elevated levels of functional components such as resistant starch and polyphenolic antioxidants may have beneficial effects on human health. Pasta incorporating whole grain either red sorghum flour (RSF) or whole grain white sorghum flour (WSF) each at 20%, 30% and 40% substitution of durum wheat semolina (DWS) was prepared. These pasta samples were compared to pasta made from 100% DWS (control) for content of starch fractions, phenolic profile and antioxidant capacity, before and after cooking. Total, digestible and resistant starch contents were determined by the AOAC method; total phenolic content by the Folin-Ciocalteu colorimetric method; individual phenolic acids and anthocyanins by reverse phase-HPLC analysis and antioxidant capacity by the ABTS colorimetric assay. The addition of both RSF and WSF to the pasta increased the resistant starch content, bound

phenolic acids, total phenolic content and antioxidant capacity at all incorporation levels compared to the control pasta while free phenolic acids and anthocyanins were higher in the RSF-containing pasta only. Cooking did not change the resistant starch content of any of the pasta formulations. Cooking did however decrease the free phenolic acids, anthocyanins, total phenolic content and antioxidant capacity and increased the bound phenolic acids of the sorghum-containing pastas. This study suggests that these sorghum flours may be very useful for the preparation of pasta with increased levels of resistant starch and polyphenolic antioxidants.

3.2 INTRODUCTION

Sorghum (*Sorghum bicolor*) is the fifth leading crop worldwide and the third most important cereal crop behind wheat and barley in Australia (Mahasukhonthachat et al., 2010). It plays an important role in sustainable grain production, particularly in semi-arid regions of the world due to its drought and high temperature tolerance and is therefore considered an important cereal crop for food security in these regions (Taylor et al., 2006). However, in Australia sorghum grain is mainly used for animal feed (up to 60% of the crop) and is still underutilised as a human food source (Mahasukhonthachat et al., 2010). Several studies have shown that sorghum is nutritionally comparable to other major cereals (Duodu et al., 2003) and is a valuable source of health functional ingredients including resistant starch (Dicko et al., 2006; Ragaee et al., 2006) and phenolic compounds (Awika & Rooney, 2004; Dykes & Rooney, 2006).

Resistant starch is considered a low-calorie functional food component that resists hydrolysis by enzymatic digestion in the small intestine (Sajilata, Singhal, & Kulkarni, 2006); undergoes complete or partial fermentation in the colon to produce beneficial short-chain fatty acids (Ferguson, Tasman-Jones, Englyst, & Harris, 2000; Henningsson, Margareta, Nyman, & Bjorck, 2003); and stimulates healthy gut microflora, and hence has potential as a prebiotic (Voragen, 1998; Young & Le Leu, 2004). The consumption of resistant starch in place of digestible starch can also reduce postprandial glycaemia and insulinaemia as unlike digestible starch it does result in glucose absorption in the small intestine (Raben et al., 1994; Reader, Johnson,

Hollander, & Franz, 1997). Despite the fact that resistant starch is physiologically beneficial, its current estimated daily intake of about 5 g/day is still lower than the recommended intake of 20 g/day (Baghurst, Baghurst, & Record, 1996).

Phenolic compounds are a health functional component of sorghum through their antioxidant properties (Dlamini et al., 2007; Dykes et al., 2005; Kamath et al., 2004). Many varieties of sorghum have higher levels of phenolic compounds compared to other widely consumed cereals such as wheat, rice, barley and millet (Ragaee et al., 2006). In sorghum these polpyhenolics are concentrated in the outer layers of the grain where they are found in both free and bound forms (Awika & Rooney, 2004). While all sorghum varieties contain phenolic compounds, the types and levels present are related to pericarp colour and the presence of pigmented testa and hence the overall grain colour. For instance, white-grained varieties have an almost transparent pericarp and contain mainly simple phenolic acids, whereas red and black-grained varieties have a red or black pigmented pericarp and contain flavonoids as well as phenolic acids. Brown and some red and black-grained varieties also have a pigmented testa and in addition to phenolic acids and flavonoids also contain condensed tannins (Awika & Rooney, 2004). Epidemiological studies have indicated that diets rich in phenolic compounds may have protective effects against various chronic diseases associated with oxidative stress such as diabetes, cancer and cardiovascular disease (Halliwell, 2008; Temple, 2000). Palatable food products containing sorghum flour as an ingredient could act as vehicles for increased dietary intake of phenolic compounds and thus provide chronic disease protective effects.

Pasta is popular worldwide and is a staple food in many countries. Conventional pasta is manufactured using durum wheat semolina as the primary ingredient. Compared to other starchy foods such as bread, pasta has more beneficial physiological effects, including inducing low postprandial glycaemic and insulinaemic responses (Aston, Gambell, Lee, Bryant, & Jebb, 2008; Bornet et al., 1989). However, conventional pasta products are neither high in resistant starch nor in polyphenolic antioxidants, both of which may further reduce the risk of chronic diseases (He, Nowson, Lucas, & MacGregor, 2007; Perez-Jimenez et al., 2008). Several studies have reported the increased resistant starch content and polyphenolic antioxidants levels of pasta containing non-durum wheat ingredients such as: unripe banana flour (Ovando-Martinez, Sayago-Ayerdi, Agama-Acevedo, Goni, & Bello-Perez, 2009); chickpea flour (Fares & Menga, 2012); common bean flour (Gallegos-Infante, et al., 2010); wakame (Prabhasankar et al., 2009); oregano and carrot leaf (Boroski et al., 2011); and barley flour (Verardo, Gomez-Caravaca, Messia, Marconi, & Caboni, 2011).

There appears however to be no studies reporting the effect of sorghum flour addition to durum wheat pasta on its resistant starch content, phenolic profile and antioxidant capacity. Therefore, the objective of this work was to evaluate the effect in both uncooked and cooked pasta, of substituting durum wheat semolina with whole grain red or white sorghum flour on resistant starch content, phenolic content and profile and antioxidant capacity.

3.3 MATERIALS AND METHODS

3.3.1 Chemicals

Diethyl ether (purity 99%), HPLC grade methanol, acetonitrile and ethanol, analytical grade acetic acid (purity 99.5%), hydrochloric acid (37%) and dimethyl sulfoxide (purity $\geq$ 99%) were obtained from Merck (Darmstadt, Germany). Folin-Ciocalteu reagent, sodium carbonate (purity $\geq$ 99%), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (purity 97%), 2,2'–azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate (purity $\geq$ 99%), ultra-pure phenolic standards including gallic acid, protocatechuic acid, gentisic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, salicylic acid, cinnamic acid and apigeninidin chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Total starch, resistant starch and amylose/amylopectin assay kits were purchased from Megazyme International Limited (Wicklow, Ireland). Luteolinidin chloride (purity 85.2%) was obtained from ChromaDex (Santa Ana, CA, USA). Milli-Q water (18.2 MΩ cm) was used in all experiments.

3.3.2 Proximate and dietary fibre analysis of raw material

Proximate and dietary fibre composition of raw materials was determined as described in **Chapter 2, Section 2.5**.

3.3.3 Pasta preparation

Pasta was prepared as described in **Chapter 2, Section 2.2**.

3.3.4 Pasta cooking and freeze drying

Pasta was cooked and freeze dried as described in **Chapter 2, Sections 2.3 and 2.4**, respectively.

3.3.5 Milling of uncooked and freeze-dried cooked pasta

Both uncooked and freeze-dried cooked pasta samples were milled as described in **Chapter 2, Section 2.4**.

3.3.6 Determination of starch fractions

The amylose content of the raw materials was determined by the method of Gibson, Solah, and McCleary (1997) using the Megazyme amylose/amylopectin assay kit (K-AMYL 04/06, Megazyme Int. Ireland Ltd., Co. Wicklow, Ireland). The total starch content of the raw materials and the uncooked and freeze-dried cooked pasta samples was determined by the Megazyme total starch assay kit, K-TSTA 04/2009 (Megazyme Int. Ireland Ltd., Co. Wicklow, Ireland) which is based on the amyloglucosidase/α-amylase method 996.11 (AOAC, 2008). Resistant starch content was determined by the Megazyme resistant starch assay kit, 05/2008 (Megazyme Int. Ireland Ltd., Co. Wicklow Ireland) according to AOAC method 2002.02 (AOAC, 2008). This method involved incubation of sample with α-amylase (37 $^{\circ}$C, 16 h) to hydrolyse digestible starch to glucose, treatment of the residues with 2 M KOH to solubilise resistant starch and finally incubation with amyloglucosidase (50 $^{\circ}$C, 30 min) to hydrolyse resistant starch to free glucose. Free glucose was then determined by colorimetric assay using glucose oxidase/peroxidase (GOPOD) reagent. In this assay GOPOD reagent oxidises

glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase enzyme couples with phenol and 4- aminoantipyrine, form quinoneimine dye. The colour developed is then measured at 510 nm. Resistant starch was calculated as: glucose (mg) x 0.9. Digestible starch was calculated as the difference between total starch and resistant starch.

3.3.7 Preparation of sample extracts for total phenolic content, antioxidant capacity and anthocyanins determination

Extracts for the determination of total phenols, antioxidant capacity and anthocyanins were prepared according to the method of Awika et al. (2004b). Briefly, 1 g samples (raw materials, uncooked pasta or freeze-dried cooked pasta) were mixed with 10 ml of 1% HCl in methanol, shaken for 1 h at low speed in an Eberbach shaker and then centrifuged at 2700 x g for 20 min. The supernatant was decanted and the residue was re-extracted as described above. The two supernatants were combined, purged with a stream of nitrogen and stored at -20 $^\circ$C until analysis for total phenolics and antioxidant capacity as described in **Sections 3.3.9 and 3.3.10**, respectively. For anthocyanins analysis, sample extracts were prepared as above and then evaporated to dryness under vacuum at 40 $^\circ$C using a Büchi Rotavapor R-215 (Büchi, Flawil, Switzerland). The residue was redissolved in 5 ml of methanol and filtered through a 0.45μm syringe filter (Fisher Scientific) prior to analysis by high performance liquid chromatography (HPLC) as described in **Section 3.3.11**.

3.3.8 Preparation of sample extracts for phenolic acids (free and bound) determination

Free phenolic acids extraction was performed according to Adom and Liu (2002) with some modification. Briefly, 2 g samples (raw materials, uncooked pasta or freeze-dried cooked pasta) were extracted with 10 ml of 80% (v/v) aqueous methanol for 1 h in a shaking water bath at 25 °C. After centrifugation at 2700 x g for 20 min, the supernatant was decanted and the extraction was repeated as described above. The two supernatants were combined, evaporated to near dryness and reconstituted with methanol to a final volume of 10 ml. The reconstituted sample was filtered through a 0.45μm syringe filter (Fisher Scientific) prior to analysis by HPLC as described in **Section 3.3.11**.

For extraction of bound phenolic acids, the residue remaining after free phenolics extraction was treated with 10 ml of 2 N HCl at 100 °C for 1 h. Ethyl ether (20 ml x 2) was added to the hydrolysate and, after partitioning the ethyl ether fraction was separated and evaporated to dryness. The residue was redissolved in 2 ml of methanol and filtered through a 0.45μm syringe filter (Fisher Scientific) prior to analysis by HPLC as described in **Section 3.3.11**.

3.3.9 Determination of total phenolic content

Total phenolic content of the raw materials and uncooked and freeze-dried cooked pasta samples was measured using the modified Folin-Ciocalteu method (Li, Cheng, et al., 2007). The Folin-Ciocalteu reagent was first diluted 10 times with milli-Q water and 0.2 ml of sample extract **(Section 3.3.7)** added to 0.8 ml of the diluted Folin-Ciocalteu reagent. After 3 min, 2 ml of 15% (w/v) sodium carbonate solution was

added, the mixture made up to 5 ml with milli-Q water, mixed and kept in darkness at room temperature for 1 h. The absorbance was then measured at 760 nm using the Synergy 2 microplate reader (BioTek, model S, Winooski, VT, USA) with milli-Q water as a blank. Gallic acid (0-0.5 mg/ml), prepared in methanol, was used as the standard and the results were expressed as mg of gallic acid equivalents (GAE)/g sample (dry basis).

3.3.10 Determination of antioxidant capacity

Antioxidant capacity of the raw materials and uncooked and freeze-dried cooked pasta was determined by the method of Van den Berg, Haenen, van den Berg, and Bast (1999) as cited by Liyana-Pathirana and Shahidi (2007) with some modifications. ABTS radical cation (ABTS$^+$) was produced by mixing 8 mM of ABTS salt with 3 mM of potassium persulfate in 25 ml of distilled water. The solution was kept at room temperature in the dark for 16 h before use. The ABTS$^+$ solution was diluted with 95% ethanol, in order to obtain an initial absorbance between 0.35 and 0.4 at 734 nm. Fresh ABTS$^+$ solution was prepared for each analysis. Trolox (0 to 500 µM) was used as the standard. Sample extracts **(Section 3.3.7)** or standards (50 µl) were mixed with 2 ml of diluted ABTS$^+$ solution and incubated at 30 ºC. Absorbance was monitored at 734 nm for 30 min using the Synergy 2 microplate reader (BioTek, model S, Winooski, VT, USA) against an ethanol/ABTS$^+$ blank (50 µl of 95% ethanol added to 2 ml of diluted ABTS$^+$ solution). The decrease in absorbance ($\Delta A = A_{t=0}$ min-$A_{t=30\ min}$) was calculated for each sample extract and standard. The antioxidant capacity of each sample extract

was calculated from the Trolox standard curve and expressed as μmoles Trolox equivalents (TE)/g sample (dry basis).

3.3.11 HPLC analysis of phenolic acids (free and bound) and anthocyanins

Reverse phase-HPLC analysis of sample extracts (raw materials, uncooked pasta and freeze-dried cooked pasta) **(Section 3.3.8)** was carried out using Agilent 1100 HPLC system equipped with an auto sampler, degasser, quaternary pump, thermostated column compartment and a diode-array detector (DAD) (Agilent Technologies, Palo Alto, CA, USA) according to the method reported by Kim, Tsao, Yang, and Cui (2006). The separation was performed on a 250×4.6 mm I.D. Allsphere ODS-2, C18 RP column with a particle size of 5 μm (Alltech, Deerfield, IL, USA) fitted with a 10×4.6 mm I.D. Allsphere ODS-2, guard column (Alltech, Deerfield, IL, USA). The mobile phase was 2% acetic acid in Milli-Q water (v/v) (solvent A) and acetonitrile (solvent B). The flow rate was kept at 1 ml/min for a total run time of 50 min and the gradient elution was: 0% B to 15% B in 15 min, 15% B to 50% B in 10 min, 50% B to 60% B in 5 min, 60% B to 70% B in 5 min and 70% B to 0% B in 5 min. There was 10 min of post-run with 100% solvent A for reconditioning. All sample extracts and standards were filtered through a 0.45 μm pore size syringe-driven filter (Fisher Scientific) before injection. The injection volume was 10 μl and 20 μl for phenolic acids and anthocyanins, respectively. Benzoic acid derivatives, cinnamic acid derivatives and anthocyanins were detected at 280 nm, 320 nm and 480 nm, respectively. Phenolic acids and anthocyanins in the samples extracts were identified by comparing their relative retention times and UV/Vis spectra with those of the standards. The

quantification was carried out using the external standard method. Stock solution of standards 1 mg/ml each were prepared in methanol, and then diluted to several concentrations (0.005, 0.01, 0.02, 0.05, 0.1 mg/ml) and injected into the HPLC system under the conditions described above. Data acquisition, peak integration and calibrations were performed with the Agilent Chemstation software. The concentrations of phenolic acids and anthocyanins were calculated from peak areas in comparison to calibration curves of the respective standards and were expressed as µg/g sample (dry basis).

3.3.12 Statistical analysis

All data were reported as means ± standard deviation of triplicate or quadruplicate analyses. One-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) *post hoc* test were used to identify significant differences. Analysis was performed using SPSS statistical software version 18 (SPSS Inc. Chicago, IL, USA). Differences were considered to be significant at $p < 0.05$.

3.4 RESULTS

3.4.1 Chemical composition of raw materials

The mean values for the proximate composition, total dietary fibre, starch fractions (total, digestible and resistant), amylose content, total phenolics and antioxidant capacity of DWS, RSF and WSF are shown in **Table 3.1**. Protein content varied significantly among the three flour samples: that for DWS being higher than that for WSF ($p < 0.05$) which in turn was higher than that for RSF ($p < 0.05$). The fat content of the RSF was significantly higher than that of the WSF ($p < 0.05$) which in turn was higher than that of DWS ($p < 0.05$). WSF was significantly higher in ash content than DWS and RSF ($p < 0.05$). The total dietary fibre content of the RSF was significantly higher than that of the WSF and DWS ($p < 0.05$). WSF was significantly higher in total and digestible starch compared to RSF and DWS ($p < 0.05$), while the resistant starch content of both RSF and WSF were significantly higher than that of the DWS ($p < 0.05$). No significant difference was observed in amylose content among all the three flour samples. Both RSF and WSF were significantly higher in total phenolic content (by 3-9 times) than the DWS ($p < 0.05$). The antioxidant capacity was also significantly higher in RSF and WSF than in the DWS (2.5-8 times) ($p < 0.05$).

Table 3.1. Proximate and dietary fibre composition, starch fractions, total phenolics and antioxidant capacity of durum wheat semolina and sorghum flours[*] (dry basis)

Component	DWS	RSF	WSF
Protein (%)	13.43 ± 0.22^a	10.05 ± 0.02^c	11.77 ± 0.04^b
Fat (%)	0.67 ± 0.10^c	2.57 ± 0.31^a	1.52 ± 0.17^b
Ash (%)	1.19 ± 0.05^b	1.18 ± 0.07^b	1.57 ± 0.08^a
Total dietary fibre (%)	4.61 ± 0.72^b	9.0 ± 0.56^a	6.46 ± 0.60^b
Total starch (%)	73.62 ± 0.93^b	76.70 ± 1.21^b	80.96 ± 1.35^a
Digestible starch (%)	73.21 ± 0.81^b	73.75 ± 1.27^b	78.75 ± 1.20^a
Resistant starch (%)	0.42 ± 0.06^c	2.95 ± 0.06^a	2.21 ± 0.15^b
Amylose (% of total starch)	23.98 ± 0.63	22.36 ± 0.46	19.26 ± 2.70
Total phenolics (mg GAE/g)	0.76 ± 0.07^c	6.65 ± 0.12^a	2.17 ± 0.05^b
Antioxidant capacity (µmol TE/g)	9.2 ± 0.31^c	71.2 ± 0.36^a	23.8 ± 0.54^b

[*] Values are expressed in means ± SD (n = 3). Different letters in the same row indicate significant differences ($p < 0.05$, LSD test). DWS: durum wheat semolina; RSF: red sorghum flour; WSF: white sorghum flour; GAE: gallic acid equivalents (Folin Ciocalteu method); TE: trolox equivalents.

3.4.2 Starch fractions of pasta samples

The starch fractions of pastas containing different percentages of RSF and WSF are shown in **Table 3.2**. In terms of total and digestible starch content, only 40% WSF pasta (cooked) showed significantly ($p < 0.05$) higher levels in comparison to the control pasta and no differences were seen in these starch fractions between uncooked and cooked forms of each formulation. Significant ($p < 0.05$) increases in resistant starch content of the uncooked pasta were observed on the addition of RSF and WSF to the pasta. Uncooked formulations with higher percentages of RSF and WSF showed

significantly ($p < 0.05$) higher resistant starch content with significant higher levels ($p < 0.05$) in the RSF compared to the WSF containing formulations at the same incorporation level. After cooking, the resistant starch content of the pasta did not differ ($p > 0.05$) from that of the equivalent uncooked formulation and differences between formulations followed the same pattern as in the uncooked samples.

Table 3.2. Starch fractions of pasta samples[*] (% dry basis)

Sample		Total starch	Digestible starch	Resistant starch
Control	Uncooked	72.51 ± 1.12^{ac}	72.13 ± 1.13^{ac}	0.39 ± 0.05^{h}
	Cooked	71.91 ± 0.94^{c}	71.48 ± 0.95^{bc}	0.43 ± 0.05^{h}
20% RSF	Uncooked	73.01 ± 3.59^{ac}	72.15 ± 3.49^{ac}	0.86 ± 0.10^{de}
	Cooked	71.82 ± 3.30^{c}	71.03 ± 3.26^{c}	0.80 ± 0.05^{ef}
30% RSF	Uncooked	73.61 ± 2.49^{ac}	72.49 ± 2.52^{ac}	1.12 ± 0.08^{b}
	Cooked	72.52 ± 3.01^{bc}	71.49 ± 2.88^{bc}	1.10 ± 0.13^{b}
40% RSF	Uncooked	74.73 ± 3.38^{ac}	73.37 ± 3.39^{ac}	1.36 ± 0.03^{a}
	Cooked	73.69 ± 0.49^{ac}	72.25 ± 0.58^{ac}	1.44 ± 0.09^{a}
20% WSF	Uncooked	73.82 ± 4.61^{ac}	73.11 ± 4.61^{ac}	0.71 ± 0.04^{fg}
	Cooked	73.30 ± 0.23^{ac}	72.67 ± 0.34^{ac}	0.64 ± 0.12^{g}
30% WSF	Uncooked	75.40 ± 3.82^{ac}	74.46 ± 3.91^{ac}	0.94 ± 0.10^{cd}
	Cooked	73.28 ± 1.15^{ac}	72.31 ± 1.03^{ac}	0.97 ± 0.13^{c}
40% WSF	Uncooked	76.19 ± 3.43^{ab}	75.08 ± 3.43^{ab}	1.11 ± 0.02^{b}
	Cooked	75.61 ± 0.88^{a}	74.45 ± 0.87^{a}	1.16 ± 0.04^{b}

[*]Values are expressed in means ± SD (n = 4). Means in the same column with different letters are significantly different (p < 0.05, LSD test). RSF: red sorghum flour; WSF: white sorghum flour.

3.4.3 Total phenolic content and antioxidant capacity of pasta samples

Table 3.3 reports the total phenolic content and antioxidant capacity of the pasta formulations before and after cooking. Compared to the control pasta, all uncooked sorghum-containing pastas had significantly ($p < 0.05$) higher total phenolic content. In addition, RSF-containing pastas had significantly ($p < 0.05$) higher total phenolic content than WSF-containing pastas at the same incorporation level mirroring the higher total phenolic content of RSF compared to WSF. Compared to the equivalent uncooked formulation, all cooked RSF-containing pastas and 30% and 40% WSF-containing pastas had significantly ($p < 0.05$) lower total phenolic content. All uncooked sorghum-containing formulations had significantly ($p < 0.05$) higher antioxidant capacity than the control pasta. The antioxidant capacity of all cooked pastas (except for the control and 20% WSF pasta) was significantly lower ($p < 0.05$) than that of the equivalent uncooked formulation.

Table 3.3. Total phenolics and antioxidant capacity of pasta samples[*] (dry basis)

Sample		Total phenol (mg GAE/g)	Antioxidant capacity (µmol TE/g)
Control	Uncooked	0.77 ± 0.07[hi]	8.50 ± 0.01[hi]
	Cooked	0.62 ± 0.03[i]	7.30 ± 0.54[i]
20% RSF	Uncooked	1.88 ± 0.11[c]	21.10 ± 0.54[c]
	Cooked	1.49 ± 0.04[d]	16.48 ± 1.62[d]
30% RSF	Uncooked	2.41 ± 0.09[b]	26.40 ± 0.54[b]
	Cooked	1.87 ± 0.05[c]	19.93 ± 1.08[c]
40% RSF	Uncooked	3.22 ± 0.21[a]	33.70 ± 1.08[a]
	Cooked	2.36 ± 0.01[b]	24.52 ± 1.08[b]
20% WSF	Uncooked	1.06 ± 0.15[eg]	11.10 ± 0.44[fg]
	Cooked	0.85 ± 0.10[gh]	9.22 ± 1.16[gh]
30% WSF	Uncooked	1.27 ± 0.21[de]	12.70 ± 0.38[e]
	Cooked	0.97 ± 0.02[fg]	10.36 ± 0.94[fg]
40% WSF	Uncooked	1.46 ± 0.17[d]	15.00 ± 0.67[d]
	Cooked	1.09 ± 0.15[ef]	11.51 ± 1.27[ef]

[*] Values are expressed in means ± SD (n = 4). Means in the same column with different letters are significantly different ($p < 0.05$, LSD test). RSF: red sorghum flour; WSF: white sorghum flour; GAE: gallic acid equivalents (Folin Ciocalteu method); TE: trolox equivalents.

3.4.4 Phenolic profile of raw materials and pasta samples

Table 3.4 reports the free phenolic acid content (PAC-free), bound phenolic acid content (PAC-bound) and anthocyanins of the DWS, RSF and WSF. Significantly ($p <$ 0.05) higher levels of PAC-free and PAC-bound were found in the RSF compared to the WSF and DWS. *p*-Hydroxybenzoic acid in DWS, ferulic acid in RSF and salicylic

acid in WSF were the dominant individual phenolic acids in the free fraction while ferulic acid was the dominant phenolic acid in bound fraction of all flour samples. Anthocyanins (luteolinidin and apigeninidin) were observed only in the RSF.

Table 3.5 reports the phenolic acids (free and bound) and anthocyanin content of uncooked and cooked pasta formulations. The addition of RSF into the uncooked pasta significantly ($p < 0.05$) increased the PAC-free at all incorporation levels compared to the control pasta; a finding not unexpected given the higher PAC-free of RSF **(Table 3.4)**. Addition of WSF to the formulations however did not change the PAC-free of the uncooked pasta ($p > 0.05$). In contrast, the addition of both RSF and WSF into the uncooked formulations significantly ($p < 0.05$) increased the PAC-bound at all incorporation levels. The uncooked 40% RSF pasta had the highest ($p < 0.05$) PAC-bound of all uncooked formulations, consistent with this formulation also having the highest ($p < 0.05$) total phenolic content and antioxidant capacity values **(Table 3.3)**. After cooking, both the control and sorghum-containing formulations showed a significant ($p < 0.05$) decrease in the PAC-free compared to the equivalent uncooked formulations. Mean differences were higher in the sorghum-containing formulations than the control (e.g. 12.2% reduction for control; 25.8% reduction for 40% RSF pasta). Cooking, however, significantly ($p < 0.05$) increased the levels of PAC-bound in all formulations.

Table 3.4. Phenolic profile of durum wheat semolina and sorghum flours[*] (µg/g dry basis)

Compound	DWS	RSF	WSF
(A) Free phenolic acids			
p-Hydroxybenzoic acid	71.82 ± 2.76^a	33.72 ± 1.41^b	13.90 ± 1.12^c
Vanillic acid	nd	16.42 ± 1.02^a	8.47 ± 0.86^b
Caffeic acid	nd	7.87 ± 0.15^b	9.93 ± 0.85^a
Syringic acid	nd	8.06 ± 0.36^a	1.96 ± 0.39^b
p-Coumaric acid	nd	14.62 ± 0.13^a	7.55 ± 1.88^b
Ferulic acid	7.83 ± 0.11^c	34.29 ± 0.75^a	15.81 ± 4.07^b
Salicylic acid	6.61 ± 0.01^c	31.08 ± 4.48^a	22.38 ± 0.94^b
Cinnamic acid	nd	4.59 ± 1.01^a	1.17 ± 0.15^b
PAC-free	86.27^b	150.67^a	81.19^b
(B) Bound phenolic acids			
Gallic acid	nd	8.64 ± 0.28	nd
Protocatechuic acid	46.22 ± 0.89^c	70.67 ± 2.43^a	55.18 ± 2.53^b
Gentesic acid	28.72 ± 0.53^b	53.80 ± 3.52^a	44.01 ± 6.04^a
Caffeic acid	10.17 ± 1.37^a	7.00 ± 1.50^a	nd
p-Coumaric acid	nd	53.82 ± 0.31^a	44.92 ± 0.29^b
Ferulic acid	48.91 ± 0.12^c	89.63 ± 2.48^a	78.87 ± 0.61^b
Salicylic acid	nd	16.93 ± 0.07^a	14.57 ± 0.99^b
PAC-bound	134.03^c	300.51^a	237.57^b
TPAC	220.28	451.17	318.76
(C) Anthocyanins			
Luteolinidin	nd	24.46 ± 1.67	nd
Apigeninidin	nd	36.78 ± 0.97	nd

[*] Values are expressed in means $\pm$ SD (n = 4). Means in the same row with different letters are significantly different (p < 0.05, LSD test). DWS: durum wheat semolina; RSF: red sorghum flour; WSF: white sorghum flour; nd: not detected; PAC-free: phenolic acid content of free extract (sum of free phenolic acids); PAC-bound: phenolic acid content of bound extract (sum of bound phenolic acids); TPAC: total phenolic acid content (sum of PAC-free and PAC-bound).

