Effect of sorghum flour addition on resistant starch content, phenolic profile and antioxidant capacity of durum wheat pasta

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ABSTRACT

Foods containing elevated levels of health functional components such as resistant starch and polyphenolic antioxidants may have beneficial effects on human health. Pasta incorporating either red sorghum flour (RSF) or white sorghum flour (WSF) each at 20%, 30% and 40% substitution of durum wheat semolina (DWS) was prepared and 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; individual phenolic acids and anthocyanins by reverse phase-HPLC analysis; total phenolic content by the Folin-Ciocalteu method and antioxidant capacity by the ABTS assay. The addition of both RSF and WSF 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-containig 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. The study suggests that these sorghum flours may be very useful for the preparation of pasta with increased levels of resistant starch and polyphenolic antioxidants.

Keywords: Sorghum, Phenolic compounds, Resistant starch, Antioxidant capacity, Pasta, Wheat

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth leading crop worldwide and the third most important cereal crop behind wheat and barley in Australia (Mahasukhonthachat, Sopade, & Gidley, 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). 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, Taylor, Belton, & Hamaker, 2003) and is a valuable source of health functional ingredients including resistant starch (Dicko, Gruppen, Traore, Voragen, & van Berkel, 2006; Ragaee, Abdel-Aal, & Noaman, 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 glycemia and insulinema 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

55 estimated daily intake of about 5 g/day is still lower than the recommended intake of 20 g/day
56 (Baghurst, Baghurst, & Record, 1996).

57 Phenolic compounds are a health functional component of sorghum through their antioxidant
58 properties (Dlamini, Taylor, & Rooney, 2007; Dykes, Rooney, Waniska, & Rooney, 2005;
59 Kamath, Chandrashekar, & Rajini, 2004). Sorghum has higher levels of phenolic compounds
60 compared to other widely consumed cereals such as wheat, rice, barley and millet (Ragaee et al.,
61 2006). In sorghum these polpyhenolics are concentrated in the outer layers of the grain where
62 they are found in both free and bound forms (Awika & Rooney, 2004). While all sorghum
63 varieties contain phenolic compounds, the types and levels present are related to pericarp colour
64 and the presence of pigmented testa and hence the overall grain colour. For instance, white-
65 grained varieties have a white pericarp and contain mainly simple phenolic acids, whereas red
66 and black-grained varieties have a red or black pericarp and contain phenolic acids and
67 anthocyanins. Some red and black-grained varieties also have a pigmented testa and in addition
68 to phenolic acids and anthycanins also contain condensed tannins (Awika & Rooney, 2004).
69 Epidemiological studies have indicated that diets rich in phenolic compounds may have
70 protective effects against various chronic diseases associated with oxidative stress such as
71 diabetes, cancer and cardiovascular disease (Halliwell, 2008; Temple, 2000). Food products
72 containing sorghum flour as an ingredient could act as vehicles for increased dietary intake of
73 phenolic compounds and thus provide chronic disease protective effects.

74 Pasta is popular worldwide and is used as a staple food in many countries. Conventional pasta
75 is manufactured using durum wheat semolina as the primary ingredient. Compared to other
76 starchy foods such as bread, pasta has beneficial physiological effects, including inducing low
77 postprandial glycemic and insulinemic responses (Aston, Gambell, Lee, Bryant, & Jebb, 2007;

Bornet et al., 1989). However, conventional pasta products are not high in resistant starch nor polyphenolic antioxidants, both of which may further reduce the risk of chronic diseases (He, Nowson, Lucas, & MacGregor, 2007; Pérez-Jiménez et al., 2008). Several studies have reported the increased resistant starch content and polyphenolic antioxidants levels of pasta through the addition of 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, 2011b).

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 red or white sorghum flour on resistant starch content, phenolic profile and antioxidant capacity.