Table 3.5. Phenolic profile of control and sorghum-containing pasta samples* (μg/g dry basis)

Compound	Control		20% RSF		30% RSF		40% RSF		20% WSF		30% WSF		40% WSF	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
(A) Free phenolic acids														
p-Hydroxybenzoic acid	71.1 ± 2.0^a	65.0 ± 1.8^{cd}	68.3 ± 1.1^b	54.1 ± 1.4^g	66.7 ± 1.3^{be}	51.0 ± 2.4^h	60.2 ± 1.2^e	43.2 ± 2.0^i	63.4 ± 1.4^d	52.0 ± 1.6^{gh}	57.1 ± 0.3^f	43.3 ± 1.7^i	50.2 ± 1.1^h	36.0 ± 2.2^j
Vanillic acid	nd	nd	4.2 ± 0.5^c	1.7 ± 0.5^{ef}	5.2 ± 0.5^b	2.2 ± 0.5^{de}	8.7 ± 0.5^a	5.3 ± 1.2^b	2.2 ± 0.7^{de}	1.0 ± 0.3^f	2.8 ± 0.5^d	1.9 ± 0.5^e	3.7 ± 0.5^c	2.9 ± 0.2^d
Caffeic acid	nd	nd	1.8 ± 0.1^{efg}	1.5 ± 0.1^g	2.2 ± 0.1^{de}	1.9 ± 0.2^{ef}	3.5 ± 0.3^{ab}	2.8 ± 0.2^c	2.4 ± 0.1^d	1.7 ± 0.3^{fg}	2.5 ± 0.2^{cd}	2.1 ± 0.3^e	3.6 ± 0.2^b	3.2 ± 0.3^b
Syringic acid	nd	nd	1.6 ± 0.2^c	1.2 ± 0.3^d	2.2 ± 0.2^b	1.9 ± 0.4^c	3.2 ± 0.1^a	3.1 ± 0.3^a	0.5 ± 0.1^f	nd	0.7 ± 0.1^{ef}	nd	0.9 ± 0.2^{de}	nd
p-Coumaric acid	nd	nd	2.4 ± 0.3^d	1.3 ± 0.1^g	3.9 ± 0.1^{bc}	2.0 ± 0.2^f	4.8 ± 0.2^a	4.2 ± 0.1^b	1.8 ± 0.2^g	0.8 ± 0.1^h	2.1 ± 0.4^{ef}	2.1 ± 0.2^{ef}	3.6 ± 0.3^c	2.3 ± 0.1^{de}
Ferulic acid	7.2 ± 0.5^i	4.3 ± 0.2^j	12.6 ± 1.0^d	10.0 ± 0.4^{fg}	15.0 ± 0.3^b	11.6 ± 0.9^e	17.5 ± 0.2^a	13.6 ± 0.6^c	8.2 ± 0.1^h	7.2 ± 0.3^i	9.3 ± 0.4^g	7.9 ± 0.4^{hi}	10.6 ± 0.2^f	9.6 ± 0.2^g
Salicylic acid	6.5 ± 0.1^g	5.0 ± 0.9^i	10.3 ± 0.9^{de}	8.7 ± 0.8^f	12.8 ± 0.6^c	10.6 ± 0.6^d	16.3 ± 0.9^a	14.1 ± 1.0^b	8.7 ± 0.2^f	4.9 ± 0.5^i	10.9 ± 0.1^d	7.4 ± 0.6^g	12.5 ± 0.8^c	9.2 ± 0.7^{ef}
Cinnamic acid	nd	nd	0.8 ± 0.1^c	0.4 ± 0.0^d	1.2 ± 0.1^b	0.8 ± 0.1^c	1.9 ± 0.1^a	1.1 ± 0.1^b	0.2 ± 0.0^e	nd	0.3 ± 0.0^{de}	nd	0.4 ± 0.0^d	nd
PAC-free	84.85^{de}	74.43^b	102.41^c	79.16^f	109.60^b	82.28^{ef}	116.42^a	87.79^d	87.26^d	67.99^g	85.29^{de}	64.93^{gh}	85.83^d	63.63^h
(B) Bound phenolic acids														
Gallic acid	nd	nd	1.7 ± 0.3^d	2.0 ± 0.1^d	2.5 ± 0.4^c	3.4 ± 0.2^b	3.3 ± 0.1^b	4.4 ± 0.3^a	nd	nd	nd	nd	nd	nd
Protocatechuic acid	43.2 ± 0.7^k	49.2 ± 1.1^{ghi}	48.2 ± 1.9^{ij}	55.4 ± 1.6^{cd}	50.4 ± 1.1^{fgh}	59.5 ± 1.3^b	53.7 ± 0.5^{de}	64.1 ± 1.1^a	46.8 ± 0.9^i	50.7 ± 0.7^{fg}	48.8 ± 0.9^{hi}	52.1 ± 1.8^{ef}	49.8 ± 1.3^{ghi}	56.0 ± 9^c
Gentesic acid	26.7 ± 1.2^i	33.0 ± 2.2^{gh}	31.9 ± 0.5^h	37.1 ± 2.5^{ef}	35.1 ± 1.5^{fg}	40.7 ± 3.1^{cd}	38.0 ± 1.5^{de}	47.6 ± 3.4^a	31.4 ± 0.7^h	36.9 ± 2.0^{ef}	33.6 ± 0.2^{gh}	43.0 ± 1.7^{bc}	35.3 ± 2.2^{fg}	44.4 ± 2.2^b
Caffeic acid	9.9 ± 0.4^c	13.3 ± 0.8^a	9.0 ± 0.5^d	9.6 ± 0.7^{cd}	8.4 ± 0.1^e	11.2 ± 0.3^b	7.7 ± 0.6^f	9.9 ± 0.5^c	nd	nd	nd	nd	nd	nd
p-Coumaric acid	nd	nd	9.2 ± 0.3^j	13.0 ± 0.1^g	14.8 ± 0.2^f	23.3 ± 0.2^b	19.0 ± 0.2^c	26.8 ± 0.3^a	7.8 ± 0.3^k	10.6 ± 0.3^i	12.3 ± 0.6^h	15.8 ± 0.8^d	15.2 ± 0.3^e	21.1 ± 0.3^b
Ferulic acid	48.0 ± 0.4^k	69.3 ± 2.5^{ef}	58.8 ± 0.2^i	80.9 ± 0.1^c	61.3 ± 0.7^h	82.7 ± 1.3^b	65.5 ± 1.1^g	84.6 ± 1.3^a	54.9 ± 0.4^j	68.4 ± 0.8^f	57.6 ± 0.4^i	70.0 ± 0.4^e	60.9 ± 0.7^h	71.8 ± 0.5^d
Salicylic acid	nd	nd	3.8 ± 0.6^f	9.2 ± 0.7^b	5.6 ± 0.9^e	12.9 ± 1.5^b	7.6 ± 0.4^d	15.9 ± 0.6^a	2.2 ± 0.3^g	4.0 ± 0.5^f	3.7 ± 0.5^f	6.2 ± 0.8^e	5.3 ± 0.5^e	10.0 ± 0.6^c
PAC-bound	128.08^k	164.96^h	163.13^h	207.58^c	178.59^f	233.98^b	195.22^d	253.49^a	143.24^j	170.80^j	156.17^i	187.27^e	166.69^h	203.44^d
(C) Anthocyanins														
Luteolinidin	nd	nd	5.2 ± 0.6^c	2.1 ± 0.5^e	7.9 ± 0.8^b	3.5 ± 0.3^d	10.2 ± 0.6^a	5.2 ± 0.1^c	nd	nd	nd	nd	nd	nd
Apigeninidin	nd	nd	6.9 ± 0.2^d	3.8 ± 0.3^e	11.5 ± 0.7^b	6.7 ± 0.6^d	14.5 ± 0.1^a	9.3 ± 0.6^c	nd	nd	nd	nd	nd	nd

*Values are expressed in means ± SD (n = 4). Means in the same row with different letters are significantly different ($p < 0.05$, LSD test). RSF: red sorghum flour; WSF: white sorghum flour; nd: not detected; PAC-free: phenolic acid content of free extract; PAC-bound: phenolic acid content of bound extract.

3.5 DISCUSSION

The aim of the present study was to evaluate the effect in both uncooked and cooked pasta, of substituting DWS with RSF or WSF on resistant starch content, phenolic profile and antioxidant capacity. The hypothesis was that the addition of RSF or WSF to durum wheat pasta at different substitution levels (20-40%) would increase its resistant starch content, polyphenolic content and antioxidant capacity in both uncooked and cooked forms. Proving the hypothesis both RSF and WSF showed significantly higher resistant starch content, polyphenolic content and antioxidant capacity compared to the DWS and their addition to durum wheat pasta significantly increased these components at all incorporation levels in both uncooked and cooked forms.

The differences in the proximate and dietary fibre contents of the sorghum flours and DWS observed in the present study may in part be due to the fact that whole grain sorghum flours were used whereas the DWS is a refined-grain wheat product. For instance the higher levels of fat in the sorghum flours may be attributed to the presence of the embryo (germ) in which oil is concentrated (Ragaee et al., 2006). The protein, fat, ash and total dietary fibre values closely matched those reported by Ovando-Martinez et al. (2009) and Petitot, Boyer, Minier, and Micard (2010) for DWS and by Liu et al. (2012) and Yousif et al. (2012) for RSF and WSF. The amylose content plays an important role in resistant starch formation. In general cereals with higher amylose content can have lower starch digestibility and higher levels of resistant starch (Sajilata et al., 2006). However in sorghum grain other factors including starch-protein interaction and enzyme inhibitory effect of sorghum polyphenols (Taylor & Emmambux, 2010) may also affect resistant starch

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content beyond effects due to amylose levels. The higher resistant starch content of the sorghum flours compared to the DWS in the present study might therefore be a result of the digestive enzyme inhibitory effect of sorghum polyphenols and sorghum starch-protein interactions. The higher antioxidant capacity values obtained for the RSF and WSF could be attributed to the higher total phenolic content in these flours compared to the DWS. Antioxidant capacity of sorghum flour has been shown to be strongly correlated with its phenolic content (Dykes et al., 2005). The total phenolic and antioxidant capacity results obtained in the present study are consistent with those of Awika, Yang, Browning, and Faraj (2009) and Fares, Platani, Baiano, and Menga (2010).

Based on the total dietary fibre, resistant starch and polyphenolic composition of the raw materials it is apparent that the addition of both types of sorghum flours to durum wheat pasta should increase the total dietary fibre, resistant starch, polyphenolics and antioxidant capacity of the pasta and thus have the potential to increase its health functional properties.

The addition of both RSF and WSF significantly increased the resistant starch content of the pasta at all incorporation levels. The experimental values for the resistant starch content of uncooked pasta in the present study were slightly less than the theoretical values calculated from the resistant starch content of the raw materials (0.42, 0.96, 1.17, 1.43, 0.82, 0.95 and 1.13 % dry basis for control, 20% RSF, 30% RSF, 40% RSF, 20% WSF, 30% WSF and 40% WSF, respectively). This discrepancy may be a result of the hydration and shear during processing rendering the starch slightly more digestible. Decrease in resistant starch content

during processing has previously been reported by Fares and Menga (2012) in pasta containing chickpea flour. Cooking did not affect the resistant starch content of the pasta. In contrast to the findings of the present study, Fares and Menga (2012) found higher resistant starch content in cooked chickpea flour-containing pasta than in uncooked; a finding they attributed to the retrogradation of the gelatinised starch after the pasta was cooled. However, in the present study the pasta was instantly frozen in liquid nitrogen immediately after cooking to prevent starch retrogradation. Vernaza et al. (2012) however observed a lower level of resistant starch content in cooked compared to uncooked pasta containing high-maize which they attributed to the leaching of resistant starch from the pasta surface during cooking.

The addition of both RSF and WSF significantly increased the total phenolic content and antioxidant capacity at all incorporation levels. The total phenolic content of the uncooked pastas was similar to the theoretical values calculated from the raw materials' composition (0.76, 1.93, 2.52, 3.31, 1.13, 1.38 and 1.51 mg GAE/g dry basis for control, 20% RSF, 30% RSF, 40% RSF, 20% WSF, 30% WSF and 40% WSF, respectively) which indicated that pasta processing did not affect total phenolic content. Aravind Session, Egan, and Fellow (2012) reported a significant decrease in total phenolic content of bran-containing pasta prepared by cold extrusion processing, possibly due to oxidative degradation in the presence of oxygen, water and heat (Fares et al., 2008). However in contrast to the study of Aravind et al. (2012), the present study used a lamination process at ambient temperature leading to only very small reductions in total phenolic content. Cooking decreased the total phenolic content of the pasta. Differences in total phenolic content between uncooked and cooked pastas may be in part due to the

leaching of these compounds into the cooking water. Lower levels of phenolic compounds in cooked compared to raw formulations have previously been reported in pasta containing seaweed (Prabhasankar et al., 2009), barley coarse fraction (Verardo, Gomez-Caravaca, et al., 2011), buckwheat pasta (Verardo, Arraez-Roman, et el., 2011) and commercial regular and whole wheat spaghetti (Hirawan, Ser, Arntfield, & Beta, 2010). According to these authors thermal treatment during cooking resulted both in leaching of these compounds into the cooking water and their degradation. The total phenolic content in the cooking water was not however analysed in the present study.

Similar to total phenolic content, the antioxidant capacity did not change during processing and the uncooked pastas had similar values of antioxidant capacity as the theoretical values calculated from the raw materials composition (9.2, 21.53, 27.74, 33.95, 12.0, 13.52 and 15.43 µmol TE/g dry basis for control, 20% RSF, 30% RSF, 40% RSF, 20% WSF, 30% WSF and 40% WSF, respectively). Cooking decreased the antioxidant capacity of the pasta. The results of the present study are in agreement with those of Prabhasankar et al. (2009), who reported lower antioxidant activity in cooked than in uncooked seaweed-containing pasta a difference they attributed to the leaching of solids into the cooking water. However, in contrast to the present results, Fares et al. (2010) observed a higher level of antioxidant activity in cooked than in uncooked wheat bran-containing pasta, an effect they attributed to the release of bound phenolic acids from the cell walls of the bran during cooking. The significantly lower levels of total phenolic content in all cooked pastas compared to uncooked might explain the lower level of antioxidant capacity in the cooked compared to the uncooked pastas. Other

antioxidant phytochemicals besides polyphenolics, for instance carotenoids might also contribute to the antioxidant capacity values of the pastas. However, these were not measured in the present study.

Phenolic profiles including free and bound phenolic acids and anthocyanins were analysed by HPLC in the flours and uncooked and cooked pasta formulations in order to determine if loss of specific polyphenols or change in their profile occurred during pasta processing and cooking. The amount and type of free and bound phenolic acids analysed were in fair agreement with those reported by Fares et al. (2010) in DWS and by N'Dri et al. (2013) in sorghum flours. In the present study, the higher concentration of both PAC-free and PAC-bound in RSF than WSF and DWS, explains the higher total phenolic content and antioxidant capacity of RSF compared to WSF and DWS. Anthocyanins (luteolinidin and apigeninidin) were observed only in the RSF. These results are in agreement with the findings of Dykes, Seitz, Rooney, and Rooney (2009) that anthocyanins were present in red sorghum only while white sorghum containing none or negligible amounts. The content of anthocyanins obtained in the present study are lower than those reported by Dykes et al. (2009), but higher than the values observed in red sorghum by N'Dri et al. (2013). These differences are linked to the variability in pericarp colour of red sorghum varieties which have been shown to affect the level of anthocyanins (Dykes et al., 2005). The presence of anthocyanins in RSF only, further explains the higher ($p < 0.05$) total phenolic content and antioxidant capacity of RSF compared to WSF and DWS in the present study.

The pasta processing did not change the PAC-free as determined from the comparison between theoretical values (data not presented) calculated from the raw materials and the corresponding experimental values of the uncooked pastas. The results from the present study contradict those of Fares et al. (2010) who reported a decrease in the free phenolic acids during pasta processing; attributed to a reduction in p-hydroxybenzoic acid. Although in the present study p-hydroxybenzoic acid was the dominant free phenolic acid in DWS, a decrease in its level was not observed, possibly due to the low processing and drying temperatures used in the present study. Likewise the PAC-bound levels in the uncooked pastas were not different to the theoretical values (data not presented). Decrease in PAC-free was observed after cooking. These results are in agreement with the data from the studies of Fares et al. (2010) and Verardo, Arraez-Roman, et al. (2011), in which reductions in free phenolic acids of pasta after cooking were reported. Unlike bound phenolic acids, free phenolic acids are not physically trapped in protein network (Naczk, Towsend, Zadernowski, & Shahidi, 2011; Prigent et al., 2009), therefore the cooking process could have resulted in leaching of these compounds into the cooking water. Cooking, however, increased the levels of PAC-bound in all formulations. This finding is in agreement with that of Fares and Menga (2012), who suggested that boiling can enhance the extractability of bound phenolic acids from the food matrix during cooking and hence can increase their apparent amount measured in pasta during chemical analysis.

The anthocyanins (luteolinidin and apigeninidin) were observed only in the RSF-containing formulations. Pasta processing did not affect the anthocyanin content. However a significant decrease in levels of the anthocyanins was observed after

cooking of up to 50% compared to the uncooked formulations, possibly as a result of thermal degradation. This finding is in agreement with N'Dri et al. (2013), who reported a loss of about 53% of anthocyanins in sorghum during cooking. The findings of the present study indicate that anthocyanins are less stable during cooking than phenolic acids within a pasta matrix. These results are in agreement with those previously reviewed by Manach, Scalbert, Morand, Remesy, and Jimenez (2004).

In conclusion, the addition of RSF and WSF into pasta at all incorporation levels effectively enhanced the antioxidant potential and resistant starch content; of possible benefit in diets to help prevention of chronic diseases related to oxidative stress such as type 2 diabetes mellitus and for improved intestinal health, respectively. The significant reduction in total phenolic content and antioxidant capacity of pasta after cooking might be due to the leaching of phenolic compounds, particularly free phenolic acids and anthocyanins, into the cooking water and their thermal degradation during cooking; however further studies are required to confirm these mechanisms. In addition studies are now required to evaluate the consumer acceptability and the *in vivo* glycaemic effect and antioxidant power of these sorghum-containing pasta formulations.

CHAPTER FOUR

STUDY 2 - EFFECT OF WHOLE GRAIN SORGHUM FLOUR ADDITION ON *IN VITRO* STARCH DIGESTIBILITY, COOKING QUALITY AND CONSUMER ACCEPTABILITY OF DURUM WHEAT PASTA

4.1 ABSTRACT

Whole grain sorghum is a valuable source of resistant starch and polyphenolic antioxidants and its addition into staple foods like pasta may reduce the starch digestibility. However, incorporating non-durum wheat materials into pasta provides a challenge in terms of maintaining cooking quality and consumer acceptability. In this study pasta was prepared from 100% durum wheat semolina (DWS) as control or by replacing DWS with either whole grain red sorghum flour (RSF) or whole grain white sorghum flour (WSF) at 20%, 30% and 40% incorporation levels. Pasta samples were evaluated for proximate composition, *in vitro* starch digestibility, cooking quality and consumer acceptability. The addition of both RSF and WSF lowered the extent of *in vitro* starch digestion at all substitution levels compared to the control pasta. The rapidly digestible starch (RDS) was lowered in all the sorghum-containing pastas compared to the control

pasta. Neither RSF nor WSF affected the pasta quality attributes (water absorption, swelling index, dry matter, adhesiveness, cohesiveness and springiness), except cooking loss, colour and hardness which were negatively affected. Consumer sensory results indicated that pasta samples containing 20% and 30% RSF or WSF had acceptable palatability based on meeting one or both of the pre-set acceptability criteria. It is concluded that the addition of whole grain sorghum flour to pasta at 30% incorporation level is possible to reduce starch digestibility whilst maintaining adequate cooking quality and consumer acceptability.

4.2 INTRODUCTION

Pasta is the second most consumed food worldwide after bread. However, in terms of health benefits pasta is considered superior to bread as it exerts favourable effects on the human body, including inducing lower postprandial blood glucose and insulin responses as compared to white bread (Aston et al., 2008). After the Food and Drug Administration (FDA) recommendation in 1940s for pasta enrichment with non-durum wheat ingredients (Marconi & Carcea, 2001), extensive research has been conducted to enhance its nutritional quality and to optimise the levels of addition of non-durum wheat ingredients into pasta through instrumental and sensory evaluation techniques. Wood (2009) found that durum wheat in pasta can be substituted with chickpea flour up to 30% incorporation level without affecting its sensory attributes. More recently, Aravind, Sissons, Fellows, Blazek, and Gilbert (2013) showed that with the addition of commercial resistant starch type II or III up to 20% level into pasta it is possible to reduce its starch digestibility without affecting cooking quality and sensory attributes. Reducing starch digestibility of foods is important because it can help lower energy intake and post-meal blood glucose levels and hence may provide protection against the development of obesity and type 2 diabetes (Barros et al., 2012). Similarly other legumes, cereals and seaweed have been successfully incorporated into pasta to enhance its nutritional properties with no or minimal effects on sensory attributes (Baiano, Lamacchia, Fares, Terracone, & Notte, 2011; Fares & Menga, 2012; Verardo, Gomez-Caravaca, et al., 2011; Prabhasankar et al., 2009).

Sorghum (*Sorghum bicolor*) is a cereal crop that is underutilised for human consumption in many developed countries. However, sorghum addition may

improve the health properties of pasta as sorghum contains a combination of beneficial health components, including slowly digestible starch, resistant starch (Dicko et al., 2006; Ragaee et al., 2006; Licata et al., 2013) and polyphenolic antioxidants (Awika & Rooney, 2004; Dykes & Rooney, 2006; Khan, Yousif, Johnson, & Gamlath, 2013). To date the consumption of food products made from sorghum alone (for example bread, cookies), are less popular than those made from more conventional cereals such as refined wheat due to the bitterness and astringency produced by the phenolic compounds in sorghum (Abdelghafor et al., 2011; Kobue-Lekalake et al., 2007), hence alternative products using sorghum as a partial ingredient are likely to be more desirable. This is illustrated by the findings of a recent study of Yousif et al. (2012) in which whole grain red and white sorghum flours incorporated into flat bread at 40% of flour weight significantly reduced the *in vitro* starch digestibility of the flat bread without major impact on sensory acceptability.

To date there is no such equivalent study that determined the effect of sorghum flour incorporation on *in vitro* starch digestibility, cooking quality and consumer acceptability of pasta. Therefore, this study was conducted with the objective to elucidate the effect of whole grain red and white sorghum flours addition to pasta on these attributes.

4.3 MATERIALS AND METHODS

4.3.1 Pasta preparation

Pasta was prepared as described in **Chapter 2, Section 2.2**.

4.3.2 Pasta cooking and freeze drying

Pasta was cooked and freeze dried as described in **Chapter 2, Sections 2.3 and 2.4**, respectively.

4.3.3 Milling of freeze-dried cooked pasta

Freeze-dried cooked pasta samples were milled as described in **Chapter 2, Section 2.4**.

4.3.4 Proximate and dietary fibre analysis of cooked pasta

Proximate and dietary fibre composition of freeze-dried cooked pasta was determined as described in **Chapter 2, Section 2.5**.

4.3.5 *In vitro* starch digestibility

The *in vitro* rate of starch digestibility was determined on freeze-dried cooked samples by the method of Sopade and Gidley (2009). Briefly, 500 mg ground sample was placed in a 50 ml screw-top plastic tube, to which 1 ml of porcine α-amylase (Sigma A-3176; 250 U/ml 0.2 M carbonate buffer, pH 7) was added before 5 ml pepsin solution (Sigma P-6887; 1 ml/ml 0.02 M HCl, pH 2) was added 15-20 s later. The mixture was incubated at 37 °C in a reciprocating water bath (85 rpm) for 30 min. The mixture was then neutralised with 5 ml 0.02 M NaOH before 25 ml 0.2 M sodium acetate buffer (pH 6) was added followed by 5 ml pancreatin (Sigma

P-1750; 2 mg/ml acetate buffer, pH 6)/amyloglucosidase (Sigma A-7420; 28 U/ml 0.2 M acetate buffer, pH 6) mixture. Incubation in the water bath (37 °C, 85 rpm) proceeded for up to 120 min, during which a small sample (10 µl) was taken at intervals of 0, 20, 45, 60, 90 and 120 min, and placed on the test strip of a hand-held glucometer (AccuCheck® Performa®, Roche Diagnostics Aust. Pty. Ltd., Castle Hill, Australia) and the glucose concentration read in duplicate. Total starch was determined by Megazyme total starch assay kit, K-TSTA 04/2009 (Megazyme Int. Ireland Ltd., Co. Wicklow, Ireland) which is based on the amyloglucosidase/α-amylase method (AOAC Method No. 996.11). Starch digestibility was expressed as digested starch (DS) in g per 100 g dry starch calculated for each time point using Eq. (1).

$$DS = \frac{0.9 \times G_G \times 180 \times V}{W \times S\,[100\text{-}M]} \qquad \text{(Sopade \& Gidley, 2009)} \qquad (1)$$

where G_G = glucometer reading (mM/L), V = volume of digesta (ml), 180 = molecular weight of glucose, W = weight of sample (g), S = total starch content of sample (g/100g dry sample), M = moisture content of sample (g/100 g sample), and 0.9 stoichiometric constant for starch from glucose contents.

Digestograms of digested starch (g/100g dry starch) versus time of digestion (min) for each sample, corrected for the value at time zero were prepared. Rapidly digestible starch (RDS) (g/100g dry starch was calculated by replacing G_G in Eq. (1) with $(G_{20} - G_o)$ representing the glucometer reading at 20 min minus the glucometer reading at 0 min. Similarly, slowly digestible starch (SDS) (g/100g dry starch) was calculated by substituting G_G for $(G_{120} - G_{20)}$ in Eq. (1).

4.3.6 Pasta cooking quality

4.3.6.1 Optimum cooking time

The optimum cooking time for each type of pasta was determined as described in **Chapter 2, Sections 2.3**.

4.3.6.2 Water absorption

Each pasta sample (10 g) was cooked to its optimum cooking time and then drained for 2 min. Water absorption was determined as [(weight of cooked pasta – weight of uncooked pasta) / weight of uncooked pasta] x 100 (Gelencser, Gal, Hodsagi, & Salgo, 2008).

4.3.6.3 Swelling index

The swelling index of cooked pasta (weight fraction of water in the cooked pasta) was determined by drying cooked pasta at 105 ºC to constant weight, expressed as (weight of cooked pasta – weight of cooked dry pasta) / weight of cooked dry pasta (Cleary & Brennan, 2006).

4.3.6.4 Dry matter

Dry matter of cooked pasta was determined after drying the samples in a laboratory oven at 105 ºC to constant weight, expressed as (weight of cooked dry pasta / weight of cooked pasta) x 100, as described in the AACC approved method 44-15A (AACC, 2000).

4.3.6.5 Cooking loss

Cooking loss was determined according to the AACC approved method 66-50 (AACC, 2000). Pasta was cooked to its optimum cooking time. The cooking water was evaporated to dryness in an air-oven at 105 ºC and the residue was weighed and reported as a percentage of the original (raw) pasta weight.

4.3.7 Texture profile analysis

Textural parameters of the cooked pasta were determined using a Texture Analyser TA-XTplus (Stable Micro Systems, Godalming, Surrey, UK), calibrated for a load cell of 25 kg. The TA-XTplus settings were as follows: pre-test speed of 1.0 mm/s, test speed of 1 mm/s, post-test speed of 10.0 mm/s, and trigger force of 0.05 N. A 35 mm cylinder probe (ref. P/35, Stable Micro Systems, Godalming, Surrey, UK) was used that compress 4 strands of pasta at a constant deformation rate (1 mm/s) to 70% of the initial pasta thickness (10 replications per sample). Each test sample was compressed two times, each compression being followed by decompression. The probe was held stationary for 10 s between the end of first compression and start of second compression was 10 s. From texture profile analysis curves, parameters of hardness, adhesiveness, springiness and cohesiveness were calculated (Epstein, Morris, & Huber, 2002). The first peak force was termed hardness and the negative area of the curve during the retraction of the probe was termed adhesiveness, representing the work required to pull the probe away from the sample. Cohesiveness was calculated by dividing the area of the second compression cycle by the area of the first compression cycle. Springiness was defined as the rate at which a compressed sample went back to its uncompressed form after the compression force is removed, calculated by dividing the distance of

the first half of the second compression cycle by the distance of the first half of the

first compression cycle.

4.3.8 Colour measurement

The colour of uncooked and cooked pasta was measured using a Minolta chroma

meter (Minolta, CR-410, Osaka, Japan). Pasta samples were placed in the sample

cup for measurement and the colour readings expressed by Hunter L^*, a^*, b^* values

(10 replications per sample). L^* values describe black to white (0-100); a^* values

describe redness (positive) and greenness (negative) and b^* values describe

yellowness (positive) and blueness (negative). The change in colour due to sorghum

addition was determined by calculating the colour differential index (ΔE) using Eq.

(2).

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \qquad\qquad (2)$$

where ΔL: $L^*_{sample} - L^*_{control}$; Δa: $a^*_{sample} - a^*_{control}$; and Δb: $b^*_{sample} - b^*_{control.}$

According to the Handbook of Colour Science (Yamauchi, 1989), ΔE is an index

for determining visual colour differences and is described in terms of related values

as follows: (0-0.5, trace difference); (0.5-1.5, slightly noticeable; hard to detect with

the human eye); (1.5-3.0, noticeable; detectable by trained people); (3.0-6.0,

appreciable; detectable by ordinary people); (6.0-12.0, large; large difference in the

same colour category) and (larger than 12, extreme; another colour category).

4.3.9 Consumer sensory evaluation of pasta

Sensory evaluation was performed on cooked pasta by an untrained consumer panel

(n = 50), 23 males and 27 females; 20-57 years old (mean ± SD = 29.3 ± 7.8).

Ethical approval for the study was granted by the Deakin University Faculty of Health, Human Ethics Advisory Group (HEAG-H 58_2011). Panel members were recruited via advertisement at Deakin University, Melbourne Australia, through which they were informed of the types of pasta used in the study. Panel members were screened for eligibility using a screening questionnaire **(Appendix I)**. Exclusion criterion included allergy to gluten.

Pasta samples were cooked for the optimum cooking time without the addition of salt, drained and kept warm until serving. Panellists assessed pasta samples (10 strands per sample) for consumer acceptability while seated isolated in sensory booths. The samples were served in plastic plates, labelled with random three digit codes, in a randomised order. The pasta samples were evaluated for acceptability of colour, flavour, texture (in mouth) and overall acceptability using the nine-point hedonic scale (1 = dislike extremely and 9 = like extremely) (Meilgaard et al., 2007) **(Appendix II)**.

Two pre-set criteria as explained by Clark and Johnson (2002), with some modification, were used to determine the acceptability of sorghum-containing pastas for the purposes of this study. RSF or WSF-containing pasta was considered acceptable if: (i) the mean sensory score for overall acceptability was equal to or greater than 6.0, representing "like slightly" on the nine-point hedonic scale and (ii) the estimated population mean ratings for overall acceptability of the RSF or WSF-containing pasta was no lower than 1 rating category below that of the control pasta; that is, if the lower 95% confidence interval for the mean difference (sorghum-containing minus control) lies above -1.0.

3.3.10 Statistical analysis

Results are presented as mean ± SD. One-way analysis of variance (ANOVA) was used to compare the effect of pasta formulation on proximate and dietary fibre composition; RDS and SDS levels; cooking quality attributes; and consumer acceptability scores. All analyses were carried out using SPSS statistical software version 20 (SPSS Inc., Chicago, IL, USA). Statistically significant differences among means were tested using Fisher's least significant difference (LSD) *post hoc* test at $p < 0.05$.

4.4 RESULTS

4.4.1 Proximate and dietary fibre composition of cooked pasta

The mean values for the proximate and dietary fibre composition of cooked pasta are presented in **Table 4.1**. Pasta protein content decreased significantly ($p < 0.05$) in 30% and 40% RSF pastas compared to the control pasta. Except 20% WSF pasta, the fat content increased significantly ($p < 0.05$) in all sorghum-containing pastas in comparison to the control pasta, while ash content did not change with the addition of both RSF and WSF. Total dietary fibre significantly ($p < 0.05$) increased in all sorghum-containing pastas in comparison to the control pasta with the highest value being observed for 40% RSF.

Table 4.1. Proximate and dietary fibre composition of control and sorghum-containing cooked pasta samples[*] (% dry basis)

Sample	Protein	Fat	Ash	Total dietary fibre
Control	13.05 ± 0.16^a	0.52 ± 0.05^d	1.27 ± 0.08^{ac}	5.10 ± 0.11^e
20% RSF	12.81 ± 0.10^a	0.79 ± 0.03^b	1.16 ± 0.08^{bc}	6.11 ± 0.12^b
30% RSF	11.96 ± 0.48^{bc}	0.81 ± 0.11^b	1.16 ± 0.07^{bc}	6.24 ± 0.09^b
40% RSF	11.57 ± 0.19^c	1.29 ± 0.03^a	1.09 ± 0.01^c	7.15 ± 0.13^a
20% WSF	13.02 ± 0.16^a	0.56 ± 0.03^{cd}	1.41 ± 0.16^a	5.47 ± 0.15^d
30% WSF	12.75 ± 0.10^{ab}	0.71 ± 0.07^{bc}	1.36 ± 0.09^{ab}	5.79 ± 0.17^c
40% WSF	12.59 ± 0.29^{ab}	0.78 ± 0.08^b	1.31 ± 0.15^{ac}	6.18 ± 0.10^b

[*]Values are expressed in means ± SD (n = 2). Means in the same columns with different letters are significantly different (p < 0.05, LSD test). RSF: red sorghum flour; WSF: white sorghum flour.

4.4.2. *In vitro* starch digestibility of pasta

The addition of both RSF and WSF into pasta decreased the rate and extent of *in vitro* starch digestion compared to the control pasta, as indicated by lower response curves in the digestogram **(Figure 4.1)**. The 40% RSF pasta exhibited the lowest values of digested starch while the control pasta exhibited the highest values at each time point. This was confirmed by the RDS and SDS values which are presented in **Table 4.2**. ANOVA demonstrated that all sorghum-containing pastas had lower levels of RDS ($p < 0.05$) than the control, but did not differ in their SDS levels. In addition pasta samples with higher percentages of RSF and WSF showed significantly ($p < 0.05$) lower levels of RDS with significant lower levels ($p < 0.05$) in the RSF compared to the WSF-containing pastas at the same incorporation level.
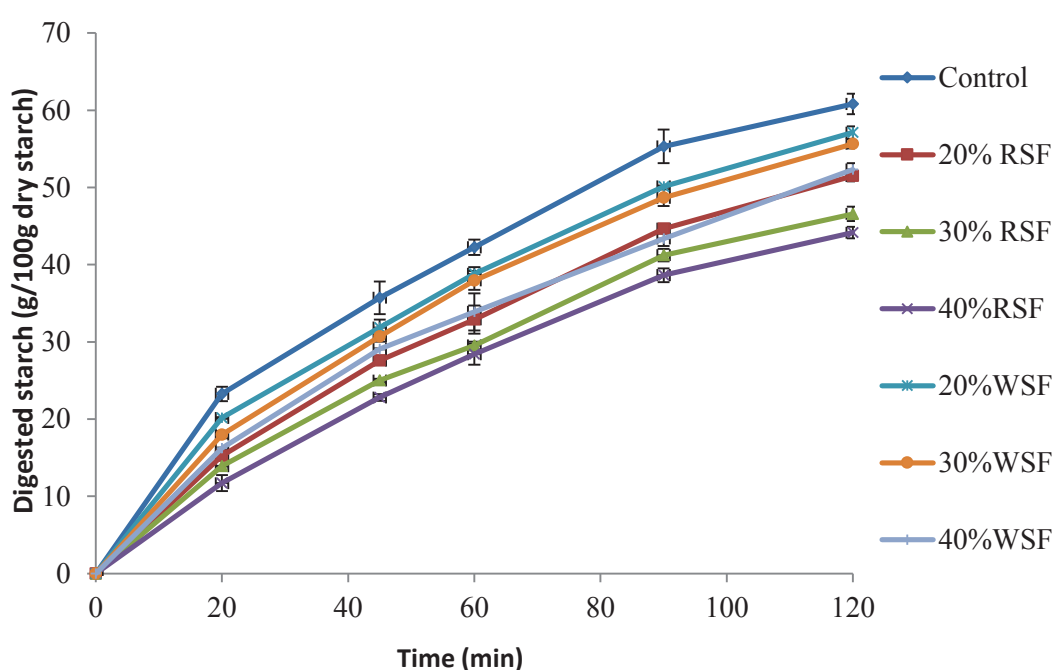
Figure 4.1. Starch digestogram of control and sorghum-containing pasta samples. Values are means ± SD of duplicate samples at each time point. RSF: red sorghum flour; WSF: white sorghum flour.

Table 4.2. RDS and SDS values of control and sorghum-containing pasta samples[*] (g/100g dry starch)

Samples	RDS	SDS
Control	23.27 ± 0.92^a	37.56 ± 0.38^{ab}
20% RSF	15.23 ± 0.38^d	36.27 ± 0.23^{ab}
30% RSF	11.91 ± 0.33^e	36.67 ± 0.98^{ab}
40% RSF	10.70 ± 1.03^e	36.44 ± 1.50^{ab}
20% WSF	20.15 ± 0.29^b	36.96 ± 0.48^{ab}
30% WSF	18.00 ± 0.45^c	37.66 ± 0.34^a
40% WSF	16.23 ± 0.59^d	36.10 ± 0.72^b

[*]Values are expressed in means $\pm$ SD (n = 2). Means in the same columns with different letters are significantly different ($p < 0.05$, LSD test). RDS: rapidly digestible starch; SDS: slowly digestible starch; RSF: red sorghum flour; WSF: white sorghum flour.

4.4.3 Cooking quality of pasta

The effects of different incorporation levels of RSF and WSF on pasta cooking quality parameters are shown in **Table 4.3**. Optimum cooking time did not change significantly ($p > 0.05$) with the addition of either RSF or WSF and was only 1 min shorter for all sorghum-containing pastas compared to the control pasta. Mean values for water absorption and swelling index of all RSF and WSF-containing pastas showed no significant difference from the control pasta ($p > 0.05$). The dry matter contents of pasta containing 30% and 40% RSF or WSF were significantly ($p < 0.05$) lower than the control pasta. Cooking loss was significantly lower ($p < 0.05$) for the control pasta than for all of the sorghum-containing pastas except 20% WSF pasta.

Table 4.3. Cooking quality parameters of control and sorghum-containing pasta samples[*]

Sample	Cooking time (min)	Water absorption (%)	Swelling index	Dry matter (%)	Cooking loss (%)
Control	15.2 ± 0.4^a	158.11 ± 0.84^a	1.99 ± 0.02^{ab}	39.42 ± 0.13^a	3.50 ± 0.34^b
20% RSF	14.3 ± 0.4^a	159.82 ± 2.53^a	2.02 ± 0.03^{ab}	38.98 ± 0.25^{ab}	4.99 ± 0.38^{ac}
30% RSF	14.1 ± 0.3^a	161.95 ± 1.59^a	2.05 ± 0.04^{ab}	38.24 ± 0.31^{bc}	5.66 ± 0.86^a
40% RSF	14.3 ± 0.2^a	161.75 ± 2.77^a	2.08 ± 0.04^a	37.52 ± 0.77^c	5.89 ± 0.20^a
20% WSF	14.2 ± 0.4^a	158.41 ± 3.45^a	1.98 ± 0.02^b	38.47 ± 0.14^{abc}	4.48 ± 0.67^{bc}
30% WSF	14.2 ± 0.3^a	159.04 ± 4.28^a	2.01 ± 0.06^{ab}	38.20 ± 0.66^{bc}	4.86 ± 0.16^{ac}
40% WSF	14.3 ± 0.4^a	160.16 ± 3.89^a	2.08 ± 0.04^a	37.64 ± 0.39^c	5.93 ± 0.03^a

[*]Values are means ± SD of duplicates. Means in the same column with different letters are significantly different ($p < 0.05$, LSD test). RSF: red sorghum flour; WSF: white sorghum flour.

4.4.4 Textural properties of pasta

Textural properties of pasta are presented in **Figure 4. 2**. The addition of both RSF and WSF significantly decreased ($p < 0.05$) the hardness of pasta at all levels compared to the control pasta. However, the hardness values did not differ ($p > 0.05$) among all the sorghum-containing pastas. Adhesiveness of pasta significantly decreased ($p < 0.05$) with the addition of both RSF and WSF. Although adhesiveness values showed a descending trend with increasing RSF or WSF concentrations, the values did not differ ($p > 0.05$) among all the sorghum-containing pastas. The addition of both RSF and WSF to pasta had no significant ($p > 0.05$) impact on cohesiveness and springiness values.
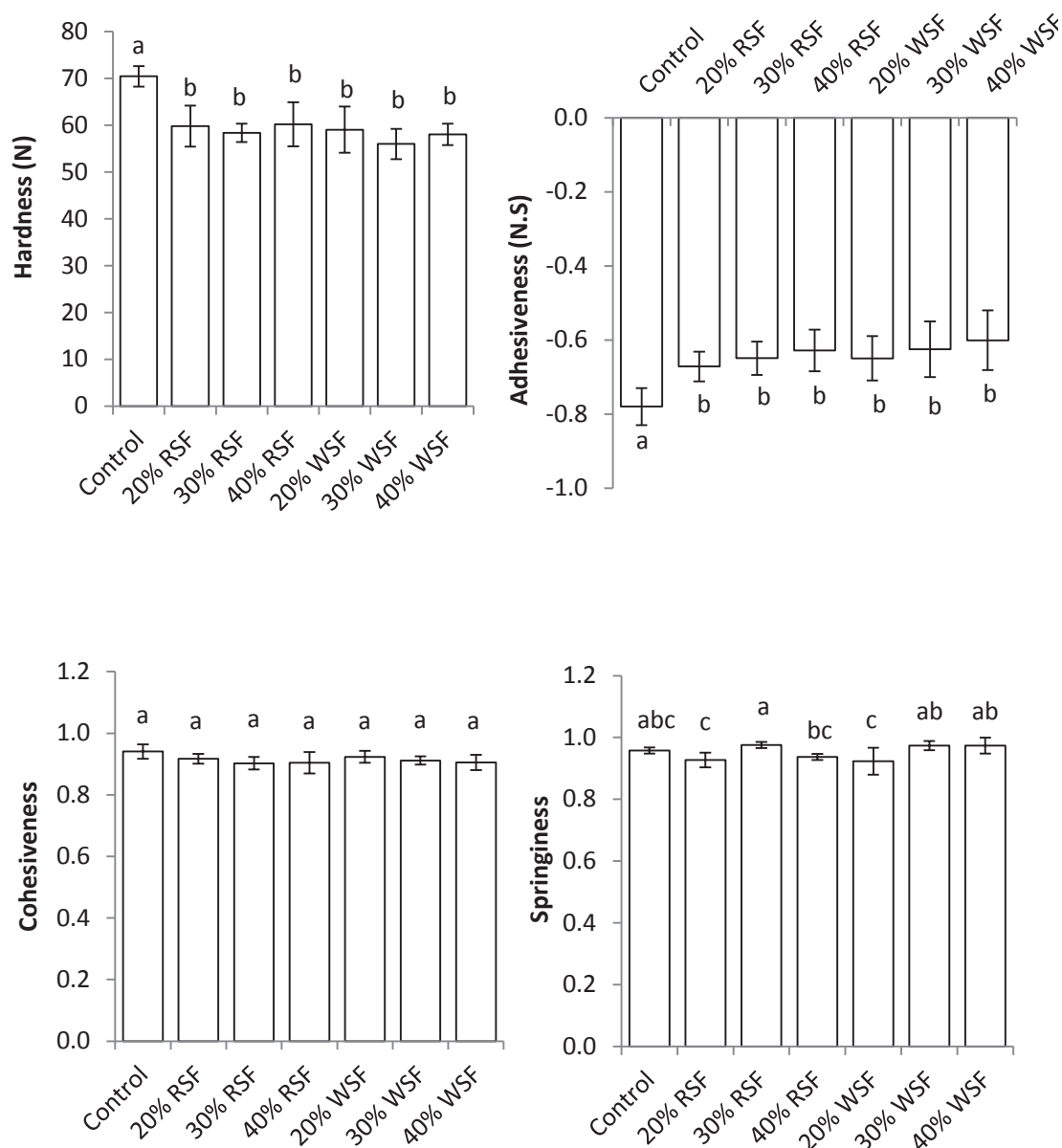
Figure 4.2. Instrumental textural characteristics of control and sorghum containing cooked pasta samples. Values are expressed in means ± SD (n = 10). Different letters above the bars within each graph indicate statistical differences (p < 0.05, LSD test). RSF: red sorghum flour; WSF: white sorghum flour.

4.4.5 Colour characteristics of pasta

Colour measurements were performed on both uncooked and cooked pasta **(Table 4.4)**. With the addition of both RSF and WSF, the colour became darker (lower L^* value). RSF addition significantly increased ($p < 0.05$) the redness (higher a^* value) whereas WSF addition did not significantly ($p > 0.05$) affect a^* value. Both RSF and WSF significantly decreased ($p < 0.05$) the yellowness (lower b^* value) at all incorporation levels. Cooked pasta had higher brightness (L^* value) and lower redness (a^* value) and yellowness (b^* value) than the equivalent raw pasta for all formulations. In addition to L^*, a^* and b^* values, ΔE was also determined to evaluate the colour difference between the control and the sorghum-containing formulations. The ΔE values of sorghum-containing pastas increased significantly ($p < 0.05$) with the increasing levels of RSF and WSF in both uncooked and cooked forms. In addition RSF-containing pastas exhibited higher ΔE ($p < 0.05$) compared to the equivalent WSF-containing pastas in uncooked form. The ΔE values were more than 12 for all sorghum-containing pastas in both uncooked and cooked forms.

Table 4.4. Instrumental colour characteristics of control and sorghum-containing pasta samples[*]

Sample	L^*	a^*	b^*	ΔE
(A) Uncooked pasta				
Control	75.36 ± 0.49[a]	2.25 ± 0.21[e]	31.71 ± 0.49[a]	
20% RSF	63.57 ± 0.61[b]	4.04 ± 0.25[c]	20.58 ± 0.71[b]	17.41 ± 0.72[e]
30% RSF	60.56 ± 0.54[d]	5.33 ± 0.19[b]	19.45 ± 0.28[c]	20.68 ± 0.64[c]
40% RSF	57.88 ± 0.50[e]	6.62 ± 0.13[a]	17.93 ± 0.24[e]	23.96 ± 0.54[a]
20% WSF	64.16 ± 0.88[b]	1.94 ± 0.14[e]	20.73 ± 0.28[b]	16.13 ± 0.46[f]
30% WSF	62.30 ± 0.89[c]	2.17 ± 0.14[e]	18.49 ± 0.18[d]	19.12 ± 0.70[d]
40% WSF	61.05 ± 0.65[d]	2.26 ± 0.18[e]	15.91 ± 0.11[f]	21.89 ± 0.59[b]
(B) Cooked pasta				
Control	79.21 ± 1.88[a]	1.87 ± 0.33[d]	29.04 ± 1.77[a]	
20% RSF	64.62 ± 0.62[b]	3.63 ± 0.60[c]	17.51 ± 0.65[c]	19.74 ± 1.97[c]
30% RSF	61.19 ± 1.51[c]	4.54 ± 0.88[b]	15.11 ± 0.66[e]	24.01 ± 2.20[b]
40% RSF	58.38 ± 0.95[d]	5.60 ± 0.86[a]	13.64 ± 0.31[f]	27.25 ± 1.89[a]
20% WSF	65.22 ± 1.33[b]	1.59 ± 0.45[d]	18.33 ± 0.16[b]	21.18 ± 1.96[bc]
30% WSF	63.82 ± 1.73[bc]	1.77 ± 0.60[d]	16.08 ± 0.63[d]	23.03 ± 2.13[b]
40% WSF	61.09 ± 1.67[c]	2.29 ± 0.58[d]	12.95 ± 0.55[f]	26.28 ± 1.52[a]

[*]Values are expressed in means ± SD (n = 10). Means in the same column for either section (A) or section (B) with different letters are significantly different ($p < 0.05$, LSD test). L^*: lightness/darkness; ± a^*: red/blue; ± b^*: yellow/green. ΔE was calculated relative to the control pasta. RSF: red sorghum flour; WSF: white sorghum flour.

4.4.6 Consumer sensory evaluation of pasta

The hedonic sensory attributes of cooked pasta evaluated by a consumer panel are given in **Table 4.5**. Panel members assigned lower (p < 0.05) scores for colour, flavour, texture (in mouth) and overall acceptability for sorghum-containing pastas than the control pasta. For acceptability of colour the control pasta scored in the range of "like moderately" while both RSF and WSF-containing pastas scored in the range of "neither like nor dislike" with the exception of 20% WSF pasta which scored in the range of "like slightly". The main comment expressed by panellists regarding colour of all sorghum-containing pastas was, "less yellow in colour". The sensory data for colour are consistent with the instrumental colour data in which control pasta scored significantly higher ($p < 0.05$) for brightness and yellowness compared to all sorghum-containing pastas. For acceptability of flavour, texture and overall acceptability, the control pasta scored in the range of "like moderately" while pastas at 20% and 30% incorporation levels of RSF or WSF scored in the range of "like slightly". Pasta at 40% incorporation level of RSF or WSF scored in the range of "neither like nor dislike".

The mean scores for overall acceptability of pasta up to 30% incorporation level of either RSF or WSF were greater than 6.0 while pasta containing RSF or WSF at 40% incorporation level did not achieve the targeted score of 6.0 and hence failed to meet the first acceptability criterion. The lower 95% confidence interval of the mean difference in overall acceptability between the sorghum-containing minus the control pasta samples lay above minus one (-1.0) for the samples containing RSF or WSF at 20%, but not for the samples containing RSF or WSF at 30% and 40% incorporation level. Hence, pasta samples containing RSF or WSF at 20% incorporation level satisfied the second pre-set acceptability criterion.

Table 4.5. Mean score, mean difference and 95% confidence intervals of difference in consumer rating of control and sorghum-containing pasta samples (n = 50)[*]

	Mean score	Mean difference (sorghum-containing – control)	95% Confidence interval of difference	
			Lower	Upper
(A) Colour				
Control	7.56[a]			
20% RSF	5.76[c]	-1.80	-2.20	-1.39
30% RSF	5.50[cd]	-2.06	-2.46	-1.65
40% RSF	5.08[e]	-2.48	-2.88	-2.07
20% WSF	6.28[b]	-1.28	-1.68	-0.87
30% WSF	5.42[cde]	-2.14	-2.54	-1.73
40% WSF	5.34[de]	-2.22	-2.62	-1.81
(B) Flavour				
Control	7.32[a]			
20% RSF	6.48[b]	-0.84	-1.23	-0.44
30% RSF	6.26[bc]	-1.60	-1.45	-0.66
40% RSF	5.42[d]	-1.90	-2.29	-1.50
20% WSF	6.40[b]	-0.92	-1.31	-0.52
30% WSF	5.98[c]	-1.34	-1.73	-0.94
40% WSF	5.12[d]	-2.20	-2.59	-1.80
(C) Texture (in mouth)				
Control	7.28[a]			
20% RSF	6.28[b]	-1.00	-1.41	-0.58
30% RSF	6.10[b]	-1.18	-1.59	-0.76
40% RSF	5.32[c]	-1.96	-2.37	-1.54
20% WSF	6.22[b]	-1.06	-1.47	-0.64
30% WSF	6.12[b]	-1.16	-1.57	-0.74
40% WSF	5.36[c]	-1.92	-2.33	-1.50
(D) Overall acceptability				
Control	7.16[a]			
20% RSF	6.50[b]	-0.66	-0.95	-0.36
30% RSF	6.38[b]	-0.78	-1.04	-0.48
40% RSF	5.26[c]	-1.90	-2.19	-1.60
20% WSF	6.56[b]	-0.60	-0.89	-0.30
30% WSF	6.34[b]	-0.82	-1.08	-0.52
40% WSF	5.34[c]	-1.82	-2.11	-1.52

Means in the same column for each section (A, B, C or D) separately, with different letters are significantly different (p < 0.05, LSD test). [*]Data collected on a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely). RSF: red sorghum flour; WSF: white sorghum flour.

4.5 DISCUSSION

The present study investigated the effect of substituting different levels of RSF or WSF for DWS on pasta *in vitro* starch digestibility, cooking quality and consumer acceptability. It was hypothesised that the addition of RSF or WSF to durum wheat pasta would decrease its starch digestibility without affecting the cooking quality and palatability. The results showed that both RSF and WSF at all substitution levels decreased the starch digestibility with no noticeable effects on most of the quality parameters. Consumer sensory results showed that pasta samples containing 20% and 30% RSF or WSF had acceptable palatability based on meeting one or both of the pre-set acceptability criteria.

Several factors may have contributed to the reduced starch digestibility of the RSF and WSF-containing pasta samples. Interaction between sorghum endosperm proteins (kafirins), which are mainly formed by intermolecular disulphide cross linking (Chandrashekar & Mazhar, 1999) and starch granules has been studied extensively as a main factor that influences sorghum starch digestibility (Ezeogu et al., 2008; Taylor & Emmambux, 2010). Ezeogu et al. (2005) found that cooking sorghum flour in the presence of a reducing agent (2-mercaptoethanol) improved flour starch digestibility by preventing or reducing disulphide-bonded polymerization of the kafirins, suggesting that sorghum proteins may act as a physical block to starch digestion.

The decrease in starch digestibility of sorghum-containing pastas might also be related to the inhibitory effect of sorghum polyphenols on digestive enzymes activity (Mkandawire et al., 2013) and interaction of sorghum polyphenols with

starch molecules (Barros et al., 2012). The RDS values found in 40% RSF or 40% WSF pasta in the present study were lower than those reported in flat bread incorporating red or white sorghum flours at the same level (Yousif et al., 2012). These differences could be attributed to the more compact structure of pasta, which is responsible for the reduced enzymatic susceptibility of starch in pasta. On the other hand the open structure of flat bread allows high accessibility to starch hydrolysis enzymes, resulting in high levels of RDS (Cavallero, Empilli, Brighenti, & Stanca, 2002). Given the relation between *in vitro* and *in vivo* starch digestion rates (Vonk et al. 2000), it would be anticipated that the addition of sorghum flour into pasta would lower glycaemic index.

The addition of RSF or WSF to pasta slightly decreased cooking time compared to the control pasta. This could possibly be due to the formation of a weaker gluten network in the pasta induced by the addition of high fibre/non-glutinous sorghum flour which may have facilitated the penetration of water to the core of pasta (Chillo, Laverse, Falcone, Protopapa, & Del Nobile, 2008). Similar response was reported previously by Aravind et al. (2013) in pasta enriched with different types of resistant starch. In the present study water absorption and swelling index values were not affected by RSF or WSF addition. Contrary to the results obtained in the present study, a decrease in these parameters for sorghum-containing pastas was expected due to the hydrophobic nature of sorghum kafirin (Duodu et al., 2003). On the other hand, an increase in these parameters of sorghum-containing pastas was expected due to the higher concentration of fibre in them. It is therefore speculated that no significant changes in water absorption and swelling index of sorghum-containing pastas could be the result of the net effect of these two factors.

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The decrease in dry matter content of RSF or WSF-containing pastas in the present study, possibly indicated increased loss of solids during cooking. Decrease in dry matter content following cooking has been previously reported by Cleary and Brennan (2006) in pasta containing barley fibre fraction.

Cooking loss, a measure of the amount of solids lost into the cooking water, is considered an important indicator of pasta quality. The cooking loss value obtained for control pasta in the present study was lower than those reported for 100% DWS pasta of 6.2% by Aravind et al. (2012), 5.6% by Petitot et al. (2010), and 4.7% by Ovanda-Martinez et al. (2009). The increase in cooking loss observed for the sorghum-containing pastas compared to the control pasta in the present study can be attributed in part to the absence of gluten protein in sorghum flour. The addition of non-gluten flour into the pasta could have diluted the gluten strength and possibly weakened the starch-gluten network which is responsible for retaining pasta physical integrity during cooking (Rayas-Duarte, Mock, & Satterlee, 1996). As a consequence, leaching of more solids from the sorghum-containing pastas into the cooking water was observed. Similar effects of increased cooking losses have been reported for pasta products incorporating non-durum ingredients such as seaweed (Prabhasankar et al., 2009), dietary fibre (Tudorica, Kuri, & Brennan, 2002), banana flour (Ovando-Martinez et al., 2009) and split pea and faba bean flours (Petitot et al., 2010). From a commercial perspective, cooking losses observed for the sorghum-containing pastas in the present study are still acceptable as losses of $\leq 8\%$ are considered desirable for good quality pasta (Dick & Youngs, 1998). In summary, the results of the cooking quality parameters, indicated that the addition

of both RSF and WSF into pasta did not affect most of the cooking quality parameters.

The addition of RSF or WSF to pasta did not affect textural parameters except hardness which was negatively affected. These results are in agreement with those of Cleary and Brennan (2006) who observed decrease in hardness of pasta incorporating barley fibre fractions. The decrease in pasta hardness is mainly associated with the disruption in protein-starch matrix induced by dietary fibre (Tudorica et al., 2002) and reduction of gluten content (Chung, Cho, & Lim, 2012; Wood, 2009). This may also be the reason for low hardness values of pasta in the present study, induced by higher level of dietary fibre and absence of gluten content in sorghum flour. Adhesiveness is another important textural parameter and high value of adhesiveness is an indicator of pasta stickiness during cooking and handling. The addition of RSF or WSF to pasta in the present study exerted beneficial effect on pasta adhesiveness by decreasing its value. These results are in line with those of Cleary and Brennan (2006) who observed decrease in pasta adhesiveness enriched with barley fibre fractions. However, contrary to the present results, Aravind et al. (2013) observed no changes in adhesiveness enriched with different types of resistant stache, while Chillo, Ranawana, and Henry (2011) observed increase in pasta adhesiveness enriched with barley β-glucan.

Instrumental colour was another quality parameter, negatively affected by the addition of RSF and WSF to durum wheat pasta. The change in colour values of sorghum-containing pastas may be attributed to the presence of coloured phenolic compounds such as anthocyanins in sorghum flours particularly in RSF (Yousif et

al., 2012). Change in pasta colour parameters (decrease in L^* and b^* values and increase in a^* value) has previously been observed by Wood (2009) in pasta enriched with chickpeas flour and by Aravind et al. (2012) in pasta substituted with durum wheat bran and germ compared to pasta made from 100% semolina only. Dry pasta colour is an important quality parameter from the consumer point of view (Wang, Kovacs, Flower, & Holley, 2004). However, in the present era, the market for composite pastas, with added non-durum materials (that are darker in colour) has grown considerably (Gallegos-Infante et al., 2010). The changes in colour parameters after pasta cooking can be attributed to the leaching and/or degradation of colour pigments such as anthocyanins (Khan et al., 2013) and carotenoids (Wood, 2009).

The results obtained in the present study on consumer acceptability of pasta show that both RSF and WSF can be successfully added into pasta at up to 30% incorporation level without affecting palatability. The results on sensory attributes obtained in the present study are consistent with those of Yousif et al. (2012) who obtained similar scores for colour, flavour, texture and overall acceptability of flat bread incorporating red and white sorghum flours. The present results on sensory attributes (flavour and overall acceptability) also closely matched those obtained for noodles substituted with banana flour and β-glucan by Choo and Aziz (2010).

Pasta samples containing RSF or WSF at 20% and 30% incorporation levels fulfilled one or both criteria, set for acceptability purposes in the present study. These pasta samples were rated more favourably than 'like slightly' by the panellists and there was enough confidence to predict that in the general population,

these samples would on average be rated no inferior than 1 rating category lower than the control pasta on the 9-point hedonic scale and hence have most promise from a commercial perspective.

In conclusion, the addition of both RSF and WSF to pasta at all levels significantly increased the dietary fibre content and reduced the RDS content compared to 100% DWS pasta. The results also suggest that addition of RSF is better able to reduce the starch digestibility of pasta than WSF. In addition, substitution of DWS by either RSF or WSF at all levels had no significant effect on most of the quality attributes, except cooking loss, colour and hardness which were affected negatively. Results on consumer acceptability indicated that panellists assigned lower scores on colour, flavour, texture and overall acceptability for all sorghum-containing pastas than the control pasta. However, pasta samples containing 20% and 30% incorporation level of either RSF or WSF fulfilled one or both pre-set acceptability criteria indicating that these formulations may have market potential. Human dietary studies are now needed to ascertain if the lower *in vitro* starch digestibility of the red and white sorghum-containing pastas than the control translates into a health beneficial lower glycaemic response *in vivo*.

CHAPTER FIVE

STUDY 3 - EFFECT OF WHOLE GRAIN SORGHUM FLOUR-CONTAINING PASTA ON POSTPRANDIAL GLYCAEMIA, APPETITE AND ENERGY INTAKE IN HEALTHY SUBJECTS

This chapter has been submitted for peer review prior to completion of this thesis.

Khan, I., Yousif, A., Johnson, S. K., & Gamlath, S. Effect of sorghum flour-containing pasta on postprandial glycaemia, appetite and energy intake in healthy humans. *British Journal of Nutrition*. (In review).

5.1 ABSTRACT

In vitro studies suggest that incorporating sorghum flour into staple foods including pasta reduces their starch digestibility and hence may suppress postprandial blood glucose levels, appetite and energy intake; however these effects in humans have yet to be reported. Therefore, the objective of this study was to investigate the effect of whole grain red and white sorghum-containing pasta on blood glucose, appetite and energy intake in humans. In a randomised crossover design, healthy subjects (n = 20) consumed the following four iso-caloric test meals as breakfast 1-2 wk apart: control (100% durum wheat) pasta (CP); 30% red sorghum pasta (RSP); 30% white sorghum pasta (WSP) and; white bread (WB) as a reference meal. Each test meals provided 50 g of available carbohydrate. Blood glucose and subjective appetite were measured postprandially for 2 and 3 h, respectively. Energy intakes from an *ad libitum* lunch consumed 3 h after breakfast and energy intake for the remainder of the day were also measured. Incremental areas under or over the curves

(iAUCs/iAOCs) for glucose and appetite parameters and glycaemic index (GI) and satiety index (SI) were calculated. The RSP meal resulted in significantly lower blood glucose response ($P = 0.005$) and GI ($P = 0.029$), significantly higher satiety rating ($P = 0.033$) and lower hunger, prospective food intake ratings and energy intake at *ad libitum* lunch ($P = 0.002$, $P < 0.001$, and $P = 0.001$, respectively) compared to CP meal. In conclusion, the results indicate that red sorghum flour addition into pasta provides a product inducing reduced glycaemia, increased satiety parameters and decreased subsequent energy intake.

5.2 INTRODUCTION

The prevalence of obesity, diabetes and CVD are increasing worldwide, with diet exerting a significant role in their development (Mann, 2002; Astrup, 2001). Epidemiological studies suggest that consumption of whole grain foods is associated with reduced risk of these conditions (Ven de Vijver, Ven den Bosch, Van den Brandt, & Goldbohm, 2007; Fung et al., 2002; Lutsey et al., 2007). The proposed mechanism behind this protective effect may be related to the suppression of appetite and avoidance of elevated blood glucose level, which are associated with excessive energy intake, decrease insulin sensitivity, oxidative stress and inflammation (Williams et al., 2008; Del Prato et al., 1994; Ceriello, 2000). Human clinical studies using whole grain cereals such as whole grain wheat bread (Kristensen et al., 2010), boiled whole grain barley kernels (Johansson, Nilsson, Ostman, Bjorck, 2013), boiled whole grain rye kernels (Rosen, Ostman, & Bjorck, 2011a) and whole grain oat-based breakfast cereals (Connolly, Tuohy, & Lovegrove, 2012) have been reported to reduce blood glucose and suppress appetite and energy intake, but there has been no equivalent study reported using sorghum grain products.

Sorghum (*Sorghum bicolor*) is a cereal grain, traditionally milled to whole grain flour for consumption (Dlamini et al., 2007). Food products made from a partial substitution of base ingredients with whole grain sorghum flour such as pasta, flat bread and extruded snack-like foods contain a combination of beneficial components, for example, slowly digestible starch, resistant starch and polyphenolic antioxidants (Yousif et al., 2012; Licata et al., 2013; Khan et al., 2013). *In vitro* studies show that polyphenols may decrease the digestibility of

starch in sorghum-based foods by inhibition of digestive enzymes such as α-amylase (Mkandawire et al., 2013; Kim et al., 2011; Hargrove et al., 2011) as well as interaction of polyphenols with starch molecules (Barros et al., 2012). In addition reduced starch digestibility of sorghum due to interaction of starch with sorghum proteins (kafirins) (Taylor & Emmambux, 2010) may play a role. Through these mechanisms the slow starch digestibility of sorghum based foods might beneficially lower blood glucose, suppress appetite and reduce energy intake in humans. *In vivo* studies conducted on non-human mammals have shown that polyphenols of sorghum decrease digestion and absorption of carbohydrates by inhibiting the activity of digestive enzymes (Al-Mamary et al., 2001) and hence decrease postprandial blood glucose (Park, Lee, Chung, & Park, 2012; Kim & Park, 2012; Chung et al., 2011) and reduce postprandial energy intake (Appleton et al., 2004). The presence of these beneficial components therefore make sorghum a promising candidate for the development of foods for obesity and diabetes management.

Despite the evidence from *in vitro* and animal model studies, the effect of sorghum based foods on postprandial blood glucose, appetite and energy intake in humans is scarce. There have been two randomised controlled studies reporting the effect of sorghum foods on glycaemic response, both in individuals with diabetes, and these studies show contradictory results. The first by Lakshmi and Vimala (1996) indicated that consumption of whole grain sorghum foods decreased glycaemic response compared to wheat and rice foods. The second by Abdelgadir et al. (2005) found that consumption of sorghum foods did not lower glycaemic response compared to wheat, millet and maize foods. However, these studies did not specify the type of sorghum used and the meals were not matched accurately for available

carbohydrate "dose". Also these studies did not measure postprandial appetite and energy intake.

Partial substitution of durum wheat semolina by whole grain sorghum flour in pasta has previously been shown to increase both the resistant starch and polyphenol contents of the product (Khan et al., 2013) and decrease the starch digestibility in vitro (Khan, Yousif, Johnson, & Gamlath, 2014). The objective of the present study was therefore to investigate the effect of partially substituting red or white whole grain sorghum flour into durum wheat semolina pasta on postprandial blood glucose, appetite and energy intake in healthy humans. It was hypothesised that pasta containing red or white whole grain sorghum flour would suppress blood glucose, appetite and energy intake compared to pasta made from durum wheat semolina only.