2. Materials and methods

2.1. 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 ≥ 99%) were obtained from Merck (Darmstadt, Germany). Total dietary fiber assay kit, Folin-Ciocalteau reagent, sodium carbonate (purity ≥ 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 ≥ 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.

2.2. Raw materials

Durum wheat semolina (DWS) (the endosperm of selected Australian durum wheat milled according to manufacturer's specifications to an average 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, was obtained from Lochabar Enterprises Pty Ltd. (Tara, QLD, Australia). White sorghum grain (var. Liberty), a commercial hybrid, was supplied by the Queensland Department of Employment, Economic Development and Innovation (Alexandra Hill, 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 to an average particle size of 206.3 µm and 198.5 µm, respectively (as determined by laser particle size analysis – full data not shown). All flours were vacuum packed and stored at 15 ^{o}C in the dark prior to use.

2.3. Proximate and dietary fiber analysis of raw materials

Moisture content was determined by oven drying at 100 ^{o}C for 16 h (AOAC, 1997). Total protein content was determined using the Kjeldahl digestion distillation procedure with a nitrogen-to-protein conversion factor 5.7 and 6.25 for durum wheat semolina and sorghum flour respectively (AACC, 2000). Ash and fat content were measured according to AOAC methods 923.03, 920.85 (AOAC, 1997). Total dietary fiber was determined by an enzymatic-gravimetric method according to AOAC method 985.29 (AOAC, 1997), using Sigma-Aldrich total dietary fibre assay kit (TDF-100A, Sigma-Aldrich, St. Louis, MO, USA).

2.4. Pasta Preparation

Formulations consisting of 100% DWS (control) or by replacing DWS with red sorghum flour (RSF) or white sorghum flour (WSF) at 20, 30 and 40% (w/w), were prepared for fettuccine-type pasta processing. The maximum inclusion level of both sorghum flours i.e. 40% was identified by measuring dough strength in preliminary experiments (data not shown). 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, 35-40 ml per 100 g of flour depending on formulation, (based on preliminary experiments, data not shown) was added to give a uniform, smooth and non-sticky dough. The dough was kneaded by hand by one researcher in a standard manner 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 at ambient temperature (21-25 ^{o}C) for 30 h to a final moisture level of $\leq$10%. Formulations were prepared in duplicate. Dried pasta was doubled bagged in moisture proof plastic bags and stored in the dark at 4 ^{o}C.

146

147 *2.5. Pasta cooking*

148 The optimum cooking time for each type of pasta was determined using AACC method 66-50
149 (AACC, 2000). Briefly, 10 g of pasta was cooked in 300 ml of boiling distilled water. Optimum
150 cooking time (Table 2) was when the white core in the pasta was still present but disappeared
151 after squeezing between two plexiglass plates.

152 Cooking loss was determined according to the AACC approved method 66-50 (AACC, 2000).
153 Pasta was cooked for optimum cooking time as above. The cooking water was evaporated to
154 dryness in an air-oven at 105 ^{o}C and the residue was weighed and reported as a percentage of the
155 original (raw) pasta weight.

156 After cooking for the optimal time, pasta was drained and immediately cooled with distilled
157 water at 20 ^{o}C. The cooked pasta was then frozen in liquid nitrogen and dried in a laboratory
158 freeze-drier (Flexi-Dry™ model FD-3-55D-MP, FTS Systems, Stone Ridge, New York, USA).
159 A sample mill (Black and Decker, Hunter Valley, MD, USA) was used to grind both the
160 uncooked and freeze dried cooked pasta to pass 100% through a 0.5 mm screen. The ground
161 samples were stored at 4 ^{o}C in sealed plastic containers in the dark.

162

163 *2.6. Starch fractions determination*

164 The Amylose content of the DWS, WSF and RSF was determined by the method of Gibson,
165 Solah, and McCleary (1997) using a Megazyme amylose/amylopectin assay kit (K-AMYL
166 04/06, Megazyme Int. Ireland Ltd., Co. Wicklow, Ireland).