5.3 MATERIALS AND METHODS

5.3.1 Subjects

Healthy subjects (both male and female) were recruited through posted flyers, newspaper advertisements and direct personal communication in Melbourne, Australia. After providing written informed consent, subjects were screened for suitability using a health questionnaire **(Appendix III)**. Exclusion criteria included a history of cardiovascular disease, diabetes, asthma, food allergies; major gastrointestinal problems, hypertension; currently dieting or breakfast skipping; pregnancy; medications known to affect glucose metabolism, appetite and weight regulation; smoking; and excessive alcohol intake. In addition, individuals with no history of diabetes but with fasting blood glucose concentration ≥ 5.6 mmol/l were excluded. A total of twenty-two subjects began the study and twenty completed all sessions. The sample size was based on a previous subjective satiety study by Flint, Raben, Blundell, and Astrup (2000). This study found that 18 subjects were needed in order to detect a 10% difference in mean appetite rating values with a power of 0.8 and $\alpha < 0.05$. To compensate for a predicted ~20% dropout rate, the number of subjects recruited in the present study were increased to 22.

5.3.2 Study design and protocol

This study was designed as a randomised, controlled, cross-over trial. Each subject attended four testing sessions, each 1-2 week apart. At each study session, subjects consumed one of the four iso-caloric meals of: reference white bread (WB), control pasta (CP), 30% red sorghum pasta (RSP) or 30% white sorghum pasta (WSP). Subjects were requested to avoid alcohol and vigorous physical activity the day before each study visit. To avoid a second meal effect, subjects were instructed to

eat and drink the same foods the evening before each study visit and to record this in a diary. Subjects consumed their evening meal no later than 9:00 pm, after which they fasted (consumption of a maximum of 500 ml of water was allowed between 9 pm and the next morning). The general layout of the experimental protocol is given in **Figure 5.1**.

Subjects attended each study visit at 08:30 hours following a 10-12 h fast. Upon arrival at the laboratory, baseline measurements including weight, height, waist circumference, blood pressure and heart rate were recorded. After 5 min rest in the supine position, fasting blood glucose was measured by finger prick and the subject assessed their appetite sensations using visual analogue scales (VAS) (time point 0). Thereafter the test meal, contributing 50 g of available carbohydrates was consumed within 10 min, during which time subjects were required to rate its palatability. Blood glucose was measured at 15, 30, 45, 60, 90, and 120 min after the beginning of the test meal. Immediately after each blood sample was taken, the subjects filled out the VAS to measure their appetite sensations. Additional VAS beyond the time frame of blood glucose measurements were completed at 150 and 180 min. The subjects were not allowed to eat or drink anything during the 3 h period, but were allowed to read, watch TV or use their computer. The subjects were allowed to talk to each other as long as the conversation did not involve food, appetite or related subjects. At 180 min after commencing the test meal, the subjects were provided a buffet-style *ad libitum* lunch comprising a variety of foods including meat balls, chicken munchies, spring rolls, pastizzis, potato chips, frozen mix berries, cheese cake and orange juice. Subjects were given 30 min to eat whatever they wanted and were instructed to eat the amount needed to reach

comfortable satiation. All food and beverage remaining were weighed and the amount of each item consumed recorded. After leaving the laboratory, subjects were given a food diary and were instructed to keep a food record of all food and drink consumed for the remainder of the day. The study was conducted according to the guidelines of the Declaration of Helsinki, and all procedures involving human subjects were approved by the Deakin University Human Research Ethics Committee (DUHREC 2012-009). This trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN: 12612000324819).

5.3.3 Test meals

The control pasta was made from 100% durum wheat semolina. The sorghum-containing pastas were prepared with semolina in which 30% of the semolina was replaced with either red or white whole grain sorghum flour. This level of sorghum incorporation was selected based on the earlier consumer sensory study described in **Chapter 4, Section 4.4.6**, which indicated that a palatable product could be made at this incorporation level. Pasta was prepared as described in **Chapter 2, Section 2.2**. All pasta samples were cooked individually in 1000 ml water at optimal time (**Chapter 2, Section 2.3**) and served with 50 g tomato sauce. A commercial white bread was used as a reference food. The test meals were served as breakfast. The composition of test meals were calculated using FoodWorks version 7 (Xyris Software, Kenmore Hills, Qld, Australia) using AusNut database (All Foods, Rev. 14, Food Standards Australia New Zealand, Canberra, Australia). The database was supplemented with direct analysis of the experimental foods and manufacturers' information for foods not found on the database. The ingredients used in all the test meals and their compositional profiles are given in **Table 5.1**. All test meals

contained equal amount of available carbohydrates (total carbohydrates − total dietary fibre), however dietary fibre content differed between the meals. All the test meals were adjusted to an equal weight with drinking water.

Table 5.1. Ingredients and energy/nutrient composition of test meals

	WB	CP	WSP	RSP
Ingredients				
Bread (g)	108	-	-	-
Uncooked pasta (g)	-	67	65	66
Tomato sauce (g)	-	50	50	50
Water (g)	292	196	200	198
Total weight (g)	400	400	400	400
Energy/nutrient values				
Energy (kJ)	1101	1119	1112	1113
Available carbohydrate (g)	50	50	50	50
Protein (g)	9.0	9.2	8.6	8.5
Fat (g)	2.2	2.6	2.7	2.8
Total dietary fibre (g)	2.9	3.4	3.8	4.2

WB: white bread; CP: control pasta; WSP: 30% white sorghum pasta; RSP: 30% red sorghum pasta.

5.3.4 Baseline measurements

Subject height was recorded to the nearest centimetre using a stadiometer (Seca Limited, Birmingham, UK), and weight was measured with a digital medical scale (model S-YB; Wedderburn, Shanghai, China) with the participant wearing light clothing and no shoes. Height and weight were used to calculate BMI. Waist circumference was measured midway between the lowest rib margin and iliac crest

and recorded to the nearest 0.1 cm. Systolic and diastolic blood pressures and heart rate were measured in the supine position with an automatic digital blood pressure monitor (UA-767, A & D Company Limited, Tokyo, Japan).

5.3.5 Blood glucose analysis

Capillary blood samples were obtained by finger-prick using an automatic, non-reusable lancet device (Accu-Chek Afe-T-Pro Plus, Roche Diagnostics, Mannheim, Germany). Before taking the blood sample, subjects were encouraged to warm their hands to increase blood flow. Blood glucose was measured using the HemoCue Glucose 201 RT analyser (HemoCue Limited, Wamberal, NSW, Australia), which expressed glucose concentration as millimoles per litre. The values were recorded in blood glucose record sheet **(Appendix IV)**. To ensure accuracy of the data, quality control tests were carried out every study morning using control solution from the manufacturer. The CV of these quality control measurements was 0.8%.

5.3.6 Subjective appetite measurements

Subjective appetite was evaluated with the use of 100 mm VAS for satiety, fullness, hunger and prospective food intake (Flint et al., 2000) **(Appendix V)**. The scales related to the following questions: How hungry do you feel? ("not hungry at all" = 0 mm to "as hungry as I have ever been" = 100 mm), How satisfied do you feel? ("completely empty" = 0 mm to "cannot eat another bite" = 100 mm), How full do you feel? ("not full at all" = 0 mm to "totally full" = 100 mm), and How much do you think you could eat? ("nothing at all" = 0 mm to "a lot" = 100 mm). The subjects were asked to place a vertical mark on each scale somewhere between the 0 and 100 mm extremes and scores were then converted to continuous variables from 0

to 100 mm. The subjects were not allowed to discuss their ratings with each other and could not refer to their previous ratings when marking the scale.

5.3.7 Palatability assessment

Palatability of test meals was assessed in terms of appearance, flavour, texture and overall acceptability using a standard nine-point hedonic scale (1 = dislike extremely and 9 = like extremely) (Meilgaard et al., 2007) **(Appendix II)**. Subjects were asked to mark a position anywhere along the scale that matched their perception. Palatability of *ad libitum* lunch was assessed in terms of overall acceptability using the same scale. The purpose of the palatability assessment of the *ad libitum* lunch was to ensure that the lunch palatability remain the same when consumed after the different test meals.

5.3.8 Post-meal energy intake

To determine energy intake differences at the *ad libitum* lunch, food items were weighed and recorded before and after the lunch intake in a food record sheet **(Appendix VI)**. The energy intake was calculated from the net weight consumed and the compositional information provided by the manufacturer of the products. Food intake for the rest of the day was determined by estimated food records method using food diary **(Appendix VII)**. Subjects were given instructions on how to accurately record all the food and drink consumed for the remainder of the day in the food diary. Estimated food records were analysed to calculate energy intake using FoodWorks version 7 (Xyris Software, Kenmore Hills, Qld, Australia) using AusNut database (All Foods, Rev. 14, Food Standards Australia New Zealand, Canberra, Australia).

5.3.9 Calculations and statistical analysis

Incremental blood glucose and appetite values for each test meal were calculated by subtracting each subject's fasting values from the values at each postprandial time point. The incremental values were then used to construct the glucose and appetite response curves. Incremental areas under the curves (iAUCs) for blood glucose and appetite parameters (satiety and fullness) were calculated using the trapezoidal method, ignoring area below zero (incremental baseline value). Similarly, incremental areas over the curves (iAOCs) for appetite parameters (hunger and prospective food intake) were calculated by the same method, ignoring the area above zero (incremental baseline value). The glycaemic index (GI) was calculated by expressing each subject's glucose iAUC for the test meal as a percentage of the same subject's response after consuming the reference white bread meal (Kendall, Josse, Esfahani, & Jenkins, 2011). The resulting individual subjects' GI values were then averaged. The satiety index (SI) of each test meal was calculated in the same manner.

Statistical analyses were performed using SPSS statistical software version 21 (SPSS Inc. Chicago, IL, USA). Two-way repeated measures analysis of variance (ANOVA) was used to determine the effects of treatments, time and time-by-treatment interaction on blood glucose and appetite parameters. A statistically significant interaction was followed by one-way repeated measures ANOVA, with Bonferroni adjustment for multiple comparisons to describe mean differences between treatments at each time point. The effects of treatments on food intake at *ad libitum* lunch and the rest of the day, and on blood glucose iAUCs and appetite parameters' iAUCs/iAOCs were determined by one-way repeated measures ANOVA followed by *post hoc* analysis with Bonferroni adjustment for multiple

comparisons. One-way repeated measures ANOVA with *post hoc* Bonferroni test was used to determine whether there was a significant difference among the palatability parameters, GI and SI of the pasta meals. All values are presented as means ± SEMs unless otherwise indicated. A $P < 0.05$ was considered to indicate statistical significance.
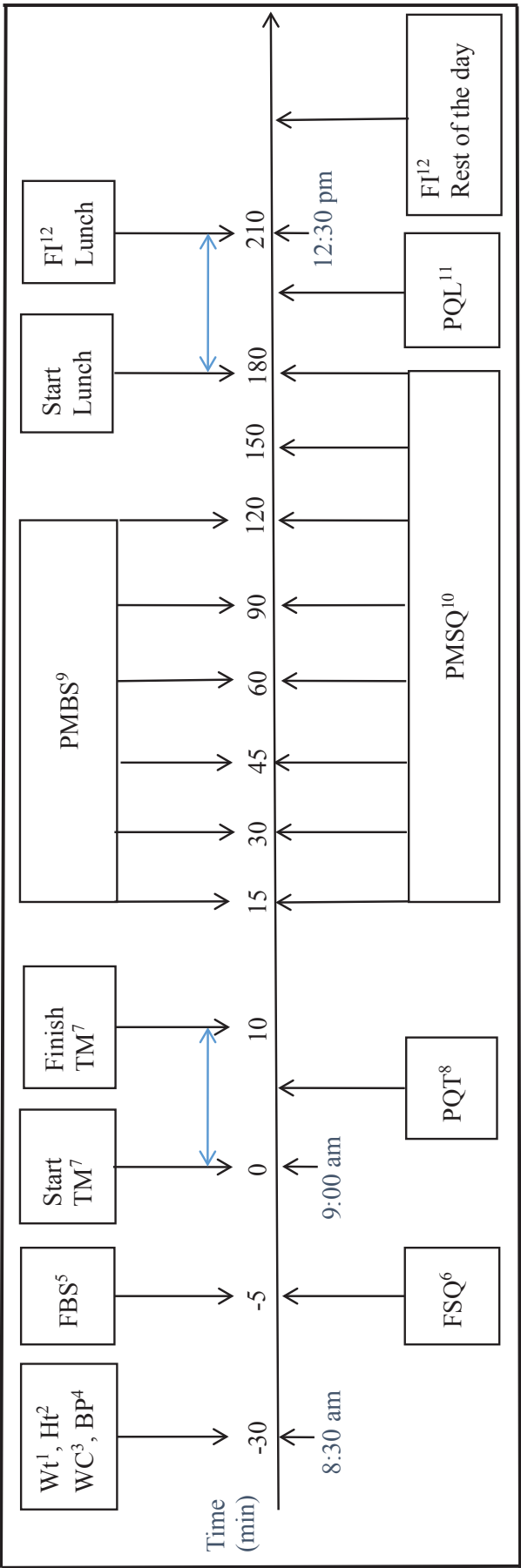
Figure 5.1. Schematic diagram of experimental protocol used in study 3.

[1]Weight, [2]Height, [3]Waist circumference, [4]Blood pressure, [5]Fasting blood sample, [6]Fasting satiety questionnaire, [7]Test meal, [8]Palatability questionnaire test meal, [9]Post meal blood sample, [10]Post meal satiety questionnaire, [11]Palatability questionnaire lunch, [12]Food intake.

5.4 RESULTS

5.4.1 Subject characteristics

Of the twenty-two subjects entering the randomisation phase of the study, twenty (six men and fourteen women) completed the study **Figure 5.2**; one subject withdrew for personal reasons; one due to illness unrelated to the study. The characteristics of the study subjects are shown in **Table 5.2**.
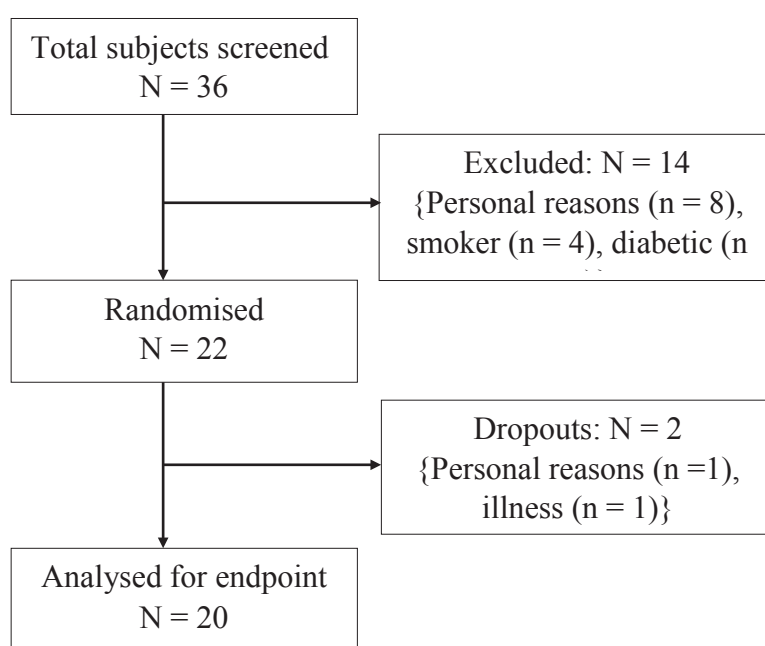
Figure 5.2. Trial profile showing the number of subjects at each stage of study recruitment and at completion.

Table 5.2. Baseline characteristics of the subjects completing the study (n = 20)[*]

Variable	Value
m/f (n)	6/14
Age (y)	23.5 ± 4.3
Weight (kg)	62.6 ± 10.5
Height (m)	1.66 ± 0.1
BMI (kg/m$^{2)}$)	22.5 ± 2.7
Waist (cm)	76.8 ± 8.4
Fasting plasma glucose (mmol/l)	4.5 ± 0.5
Systolic blood pressure (mm Hg)	115.4 ± 7.9
Diastolic blood pressure (mm Hg)	73.0 ± 6.2
Heart rate (bpm)	80.7 ± 12.4

[*]Values are means ± SD

5.4.2 Palatability of the test and *ad libitum* meals

There were no significant differences among the three pasta meals in terms of acceptability of appearance, flavour, texture and overall acceptability **(Table 5.3)**. The palatability of the *ad libitum* meal, assessed in terms of overall acceptability was rated the same when consumed after any of the three pasta meals **(Table 5.4)**.

Table 5.3. Acceptability ratings of the three pasta test meals (mean ± SEM)[*]

Test meal	Appearance	Flavour	Texture	Overall acceptability
CP	6.40 ± 0.34	6.85 ± 0.23	7.20 ± 0.21	6.95 ± 0.25
WSP	6.35 ± 0.32	6.80 ± 0.26	6.30 ± 0.34	7.00 ± 0.24
RSP	6.20 ± 0.39	6.85 ± 0.26	6.55 ± 0.33	6.90 ± 0.23

[*]No significant differences were observed among the test meals for any of the palatability parameters using 9-point hedonic scale. $P < 0.05$ (one-way repeated ANOVA, Bonferroni adjustment). CP: control pasta; WSP: 30% white sorghum pasta; RSP: 30% red sorghum pasta.

Table 5.4. Palatability assessments of the lunch buffet (mean ± SEM)[*]

Food item	Visit-I	Visit-II	Visit-III	Visit-IV
Meat balls	6.95 ± 0.34	6.90 ± 0.38	6.90 ± 0.33	6.85 ± 0.28
Chicken munchies	6.90 ± 0.39	6.95 ± 0.34	7.20 ± 0.24	6.90 ± 0.25
Spring rolls	6.85 ± 0.32	6.65 ± 0.32	6.35 ± 0.36	6.30 ± 0.31
Pastizzis	7.10 ± 0.34	7.35 ± 0.32	7.25 ± 0.31	7.30 ± 0.33
Potato chips	5.95 ± 0.43	5.90 ± 0.45	5.65 ± 0.32	5.60 ± 0.34
Frozen mix berries	6.50 ± 0.41	6.55 ± 0.35	6.85 ± 0.41	7.10 ± 0.31
Cheese cake	7.75 ± 0.22	7.60 ± 0.32	7.75 ± 0.24	7.55 ± 0.27
Orange juice	7.40 ± 0.33	7.35 ± 0.31	7.25 ± 0.31	7.00 ± 0.35

[*]No significant differences were observed in any of the lunch food item for overall acceptability parameter, over repeated consumption, using 9-point hedonic scale.

5.4.3 Blood glucose response

The postprandial blood glucose responses after consumption of the three pasta test meals with corresponding iAUCs are presented in **Figure 5.3**. There was significant overall effect of treatment ($P < 0.001$), time ($P < 0.001$), and time x treatment interaction ($P = 0.024$) on blood glucose concentrations. *Post* hoc pair-wise comparison showed that RSP meal resulted in significantly lower blood glucose concentration compared to the CP meal ($P = 0.005$). To further investigate the response to the treatments over time, the effect of treatment was determined at each time point by one-way repeated measure ANOVA. The RSP meal resulted in significantly lower blood glucose concentrations at time points 30 and 45 min compared to the CP meal ($P = 0.049$ and $P = 0.005$, respectively). In addition, the RSP meal resulted in significantly lower glucose iAUC (0-120min) compared to the CP meal ($P = 0.017$). The GI **(Table 5.5)** of the RSP meal was significantly lower compared to the CP meal ($P = 0.029$).
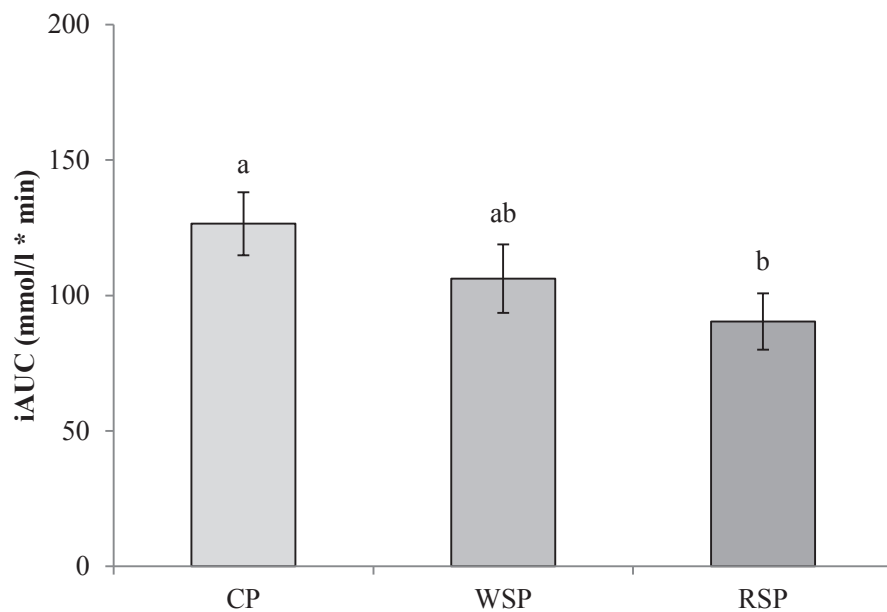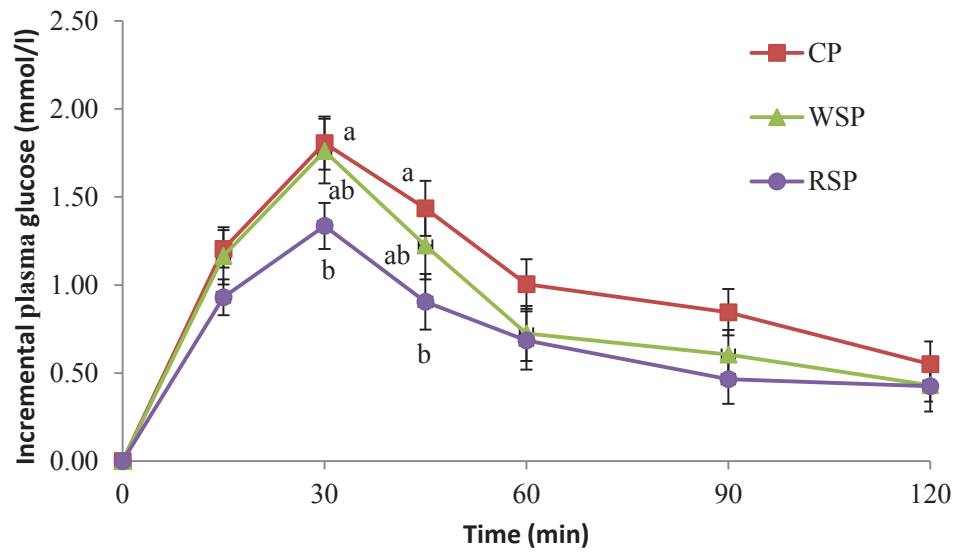
Figure 5.3. Mean (± SEM) changes from baseline in plasma glucose and incremental areas under the curve (iAUCs) in healthy subjects (n = 20) after consumption of pasta meals (CP: control pasta; WSP: 30 % white sorghum pasta; RSP: 30% red sorghum pasta). Values with different superscript letters are significantly different at each time point: two-way repeated measure ANOVA, followed by one-way repeated measure ANOVA, Bonferroni adjustment ($P < 0.05$). Bars with different letters are significantly different, $P < 0.05$ (one-way repeated ANOVA, Bonferroni adjustment).

Table 5.5. Glycaemic index, satiety index, *ad libitum* lunch energy intake and remainder of day energy intake of test meals[*]

Test meal	Glycaemic index[**]	Satiety index	EI at lunch (kJ)[**]	EI remainder of day (kJ)
CP	92.5 ± 5.5^a	138.8 ± 24.1	4747 ± 245^a	4170 ± 323
WSP	77.9 ± 8.6^{ab}	165.5 ± 14.6	4267 ± 204^{ab}	3659 ± 247
RSP	66.6 ± 6.7^b	173.0 ± 16.5	3953 ± 202^b	3412 ± 240

EI: energy intake; CP: control pasta; WSP: 30% white sorghum pasta; RSP: 30% red sorghum pasta. *All values are means ± SEMs (n = 20). **Values in the same column with different superscript letters are significantly different from each other, $P < 0.05$ (one-way repeated ANOVA, Bonferroni adjustment).

5.4.4 Subjective appetite measures

Responses in ratings of satisfaction, fullness, hunger and prospective food intake with corresponding iAUCs/iAOCs are presented in **Figure 5.4 A-D**. A significant overall effect of treatment ($P < 0.001$) and time ($P < 0.001$) on the postprandial response of all appetite parameters was found, but there was no significant interaction (time x treatment) effect on these measures ($P > 0.05$). *Post hoc* pair-wise comparison showed that the RSP meal resulted in higher rating of satiety ($P = 0.033$) as well as lower ratings of hunger ($P = 0.002$) and prospective food intake ($P < 0.001$) compared to the CP meal. In addition the RSP meal resulted in significantly higher satiety iAUC *($P = 0.029$)* and significantly lower hunger and prospective food intake iAOCs ($P = 0.004$ and $P < 0.001$, respectively) compared to the CP meal. The SI of RSP and WSP meals were not significantly different ($P > 0.05$) compared to the CP meal **(Table 5.5)**.

5.4.5 Post-meal energy intake

Energy intakes at the *ad libitum* lunch and for the rest of the day are presented in

Table 5.5. At the *ad libitum* lunch the RSP meal led to significantly lower energy

intake compared to the CP meal ($P = 0.001$). Energy intake for the rest of the day

was similar for all the pasta meals ($P > 0.05$).

A
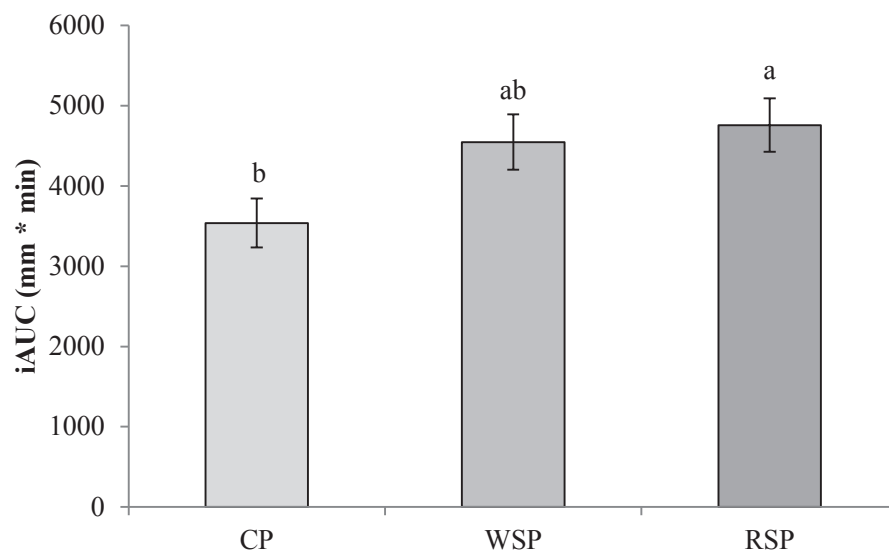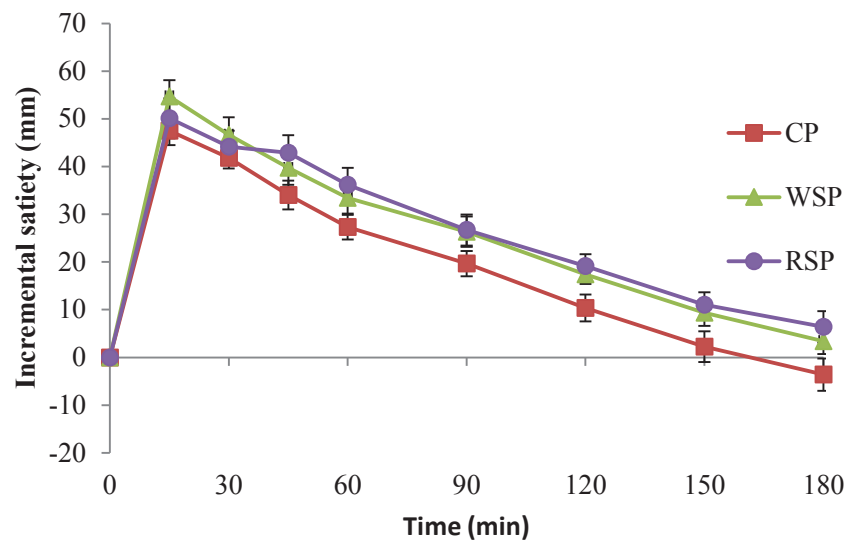
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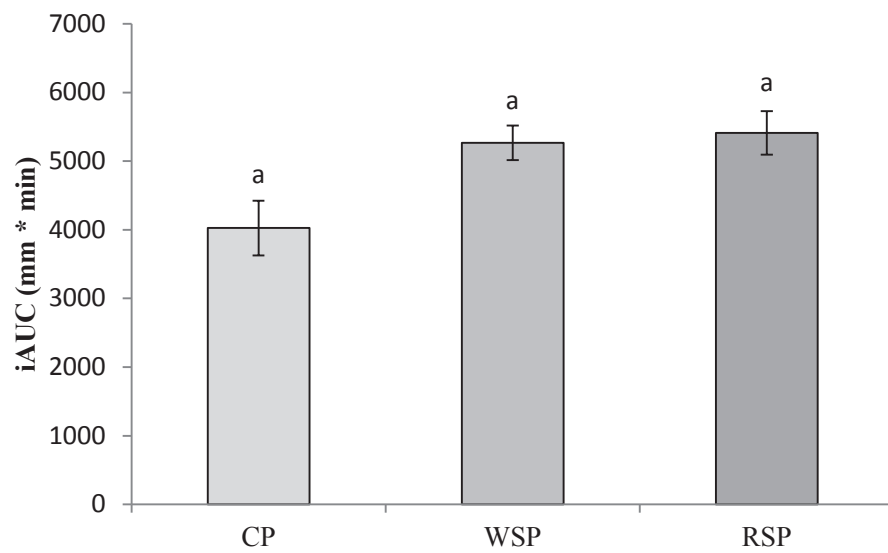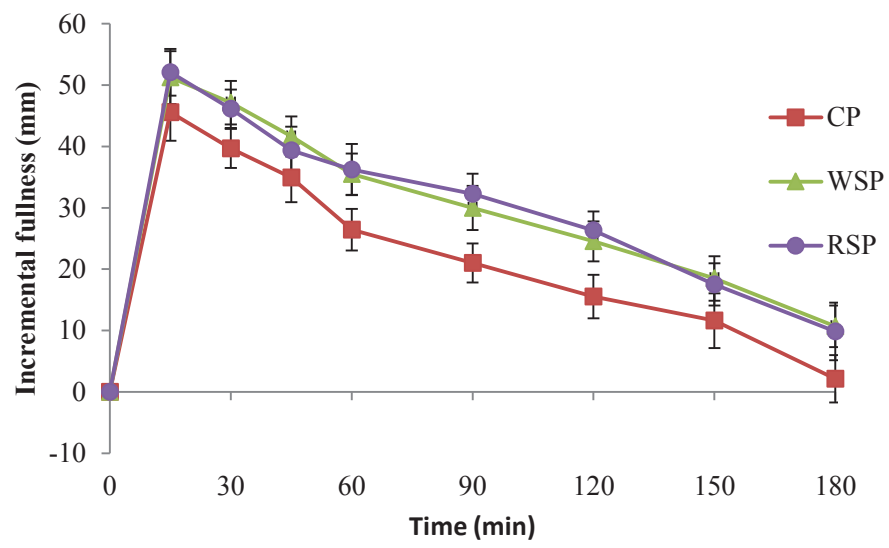
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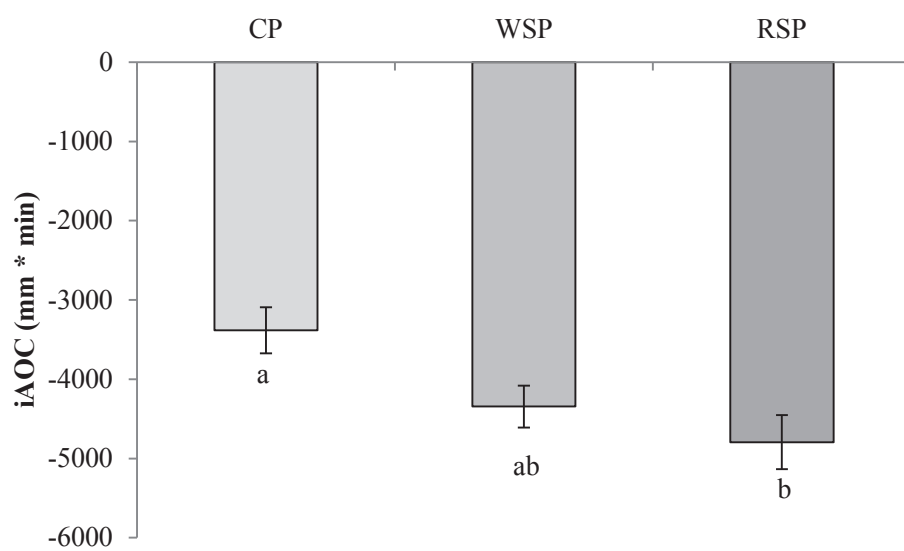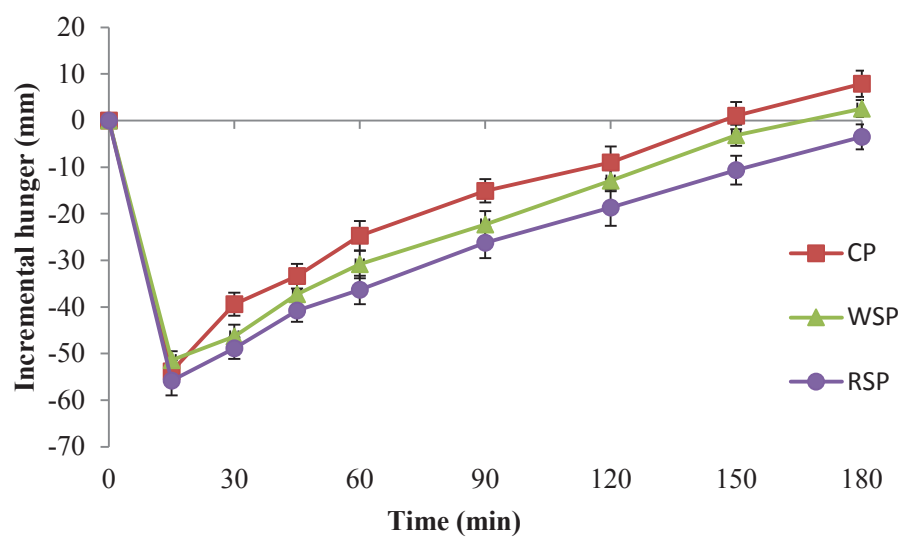
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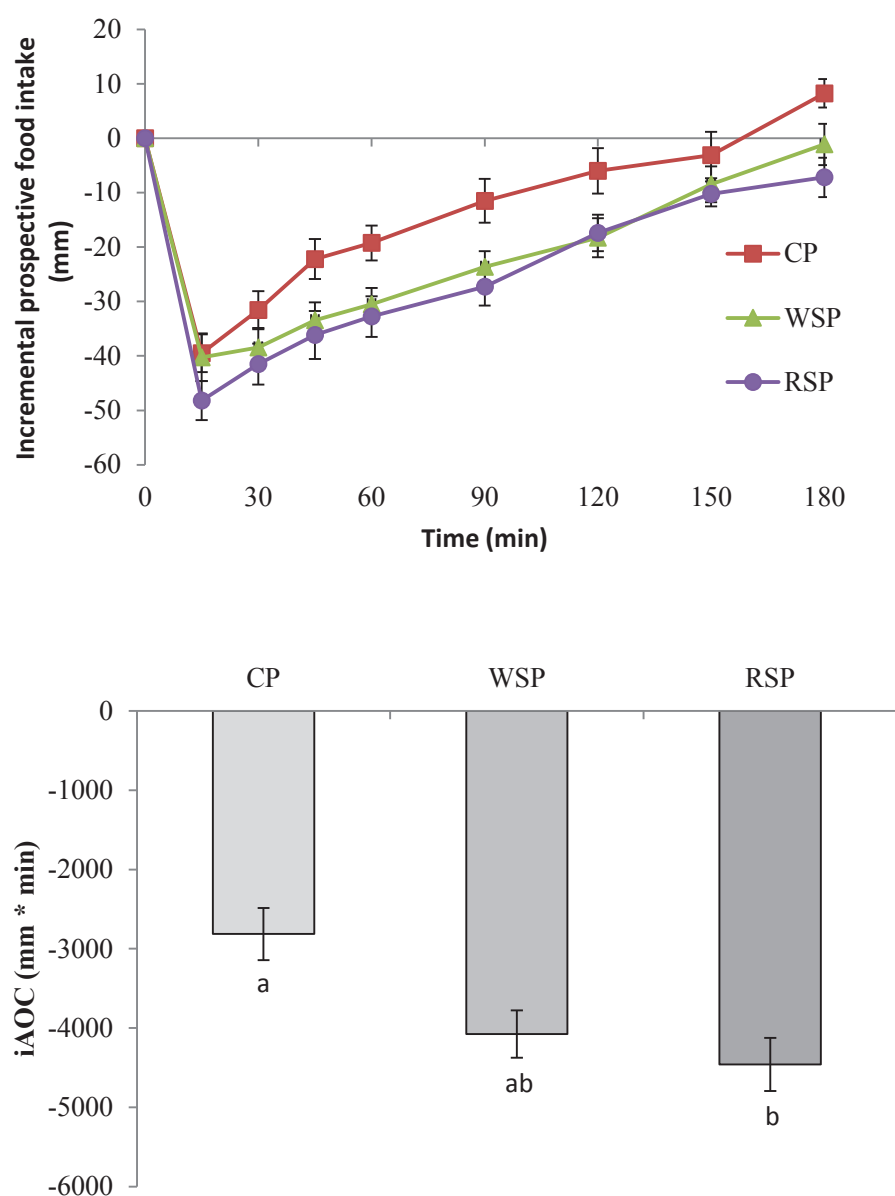
Figure 5.4. Mean (± SEM) changes from baseline in responses of satiety-related parameters of: (A) satiety; (B) fullness; (C) hunger; (D) prospective food intake with corresponding incremental areas under or over the curve (iAUCs/iAOCs) in healthy subjects (n = 20) after consumption of iso-caloric pasta meals (CP: control pasta; WSP: 30 % white sorghum pasta; RSP: 30% red sorghum pasta). Two-way repeated measure ANOVA, followed by one-way repeated measure ANOVA, Bonferroni adjustment ($P < 0.05$). Bars with different letters are significantly different, $P < 0.05$ (one-way repeated ANOVA, Bonferroni adjustment).

5.5 DISCUSSION

This is the first study to report the effect on postprandial glycaemia, subjective appetite and energy intake of sorghum flour addition to durum semolina pasta in healthy humans. The hypothesis was that pasta containing whole grain red or white sorghum flour would decrease glycaemia, appetite and energy intake compared to control pasta. However, the hypothesis was substantiated in the case of the RSP only. The results showed that RSP but not WSP suppressed postprandial glycaemia compared to CP, with a concurrent decrease in appetite and subsequent energy intake from an *ad libitum* lunch.

The present study justify the findings of a previous *in vitro* study in which pasta containing whole grain red sorghum flour at 30% incorporation level showed reduced starch digestibility compared to control pasta (Khan et al., 2014). The reduced glycaemic response of the RSP meal may have been a result of its higher content of polyphenols. Previously it was observed that pasta containing whole grain red sorghum flour at 30% incorporation level contained significantly higher content of polyphenols compared to control 100% durum wheat pasta (Khan et al., 2013). Several *in vitro* (Kim et al., 2011; Mkandawire et al. 2013; Hargrove et al., 2011) and animal studies (Al-Mamary et al., 2001; Kim & Park, 2012) have shown that sorghum polyphenols inhibit α-amylase and α-glucosidase activities. In addition polyphenols have been shown to inhibit absorption of glucose from the small intestine in animals (Welsch, Lachance, & Wasserman, 1989; Cermak, Landgraf, & Wolffram, 2004). Besides inhibitory effects of polyphenols on digestive enzymes and intestinal glucose absorption, chemical interaction of sorghum polyphenols with starch molecules may potentially have lowered the

postprandial glycaemic response to the RSP meal. It has been recently reported that tea polyphenols interact with high-amylose maize starch *in vitro* and reduce postprandial glycaemic responses in mice (Chai, Wang, & Zhang, 2013). Therefore, inhibition of digestive enzymes, decreased absorption of glucose from the small intestine and interaction of polyphenols with starch are the potential mechanisms underlying the improved postprandial glycaemic response observed after consumption of the RSP meal.

The low postprandial glycaemic response seen with the RSP meal may also in part be due to its higher dietary fibre and resistant starch content. As reviewed by Russell et al. (2013), many human clinical studies have shown that soluble dietary fibre decrease postprandial glycaemia, possibly by the formation of high viscous solution in the stomach, leading to a reduce gastric emptying rate and a lowered rate of small intestinal glucose absorption. Colonic fermentation of insoluble fibre to short chain fatty acids may have been another factor in the postprandial glycaemic response. Short chain fatty acids have been shown to reduce postprandial glycaemic response in healthy individuals (Brighenti et al., 2006). In addition, interaction of sorghum endosperm proteins (kafirins) with starch, that may reduce sorghum starch digestibility (Taylor and Emmambux 2010), may have played a role in the reduced glycaemic response of the RSP meal.

The hypothesis of the present study was however, not substantiated in the case of WSP as the WSP did not improve postprandial glycaemia compared to the CP meal. A possible explanation for this may be the differences between the concentration and composition of polyphenols of the two sorghum types. The whole grain red

sorghum flour had a higher content of phenolic acids and the phenolic acids composition of these two sorghum types is different (Khan et al., 2013). Phenolic acids were found to be negatively related with postprandial glycaemic responses in healthy humans following consumption of whole grain rye breads (Rosen et al., 2011b) and have been shown to inhibit α-glucosidase activity in rats (Adisakwattana, Chantarasinlapin, Thammarat, & Yibchok-Anun, 2009). In addition flavonoids (anthocyanins) were detected in red sorghum only (Khan et al., 2013). It has been previously reported that sorghum flavonoids inhibits α-amylase activity *in vitro* (Hargrove et al., 2011). The second aspect to be considered is the presence of higher dietary fibre and resistant starch content in red sorghum compared to white sorghum (Khan et al., 2013). Thus, these differences in the polyphenols and dietary fibre concentration of the two sorghum types may be related to the different glycaemic responses of the RSP and WSP meals.

The RSP meal resulted in significantly increased perception of satiety and decreased hunger and prospective food intake responses and a trend towards greater feeling of fullness response compared to the CP meal. The improved appetite ratings of the RSP meal were associated with decreased energy intake from the subsequent *ad libitum* lunch, but did not result in decreased energy intake for the rest of the day.

The biochemical/physiological mechanism underlying the satiating effects of the foods were not investigated in the present study. However, several factors may have contributed to the improved appetite sensations and decrease energy intake following the consumption of RSP meal. The decrease in appetite and energy intake following RSP meal may be related to its lower glycaemic response. In their review

of randomised controlled studies on glycaemic and appetite responses, Bornet, Jardy-Gennetier, Jacquet, and Stowell (2007) concluded that low glycaemic foods increase satiety and decrease energy intake compared to high glycaemic foods. Similarly, Sloth and Astrup (2006) reported that a high glycaemic response following a high-glycaemic meal is hypothesised to result in a higher immediate insulinaemic response, which will increase hunger and decrease satiety as compared to low-glycaemic meal.

A second means by which RSP meal may have suppressed appetite and decreased energy intake is through the presence of high content of indigestible carbohydrates (dietary fibre and resistant starch) in the RSP meal compared to the CP and WSP meals. Dietary fibre has been shown to affect appetite and energy intake through several mechanisms. It has recently been demonstrated that the consumption of foods high in dietary fibre requires longer oral exposure times than that of low fibre foods, which results in increased satiety and decrease *ad libitum* energy intake (Wanders et al., 2013). However oral exposure time was not measured in the present study. Colonic fermentation of dietary fibre may also enhance satiety and decrease energy intake. Increased fermentation measured by breath hydrogen following consumption of whole grain rye products was shown to be associated with lowered energy intake at a subsequent *ad libitum* meal (Rosen et al., 2011a). Fermentation of dietary fibre and resistant starch yields short chain fatty acids such as acetate and propionates (Topping & Clifton, 2001), which have been proposed to affect satiety through a relaxation of the gastric tone and a slower gastric motility (Piche et al., 2003; Cuche, Cuber, & Malbert, 2000). Other mechanisms related to specific dietary fibre properties, such as delay of gastric emptying (Clegg & Shafat, 2014),

prolong intestinal transit time, which has the effect of reducing starch absorption (Samra & Anderson 2007), secretion of appetite suppressant gut hormones such as glucagon-like peptide-1 and peptide YY (Aleixandre & Miguel, 2008) and enhanced insulin sensitivity, as insulin response is inversely proportional to satiety (Rosen et al., 2011a), may have played a role in enhanced satiety of the RSP meal. However, since the RSP meal provided 4.2 g of total dietary fibre and the two sorghum-containing pasta meals only differed in fibre content by ~ 0.4 g, it is unlikely that the increased satiety and reduced energy intake could be solely explained by the slightly higher fibre content of the RSP meal.

Finally the presence of high content of polyphenols in the RSP meal compared to the CP and WSP meals may have contributed to the improved appetite sensations and reduced energy intake at the subsequent lunch. In a recent review by Panickar (2013) polyphenols have been shown to increase the secretion of neuropeptide hormones such as glucagon-like peptide-1, peptide YY, leptin and melanocortins involved in the suppression of appetite and reduction of energy intake.

In addition to the nutrient composition of food, several other factors including weight or volume of a food (portion size), energy density and palatability could also affect satiety and energy intake (Ello-Martin, Ledikwe, & Rolls, 2005; Rosen et al., 2011a; Yeomans, Weinberg, & James, 2005). However, in the present study the portion size, energy density and macronutrient content of the pasta meals were matched and there were no significant differences in the palatability among the test meals. The present results were therefore unlikely to have been influenced by portion size, energy density and palatability.

Future research is now warranted on the effect of sorghum foods on hormones that regulate glycaemia, appetite and food intake such as insulin, ghrelin, glucagon-like peptide-1 and peptide YY. These investigations will provide supporting evidence and additional mechanistic insight into the reduce glycaemic and increased satiety effects of RSP meal observed in the present study. The generalisability of this study is limited due to the sample including only normal weight and healthy subjects. The results may differ in obese and diabetic subjects and these cohorts should be investigated. Finally, the present study assessed only the acute effects of sorghum-containing pasta on glycaemia, satiety and energy intake. These acute effects may not be indicative of glycaemic control, insulin sensitivity and energy balance in the long term which requires further investigation. In addition studies are required to evaluate the effect of polyphenols of the sorghum-containing pasta on antioxidant status and oxidative stress biomarkers in humans.

In conclusion, the results of the present study show that whole grain red sorghum flour addition into durum semolina pasta reduced its effect on glycaemia, improved its satiety effect and decreased energy intake. It may have potential benefit for type 2 diabetes and obesity risk reduction. However, white sorghum flour addition did not change these parameters compared to the control pasta. The differences in effect between the two sorghum types on these postprandial measures could be related to the higher polyphenol and dietary fibre content of the red sorghum flour compared to the white sorghum flour.

CHAPTER SIX

STUDY 4 - ACUTE EFFECT OF WHOLE GRAIN SORGHUM FLOUR-CONTAINING PASTA ON PLASMA TOTAL POLYPHENOLS, ANTIOXIDANT CAPACITY AND OXIDATIVE STRESS MARKERS IN HEALTHY SUBJECTS

This chapter has been submitted for peer review prior to completion of this thesis.

6.1 ABSTRACT

It has been previously reported that pasta containing whole grain sorghum flour exhibits high content of polyphenols and antioxidant capacity and hence might enhance antioxidant status and reduce markers of oxidative stress *in vivo*; however no clinical studies have yet been reported. Therefore, the present study assessed the effect of pasta containing whole grain red or white sorghum flour on plasma total polyphenols, antioxidant capacity and oxidative stress markers in humans. In a randomised crossover design, healthy subjects (n = 20) consumed three test meals of control pasta (CP), 30% red sorghum pasta (RSP) or 30% white sorghum pasta (WSP), 1-2 wk apart. The test meals were consumed as breakfast after an overnight fast. Blood samples were obtained at fasting and 2 h after consumption of the test meals and analysed for total polyphenols, antioxidant capacity, superoxide

dismutase (SOD) activity, protein carbonyl and 8-isoprostane. Compared to baseline, the 2 h postprandial levels following the RSP meal of plasma polyphenols, antioxidant capacity and superoxide dismutase (SOD) activity were significantly ($P < 0.001$) higher while the protein carbonyl level was significantly lower ($P = 0.035$). Furthermore, net changes in polyphenols, antioxidant capacity and SOD activity were significantly ($P < 0.001$) higher while protein carbonyl were significantly ($P = 0.035$) lower following consumption of the RSP meal than after the CP meal. In conclusion, the results demonstrated that pasta containing whole grain red sorghum flour enhanced antioxidant status and diminished marker of oxidative stress in healthy subjects.

6.2 INTRODUCTION

Oxidative stress, an imbalance between the generation of free radicals such as reactive oxygen/nitrogen species and the antioxidant defences, plays a central role in the development and progression of many chronic diseases such as cardiovascular disease (Bagatini et al., 2011; Davi & Falco, 2005), diabetes (Maritim et al., 2003; Matteucci & Giampietro, 2000) and cancer (Sener, Gonenc, Akinci, & Torun, 2007; Neuhouser, 2004). The antioxidant defence system consists of endogenous antioxidants such as glutathione, catalase and superoxide dismutase (SOD) and exogenous antioxidants obtained by dietary intake such as polyphenols, α-tocopherol and β- carotene (Scalbert & Williamson, 2000). Dietary polyphenols have been identified as both powerful chemical antioxidants and have also been shown to up-regulate the synthesis of endogenous antioxidants (Moskaug, Carlsen, Myhrstad, & Blomhoff, 2005).

Epidemiological studies have consistently shown an inverse association between the consumption of polyphenolic-rich foods and the risk of chronic diseases associated with oxidative stress (Scalbert et al., 2005; Arts & Hollman, 2005; Wang et al., 2013). This is supported by data from cross sectional studies, which show that markers of oxidative stress are inversely related to intake of polyphenolic-rich foods (Hermsdorff et al., 2012; Wang et al., 2012; Yang et al., 2013). Furthermore, polyphenolic-rich foods such as apple juice (Vieira et al., 2012), various nuts such as walnut, almond and pecan (Torabian et al., 2009; Haddad et al., 2014; Li, Jia, et al., 2007; Hudthagosol et al., 2011), green tea (Basu et al., 2013; Bogdanski et al., 2012), oolong tea (Villaño et al., 2012), dark chocolate (Davison, Callister, Williamson, Cooper, & Gleeson, 2012), red wine (Modun et al., 2008), Korean red

ginseng (Kim, Park, Kang, Kim, & Lee, 2012) and whole grain foods such as wheat aleurone-rich bread and extruded cereals (Price et al., 2012) have been demonstrated to enhance antioxidant status and improve markers of oxidative stress in human clinical studies.

Consumption of foods containing whole grain sorghum flour as an ingredient have potential to enhance the antioxidant status and beneficially modulate marker of oxidative stress. Among cereal grains some varieties of sorghum grain have the highest polyphenolic content (Ragaee et al., 2006). These sorghum polyphenols are known to function as strong antioxidants, at least *in vitro* (Dlamini et al., 2007; Dykes et al., 2005). In vivo studies on the oxidative stress related properties of sorghum foods are limited to just one study conducted on Wistar rats which demonstrated that a diet containing sorghum flour reduced markers of oxidative stress and inflammation (Moraes et al., 2012).

Studies on the effect of foods containing sorghum flour on antioxidant status and markers of oxidative stress in humans is lacking and the bioavailability of polyphenols from such foods has not yet determined. Therefore, the present study aimed to investigate the acute effect of pasta containing whole grain red or white sorghum flour on plasma total polyphenols, antioxidant capacity and markers of oxidative stress in healthy human subjects. It was hypothesised that pasta containing whole grain red or white sorghum flour would enhance plasma polyphenols and antioxidant status (enzymatic and/or non-enzymatic) and would suppress markers of oxidative stress compared to pasta made from durum wheat semolina only.

6.3 MATERIALS AND METHODS

6.3.1 Subjects

This study used the same subjects that undertook Study 3, presented in **Chapter 5** and their details appear in **Section 5.3.1**.

6.3.2 Study design and protocol

The data for this study was collected whilst conducting Study 3, presented in **Chapter 5** and the details of the general experimental design are contained within **Section 5.3.2**. The specific experimental design of this study is shown in **Figure 6.1**. Two venous blood samples were collected from the subjects on CP, RSP and WSP meals administration days at baseline and at 2 h after starting consuming the meal. This time point was chosen to correspond to the maximal postprandial antioxidant capacity and polyphenols concentration in blood reported in the literature (Hudthagosol et al., 2011; Torabian et al., 2009; Briviba et al., 2007). The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Deakin University Human Research Ethics Committee (DUHREC 2012-009). This trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN: 12612000324819).
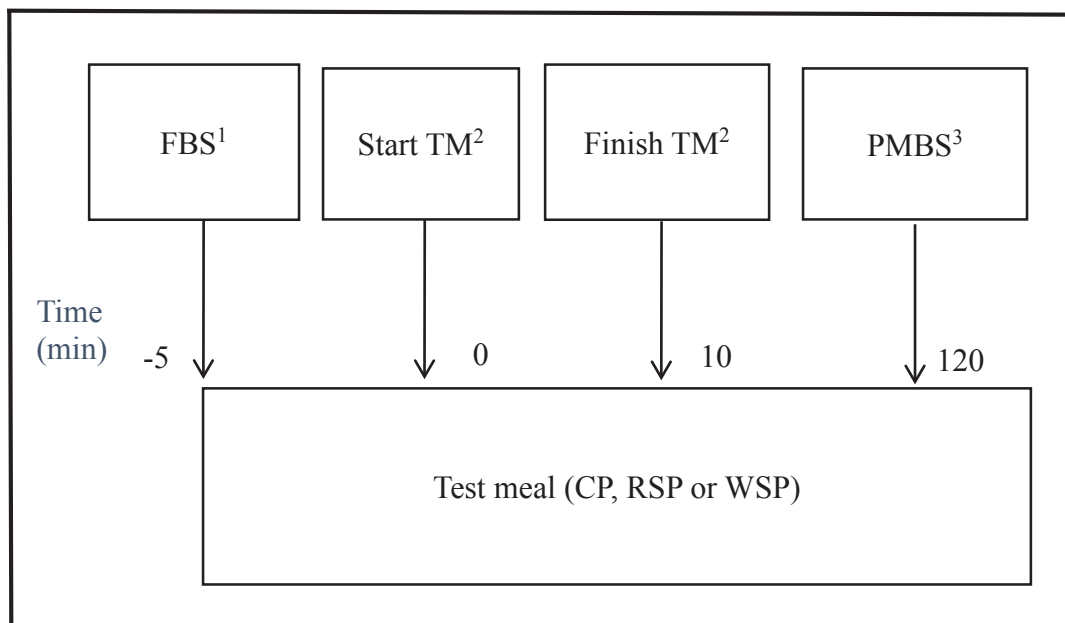
Figure 6.1. Schematic diagram of experimental protocol used in study 4.

[1]Fasting blood sample, [2]Test meal, [3]Post meal blood sample.

6.3.3 Blood collection and processing

Blood samples were collected in EDTA vacutainers by venepuncture by a qualified phlebotomist. Immediately after blood collection, samples were centrifuged at 1500 x g for 10 minutes at 4 °C. The plasma was then removed and stored in aliquots in cryovials at -80 °C until analysis of plasma total polyphenols, total antioxidant capacity, SOD activity, protein carbonyl and 8-isoprostane.

6.3.4 Measurement of plasma total polyphenols

Total phenolic compounds in plasma were determined by the method of Serafini, Maiani, and Ferro-Luzzi (1998) with some modifications. Briefly, 200 µl of plasma was mixed with 400 µl of 1 mol HCl, vortexed for 1 min and incubated at 37 °C for 30 min. Then 400 µl of 2 mol NaOH in 75% methanol was added and the resulting mixture was vortexed for 2 min and incubated at 37 °C for 30 min. Following this

400 µl of 0.75 mol meta-phosphoric acid was added; the resulting mixture was vortexed for 2 min centrifuged at 1700 x g for 10 min at 4 °C. The supernatant was collected and kept on ice in the dark, while any polyphenols remaining in the pellet were extracted again by adding 400 µl of a 1:1 (v/v) solution of acetone:water. The resulting mixture was vortexed for 1 min and centrifuged at 1700 x g for 10 min at 4 °C to collect the supernatant. The two supernatants were combined and centrifuged at 1700 x g for 5 min at 4 °C. After centrifugation the final supernatant was collected and used for analysis. For the assay, 500 µl of 0.2 N Folin-Ciocalteu's phenol reagent and 400 µl of a 2 mol Na_2CO_3 solution were added to 100 µl of the final supernatant. After 90 min of incubation in darkness at room temperature, absorbance was measured at 750 nm using the Synergy 2 microplate reader (BioTek, model S, Winooski, VT, USA). A blank received 100 µl of milli-Q water instead of plasma. Gallic acid (0-500 mg/L), prepared in milli-Q water, was used as the standard to prepare a calibration curve. The results were expressed as mg of gallic acid equivalents (GAE) per litre.

6.3.5 Measurement of total antioxidant capacity

The total antioxidant capacity in plasma samples was determined using a commercially available assay kit (ImAnOx TAS/TAC kit; Immundiagnostik, Stubenwald-Allee, Bensheim, Germany), following the manufacturer's instructions. Briefly, 10 µl sample or calibrator was added to appropriate wells of 96-well mircoplate. Then, 100 µl of reagent 1 (reaction buffer A + peroxide solution) was added to each well and incubated for 10 min at 37 °C. Following this, 100 µl of reagent 2a (reaction buffer A + reaction buffer B + enzyme solution) and 100 µl of reagent 2b (reaction buffer A + reaction buffer B) were added to each well

and incubated for 5 min at room temperature. Finally, stop solution was added to each well and the plate was read immediately using the Synergy 2 microplate reader (BioTek, model S, Winooski, VT, USA) at 450 nm. Quantification was performed with the calibrator data obtained and the results were expressed as µmol of hydrogen peroxide (H_2O_2) equivalents per litre.

6.3.6   Measurement of SOD activity

SOD activity was determined by the superoxide dismutase assay kit (Cayman Chemical, Ann Arbor, MI, USA), according to the manufacturer's instructions. This assay utilizes xanthine oxidase and hypoxanthine to generate superoxide radicals detected by tetrazolium salt. One unit of SOD is defined as the amount of enzyme required to inhibit the distmutation of the superoxide radical by 50%. Briefly, 10 µl of plasma or standard was mixed with 200 µl of the diluted radical detector in a 96-well microplate, and the reaction was initiated by adding 20 µl diluted xanthine oxidase. The plate was covered and incubated with shaking for 20 min. The absorbance was then read at 450 nm using the Synergy 2 microplate reader (BioTek, model S, Winooski, VT, USA). Bovine erythrocyte SOD prepared in sample buffer (0-0.25, final SOD activity U/ml) was used as the standard to prepare the calibration curve. The results were expressed as units per millilitre (U/ml) of SOD activity.

6.3.7   Measurement of protein carbonyl

The levels of protein carbonyls in plasma were determined using protein carbonyl assay kit (Cayman Chemical, Ann Arbor, MI, USA) following the protocol provided by the manufacturer. Briefly, 200 µl of plasma sample was added into 2 ml plastic tubes, to which 800 µl of 2,4-dinitrophenylhydrazine (DNPH) in 2.5 M

HCl was added. The control tube received 2.5 M HCl instead of DNPH. Tubes were then incubated for 1 h at room temperature and vortexed every 15 min. Samples were then precipitated with 1 ml 20% trichloroacetic acid (TCA) and centrifuged at 10,000 x g for 10 min at 4 °C. The pellets were washed 3 times with 1 ml ethanol/ethyl acetate mixture (1:1) to remove the DNPH and lipid contaminants. The final pellet was dissolved in 500 µl of guanidine hydrochloride and insoluble substances were removed by centrifugation (10,000 x g for 10 min at 4 °C). The supernatant (220 µl) from the sample and control tubes were transferred to a 96-well microplate and the absorbance was read at 370 nm using the Synergy 2 microplate reader (BioTek, model S, Winooski, VT, USA). The protein carbonyl content were determined from the difference in absorbance between the DNPH-treated and the HCl-treated samples using the molar extinction coefficient of 22000 $M^{-1}cm^{-1}$ and were expressed as nmol protein carbonyl per litre.

6.3.8 Measurement of plasma 8-isoprostane

The 8-isoprostanes (8-iso-prostaglandin $F_{2\alpha}$) were analysed in plasma by means of a competitive immunoassay kit (8-iso-$PGF_{2\alpha}$ EIA kit, Enzo Life Sciences, Farmingdale, NY, USA) following the manufacturer's instructions. Prior to analysis plasma samples were hydrolysed by addition of 50 µl 10 N NaOH to each 200 µl plasma sample. The samples were incubated at 45°C for 2 hours. Following this, 50 µl 12.1N HCl was added and the samples were centrifuged for 5 min at 12,000 × g. The supernatant was removed and used for the assay. Briefly, 50 µl of neutralizing reagent was added to all wells of a goat anti-rabbit IgG coated microplate, except for the total activity (TA) and blank wells. To the non-specific binding (NSB) and maximum binding (B0) wells 50 µl sample diluent was added.

Next, 50 μl of standard or sample were added to the appropriate wells. Following this, 50 μl conjugate solution was added to each well except TA and blank wells. Then, 50 μl EIA antibody was added to each well except TA, NSB and blank wells. The plate was incubated at room temperature with shaking for 2 h. The wells were then emptied and rinsed 3 times with wash buffer. After the final wash, 200 μl of p-nitrophenyl phosphate (pNpp) was added to each well and to the TA wells 5 μl of conjugate solution was also added. The plate was covered and incubated at room temperature for 45 min. Finally, stop solution was added to all wells and the absorbance was read immediately at 405 nm using the Synergy 2 microplate reader (BioTek, model S, Winooski, VT, USA). 8-iso-prostaglandin $F_{2\alpha}$ (10,0000-160 pg/ml), prepared in sample diluent was used as the standard to prepare the calibration curve. The results were expressed as picogram (pg) 8-iso-prostaglandin $F_{2\alpha}$ per millilitre.

6.3.9 Statistical analysis

All results are reported as means ± SEMs unless otherwise indicated. The effect of meal (CP, RSP and WSP) on plasma total polyphenols and oxidant/antioxidant biomarkers (antioxidant capacity, SOD activity, protein carbonyl and 8-isoprostane) for intra-group comparison (baseline vs. 2 h values for each meal) and inter-group comparison (net change = post meal – pre meal) were evaluated by one-way repeated measures ANOVA with *post hoc* Bonferroni test. Pearson's correlation test between polyphenols and oxidant/antioxidant biomarkers was performed using the data of difference (post meal – pre meal) of all the subjects for all the meals. A $P < 0.05$ was considered to indicate statistical significance. Statistical analyses were performed by using SPSS statistical software version 21 (SPSS Inc. Chicago, IL, USA).

6.4 RESULTS

6.4.1 Plasma total polyphenols

Plasma polyphenol concentration 2 hours after consumption of the RSP meal was significantly ($P < 0.001$) higher than baseline (0 min) **(Table 6.1)** and the net change was significantly ($P < 0.001$) higher for the RSP meal compared to the CP meal **(Figure 6.2)**. No significant difference in plasma polyphenol was observed after consumption of either WSP meal ($P = 0.92$) or CP meal ($P = 1.00$).