167 The total starch content of the raw materials and the uncooked and cooked pasta samples was
168 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 996.11 (AOAC, 2008). Resistant starch content was determined by 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 ^{o}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 ^{o}C, 30 min) to hydrolyse resistant starch to free glucose. Free glucose was 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 to 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.

2.7. Extraction of samples for total phenolic, antioxidant capacity and anthocyanin determination

Extracts for the determination of total phenols, antioxidant capacity and anthocyanins were prepared according to the method of Awika, Rooney, and Waniska (2004). Briefly, 1 g samples (raw materials, uncooked pasta or 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 3000 rpm 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 ^{o}C until analysis for total phenolics and antioxidant capacity. For anthocyanins analysis, sample extracts were prepared as above and then evaporated to dryness under vacuum at 40 ^{o}C using a Büchi

192 Rotavapor R-215 (Büchi, Flawil, Switzerland). The residue was redissolved in 5 ml of methanol
193 and filtered through a 0.45µm syringe filter (Fisher Scientific) prior to analysis by high
194 performance liquid chromatography (HPLC).
195
196 *2.8. Extraction of samples for phenolic acid (free and bound) determination*
197 Free phenolic acids extraction was performed according to Adom and Liu (2002) with some
198 modification. Briefly, 2 g samples (raw materials, uncooked pasta or cooked pasta) were
199 extracted with 10 ml of 80% (v/v) aqueous methanol for 1 h in a shaking water bath at 25 ^{o}C.
200 After centrifugation at 3000 rpm for 20 min, the supernatant was decanted and the extraction was
201 repeated as described above. The two supernatants were combined, evaporated to near dryness
202 and reconstituted with methanol to a final volume of 10 ml. The reconstituted sample was
203 filtered through a 0.45µm syringe filter (Fisher Scientific) prior to analysis by HPLC.
204 For extraction of bound phenolic acids, the residue remaining after free phenolics extraction
205 was treated with 10 ml of 2 N HCl at 100 ^{o}C for 1 h. Ethyl ether (20 ml x 2) was added to the
206 hydrolysate and, after partitioning the ethyl ether fraction was separated and evaporated to
207 dryness. The residue was redissolved in 2 ml of methanol and filtered through a 0.45µm syringe
208 filter (Fisher Scientific) prior to analysis by HPLC.
209
210 *2.9. Determination of total phenolic content*
211 Total phenolic content of raw materials and uncooked and cooked pasta samples was
212 measured using the modified Folin-Ciocalteu method (Li et al., 2007). The Folin-Ciocalteu
213 reagent was first diluted 10 times with milli-Q water and 0.2 ml of sample extract (section 2.7)
214 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 a standard and the results were expressed as mg of gallic acid equivalents (GAE)/g sample (dry basis).

2.10. Determination of antioxidant capacity

Antioxidant capacity of the raw materials and uncooked and 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 ($ABTS^{\cdot+}$) 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 $ABTS^{\cdot+}$ solution was diluted with 95% ethanol, in order to obtain an initial absorbance between 0.35 and 0.4 at 734 nm. Fresh $ABTS^{\cdot+}$ solution was prepared for each analysis. Trolox (0 to 500 µM) was used as a standard. Sample extracts (section 2.7) or standards (50 µl) were mixed with 2 ml of diluted $ABTS^{\cdot+}$ solution and incubated at 30 ^{o}C. Absorbance was monitored at 734 nm for 30 min using the Synergy 2 microplate reader (BioTek, model S, Winooski, VT, USA) against an ethanol/$ABTS^{\cdot+}$ blank (50 µl of 95% ethanol added to 2 ml of diluted $ABTS^{\cdot+}$ solution). The decrease in absorbance ($\Delta A = A_{t=0\ \text{min}} - A_{t=30\ \text{min}}$) 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).

2.11. HPLC analysis of phenolic acids (free and bound) and anthocyanins

Reverse phase-HPLC analysis of sample extracts 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 proposed 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 was 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 concentration 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).

261

262 *2.12. Statistical analysis*

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

268

269 **3. Results and discussion**

270

271 *3.1. Proximate and dietary fiber composition of raw materials*

272 The mean values for the proximate composition and total dietary fiber of DWS, RSF and
273 WSF are shown in Table 1. Protein content varied significantly among the three flour samples:
274 that for DWS being higher than WSF ($p < 0.05$) which in turn was higher than RSF ($p < 0.05$).
275 The fat content of the RSF was significantly higher than that of the WSF ($p < 0.05$) which in turn
276 was higher than the DWS ($p < 0.05$). WSF was significantly higher in ash content than DWS and
277 RSF ($p < 0.05$). The total dietary fiber content of the RSF was significantly higher than that of
278 the WSF ($p < 0.05$), which in turn was higher than that for the DWS ($p < 0.05$). The differences
279 in the proximate and dietary fiber contents of the sorghum flours and DWS may in part be due to
280 the fact that whole grain sorghum flours were used whereas the DWS is a refined-grain wheat
281 product. For instance the higher levels of fat in the sorghum flours may be attributed to the
282 presence of the embryo (germ) in which oil is concentrated (Ragaee et al., 2006). These protein,
283 fat, ash and total dietary fiber 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, Nhepera, and Johnson (2012) for RSF and WSF.

Based on the total dietary fiber 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 fiber content of the pasta and thus have potential to increase its health functional properties.

3.2. Effect of sorghum addition on pasta cooking loss

Cooking loss, a measure of the amount of solids lost into the cooking water, is considered an important indicator of pasta quality. Cooking loss was significantly lower ($p < 0.05$) for the control pasta than for all of sorghum-containing pastas except 20% WSF pasta (Table 2). 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, Sessions, Egan and Fellows (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 pasta compared to the control 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 pasta into the cooking water was observed. Similar effects on increasing 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

306 losses observed for the sorghum-containing pasta in the present study are still acceptable as
307 losses of ≤8% are considered desirable for good quality pasta (Dick & Youngs, 1998).
308
309 *3.3. Effect of sorghum addition on starch fractions of pasta*
310 The amylose content of the starches of the three flours (mean ± SD, n = 3); DWS (23.0 ±
311 0.83%), RSF (22.4 ± 1.46%) and WSF (19.3 ± 2.70 %) were not significantly different ($p >$
312 0.05). The amylose content plays an important role in resistant starch formation. In general
313 cereals with higher amylose content can have lower starch digestibility and higher levels of
314 resistant starch (Sajilata et al., 2006). However in sorghum grain other factors including starch-
315 protein interaction and enzyme inhibitory effect of sorghum polyphenols (Taylor & Emmambux,
316 2010) may also affect resistant starch content beyond effects due to amylose levels.
317 The starch fractions (total, digestible and resistant) of the flours (DWS, RSF and WSF) and
318 pastas containing different percentages of RSF and WSF are shown in Table 3. WSF had a
319 significantly higher ($p < 0.05$) total starch and digestible starch content than RSF and DWS.
320 However the resistant starch content of the RSF was significantly higher ($p < 0.05$) than the WSF
321 which in turn was higher ($p < 0.05$) than the DWS. The higher resistant starch content of the
322 sorghum flours compared to the DWS might be a result of the digestive enzyme inhibitory effect
323 of sorghum polyphenols and sorghum starch-protein interactions (Austin, Turner, McDonough,
324 & Rooney, 2012; Taylor & Emmambux, 2010).
325 In terms of total and digestible starch content, only 40% WSF pasta (cooked) showed
326 significantly higher ($p < 0.05$) levels in comparison to the control pasta and no differences were
327 seen in these starch fractions between uncooked and cooked forms of each formulation. The
328 values for total and digestible starch content obtained in the present study are comparable to