6.4.2 Plasma total antioxidant capacity

After the consumption of RSP meal, a significant increase in the total antioxidant capacity of plasma was found in relation to baseline (0 min) value ($P < 0.001$, **Table 6.1**) and the net change was significantly ($P < 0.001$) higher for the RSP meal compared to the CP meal **(Figure 6.3)**. There was no significant change in plasma antioxidant capacity after consumption of either WSP meal ($P = 0.78$) or CP meal ($P = 1.00$).

6.4.3 Antioxidant enzyme and oxidative stress markers

Markers of antioxidant status and oxidative stress are represented in **Table 6.1**. Plasma SOD activity 2 hours following the RSP meal was significantly ($P < 0.001$) higher than baseline (0 min) activity and the net change post-prandially (120 min – 0 min) of the RSP meal was significantly ($P = 0.003$) higher than that of the CP meal **(Figure 6.4 A)**. No significant increase in plasma SOD activity was observed after consumption of either WSP meal ($P = 0.93$) or CP meal ($P = 1.00$).

A significant decrease in the protein carbonyl content of plasma was found 120 mins after the consumption of RSP meal in relation to baseline (0 mins) value ($P = 0.035$, **Table 6.1**) and the net change was significantly ($P =0.35$) lower for the RSP meal compared to the CP meal **(Figure 6.4 B)**. There was no significant decrease in plasma protein carbonyl content after consumption of either WSP meal or CP meal (both, $P = 1.00$). Plasma 8-isoprostane level did not differ 2 hours following consumption of any of the test meals compared to the baseline (0 mins) value (RSP, $P = 0.638$; WSP, $P = 1.00$; CP, $P = 1.00$, **Table 6.1**). The net changes in 8-isoprostane levels post-prandially of RSP and WSP meals were also not significantly different compared to the CP meal ($P = 0.071$ and $P = 1.00$, respectively, **Figure 6.4 C**).

Table 6.1. Plasma total polyphenols (TP), total antioxidant capacity (TAC), SOD activity, protein carbonyl and 8-isoprostane levels in healthy subjects before and after consuming the test meals

Test meal	Time (min)	TP (mg GAE/L)	TAC (µmol/l)	SOD activity (U/ml)	PC (nmol/l)	8-isoprostane (pg/ml)
CP	0	215.16 ± 3.97	307.86 ± 2.49	8.82 ± 0.52	37.92 ± 2.41	51.22 ± 0.36
	120	212.26 ± 3.85	312.83 ± 3.32	8.88 ± 0.25	41.05 ± 3.90	51.56 ± 0.58
WSP	0	215.19 ± 2.99	305.44 ± 4.35	9.22 ± 0.40	34.91 ± 3.02	51.47 ± 0.46
	120	224.41 ± 3.02	318.09 ± 3.56	10.98 ± 0.70	30.66 ± 1.73	51.87 ± 0.52
RSP	0	216.90 ± 2.62	297.08 ± 5.13	10.16 ± 0.51	38.01 ± 2.93	51.96 ± 0.37
	120	269.40 ± 2.33[*]	375.44 ± 2.11[*]	13.66 ± 0.67[*]	28.23 ± 1.63[*]	50.59 ± 0.51

All values are means ± SEMs (n = 20). [*]Significantly different from 0 mins, $P < 0.05$ (one-way repeated ANOVA, Bonferroni adjustment). TP: total polyphenols; TAC: total antioxidant capacity; PC: protein carbonyl; CP: control pasta; WSP: 30% white sorghum pasta; RSP: 30% red sorghum pasta.

6.4.4 **Association between postprandial changes in total polyphenols, antioxidant capacity and markers of oxidative stress**

Correlation coefficients among postprandial changes in polyphenols, antioxidant capacity and markers of oxidative stress are showan in **Table 6. 2**. Changes in polyphenol level from baseline correlated positively with changes in antioxidant capacity ($r = 0.73$, $P = 0.001$) and SOD activity ($r = 0.47$, $P = 0.001$). Changes in antioxidant capacity correlated positively with changes in SOD activity ($r = 0.44$, $P = 0.001$) and negatively with changes in protein carbonyl ($r = -0.28$, $P = 0.014$).

Table 6.2. Association between plasma total polyphenols (TP), total antioxidant capacity (TAC) and oxidative stress biomarkers

	SOD activity	8-isoprostane	PC	TAC
TP	0.47 (p = 0.001)[**]	-0.14 (p = 0.13)	-0.20 (p = 0.056)	0.73 (p = 0.001)[**]
TAC	0.44 (p = 0.001)[**]	-0.19 (p = 0.066)	-0.28 (p = 0.014)[*]	
PC	-0.05 (p = 0.347)	0.06 (p = 0.336)		
8-isoprostane	-0.07 (p = 0.286)			

Values are correlation coefficients. [*]Correlation coefficients are significant ($p \leq 0.05$). [**]Correlation coefficients are significant ($p \leq 0.01$). TP: total polyphenols; PC: protein carbonyl; TAC: total antioxidant capacity.
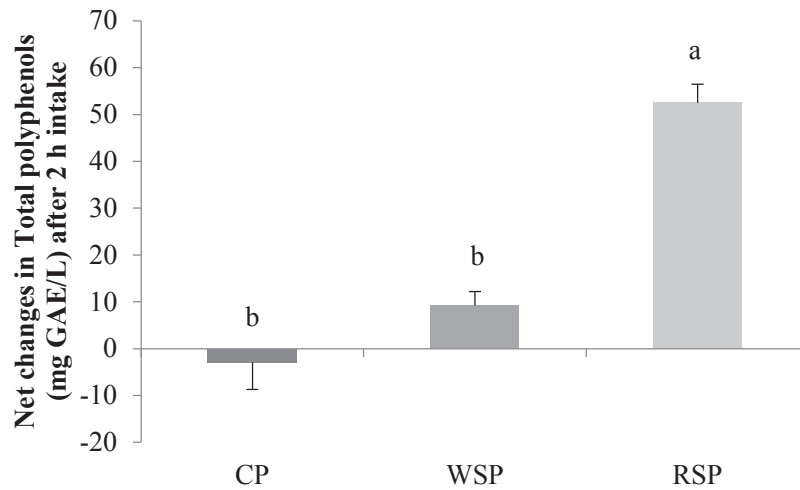
Figure 6.2. Net changes (120 - 0 min) in total polyphenols. Values are means ± SEMs (n = 20). CP: control pasta; WSP: 30 % white sorghum pasta; RSP: 30% red sorghum pasta. Bars with different letters are significantly different, $P < 0.05$ (one-way repeated ANOVA, Bonferroni adjustment).
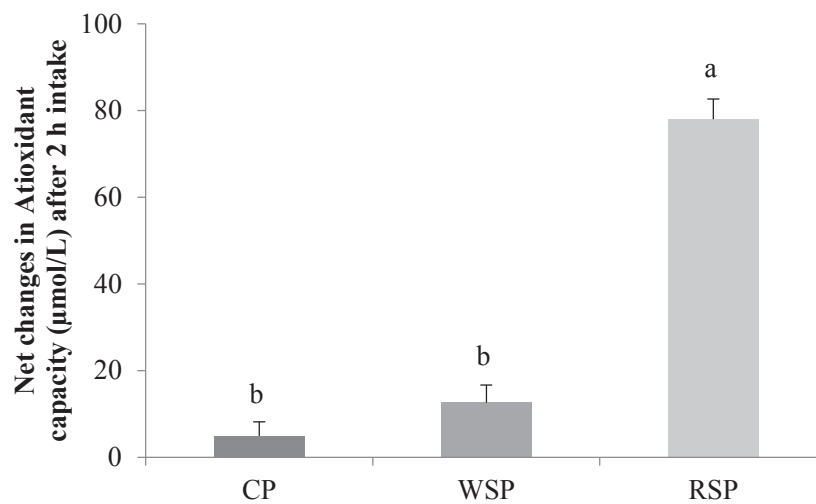
Figure 6.3. Net changes (120 - 0 min) in antioxidant capacity. Values are means ± SEMs (n = 20). CP: control pasta; WSP: 30 % white sorghum pasta; RSP: 30% red sorghum pasta. Bars with different letters are significantly different, $P < 0.05$ (one-way repeated ANOVA, Bonferroni adjustment).

A
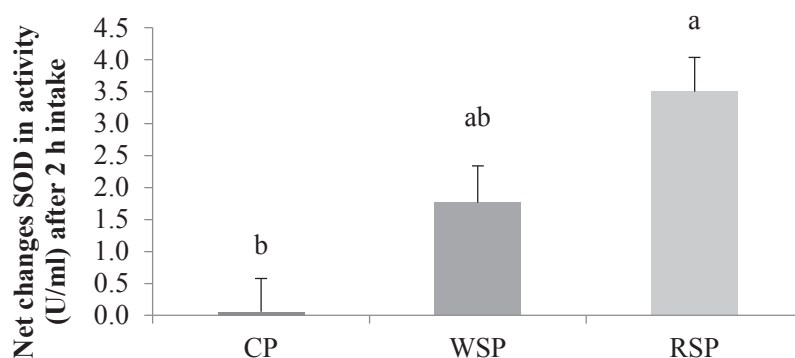
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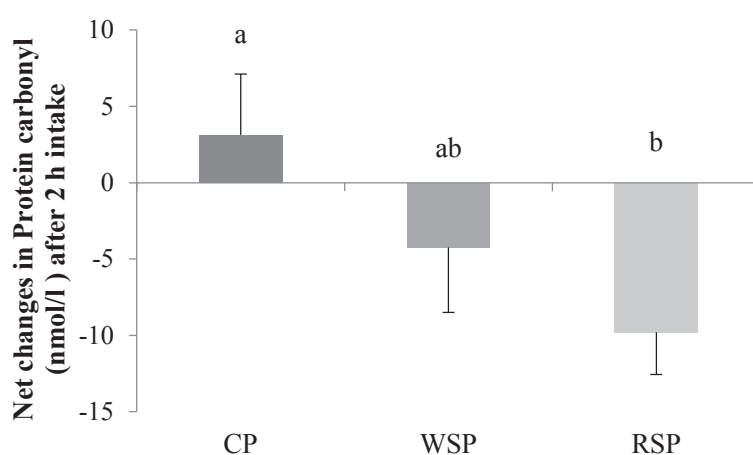
C
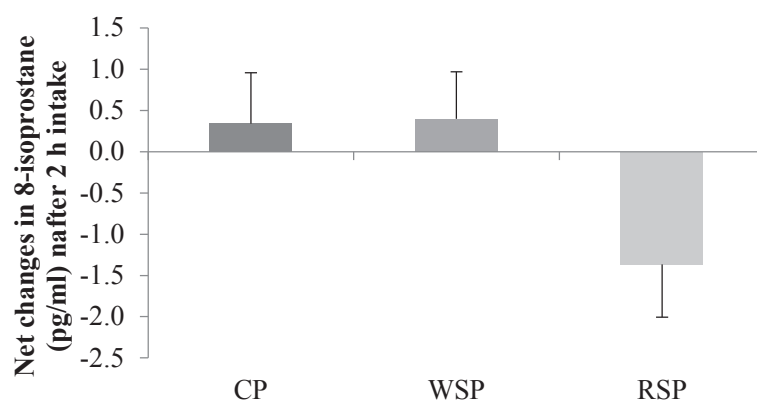
Figure 6.4. Net changes (120 - 0 min): (A) SOD activity; (B) protein carbonyl; (C) 8-isoprostane level. All values are means ± SEMs (n = 20). CP: control pasta; WSP: 30 % white sorghum pasta; RSP: 30% red sorghum pasta. Bars with different letters are significantly different, $P < 0.05$ (one-way repeated ANOVA, Bonferroni adjustment).

6.5 DISCUSSION

This study investigated the acute effect of a meal pasta containing whole grain red or white sorghum flour at 30% incorporation level on plasma total polyphenols, antioxidant capacity and markers of oxidative stress in healthy human subjects. The hypothesis was that the pasta containing red or white whole grain sorghum flour would increase plasma polyphenol level, antioxidant capacity and beneficially modulate markers of oxidative stress. The results demonstrated that the RSP meal but not the WSP meal increased plasma polyphenol level, antioxidant capacity, endogenous antioxidant enzyme (SOD) activity and decreased protein carbonyl level compared to the CP meal.

The increase in plasma polyphenols by the RSP meal may have been a result of its higher content of polyphenols. Previously it was observed that pasta containing red whole grain sorghum flour at 30% incorporation level contained significantly higher content of polyphenols compared to control pasta made from durum wheat semolina only (Khan et al., 2013). The present results agree with previous acute studies in other polyphenol-rich foods in which increase in plasma polyphenol levels following consumption was observed (Haddad et al., 2014; Torabian et al., 2009; Modun et al., 2008; Fuhrman, Volkova, Coleman, & Aviram, 2005). However, some studies showed no significant increase in plasma polyphenol levels following consumption of polyphenol-rich foods (Godycki-Cwirko et al., 2010; Liu et al., 2013). These contrasting results may be due to several factors including poor bioavailability of polyphenols from the intestinal tract (Manach et al., 2005), large inter-individual variations in polyphenols absorption efficiency (Garcia-Alonso, Ros, Vidal-Guevara, & Periago, 2006) and differences in rate of absorption among

different phenolic compounds (Scalbert, & Williamson, 2000). Based on the total polyphenolic data from the previous study (Khan et al., 2013), the absorption of polyphenols from the RSP meal calculated in the present study is ~5%. It has been previously reported that the absorption of polyphenols from the intestinal tract is around 2% to 8% (Manach et al., 2005) and most polyphenols undergo extensive metabolism by intestinal bacteria prior to absorption and in intestinal tissues during absorption (Sang, Lambert, Ho, & Yang, 2011).

In previously reported studies, an increase in plasma polyphenol level following consumption of polyphenol-rich foods was accompanied by a significant increase in plasma total antioxidant capacity (Panza et al., 2008; Torabian et al., 2009; Hudthagosol et al., 2011). The present study demonstrated an increase in total antioxidant capacity along with an increase in plasma polyphenol level only following consumption of the RSP meal, implying that polyphenols are the major contributor to the increased plasma antioxidant capacity observed. This is further supported by the significant positive correlation between polyphenols and antioxidant capacity observed in the present study. Antioxidant capacity following consumption of the WSP meal did not increase compared to the CP meal possibly due to the higher concentration of polyphenols of the RSF and differences in polyphenolic composition, e.g. anthocyanins have been detected in RSF only (Khan et al., 2013) and are strong antioxidants *in vitro* (Awika et al., 2004b).

Antioxidant enzymes such as SOD also contribute to the antioxidant defence system of the body providing protection against reactive oxygen species by eliminating superoxide anion radicals, which have been shown to be involved in damage to

cellular membranes (Saraswathi & Devaraj, 2013). In the present study the increase in SOD activity observed after the consumption of the RSP meal may be due to its high levels of polyphenols. A polyphenol-rich diet has previously been reported to increase activities of endogenous antioxidant enzymes in animal model and human studies (Li, Jia, et al., 2007; Babu, Sabitha, & Shyamaladevi, 2006).

Reactive oxygen species such as superoxide anions and hydroxyl radicals have been shown to result in protein oxidation in the body (Dalle-Donne, Rossi, Giustarini, Milzani, & Colombo, 2003). Consumption of foods rich in polyphenols have been shown to decrease protein carbonyl levels, a marker of protein oxidation, in both diabetic and healthy human subjects (Liu et al., 2013; Morillas-Ruiz, Villegas Garcia, Lopez, Vidal-Guevara, & Zafrilla, 2006). In line with these findings, the present study observed a significant reduction in protein carbonyl in plasma following consumption of the RSP meal compared to the CP meal. The decrease in protein carbonyl may due to the high levels of polyphenols and presence of flavonoids in the RSP meal, leading to increased radical scavenging and reduced generation of superoxide anions and hydroxyl radicals (Bravo, 1998).

The level of 8-isoprostane was measured as a marker of lipid peroxidation in the present study. The lack of effect of any of the test meals on the level of 8-isoprostane in the present study is consistent with previous acute study in which consumption of dark chocolate as a source of polyphenols did not affect the level of 8-isoprostane (Taubert, Roesen, Lehmann, Jung, & Schömig, 2007). A possible reason for this lack of effect on isoprostane level may be due to the short half-life time of isoprostanes in the plasma. It has previously been reported that urine is a

better biological fluid than plasma for determination of the effects of dietary interventions on isoprostane level, because in urine accumulation of the isoprostanes can be measured, whereas isoprostanes in plasma have a short half-life time and levels vary widely across the day (Hollman et al., 2011).

The short duration and use of only one postprandial blood collection are the potential limitation of the present study and the timeline and number of samples should be increased in future studies. The use of non-specific method for the determination of plasma total polyphenols is another potential limitation of the present study. In future studies the levels of specific phenolic compounds and their key metabolites in plasma should be determined using mass spectrometry based methods. The activities of other endogenous antioxidant enzymes such glutathione peroxidase and catalase were not measured in the present study. Furthermore, this study was conducted on healthy subjects only and the results may differ in obese or diabetic subjects. In addition studies are required to evaluate the effect of longer term consumption of polyphenol-rich sorghum foods on biomarkers of chronic disease risk.

In conclusion, the results of the present study show that acute consumption of pasta containing whole grain red sorghum flour at 30% incorporation level, but not that containing whole grain white sorghum flour improved antioxidant status by increasing plasma polyphenols, antioxidant capacity and SOD activity and decreasing a marker of protein oxidation compared to the control (durum wheat semolina) pasta. These differences in effect between the two sorghum grain types could be related to the higher polyphenolic content and different profile of polyphenolic species in red compared to white sorghum flour.

CHAPTER SEVEN

CONCLUSIONS AND FUTURE DIRECTIONS

7.1 OVERVIEW

Sorghum (*Sorghum bicolor*) is a grain cereal crop that plays an important role in sustainable grain production, particularly in semi-arid regions of the world due to its drought and high temperature tolerance and is therefore considered an important cereal crop for food security in these regions (Taylor et al., 2006). In Australia sorghum grain is mainly grown for animal consumption (up to 60% of the crop) and is still underutilised as a human food source (Mahasukhonthachat et al., 2010). For human consumption, sorghum grain is usually decorticated or milled to whole grain flour (Dlamini et al., 2007) which contain substantial level of beneficial components, for example, slowly digestible starch, resistant starch and polyphenols (Yousif, et al., 2012; Licata et al., 2013; Ragaee et al., 2006; Awika & Rooney, 2004; Dykes & Rooney, 2006). In other foods these components have been shown to be of benefit in improving biomarkers for type 2 diabetes, obesity and CVD (Buyken, Mitchell, Ceriello, & Brand-Miller, 2010; Raben et al., 1994; Halliwell, 2008). The presence of these beneficial components therefore make sorghum flour a promising ingredient for the development of foods to assist in the prevention of diet related chronic diseases. Nevertheless, for such foods to have a beneficial effect on human health, they need to demonstrate both physiological benefit and high palatability when consumed repeatedly (Augustin, 2001). Currently there is scant data demonstrating the role of sorghum foods in regards to their palatability and physiological functionality. The overall aim of this thesis was therefore to evaluate the potential of whole grain sorghum flour as a palatable health beneficial

ingredient through a series of products development, *in vitro* and clinical studies using pasta as a model to examine the effect of sorghum on biomarkers for type 2 diabetes and obesity. The specific aims of this thesis were to:

1. To evaluate the effect of substituting durum wheat semolina with whole grain red or white sorghum flour on resistant starch content, phenolic profile and antioxidant capacity in both uncooked and cooked pasta.

2. To elucidate the effect of whole grain red or white sorghum flour addition into durum wheat semolina pasta on its *in vitro* starch digestibility, cooking quality and consumer acceptability.

3. To investigate the effect of pasta containing whole grain red or white sorghum flour on postprandial glycaemia, appetite and energy intake in healthy humans.

4. To examine the effect of pasta containing whole grain red or white sorghum flour on postprandial plasma total polyphenols, antioxidant capacity and markers of oxidative stress in healthy human subjects.

7.2 SUMMARY OF MAJOR FINDINGS

Broadly the studies presented in this thesis evaluated the potential of whole grain sorghum flour as a palatable health beneficial ingredient through a series of product development, *in vitro* and clinical studies examining the effect of sorghum pasta consumption on biomarkers for chronic diseases.

Study 1 (Chapter 3) hypothesised that the addition of RSF or WSF to durum wheat pasta at different substitution levels (20, 30 and 40%) would increase its resistant starch content, polyphenolic content and antioxidant capacity in both uncooked and cooked forms. The results of this study demonstrated that the addition of both RSF and WSF at all incorporation levels significantly increased the resistant starch content, polyphenolic content and antioxidant capacity compared to the control pasta in both uncooked and cooked forms. In addition pasta containing RSF exhibited higher resistant starch content, polyphenolic content and antioxidant capacity than pasta containing WSF at equivalent incorporation levels. To exert beneficial physiological effects the daily intake of resistant starch is estimated to be 20 g/day (Baghurst et al., 1996). The increase in resistant starch content of sorghum-containing pastas may help to achieve this level which is currently about 5 g/day. Cooking decreased total phenolic content, antioxidant capacity, free phenolic acids and anthocyanins and increased the bound phenolic acids. The reduction in polyphenolic content and antioxidant capacity of pasta after cooking may be attributed to the leaching of phenolic compounds into the cooking water and thermal degradation of these compounds during cooking (Prabhasankar et al., 2009; Hirawan et al., 2010). However, the final cooked sorghum-containing pasta samples still had considerably higher levels of polyphenolic content and possessed

high antioxidant capacity compared to the cooked control pasta and thus the consumption of these products may enhance the post-prandial antioxidant status.

Study 2 (Chapter 4) hypothesised that the addition of RSF or WSF to durum semolina pasta at different substitution levels would decrease its starch digestibility without affecting the cooking quality and consumer acceptability. The results of this study showed that both RSF and WSF at all substitution levels decreased the *in vitro* starch digestibility with no deleterious effects on most of the cooking quality attributes. In addition pasta samples containing RSF showed lower rate of starch digestion than pasta sample containing WSF at the same incorporation level. The reduced starch digestibility of the RSF and WSF-containing pasta samples may be due to several factors including the interaction between sorghum proteins (kafirins) and starch (Ezeogu et al., 2008; Taylor & Emmambux, 2010), inhibition of the effect of digestive enzymes by sorghum polyphenols (Mkandawire et al., 2013) and interaction of sorghum polyphenols with starch molecules (Barros et al., 2012). Based on the positive association observed between *in vitro* starch digestion and *in vivo* glycaemic response in previous studies (Vonk et al. 2000; Rosen et al. 2011b), it would be anticipated that the RSF and WSF-containing pasta might lower postprandial glycaemia than the durum semolina control. The results on consumer acceptability have shown that RSF or WSF at all incorporation levels reduced the sensory attributes of colour, flavour, texture and overall acceptability compared to the control pasta. These results concur with those of Yousif et al. (2012) who reported similar scores for colour, flavour, texture and overall acceptability of flat bread incorporating red and white whole grain sorghum flours. However, the two criteria pre-set for the overall acceptability in this study were fulfilled by pasta

samples containing RSF or WSF up to 30% incorporation levels. These results indicate that pasta can be substituted with RSF and WSF up to 30% incorporation level without affecting its palatability and hence can be used in future clinical studies and may have market potential.

Based on the *in vitro* starch digestibility results observed in Study 2 (Chapter 4), it was hypothesised in Study 3 (Chapter 5) that pasta containing RSF or WSF at 30% incorporation level would decrease glycaemia, appetite and energy intake compared to the control pasta. The hypothesis was successfully substantiated in the case of the RSP meal. The results showed that RSP meal suppressed postprandial glycaemia compared to the CP meal, with a concurrent decrease in appetite and subsequent energy intake. However, WSP meal did not affect these post-prandial measures. Based on the GI value obtained for the RSP meal in the present study it would be considered a low-GI food (GI < 55, using glucose as the standard) (Foster-Powell, Holt & Brand-Miller, 2002). The present results obtained for the RSP meal are in line with those of Bornet et al. (2007) who concluded in their review of randomised controlled studies that low GI foods increase satiety and decrease energy intake compared to high GI foods. The consumption of a diet containing a higher proportion of whole grain and low-GI foods is suggested to be beneficial in the suppression of appetite and avoidance of elevated blood glucose level, which are associated with excessive energy intake, decrease insulin sensitivity, oxidative stress and inflammation (Williams et al., 008; Del Prato et al., 1994; Ceriello, 2000). These conditions in turn are associated with the progression of diet related chronic diseases such as type 2 diabetes, obesity and CVD (Willett et al., 2002; Maritim et al., 2003; Bagatini et al., 2011; Liu et al., 2000; Brand-Miller et al., 2002). These

results suggest that the consumption of food containing red whole grain sorghum flour might reduce the risk of type 2 diabetes and obesity. However, the present results do not suggest that foods containing white whole grain sorghum flour could assist in reducing the risk of these conditions.

Study 4 (Chapter 6) hypothesised that pasta containing the red or white whole grain sorghum flour would increase plasma polyphenol, antioxidant capacity and decrease markers of oxidative stress. The results showed that the RSP meal but not the WSP meal increased plasma polyphenol, antioxidant capacity, endogenous antioxidant enzyme (SOD) activity and decreased protein carbonyl level compared to the CP meal. The consumption of a diet containing polyphenolic-rich foods has been suggested to be beneficial in the prevention and progression of oxidative stress related diseases such as type 2 diabetes, CVD and cancer (Scalbert et al., 2005; Arts & Hollman, 2005). The results of the present study therefore suggest the consumption of foods containing whole grain red sorghum flour that may decrease the risk of chronic diseases by enhancing antioxidant status and decreasing oxidative stress. No evidence was found to suggest that foods containing white whole grain sorghum flour could assist in reducing the risk of these diseases.

7.3 CONCLUSIONS

From the results of the present studies the following conclusions can be drawn:

1.   The addition of both RSF and WSF into durum semolina pasta at all incorporation levels effectively increased the polyphenolic content, antioxidant capacity and resistant starch content of the pasta.

2.   Cooking decreased the total phenolic content, antioxidant capacity, free phenolic acids and anthocyanins and increased bound phenolic acids; cooking however did not change the resistant starch content.

3.   The addition of both RSF and WSF to pasta at all levels (20-40%) increased dietary fibre content and reduced the *in vitro* starch digestibility. It had no effect on most of the quality attributes except cooking loss, colour and firmness which were negatively affected.

4.   Consumer sensory results indicated that pasta containing up to 30% RSF or WSF were acceptable to consumers; however all the RSF and WSF containing samples were less preferred to control pasta.

5.   The addition of RSF at 30% level into durum semolina pasta reduced its effect on postprandial glycaemia, improved its satiety effect and resulted in decreased energy intake from a subsequent lunch; however WSF addition to pasta had no effect on these parameters.

6.   The addition of RSF and WSF to pasta had no effect on the palatability of the product in the postprandial setting.

7.   The addition of RSF into durum semolina pasta improved its effect on postprandial antioxidant status by increasing plasma polyphenols, antioxidant capacity, SOD activity and decreasing a marker of protein oxidation; however WSF addition to pasta did not affect these parameters.

7.4 LIMITATIONS

There are a number of limitations associated with the studies presented in this thesis. In Study 1 the level of phenolic compounds in the cooking water was not measured. Therefore it was not possible to demonstrate whether the loss in polyphenolic content and antioxidant capacity were due to leaching of phenolic compounds into the cooking water or due to thermal degradation during cooking. Further studies are required to confirm these mechanisms of polyphenolics loss.

In Study 3, the mechanisms underlying the beneficial effect of RSP meal on postprandial glycaemia, satiety and energy intake were not measured. The possible mechanisms include effects on satiety related hormones, reduced gastric emptying rate, increased colonic fermentation and inhabitation of digestive enzymes by RSP meal's polyphenols. These mechanisms should be investigated to fully understand the beneficial effect of the RSP meal on postprandial glycaemia, satiety and energy intake. This study was conducted only on healthy subjects and the results may vary in diabetic and obese subjects and should be investigated. Finally, the present study assessed only the acute effects of sorghum-containing pasta on glycaemia, satiety and energy intake. These acute effects may not be indicative of glycaemic control and energy balance in the long term which requires further investigation.

The short duration and use of only one postprandial blood collection are the potential limitation of the present study and the timeline and number of samples should be increased in future studies. The activities of other important endogenous antioxidant enzymes such glutathione peroxidase and catalase were not measured in the present study. Furthermore, this study was conducted on healthy subjects only

and the results may differ in diabetic subjects. The acute effect of sorghum-containing pasta on antioxidant status and markers of oxidative stress may not be indicative of its longer term beneficial effect. Therefore the effect of polyphenol-rich sorghum foods on biomarkers of chronic disease risk should be investigated in long term dietary intervention studies.

7.5 FUTURE DIRECTIONS

The studies in this thesis have provided new insights into the potential applications of sorghum flour in staple foods. The effect of whole grain sorghum flour addition to pasta on resistant starch, phenolic profile, antioxidant capacity, cooking quality and *in vitro* starch digestibility was investigated. Next, palatability of the pasta samples containing different levels of RSF or WSF was determined using consumer sensory evaluation panel. Finally, the effect of pasta containing the highest acceptable levels of RSF or WSF on biomarkers of type 2 diabetes and obesity risk was studied in a postprandial clinical sitting. Collectively, these studies demonstrated beneficial effects of pasta containing RSF on biomarkers of chronic diseases. However, considerable further work is needed to investigate the mechanisms by which RSP meal has decreased the postprandial glycaemia, satiety and energy intake. Further studies are now required to investigate the effect of RSF-containing foods on postprandial glycaemia and oxidative stress in individuals having or at risk of developing type 2 diabetes. Finally long term dietary intervention studies are required to determine the effect of RSF-containing foods on body weight and composition, glucose control, insulin sensitivity, lipid profile and oxidative stress in overweight/obese subjects. The following section outlines the proposed studies designed to address these new areas of research.

7.5.1 Mechanisms of the low glycaemic and satiety enhancing effects of the RSP meal

Background

Study 3 demonstrated that the RSP meal reduced postprandial glycaemia, appetite and energy intake compared to the control pasta; however the physiological

mechanism underlying this beneficial effect is unknown. It was proposed in this thesis that the higher content of dietary fibre and polyphenols in the RSP meal may have resulted in the reduced glycaemia, appetite and energy intake. Previous studies have shown that dietary fibre and polyphenols increase secretion of appetite suppressant hormones such as glucagon-like peptide-1 and peptide YY (Aleixandre & Miguel, 2008; Panickar, 2013). Dietary fibre has also been shown to reduce glycaemia and appetite by decreasing gastric emptying rate and enhancing insulin sensitivity (Clegg & Shafat, 2013; Rosen et al., 2011a). Colonic fermentation of dietary fibre and resistant starch yields short chain fatty acids which have been proposed to affect satiety through a relaxation of gastric tone and a slower gastric motility (Piche et al., 2003; Cuche, Cuber, & Malbert, 2000).

Aims

1. To determine the acute effect the RSP meal on satiety related hormones such as insulin, ghrelin, glucagon-like peptide-1 and peptide YY.

2. To determine the effect of RSP meal on gastric emptying rate and colonic fermentation.

Hypothesis

1. That the RSP meal will beneficially modulate satiety related hormones.

2. That the RSP meal will decrease gastric emptying rate and enhance colonic fermentation.

Key methods

Healthy subjects will participate in a randomised double blind cross-over postprandial study. Subjects will receive two test meals of control pasta (CP) and

30% red sorghum pasta (RSP) in a randomised order. Blood samples will be collected at baseline and at 15, 30, 45, 60, 90,120, 150 and 180 min after consumption of the test meals. Concentration of insulin, ghrelin, glucagon-like peptide-1 and peptide YY will be determined using a Human Metabolic Hormone Multiplex assay kit (Millipore). Subjective appetite will be measured with the use of 100 mm VAS for satiety, fullness, hunger, prospective food intake and desire to eat (Flint et al., 2000). Energy intake will be measured as described in this thesis (Study 3, Chapter 5). Gastric emptying rate will be measured by the method of Thondre, Shafat and Clegg (2013) using $1^{-13}C$ sodium acetate. Breath hydrogen will be measured as a marker of colonic fermentation by the method of Rosen et al. (2011a).

Significance

This study is expected to find out various mechanisms responsible for the reduced glycaemic and appetite responses of the RSP meal observed in study 3.

7.5.2 Acute effect of RSF-containing foods on postprandial glycaemia, insulinaemia and oxidative stress in type 2 diabetic subjects

Background

Elevated level of blood glucose has been shown to be associated with oxidative stress (Ceriello, 2000) which in turn result in insulin resistance in tissues (Kaneto et al., 2006). The results of this thesis (Study 3 & 4) demonstrated that pasta containing RSF decreased blood glucose and oxidative stress compared to control pasta in healthy subjects and hence may enhance insulin sensitivity. However, the

effect of foods containing RSF on glycaemia, insulinaemia and oxidative stress in high risk populations such as type 2 diabetes has not been investigated.

Aims

1. To determine the acute effect of RSF-containing foods on glycaemia, insulinaemia and oxidative stress in type 2 diabetic individuals.

Hypothesis

1. That foods containing RSF would decrease postprandial glycaemia and oxidative stress and would therefore enhance insulin sensitivity in type 2 diabetic individuals.

Key methods

Type 2 diabetic subjects would participate in a double blind randomised cross-over study. Subject will consume RSF-containing food and a control food in a randomised order. Blood samples will be collected at baseline and at 15, 30, 45, 60, 90, 120, 150 and 180 min after consumption of the test foods. Glycaemic response and blood glucose area under the curve will be determined as described in this thesis (Study 3, Chapter 5). Blood insulin will be determined by a Human Metabolic Hormone Multiplex assay kit ((Millipore). Insulin concentration will be used to construct insulin response curve and to calculate insulin area under the curve. Various markers of oxidative stress will be measured using the commercially available assay kits.

Significance

The results of this study would help to design a diet for type 2 diabetic subjects that would prevent frequent and elevated blood glucose excursions and hence would enhance insulin sensitivity.

7.5.3 Effect of RSF-containing foods on body weight and composition, lipid profile and oxidative stress in overweight/obese subjects: a double blind randomised placebo controlled intervention trial

Background

Study 3 and 4 presented in this thesis demonstrated that partial substitution of durum wheat semolina in pasta with RSF can reduce appetite, energy intake and oxidative stress acutely compared to control pasta made from durum semolina only. In addition, several animal studies have suggested that sorghum may reduce cholesterol concentrations (Lee et al., 2014; Hoi et al., 2009; Carr et al., 2005). Regular consumption of foods containing red whole grain sorghum flour may therefore benefit body weight and composition and reduce cardiovascular disease risk factors.

Aims

1. To investigate the effect of RSF-containing foods on body weight and composition, lipid profile and oxidative stress in overweight/obese individuals.

Hypothesis

1. That regular consumption of RSF-containing foods will reduce body weight and improve body composition, lipid profile, blood glucose, insulin sensitivity and oxidative stress markers.

Key methods

The proposed study will be a parallel double blind placebo randomised controlled intervention trial of 16-wk duration in which study participants (overweight/obese) will be randomly assigned into one of the two groups: RSF or control. Participants in both groups will required to replace approximately 15-20% of their usual daily energy intake with either RSF-containing foods or with control foods for intervention period. During the intervention dietary intake will be assessed using a diet history questionnaire (Barnard, Tapsell, Brenniger, Storlien, & Davies, 2002) at base line, mid-point and at the end of intervention. Anthropometric measurements including weight, height and waist circumference will performed at baseline and every two weeks using the procedures described in Study 3. Body composition will be measured at baseline, mid-point and at the end of intervention using dual-energy X-ray absorptiometry. At baseline, mid-point and at the end of intervention, venous blood samples will be taken after a 12-h fast and will be analysed for glucose, insulin, triglycerides, Total cholesterol, HDL-cholesterol and oxidative stress markers.

Significance

The results of this study would help to design foods for overweight/obese subjects that would facilitate weight loss and improve biomarkers of chronic diseases.

REFERENCES

AACC (2000). *Approved methods of the American association of cereal chemists* (10th ed.). St. Paul, MN, USA: American Association of Cereal Chemists.

Abdelgadir, M., Abbas, M., Järvi, A., Elbagir, M., Eltom, M., & Berne, C. (2005). Glycaemic and insulin responses of six traditional Sudanese carbohydrate-rich meals in subjects with Type 2 diabetes mellitus. *Diabetic Medicine*, *22*(2), 213-217.

Abdelghafor, R. F., Mustafa, A. I., Ibrahim, A. M. H., & Krishnan, P. G. (2011). Quality of bread from composite flour of sorghum and hard white winter wheat. *Advance Journal of Food Science and Technology, 3*(1), 9-15.

ABS (Australian Bureau of Statistics) (2013). *4842.0.55.001 - Overweight and Obesity in Adults in Australia: A Snapshot, 2007–08*. Canberra. Retrieved fromhttp://www.abs.gov.au/AUSSTATS/abs@.nsf/0/73E036F555CE4C11 CA25789C0023DAF8?opendocument.

Adisakwattana, S., Chantarasinlapin, P., Thammarat, H., & Yibchok-Anun, S. (2009). A series of cinnamic acid derivatives and their inhibitory activity on intestinal α-glucosidase. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *24*(5), 1194-1200.

Adom, K. K., & Liu, R. H. (2002). Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry, 50*(21), 6182-6187.

AIHW (Australian Institute of Health and Welfare) (2013). *Diabetes*. Canberra. Retrieved from http://www.aihw.gov.au/diabetes.

Akerberg, A., Liljeberg, H., Granfeldt, Y., Drews, A., & Björck, I. (1998). An *in vitro* method based on chewing to predict resistant starch content in foods,

allows parallel determination of potentially available starch and dietary fiber. *Journal of Nutrition, 128*, 651–660.

Aleixandre, A., & Miguel, M. (2008). Dietary fiber in the prevention and treatment of metabolic syndrome: a review. *Critical Reviews in Food Science and Nutrition*, *48*(10), 905-912.

Al-Mamary, M., Molham, A. H., Abdulwali, A. A., & Al-Obeidi, A. (2001). In vivo effects of dietary sorghum tannins on rabbit digestive enzymes and mineral absorption. *Nutrition Research*, *21*(10), 1393-1401.

Anderson, G. H., Cho, C. E., Akhavan, T., Mollard, R. C., Luhovyy, B. L., & Finocchiaro, E. T. (2010). Relation between estimates of cornstarch digestibility by the Englyst in vitro method and glycemic response, subjective appetite, and short-term food intake in young men. *American Journal of Clinical Nutrition*, *91*(4), 932-939.

Andreasen, M. F., Kroon, P. A., Williamson, G., & Garcia-Conesa, M. T. (2001). Intestinal release and uptake of phenolic antioxidant diferulic acids. *Free Radical Biology and Medicine*, *31*(3), 304-314.

AOAC (1997). *Official methods of analysis* (16th ed.). Gaithersburg, MD, USA: Assocaition of Official Analytical Chemists.

AOAC (2008). *Official methods of analysis* (17th ed.). Gaithersburg, MD, USA: Assocaition of Official Analytical Chemists.

Appleton, D. J., Rand, J. S., Priest, J., Sunvold, G. D., & Vickers, J. R. (2004). Dietary carbohydrate source affects glucose concentrations, insulin secretion, and food intake in overweight cats. *Nutrition Research*, *24*(6), 447-467.

Aravind, N., Sissons, M., Egan, N., & Fellows, C. (2012). Effect of insoluble dietary fibre addition on technological, sensory, and structural properties of durum wheat spaghetti. *Food Chemistry, 130*(2), 299-309.

Aravind, N., Sissons, M., Fellows, C. M., Blazek, J., & Gilbert, E. P. (2013). Optimisation of resistant starch II and III levels in durum wheat pasta to reduce in vitro digestibility while maintaining processing and sensory characteristics. *Food Chemistry, 136*(2), 1100-1109.

Arts, I. C., & Hollman, P. C. (2005). Polyphenols and disease risk in epidemiologic studies. *American Journal of Clinical Nutrition, 81*(1), 317S-325S.

Aston, L. M. (2006). Glycaemic index and metabolic disease risk. *Proceedings of the Nutrition Society, 65*, 125–134.

Aston, L. M., Gambell, J. M., Lee, D. M., Bryant, S. P., & Jebb, S. A. (2008). Determination of the glycaemic index of various staple carbohydrate-rich foods in the UK diet. *European Journal of Clinical Nutrition, 62*(2), 279-285.

Astrup, A. (2001). Healthy lifestyles in Europe: prevention of obesity and type II diabetes by diet and physical activity. *Public Health Nutrition, 4*(2b), 499-515.

Augustin, M. A. (2001). Functional foods: an adventure in food formulation. *Food Australia, 53*, 428-432.

Awika, J. M., McDonough, C. M., & Rooney, L. W. (2005). Decorticating sorghum to concentrate healthy phytochemicals. *Journal of Agricultural and Food Chemistry, 53*, 6230-6234.

Awika, J. M., & Rooney, L. W. (2004). Sorghum phytochemicals and their potential impact on human health. *Phytochemistry, 65*(9), 1199-1221.

Awika, J. M., Rooney, L. W., & Waniska R. D. (2004a). Anthocyanins from black sorghum and their antioxidant properties. *Food Chemistry, 90*, 293-301.

Awika, J. M., Rooney, L. W., & Waniska, R. D. (2004b). Properties of 3-deoxyanthocyanins from sorghum. *Journal of Agricultural and Food Chemistry, 52*(14), 4388-4394.

Awika, J. M., Yang, L., Browning, J. D., & Faraj, A. (2009). Comparative antioxidant, antiproliferative and phase II enzyme inducing potential of sorghum (*Sorghum bicolor*) varieties. *LWT - Food Science and Technology, 42*(6), 1041-1046.

Babu, P. V. A., Sabitha, K. E., & Shyamaladevi, C. S. (2006). Therapeutic effect of green tea extract on oxidative stress in aorta and heart of streptozotocin diabetic rats. *Chemico-Biological Interactions*, *162*(2), 114-120.

Bagatini, M. D., Martins, C. C., Battisti, V., Gasparetto, D., da Rosa, C. S., Spanevello, R. M., et al. (2011). Oxidative stress versus antioxidant defences in patients with acute myocardial infarction. *Heart and Vessels*, *26*(1), 55-63.

Baghurst, P. A., Baghurst, K. I., & Record, S. J. (1996). Dietary fibre, non-starch polysaccharides and resistant starch – a review. *Food Australia, 48*, 3–35.

Baiano, A.,Lamacchia, C., Fares, C., Terracone, C., & La Notte, E. (2011). Cooking behaviour and acceptability of composite pasta made of semolina and toasted or partially defatted soy flour. *LWT-Food Science and Technology, 44*, 1226-1232.

Barnard, J., Tapsell, L. C., Brenniger, V., Storlien, L. H., & Davies, P. S. W. (2002). Relationship of high energy expenditure and variation in dietary intake with reporting accuracy on 7 day food records and diet histories in a group of

healthy adult volunteers. *European Journal of Clinical Nutrition*, *56*, 358-367.

Barros, F., Awika J. M., & Rooney, L. W. (2012). Interaction of tannins and other sorghum phenolic compounds with starch and effects on in vitro starch digestibility. *Journal of Agricultural and Food Chemistry, 60*, 11609-11617.

Basu, A., Betts, N. M., Mulugeta, A., Tong, C., Newman, E., & Lyons, T. J. (2013). Green tea supplementation increases glutathione and plasma antioxidant capacity in adults with the metabolic syndrome. *Nutrition Research, 33*, 180-87

BNF (British Nutrition Foundation) (2004). *Nutritional aspects of cereals*. London: BNF.

Bogdanski, P., Suliburska, J., Szulinska, M., Stepien, M., Pupek-Musialik, D., & Jablecka, A. (2012). Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves parameters associated with insulin resistance in obese, hypertensive patients. *Nutrition Research*, *32*(6), 421-427.

Bornet, F. R. J., Fontvieille, A. M., Rizkalla, S., Collona, P., Blayo, A., Mercier, C., & Stama, G. (1989). Insulin and glycemic responses in healthy humans to native starches processed in different ways: Correlation with in vitro α-amylase hydrolysis. *American Journal of Clinical Nutrition, 50*, 315-323.

Bornet, F. R., Jardy-Gennetier, A. E., Jacquet, N., & Stowell, J. (2007). Glycaemic response to foods: impact on satiety and long-term weight regulation. *Appetite*, *49*(3), 535-553.

Boroski, M., de Aguiar, A. C., Boeing, J. S., Rotta, E. M., Wibby, C. L., Bonafé, E. G., et al. (2011). Enhancement of pasta antioxidant activity with oregano and carrot leaf. *Food Chemistry, 125*(2), 696-700.

Bors, W., Michel, C., & Stettmaier, K. (2000). Electron paramagnetic resonance studies of radical species of proanthocyanidins and gallate esters. *Archives of Biochemistry and Biophysics, 374*, 347-355.

Brand-Miller, J. C., Holt, S. H., Pawlak, D. B., & McMillan, J. (2002). Glycemic index and obesity. *American Journal of Clinical Nutrition*, *76*(1), 281S-285S.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, *56*(11), 317-333.

Brighenti, F., Benini, L., Del Rio, D., Casiraghi, C., Pellegrini, N., Scazzina, F., et al. (2006). Colonic fermentation of indigestible carbohydrates contributes to the second-meal effect. *American Journal of Clinical Nutrition*, *83*(4), 817-822.

Briviba, K., Stracke, B. A., Rüfer, C. E., Watzl, B., Weibel, F. P., & Bub, A. (2007). Effect of consumption of organically and conventionally produced apples on antioxidant activity and DNA damage in humans. *Journal of Agricultural and Food Chemistry*, *55*(19), 7716-7721.

Burda, S., & Oleszek, W. (2001). Antioxidant and antiradical activities of flavonoids. *Journal of Agriculture and Food Chemistry, 49*, 2774-2779.

Buyken, A. E., Mitchell, P., Ceriello, A., & Brand-Miller, J. (2010). Optimal dietary approaches for prevention of type 2 diabetes: a life-course perspective. *Diabetologia, 53*, 406–418.

Cao, G., Muccitelli, H. U., Sánchez-Moreno, C., & Prior, R. L. (2001). Anthocyanins are absorbed in glycated forms in elderly women: a pharmacokinetic study. *American Journal of Clinical Nutrition*, *73*(5), 920-926.

Carr, T. P., Weller, C. L., Schlegel, V. L., Cuppett, S. L., Guderian, D. M., & Johnson, K. R. (2005). Grain sorghum lipid extract reduces cholesterol absorption and plasma non-HDL cholesterol concentration in hamsters. *Journal of Nutrition*, *135*(9), 2236-2240.

Carson, L., Setser, C., & Sun, X. S. (2000). Sensory characteristics of sorghum composite bread. *International Journal of Food Science and Technology, 35*, 465-471.

Cavallero, A., Empilli, S., Brighenti, F., & Stanca, A. M. (2002). High (1→3, 1→4)-β-glucan barley fractions in bread making and their effects on human glycemic response. *Journal of Cereal Science, 36*(1), 59–66.

Ceriello, A. (2000). The post-prandial state and cardiovascular disease: relevance to diabetes mellitus. *Diabetes/Metabolism Research and Reviews*, *16* (2), 125-132.

Cermak, R., Landgraf, S., & Wolffram, S. (2004). Quercetin glucosides inhibit glucose uptake into brush-border-membrane vesicles of porcine jejunum. *British Journal of Nutrition*, *91*(06), 849-855.

Chai, Y., Wang, M., & Zhang, G. (2013). Interaction between amylose and tea polyphenols modulates the postprandial glycemic response to high-amylose maize starch. *Journal of Agricultural and Food Chemistry*, *61*(36), 8608-8615.

Chandrashekar, A., & Mazhar, H. (1999). The biochemical basis and implications of grain strength in sorghum and maize. *Journal of Cereal Science*, *30*(3), 193-207.

Chillo, S., Laverse, J., Falcone, P. M., Protopapa, A., & Del Nobile, M. A. (2008). Influence of the addition of buckwheat flour and durum wheat bran on spaghetti quality. *Journal of Cereal Science*, *47*(2), 144-152.

Chillo, S., Ranawana, D. V., & Henry, C. J. K. (2011). Effect of two barley β-glucan concentrates on in vitro glycaemic impact and cooking quality of spaghetti. *LWT - Food Science and Technology, 44*(4), 940-948.

Choo, C. L., & Aziz, N. A. A. (2010). Effects of banana flour and β-glucan on the nutritional and sensory evaluation of noodles. *Food Chemistry, 119*(1), 34-40.

Chung, H. J., Cho, A., & Lim, S. T. (2012). Effect of heat-moisture treatment for utilization of germinated brown rice in wheat noodle. *LWT - Food Science and Technology, 47*(2), 342-347.

Chung, I. M., Kim, E. H., Yeo, M. A., Kim, S. J., Seo, M. C., & Moon, H. I. (2011). Antidiabetic effects of three Korean sorghum phenolic extracts in normal and streptozotocin-induced diabetic rats. *Food Research International*, *44*(1), 127-132.

Circu, M. L., & Aw, T. Y. (2010). Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radical Biology and Medicine*, *48*(6), 749-762.

Clark, R., & Johnson, S. (2002). Sensory acceptability of foods with added lupin (Lupinus angustifolius) kernel fiber using pre-set criteria. *Journal of Food Science, 67*, 356-362.

Cleary, L., & Brennan, C. (2006). The influence of a (1→3)(1→4)-β-D-glucan rich fraction from barley on the physico-chemical properties and in vitro reducing sugars release of durum wheat pasta. *International Journal of Food Science and Technology, 41*(8), 910-918.

Clegg, M. E., & Shafat, A. (2014). The effect of agar jelly on energy expenditure, appetite, gastric emptying and glycaemic response. *European Journal of Nutrition, 53*, 533-539.

Connolly, M. L., Tuohy, K. M., & Lovegrove, J. A. (2012). Wholegrain oat-based cereals have prebiotic potential and low glycaemic index. *British Journal of Nutrition, 108*(12), 2198-2206.

Cook, N. C., & Samman, S. (1996). Flavonoids: chemistry, metabolism, cardio protective effects and dietary sources. *Journal of Nutritional Biochemistry, 7*, 66-76.

Cuche, G., Cuber, J. C., & Malbert, C. H. (2000). Ileal short-chain fatty acids inhibit gastric motility by a humoral pathway. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 279*(5), G925-G930.

DAFF (Department of Agriculture, Fisheries and Forestry) (2012). *Overview of the sorghum industry*. Retrieved from http://www.daff.qld.gov.au/plants/field-crops-and-pastures/broadacre-field-crops/sorghum/overview.

Dalle-Donne, I., Rossi, R., Giustarini, D., Milzani, A., & Colombo, R. (2003). Protein carbonyl groups as biomarkers of oxidative stress. *Clinica Chimica Acta, 329*(1), 23-38.

Davi, G., & Falco, A. (2005). Oxidant stress, inflammation and atherogenesis. *Lupus, 14*(9), 760-764.

Davison, G., Callister, R., Williamson, G., Cooper, K. A., & Gleeson, M. (2012). The effect of acute pre-exercise dark chocolate consumption on plasma antioxidant status, oxidative stress and immunoendocrine responses to prolonged exercise. *European Journal of Nutrition*, *51*(1), 69-79.

Del Prato, S., Leonetti, F., Simonson, D. C., Sheehan, P., Matsuda, M., & DeFronzo, R. A. (1994). Effect of sustained physiologic hyperinsulinaemia and hyperglycaemia on insulin secretion and insulin sensitivity in man. *Diabetologia*, *37*(10), 1025-1035.

Dick, J. W., & Youngs, V. L. (1998). Evaluation of durum wheat semolina and pasta in the United States. In G. Fabriani & C. Lintas (Eds.), *Durum wheat, chemistry and technology* (pp. 238–248). St. Paul, MN: American Association of Cereal Chemists.

Dicko, M. H., Gruppen, H., Traore, A. S., Voragen, A. G. J., & van Berkel, W. J. H. (2006). Sorghum grain as human food in Africa: relevance of content of starch and amylase activities. *African Journal of Biotechnology, 5*(5), 384-395.

Dlamini, N. R., Taylor, J. R. N., & Rooney, L. W. (2007). The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. *Food Chemistry, 105*(4), 1412-1419.

Duodu, K. G., Taylor, J. R. N., Belton, P. S., & Hamaker, B. R. (2003). Factors affecting sorghum protein digestibility. *Journal of Cereal Science, 38*(2), 117-131.

Dykes, L., & Rooney, L. W. (2006). Sorghum and millet phenols and antioxidants. *Journal of Cereal Science, 44*(3), 236-251.

Dykes, L., Rooney, W. L., & Rooney, L. W. (2013). Evaluation of phenolics and antioxidant activity of black sorghum hybrids. *Journal of Cereal Science*, *58*(2), 278-283.

Dykes, L., Rooney, L. W., Waniska, R. D., & Rooney, W. L. (2005). Phenolic compounds and antioxidant activity of sorghum grains of varying genotypes. *Journal of Agricultural and Food Chemistry, 53*(17), 6813-6818.

Dykes, L., Seitz, L. M., Rooney, W. L., & Rooney, L. W. (2009). Flavonoid composition of red sorghum genotypes. *Food Chemistry, 116*(1), 313-317.

Eelderink, C., Schepers, M., Preston, T., Vonk, R. J., Oudhuis, L., & Priebe, M. G. (2012). Slowly and rapidly digestible starchy foods can elicit a similar glycemic response because of differential tissue glucose uptake in healthy men. *American Journal of Clinical Nutrition*, *96*(5), 1017-1024.

Ello-Martin, J. A., Ledikwe, J. H., & Rolls, B. J. (2005). The influence of food portion size and energy density on energy intake: implications for weight management. *American Journal of Clinical Nutrition*, *82*(1), 236S-241S.

Ells, L. J., Seal, C. J., Kettlitz, B., Bal, W., & Mathers, J. C. (2005). Postprandial glycaemic, lipaemic and haemostatic responses to ingestion of rapidly and slowly digested starches in healthy young women. *British Journal of Nutrition*, *94*(06), 948-955.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, *46*, 33-50

Epstein, J., Morris, C.F., & Huber, K.C. (2002). Instrumental texture of white salted noodles prepared from recombinant inbred lines of wheat differing in

the three granule bound starch synthase (Waxy) genes. *Journal of Cereal Science, 35*, 51-63.

Ezeogu, L. I., Duodu, K. G., & Taylor, J. R. N. (2005). Effects of endosperm texture and cooking conditions on the in vitro starch digestibility of sorghum and maize flours. *Journal of Cereal Science, 42*(1), 33-44.

Ezeogu, L. I., Duodu, K. G., Emmambux, M. N., & Taylor, J. R. (2008). Influence of cooking conditions on the protein matrix of sorghum and maize endosperm flours. *Cereal Chemistry, 85*(3), 397-402.

FAO (1995). *Sorghum and millet in human nutrition*. Rome: FAO.

Fares, C., & Menga, V. (2012). Effects of toasting on the carbohydrate profile and antioxidant properties of chickpea (*Cicer arietinum* L.) flour added to durum wheat pasta. *Food Chemistry, 131*(4), 1140-1148.

Fares, C., Codianni, P., Nogro, F., Platani, C., Scazzina, F., & Pellegrini, N. (2008). Processing and cooking effects on chemical, nutritional and functional properties of pasta obtained from selected emmer genotypes. *Journal of the Science of Food and Agriculture, 88*, 2435-2444.

Fares, C., Platani, C., Baiano, A., & Menga, V. (2010). Effect of processing and cooking on phenolic acid profile and antioxidant capacity of durum wheat pasta enriched with debranning fractions of wheat. *Food Chemistry, 119*(3), 1023-1029.

Ferguson, L. R., Tasman-Jones, C., Englyst, H., & Harris, P. J. (2000). Comparative effects of three resistant starch preparations on transit time and short-chain fatty acid production in rats. *Nutrition and Cancer, 36*(2), 230-237.

Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations

in single test meal studies. *International Journal of Obesity and Related Metabolic Disorders, 24*(1), 38-48.

Foster-Powell, K., Holt, S. H., & Brand-Miller, J. C. (2002). International table of glycemic index and glycemic load values: 2002. *American Journal of Clinical Nutrition, 76*(1), 5-56.

Fuhrman, B., Volkova, N., Coleman, R., & Aviram, M. (2005). Grape powder polyphenols attenuate atherosclerosis development in apolipoprotein E deficient (E0) mice and reduce macrophage atherogenicity. *Journal of Nutrition, 135*(4), 722-728.

Fung, T. T., Hu, F. B., Pereira, M. A., Liu, S., Stampfer, M. J., Colditz, G. A., & Willett, W. C. (2002). Whole-grain intake and the risk of type 2 diabetes: a prospective study in men. *American Journal of Clinical Nutrition, 76*(3), 535-540.

Gallegos-Infante, J. A., Rocha-Guzman, N. E., Gonzalez-Laredo, R. F., Ochoa-Martínez, L. A., Corzo, N., Bello-Perez, L. A., et al. (2010). Quality of spaghetti pasta containing Mexican common bean flour (*Phaseolus vulgaris* L.). *Food Chemistry, 119*(4), 1544-1549.

Garcia-Alonso, J., Ros, G., Vidal-Guevara, M. L., & Periago, M. J. (2006). Acute intake of phenolic-rich juice improves antioxidant status in healthy subjects. *Nutrition Research, 26*(7), 330-339.

Gelencser, T., Gál, V., Hodsagi, M., & Salgó, A. (2008). Evaluation of quality and digestibility characteristics of resistant starch-enriched pasta. *Food and Bioprocess Technology, 1*, 171-179.

Gibson, T. S., Solah, V. A., & McCleary, B. V. (1997). A procedure to measure amylose in cereal starches and flours with concanavalin A. *Journal of Cereal Science, 25*(2), 111-119.

Godycki-Cwirko, M., Krol, M., Krol, B., Zwolinska, A., Kolodziejczyk, K., Kasielski, M., et al. (2010). Uric acid but not apple polyphenols is responsible for the rise of plasma antioxidant activity after apple juice consumption in healthy subjects. *Journal of the American College of Nutrition*, *29*(4), 397-406.

Haddad, E. H., Gaban-Chong, N., Oda, K., & Sabaté, J. (2014). Effect of a walnut meal on postprandial oxidative stress and antioxidants in healthy individuals. *Nutrition Journal*, *13*(1), 1-9.

Halliwell, B. (2008). Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? *Archives of Biochemistry and Biophysics, 476*(2), 107-112.

Hanhineva, K., Törrönen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkänen, H., & Poutanen, K. (2010). Impact of dietary polyphenols on carbohydrate metabolism. *International Journal of Molecular Sciences*, *11*(4), 1365-1402.

Harbis, A., Perdreau, S., Vincent-Baudry, S., Charbonnier, M., Bernard, M. C., Raccah, D., et al. (2004). Glycemic and insulinemic meal responses modulate postprandial hepatic and intestinal lipoprotein accumulation in obese, insulin-resistant subjects. *American Journal of Clinical Nutrition*, *80*(4), 896-902.

Hargrove, J. L., Greenspan, P., Hartle, D. K., & Dowd, C. (2011). Inhibition of aromatase and α-amylase by flavonoids and proanthocyanidins from

Sorghum bicolor bran extracts. *Journal of Medicinal Food*, *14*(7-8), 799-807.

He, F. J., Nowson, C. A., Lucas, M., & MacGregor, G. A. (2007). Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. *Journal of Human Hypertension, 21*(9), 717-728.

Henningsson, A. M., Margareta, E., Nyman, G. L., & Bjorck, I. M. E. (2003). Influences of dietary adaptation and source of resistant starch on short-chain fatty acids in the hindgut of rats. *British Journal of Nutrition, 89*(3), 319-327.

Henriksen, E. J., Diamond-Stanic, M. K., & Marchionne, E. M. (2011). Oxidative stress and the aetiology of insulin resistance and type 2 diabetes. *Free Radical Biology and Medicine*, *51*(5), 993-999.

Hermsdorff, H. H., Barbosa, K. B., Volp, A. C., Puchau, B., Bressan, J., Zulet, M. Á., & Martínez, J. A. (2012). Vitamin C and fibre consumption from fruits and vegetables improves oxidative stress markers in healthy young adults. *British Journal of Nutrition*, *107*(8), 1119-1127.

Hirawan, R., Ser, W. Y., Arntfield, S. D., & Beta, T. (2010). Antioxidant properties of commercial, regular- and whole-wheat spaghetti. *Food Chemistry, 119*(1), 258-264.

Hoi, J. T., Weller, C. L., Schlegel, V. L., Cuppett, S. L., Lee, J. Y., & Carr, T. P. (2009). Sorghum distillers dried grain lipid extract increases cholesterol excretion and decreases plasma and liver cholesterol concentration in hamsters. *Journal of Functional Foods*, *1*(4), 381-386.

Hollman, P. C., Cassidy, A., Comte, B., Heinonen, M., Richelle, M., Richling, E., et al. (2011). The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established. *Journal of Nutrition*, *141*(5), 989S-1009S.

Hudthagosol, C., Haddad, E. H., McCarthy, K., Wang, P., Oda, K., & Sabaté, J. (2011). Pecans acutely increase plasma postprandial antioxidant capacity and catechins and decrease LDL oxidation in humans. *Journal of Nutrition*, *141*(1), 56-62.

IDF (International Diabetes Federation) (2013). *IDF diabetes atlas* (6th Ed.). Retrieved from http://www.idf.org/diabetesatlas.

Johansson, E. V., Nilsson, A. C., Ostman, E. M., & Bjorck, I. M. (2013). Effects of indigestible carbohydrates in barley on glucose metabolism, appetite and voluntary food intake over 16 h in healthy adults. *Nutrition Journal*, *12*(1), 46.

Kamath, V. G., Chandrashekar, A., & Rajini, P. S. (2004). Antiradical properties of sorghum (*Sorghum bicolor* L. Moench) flour extracts. *Journal of Cereal Science, 40*(3), 283-288.

Kaneto, H., Nakatani, Y., Kawamori, D., Miyatsuka, T., Matsuoka, T. A., Matsuhisa, M., & Yamasaki, Y. (2006). Role of oxidative stress, endoplasmic reticulum stress, and c-Jun N-terminal kinase in pancreatic β-cell dysfunction and insulin resistance. *International Journal of Biochemistry & Cell Biology*, *38*(5), 782-793.

Kasarda, D. D. (2001). Grains in relation to celiac disease. *Cereal Foods World*, *46*, 209-210.

Kendall, C. W. C., Josse, A. R., Esfahani, A., & Jenkins, D. J. A. (2011). The impact of pistachio intake alone or in combination with high-carbohydrate foods on post-prandial glycemia. *European Journal of Clinical Nutrition*, *65*(6), 696-702.

Khan, I., Yousif., A., Johnson., S. K., & Gamlath, S. (2013). Effect of sorghum flour addition on resistant starch content, phenolic profile and antioxidant capacity of durum wheat pasta. *Food Research International, 54*(1), 578-586.

Khan, I., Yousif., A., Johnson., S. K., & Gamlath, S. (2014). Effect of sorghum flour addition on *in vitro* starch digestibility, cooking quality and consumer acceptability of durum wheat pasta. *Journal of Food Science* (in press).

Kim, J. S., Hyun, T. K., & Kim, M. J. (2011). The inhibitory effects of ethanol extracts from sorghum, foxtail millet and proso millet on α-glucosidase and α-amylase activities. *Food Chemistry*, *124*(4), 1647-1651.

Kim, J. Y., Park, J. Y., Kang, H. J., Kim, O. Y., & Lee, J. H. (2012). Beneficial effects of Korean red ginseng on lymphocyte DNA damage, antioxidant enzyme activity, and LDL oxidation in healthy participants: a randomized, double-blind, placebo-controlled trial. *Nutrition Journal*, *11*(1), 47.

Kim, J., & Park, Y. (2012). Anti-diabetic effect of sorghum extract on hepatic gluconeogenesis of streptozotocin-induced diabetic rats. *Nutrition & Metabolism*, *9*(1), 106.

Kim, K., Tsao, R., Yang, R., & Cui, S. (2006). Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chemistry, 95*(3), 466-473.

Klosterbuer, A. S., Thomas, W., & Slavin, J. L. (2012). Resistant starch and pullulan reduce postprandial glucose, insulin, and GLP-1, but have no effect on satiety in healthy humans. *Journal of Agricultural and Food Chemistry*, *60*(48), 11928-11934.

Kobue-Lekalake, R. I., Taylor, J., & De Kock, H. L. (2007). Effects of phenolics in sorghum grain on its bitterness, astringency and other sensory properties. *Journal of the Science of Food and Agriculture*, *87*(10), 1940-1948.

Koehler, P., & Wieser, H. (2013). Chemistry of Cereal Grains. In *Handbook on Sourdough Biotechnology* (pp. 11-45). Springer US.

Kristensen, M., Jensen, M. G., Riboldi, G., Petronio, M., Bügel, S., Toubro, S., Tetens, I., & Astrup, A. (2010). Wholegrain vs. refined wheat bread and pasta. Effect on postprandial glycemia, appetite, and subsequent *ad libitum* energy intake in young healthy adults. *Appetite*, *54*(1), 163-169.

Krueger, C. G., Vestling, M. M., & Reed, J. D. (2003). Matrix- assisted laser desorption/ionization time- of- flight mass spectrometry of heteropolyfl avan- 3- ols and glucosylated heteropolyfl avans in sorghum [Sorghum bicolor (L.) Moench]. *Journal of Agricultural and Food Chemistry, 51*, 538– 543.

Lakshmi, K. B., & Vimala, V. (1996). Hypoglycemic effect of selected sorghum recipes. *Nutrition Research*, *16*(10), 1651-1658.

Lamb, R. E., & Goldstein, B. J. (2008). Modulating an oxidative-inflammatory cascade: potential new treatment strategy for improving glucose metabolism, insulin resistance, and vascular function. *International Journal of Clinical Practice*, *62*(7), 1087-1095.

Lee, B. H., Carr, T. P., Weller, C. L., Cuppett, S., Dweikat, I. M., & Schlegel, V. (2014). Grain sorghum whole kernel oil lowers plasma and liver cholesterol in male hamsters with minimal wax involvement. *Journal of Functional Foods*, *7*, 709-718.

Li, H., Cheng, K., Wong, C., Fan, K., Chen, F., & Jiang, Y. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry, 102*(3), 771-776.

Li, N., Jia, X., Chen, C. Y. O., Blumberg, J. B., Song, Y., Zhang, W., et al. (2007). Almond consumption reduces oxidative DNA damage and lipid peroxidation in male smokers. *Journal of Nutrition*, *137*(12), 2717-2722.

Licata, R., Chu, J., Wang, S., Coorey, R., James, A., Zhao, Y., & Johnson, S. (2013). Determination of formulation and processing factors affecting slowly digestible starch, protein digestibility and antioxidant capacity of extruded sorghum–maize composite flour. *International Journal of Food Science & Technology*, *49*(9), 1408-1419.

Liu, J. F., Liu, Y. H., Chen, C. M., Chang, W. H., & Chen, C. O. (2013). The effect of almonds on inflammation and oxidative stress in Chinese patients with type 2 diabetes mellitus: a randomized crossover controlled feeding trial. *European Journal of Nutrition*, 52, 927-35.

Liu, L., Herald, T. J., Wang, D., Wilson, J. D., Bean, S. R., & Aramouni, F. M. (2012). Characterization of sorghum grain and evaluation of sorghum flour in a Chinese egg noodle system. *Journal of Cereal Science, 55*(1), 31-36.

Liu, S., Willett, W. C., Stampfer, M. J., Hu, F. B., Franz, M., Sampson, L., et al. (2000). A prospective study of dietary glycemic load, carbohydrate intake,

and risk of coronary heart disease in US women. *American Journal of Clinical Nutrition*, *71*(6), 1455-1461.

Liyana-Pathirana, C. M., & Shahidi, F. (2007). Antioxidant and free radical scavenging activities of whole wheat and milling fractions. *Food Chemistry, 101*(3), 1151-1157.

Lutsey, P. L., Jacobs, D. R., Kori, S., Mayer-Davis, E., Shea, S., Steffen, L. M., Szklo, M., & Tracy, R. (2007). Whole grain intake and its cross-sectional association with obesity, insulin resistance, inflammation, diabetes and subclinical CVD: The MESA Study. *British Journal of Nutrition*, *98*(2), 397-405.

Mahasukhonthachat, K., Sopade, P. A., & Gidley, M. J. (2010). Kinetics of starch digestion in sorghum as affected by particle size. *Journal of Food Engineering, 96*(1), 18-28.

Manach, C., Scalbert, A., Morand, C., Remesy, C., & Jimenez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition, 79*, 727-747.

Manach, C., Williamson, G., Morand, C., Scalbert, A., & Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition*, *81*(1), 230S-242S.

Mann, J. I. (2002). Diet and risk of coronary heart disease and type 2 diabetes. *Lancet*, *360*(9335), 783-789.

Marconi, E., & Carcea, M. (2001). Pasta from non-traditional materials. *Cereal Foods World*, *46*, 522-530.

Maritim, A. C., Sanders, R. A., & Watkins, J. B. (2003). Diabetes, oxidative stress, and antioxidants: a review. *Journal of Biochemical and Molecular Toxicology*, *17*(1), 24-38.

Matteucci, E., & Giampietro, O. (2000). Oxidative stress in families of type 1 diabetic patients. *Diabetes Care*, *23*(8), 1182-1186.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007). In M. C. Meilgaard, G. V. Civille, & B. T. Carr (Eds.), *Sensory evaluation techniques* (4th ed.). (pp. 255–311). Boca Raton, FL: CRC Press.

Miao, M., Jiang, B., Cui, S. W., Zhang, T., & Jin, Z. (2013). Slowly Digestible Starch–A Review. *Critical Reviews in Food Science and Nutrition*, (in press).

Mkandawire, N. L., Kaufman, R. C., Bean, S. R., Weller, C. L., Jackson, D. S., & Rose, D. J. (2013). Effects of sorghum (*Sorghum bicolor* (L.) Moench) tannins on α-amylase activity and in vitro digestibility of starch in raw and processed flours. *Journal of Agricultural and Food Chemistry, 61*, 4448-4454.

Modun, D., Music, I., Vukovic, J., Brizic, I., Katalinic, V., Obad, A., et al. (2008). The increase in human plasma antioxidant capacity after red wine consumption is due to both plasma urate and wine polyphenols. *Atherosclerosis*, *197*(1), 250-256.

Moraes, É. A., Natal, D. I. G., Queiroz, V. A. V., Schaffert, R. E., Cecon, P. R., de Paula, S. O., et al. (2012). Sorghum genotype may reduce low-grade inflammatory response and oxidative stress and maintains jejunum morphology of rats fed a hyperlipidic diet. *Food Research International. 49*, 553-59

Morillas-Ruiz, J. M., Villegas Garcia, J. A., Lopez, F. J., Vidal-Guevara, M. L., & Zafrilla, P. (2006). Effects of polyphenolic antioxidants on exercise-induced oxidative stress. *Clinical Nutrition*, *25*(3), 444-453.

Moskaug, J. O., Carlsen, H., Myhrstad, M. C., & Blomhoff, R. (2005). Polyphenols and glutathione synthesis regulation. *American Journal of Clinical Nutrition*, *81*(1), 277S-283S.

Mulleder, U., Murkovic, M., & Pfannhauser, W. (2002). Urinary excretion of cyanidin glycosides. *Journal of Biochemical and Biophysical Methods*, *53*(1), 61-66.

Muriu, J. I., Njoka-Njiru, E. N., Tuitoek, J. K., & Nanua, J. N. (2002). Evaluation of sorghum (Sorghum bicolor) as replacement for maize in the diet of growing rabbits (Oryctolagus cuniculus). *Asian- Australian Journal of Animal Science, 15*, 565–569.

Nathan, D. M. (1993). Long-term complications of diabetes mellitus. *New England Journal of Medicine*, *328*(23), 1676-1685.

N'Dri, D., Mozzeo, T., Zaupa, M., Ferracane, R.,Fogliano, V., & Pellegrini, N. (2013). Effetc of cooking on the total antioxidant capacity and phenolic profile of some whole-meal African cereals. *Journal of the Science of Food and Agriculture, 93*, 29-36.

Naczk, M., Towsend, M., Zadernowski, R., & Shahidi, F. (2011). Protein-binding and antioxidant potential of phenolics of mangosteen fruit (*Garcinia mangostana*). *Food Chemistry, 128*(2), 292-298.

Nemzer, B. V., Rodriguez, L. C., Hammond, L., DiSilvestro, R., Hunter, J. M., & Pietrzkowski, Z. (2012). Acute reduction of serum 8-iso-PGF2-alpha and advanced oxidation protein products in vivo by a polyphenol-rich beverage;

a pilot clinical study with phytochemical and in vitro antioxidant characterization. *Nutrition Journal*, *10*(1), 67-75.

Neuhouser, M. L. (2004). Review: dietary flavonoids and cancer risk: evidence from human population studies. *Nutrition and Cancer*, *50*(1), 1-7.

Nichols, B. L., Avery, S., Sen, P., Swallow, D. M., Hahn, D., & Sterchi, E. (2003). The maltase-glucoamylase gene: common ancestry to sucrase-isomaltase with complementary starch digestion activities. *Proceedings of the National Academy of Science, 100*, 1432-1437.

Nilsson, A., Johansson, E., Ekström, L., & Björck, I. (2013). Effects of a brown beans evening meal on metabolic risk markers and appetite regulating hormones at a subsequent standardized breakfast: a randomized cross-over study. *Plos One*, *8*(4), e59985.

Nishida, C., Uauy, R., Kumanyika, S., & Shetty, P. (2004). The joint WHO/FAO expert consultation on diet, nutrition and the prevention of chronic diseases: process, product and policy implications. *Public Health Nutrition, 7*, 245-250.

Ovando-Martinez, M., Sáyago-Ayerdi, S., Agama-Acevedo, E., Goñi, I., & Bello-Pérez, L. A. (2009). Unripe banana flour as an ingredient to increase the undigestible carbohydrates of pasta. *Food Chemistry, 113*(1), 121-126.

Pale, E., Kouda-Bonafos, M., Nacro, M., Vanhaelen-Fastre, R., & Ottinger, R. (1997). 7-O-methylapigeninidin, an anthocyanidin from Sorghum caudatum. *Phytochemistry, 45*, 1091-1093.

Panickar, K. S. (2013). Effects of dietary polyphenols on neuroregulatory factors and pathways that mediate food intake and energy regulation in obesity. *Molecular Nutrition & Food Research*, *57*(1), 34-47.

Panza, V. S. P., Wazlawik, E., Ricardo Schütz, G., Comin, L., Hecht, K. C., & da Silva, E. L. (2008). Consumption of green tea favorably affects oxidative stress markers in weight-trained men. *Nutrition*, *24*(5), 433-442.

Park, J. H., Lee, S. H., Chung, I. M., & Park, Y. (2012). Sorghum extract exerts an anti-diabetic effect by improving insulin sensitivity via PPAR-γ in mice fed a high-fat diet. *Nutrition Research and Practice*, *6*(4), 322-327.

Pérez-Jiménez, J., Serrano, J., Tabernero, M., Arranz, S., Díaz-Rubio, M. E., García-Diz, L., et al. (2008). Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. *Nutrition, 24*(7-8), 646-653.

Perez-Matute, P., Zulet, M., & Martínez, J. A. (2009). Reactive species and diabetes: counteracting oxidative stress to improve health. *Current Opinion in Pharmacology*, *9*(6), 771-779.

Petitot, M., Boyer, L., Minier, C., & Micard, V. (2010). Fortification of pasta with split pea and faba bean flours: Pasta processing and quality evaluation. *Food Research International, 43*(2), 634-641.

Piche, T., des Varannes, S. B., Sacher-Huvelin, S., Holst, J. J., Cuber, J. C., & Galmiche, J. P. (2003). Colonic fermentation influences lower esophageal sphincter function in gastroesophageal reflux disease. *Gastroenterology*, *124*(4), 894-902.

Pietta, P. G. J. (2000). Flavonoids as antioxidants. *Journal of Natural Products, 63*, 1035-1042.

Pontieri, P., Mamone, G., De Caro, S., Tuinstra, M. R., Roemer, E., Okot, J., et al. (2013). Sorghum, a healthy and gluten-free food for celiac patients as demonstrated by genome, biochemical, and immunochemical analyses. *Journal of Agricultural and Food Chemistry*, *61*(10), 2565-2571.

Prabhasankar, P., Ganesan, P., Bhaskar, N., Hirose, A., Stephen, N., Gowda, L. R., et al. (2009). Edible Japanese seaweed, wakame (*Undaria pinnatifida*) as an ingredient in pasta: Chemical, functional and structural evaluation. *Food Chemistry, 115*(2), 501-508.

Price, R. K., Wallace, J. M., Hamill, L. L., Keaveney, E. M., Strain, J. J., Parker, M. J., & Welch, R. W. (2012). Evaluation of the effect of wheat aleurone-rich foods on markers of antioxidant status, inflammation and endothelial function in apparently healthy men and women. *British Journal of Nutrition*, *108*(9), 1644.

Prigent, S. V. E., Voragen, A. G. J., van Koningsveld, G. A., Baron, A., Renard, C. M. G. C., & Gruppen, H. (2009). Interactions between globular proteins and procyanidins of different degrees of polymerization. *Journal of Dairy Science, 92*(12), 5843-5853.

Raben, A., Tagliabue, A., Christensen, N. J., Madsen, J., Holst, J. J., & Astrup, A. (1994). Resistant starch - the effect on postprandial glycemia, hormonal response, and satiety. *American Journal of Clinical Nutrition, 60*(4), 544-551.

Ragaee, S., Abdel-Aal, E. M., & Noaman, M. (2006). Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry, 98*(1), 32-38.

Ragaee, S., Seetharaman, K., & Abdel-Aal, E. S. M. (2012). Impact of milling and thermal processing on phenolic compounds in cereal grains. *Critical Reviews in Food Science and Nutrition, 54*, 837-849.

Rahman, S., Bird, A., Regina, A., Li, Z., Ral, J. P., McMaugh, S., Topping, D., & Morell, M. (2007). Resistant starch in cereals: exploiting genetic engineering and genetic variation. *Journal of Cereal Science, 46*, 251-260.

Rayas-Duarte, P., Mock, C. M., & Satterlee, L. D. (1996). Quality of spaghetti containing buckwheat, amaranth, and lupin flours. *Cereal Chemistry, 73*, 381-387.

Reader, D., Johnson, M. L., Hollander, P., & Franz, M. (1997). The glycemic and insulinemic response of resistant starch in a food bar vs. two commercially available food bars in persons with type II diabetes mellitus. *Diabetes, 46*, 975-975.

Rice-Evans, C. J., Miller, J. N., & Paganga, G. (1996). Structure antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*, 933-956.

Rooney, L. W., & Awika, J. M. (2005). Overview of products and health benefits of specialty sorghums. *Cereal Foods World*, *50*, 109-115.

Rooney, L. W., & Pflugfelder, R. L. (1986). Factor affecting starch digestibility with special emphasis on sorghum and corn. *Journal of Animal Science*, *63*, 1607-1623.

Rooney, L. W., & Serna-Saldivar, S. O. (2000). Sorghum. In K. Kulp & J.G. Ponte (Eds.). *Handbook of cereal science and technology* (pp. 149-175). New York, NY: Marcel Dekker.

Rosen, L. A., Östman, E. M., & Björck, I. M. (2011a). Effects of cereal breakfasts on postprandial glucose, appetite regulation and voluntary energy intake at a subsequent standardized lunch; focusing on rye products. *Nutrition Journal*, *10*(7).

Rosen, L. A., Ostman, E. M., Shewry, P. R., Ward, J. L., Andersson, A. A., Piironen, V., et al. (2011b). Postprandial glycemia, insulinemia, and satiety responses in healthy subjects after whole grain rye bread made from different rye varieties. 1. *Journal of Agricultural and Food Chemistry*, *59*(22), 12139-12148.

Ruskovska, T., & Bernlohr, D. A. (2013). Oxidative stress and protein carbonylation in adipose tissue—Implications for insulin resistance and diabetes mellitus. *Journal of Proteomics*, *92*, 323-334.

Russell, W. R., Baka, A., Björck, I., Delzenne, N., Gao, D., Griffiths, H. R., et al. (2013). Impact of diet composition on blood glucose regulation. *Critical Reviews in Food Science and Nutrition* (in press).

Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R. (2006). Resistant starch - A review. *Comprehensive Reviews in Food Science and Food Safety, 5*(1), 1-17.

Samra, R. A., & Anderson, G. H. (2007). Insoluble cereal fiber reduces appetite and short-term food intake and glycemic response to food consumed 75 min later by healthy men. *American Journal of Clinical Nutrition*, *86*(4), 972-979.

Sands, A. L., Leidy, H. J., Hamaker, B. R., Maguire, P., & Campbell, W. W. (2009). Consumption of the slow-digesting waxy maize starch leads to blunted plasma glucose and insulin response but does not influence energy expenditure or appetite in humans. *Nutrition Research*, *29*(6), 383-390.

Sang, S., Lambert, J. D., Ho, C. T., & Yang, C. S. (2011). The chemistry and biotransformation of tea constituents. *Pharmacological Research*, *64*(2), 87-99.

Santhakumar, A. B., Bulmer, A. C., & Singh, I. (2014). A review of the mechanisms and effectiveness of dietary polyphenols in reducing oxidative stress and thrombotic risk. *Journal of Human Nutrition and Dietetics*, *27*(1), 1-21.

Saraswathi, R., & Devaraj, S. N. (2013). Oxidative stress in skeletal muscle impairs mitochondrial function in alloxan induced diabetic rats: Role of alpha lipoic acid. *Biomedicine & Preventive Nutrition, 3*, 213-19.

Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition*, *130*(8), 2073S-2085S.

Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*, *45*(4), 287-306.

Schofield, P., Mbugua, D. M., & Pell, A. N. (2001). Analysis of condensed tannins: A review. *Animal Feed Science and Technology, 91*, 21-40.

Seal, C. J., Daly, M. E., Thomas, L. C., Bal, W., Birkett, A. M., Jeffcoat, R., & Mathers, J. C. (2003). Postprandial carbohydrate metabolism in healthy subjects and those with type 2 diabetes fed starches with slow and rapid hydrolysis rates determined in vitro. *British Journal of Nutrition*, *90*(05), 853-864.

Sener, D. E., Gonenc, A., Akinci, M., & Torun, M. (2007). Lipid peroxidation and total antioxidant status in patients with breast cancer. *Cell Biochemistry and Function*, *25*(4), 377-382.

Serafini, M., Maiani, G., & Ferro-Luzzi, A. (1998). Alcohol-free red wine enhances plasma antioxidant capacity in humans. *Journal of Nutrition*, *128*(6), 1003-1007.

Sloth, B., & Astrup, A. (2006). Low glycemic index diets and body weight. *International Journal of Obesity*, *30*, S47-S51.

Sopade, P. A., & Gidley, M. J. (2009). A rapid in-vitro digestibility assay based on glucometry for investigating kinetics of starch digestion. *Starch/Stärke, 61*, 245–255.

Taubert, D., Roesen, R., Lehmann, C., Jung, N., & Schömig, E. (2007). Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide. *JAMA: Journal of the American Medical Association*, *298*(1), 49-60.

Taylor, J. R. N., & Emmambux, M. N. (2010). Developments in our understanding of sorghum polysaccharides and their health benefits. *Cereal Chemistry, 87*(4), 263-271.

Taylor, J. R. N., Schober, T. J., & Bean, S. R. (2006). Novel food and non-food uses for sorghum and millets. *Journal of Cereal Science, 44*(3), 252-271.

Taylor, J., Belton, P. S., Beta, T., & Duodu, K. G. (2013). Increasing the utilisation of sorghum, millets and pseudocereals: Developments in the science of their phenolic phytochemicals, biofortification and protein functionality. *Journal of Cereal Science*, *59*(3), 257-275.

Temple, N. J. (2000). Antioxidants and disease: More questions than answers. *Nutrition Research, 20*(3), 449-459.

Thondre, P. S., Shafat, A., & Clegg, M. E. (2013). Molecular weight of barley β-glucan influences energy expenditure, gastric emptying and glycaemic response in human subjects. *British Journal of Nutrition*, *100*, 2173-2179.

Topping, D. L., & Clifton, P. M. (2001). Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews*, *81*(3), 1031-1064.

Torabian, S., Haddad, E., Rajaram, S., Banta, J., & Sabate, J. (2009). Acute effect of nut consumption on plasma total polyphenols, antioxidant capacity and lipid peroxidation. *Journal of Human Nutrition and Dietetics*, *22*(1), 64-71.

Tudorica, C. M., Kuri, V., & Brennan, C. S. (2002). Nutritional and physicochemical characteristics of dietary fiber enriched pasta. *Journal of Agricultural and Food Chemistry, 50*(2), 347-356.

van Can, J. G., van Loon, L. J., Brouns, F., & Blaak, E. E. (2012). Reduced glycaemic and insulinaemic responses following trehalose and isomaltulose ingestion: implications for postprandial substrate use in impaired glucose-tolerant subjects. *British Journal of Nutrition*, *108*(07), 1210-1217.

Van de Vijver, L. P. L., Van den Bosch, L. M. C., Van den Brandt, P. A., & Goldbohm, R. A. (2007). Whole-grain consumption, dietary fibre intake and body mass index in the Netherlands cohort study. *European Journal of Clinical Nutrition*, *63*(1), 31-38.

Van den Berg, R., Haenen, G. R. M. M., van den Berg, H., & Bast, A. (1999). Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chemistry, 66*, 511–517.

Verardo, V., Arraez-Roman, D., Segura-Carretero, A., Marconi, E., Fernandez-Gutierrez, A., & Caboni, M. F. (2011). Determination of free and bound phenolic compounds in buckwheat spaghetti by RP-HPLC-ESI-TOF-MS: Effect of thermal processing from farm to fork. *Journal of Agricultural and Food Chemistry, 59*(14), 7700-7707.

Verardo, V., Gomez-Caravaca, A. M., Messia, M. C., Marconi, E., & Caboni, M. F. (2011). Development of functional spaghetti enriched in bioactive

compounds using barley coarse fraction obtained by air classification. *Journal of Agricultural and Food Chemistry, 59*(17), 9127-9134.

Vernaza, M. G., Biasutti, E., Schmiele, M., Jaekel, L. Z., Bannwart, A., & Chang, Y. K. (2012). Effect of supplementation of wheat flour with resistant starch and monoglycerides in pasta dried at high temperatures. *International Journal of Food Science & Technology, 47*(6), 1302-1312.

Vieira, F. G., Di Pietro, P. F., da Silva, E. L., Borges, G. S., Nunes, E. C., & Fett, R. (2012). Improvement of serum antioxidant status in humans after the acute intake of apple juices. *Nutrition Research*, *32*(3), 229-232.

Villaño, D., Lettieri-Barbato, D., Guadagni, F., Schmid, M., & Serafini, M. (2012). Effect of acute consumption of oolong tea on antioxidant parameters in healthy individuals. *Food Chemistry*, *132*(4), 2102-2106.

Visioli, F., Lastra, C. A. D. L., Andres-Lacueva, C., Aviram, M., Calhau, C., Cassano, A., et al. (2011). Polyphenols and human health: a prospectus. *Critical Reviews in Food Science and Nutrition*, *51*(6), 524-546.

Vonk, R. J., Hagedoorn, R. E., Graaff, R. D., Elzinga, H., Tabak, S., Yang, Y. X., et al. (2000). Digestion of so-called resistant starch sources in the human small intestine. *American Journal of Clinical Nutrition, 72*, 432–438.

Voragen, A. G. J. (1998). Technological aspects of functional food-related carbohydrates. *Trends in Food Science & Technology, 9*(8-9), 328-335.

Wanders, A. J., Jonathan, M. C., van den Borne, J. J., Mars, M., Schols, H. A., Feskens, E. J., & de Graaf, C. (2013). The effects of bulking, viscous and gel-forming dietary fibres on satiation. *British Journal of Nutrition*, *1*(1), 1-8.

Wang, C., Kovacs, M. I. P., Flower, D. B., & Holley, R. (2004). Effects of protein content and composition on white noodle making quality: Color. *Cereal Chemistry, 81*, 777-784.

Wang, Y., Chun, O. K., & Song, W. O. (2013). Plasma and dietary antioxidant status as cardiovascular disease risk factors: A Review of Human Studies. *Nutrients, 5*(8), 2969-3004.

Wang, Y., Yang, M., Lee, S. G., Davis, C. G., Kenny, A., Koo, S. I., & Chun, O. K. (2012). Plasma total antioxidant capacity is associated with dietary intake and plasma level of antioxidants in postmenopausal women. *Journal of Nutritional Biochemistry, 23*(12), 1725-1731.

Welsch, C. A., Lachance, P. A., & Wasserman, B. P. (1989). Dietary phenolic compounds: inhibition of Na+-dependent D-glucose uptake in rat intestinal brush border membrane vesicles. *Journal of Nutrition, 119*(11), 1698.

WHO (2013). *Obesity and overweight*. Retrieved from http://www.who.int/mediacentre/factsheets/fs311/en.

Willett, W., Manson, J., & Liu, S. (2002). Glycemic index, glycemic load, and risk of type 2 diabetes. *American Journal of Clinical Nutrition, 76*(1), 274S-280S.

Williams, P. G., Grafenauer, S. J., & O'Shea, J. E. (2008). Cereal grains, legumes, and weight management: a comprehensive review of the scientific evidence. *Nutrition Reviews, 66*(4), 171-182.

Willis, H. J., Eldridge, A. L., Beiseigel, J., Thomas, W., & Slavin, J. L. (2009). Greater satiety response with resistant starch and corn bran in human subjects. *Nutrition Research, 29*(2), 100-105.

Wood, J. A. (2009). Texture, processing and organoleptic properties of chickpea fortified spaghetti with insights to the underlying mechanisms of traditional durum pasta quality. *Journal of Cereal Science, 49*, 128–133.

Yamauchi, J. (1989). *Handbook of colour ccience*. Japanase Academy of Colour Science, Tokyo, Japan.

Yang, M., Chung, S. J., Floegel, A., Song, W. O., Koo, S. I., & Chun, O. K. (2013). Dietary antioxidant capacity is associated with improved serum antioxidant status and decreased serum C-reactive protein and plasma homocysteine concentrations. *European Journal of Nutrition*, *52*, 1901-11.

Yeomans, M. R., Weinberg, L., & James, S. (2005). Effects of palatability and learned satiety on energy density influences on breakfast intake in humans. *Physiology & Behavior*, *86*(4), 487-499.

Young, G. P., & Le Leu, R. K. (2004). Resistant starch and colorectal neoplasia. *Journal of AOAC International, 87*(3), 775-786.

Yousif, A., Nhepera, D., & Johnson, S. (2012). Influence of sorghum flour addition on flat bread in vitro starch digestibility, antioxidant capacity and consumer acceptability. *Food Chemistry, 134*(2), 880-887.

Zhang, G., & Hamaker, B. R. (1998). Low α-amylase starch digestibility of cooked sorghum flours and the effect of protein 1. *Cereal Chemistry*, *75*(5), 710-713.

APPENDIX I

RECRUITMENT SCREENING QUESTIONNAIRE USED FOR STUDY 2

DEAKIN
UNIVERSITY AUSTRALIA

SCREENING QUESTIONNAIRE

Please circle/complete the following questions:

1. Name: _____

2. Age :_____(In years)

3. Gender: Male / Female

4. What is your current status?

   Staff member   Student   Other

5. How often do you eat pasta?

   Every day   Once a week   Once a month   Never

6. Are you allergic to gluten?

   Yes   No

I believe the information I have provided to be true and correct.

Signed: _____

Date: _____

APPENDIX II

PALATABILITY QUESTIONNAIRE USED FOR STUDY 2 AND 3

Subject #: _____   **Date** _____

Sample Code: _____

PALATABILITY QUESTIONNAIRE

Please rate the palatability of the food in terms of the following characteristics

Scale / Characteristics	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like or Dislike	Like slightly	Like moderately	Like very much	Like extremely
Appearance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Flavour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Texture (in mouth)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Overall Acceptance	☐	☐	☐	☐	☐	☐	☐	☐	☐

APPENDIX III

RECRUITMENT HEALTH AND SCREENING QUESTIONNAIRE USED FOR STUDY 3 AND 4

DEAKIN UNIVERSITY
ETHICS COMMITTEE
SCREENING QUESTIONNAIRE

Name: _____Age:_____Years

Weight (kg):_____Height (cm) _____Gender:_____

Are you a smoker?	YES	NO
Do you eat breakfast regularly?	YES	NO
Are you currently dieting?	YES	NO
Are you pregnant?	YES	NO	NOT APPLICABLE

Has anyone ever told you that you:

- are overweight?   YES   NO   DON'T KNOW
- have high blood pressure?   YES   NO   DON'T KNOW
- are diabetic?   YES   NO   DON'T KNOW
- are asthmatic?   YES   NO   DON'T KNOW

Have you, or anyone of your family a history of cardiovascular disease?   YES   NO

(E.g. Heart attack, chest pain, stroke, rheumatic vascular disease)

If YES, please elaborate: _____

Are you sensitive to gluten?   YES   NO   DON'T KNOW

If YES, please elaborate: _____

Do you have any gastrointestinal disease?   YES   NO   DON'T KNOW

If YES, please elaborate: _____

Are you currently on medication?   YES   NO

If YES, what is the medication: _____

Do you drink alcohol?   YES   NO

If YES, how much (ml or gram per week):_____ _____

I believe the information I have provided to be true and correct.

Signed: _____

Date: _____

APPENDIX IV

BLOOD GLUCOSE RECORD SHEET USED IN STUDY 3

Subject #:_____   **Date** _____

Sample Code: _____

Blood Glucose Record

Time (min)	mmol/l
Fasting	
15	
30	
45	
60	
90	
120	

APPENDIX V

VISUAL ANALOGUE SCALES FOR APPETITE MEASUREMENT USED IN STUDY 3

Subject #:_____   **Date** _____

Sample Code: _____   **Time**_____

Satiety Questionnaire

(100 mm Visual Analogue Scales)

For each question, please clearly place a vertical line at the point which best represent how you are feeling.

How hungry do you feel?

I am not ——————————————————————— I have never
hungry at all   been more hungry

How satisfied do you feel?

I am ——————————————————————— I cannot eat
completely empty   another bite

How full do you feel?

Not at ——————————————————————— Totally full
all full

How much do you think you can eat?

Nothing ——————————————————————— A lot
at all

APPENDIX VI

FOOD INTAKE RECORD SHEET USED FOR FOOD INTAKE AT LUNCH IN STUDY 3

Subject #:_____   Date _____

Sample Code: _____

Food Intake Record (from lunch)

Food	Quantity	Weight of food before serving (g)	Weight of food after serving (g)
1. Meat balls			
2. Spring rolls			
3. Chicken munchies			
4. Pastizzis			
5. Chips			
6. Juice			
7. Fruit s (frozen)			
8. Tomato sauce			
9. Cheese cake			

APPENDIX VII

FOOD INTAKE RECORD SHEET USED FOR REMAINING DAY FOOD INTAKE IN STUDY 3

Subject #:_____   **Date** _____

Sample Code: _____

Food Intake Record (reminder of the day)

Time	Amount	Description of Food