329 those reported by Fares and Menga (2012) in chickpea flour-enriched pasta and Ovando-
330 Martinez et al. (2009) in unripe banana flour-enriched pasta.
331 Significant ($p < 0.05$) increases in resistant starch content of the uncooked pasta were
332 observed on the addition of RSF and WSF to the pasta. Uncooked formulations with higher
333 percentages of RSF and WSF showed significantly higher ($p < 0.05$) resistant starch content with
334 significant higher levels ($p < 0.05$) in the RSF compared to the WSF containing formulations at
335 the same incorporation level. The experimental values for the resistant starch content (Table 3B)
336 were slightly less than the theoretical values calculated from the resistant starch content of the
337 raw materials (0.42, 0.96, 1.17, 1.43, 0.82, 0.95 and 1.13 % dry basis for control, 20% RSF, 30%
338 RSF, 40% RSF, 20% WSF, 30% WSF and 40% WSF, respectively). This discrepancy may be a
339 result of the hydration and shear during processing rendering the starch slightly more digestible.
340 Decrease in resistant starch content during processing has previously been reported by Fares and
341 Menga (2012) in pasta containing chickpea flour. After cooking, the resistant starch content of
342 the pasta did not differ ($p > 0.05$) from that of the equivalent uncooked formulation and
343 differences between formulations followed the same pattern as in the uncooked samples. In
344 contrast to the findings of the present study, Fares and Menga (2012) found higher resistant
345 starch content in cooked chickpea flour-containing pasta than uncooked; a finding they attributed
346 to the retrogradation of the gelatinised starch after the pasta was cooled. However, in the present
347 study the pasta was instantly frozen in liquid nitrogen immediately after cooking to prevent
348 starch retrogradation. Vernaza et al. (2012) however observed a lower level of resistant starch
349 content in cooked compared to uncooked pasta containing high-maize which they attributed to
350 the leaching of resistant starch from the pasta surface during cooking.
351

352 *3.4. Effect of sorghum addition on total phenolic content and antioxidant capacity of pasta*

353 Table 4 reports the total phenolic content and antioxidant capacity of the flours and of the
354 pasta formulations before and after cooking. The total phenolic content and antioxidant capacity
355 of both RSF and WSF were significantly ($p < 0.05$) higher than DWS. These values are in
356 agreement with those reported by Awika, Yang, Browning, and Faraj (2009) and Fares, Platani,
357 Baiano, and Menga (2010).

358 Compared to the control pasta, all sorghum-containing pastas had significantly ($p < 0.05$)
359 higher total phenolic content (Table 4B). In addition, RSF-containing pastas had significantly (p
360 < 0.05) higher total phenolic content than WSF-containing pastas at the same incorporation level
361 mirroring the higher total phenolic content of RSF compared to WSF (Table 4A). The total
362 phenolic content of the uncooked pastas were slightly lower than the theoretical values
363 calculated from the raw materials composition (0.76, 1.93, 2.52, 3.31, 1.13, 1.38 and 1.51 mg
364 GAE/g dry basis for control, 20% RSF, 30% RSF, 40% RSF, 20% WSF, 30% WSF and 40%
365 WSF, respectively). Aravind et al. (2012) reported a significant decrease in total phenolic content
366 of bran-containing pasta prepared by extrusion processing, possibly due to oxidative degradation
367 in the presence of oxygen, water and heat (Fares et al., 2008). However in contrast to the study of
368 Aravind et al. (2012), the present study used a lamination process at ambient temperature leading
369 to only very small reductions in total phenolic content.

370 Compared to the equivalent uncooked formulation, all cooked RSF-containing pastas and
371 30% and 40% WSF-containing pastas had significantly ($p < 0.05$) lower total phenolic content.
372 Differences in total phenolic content between uncooked and cooked pastas may be in part due to
373 the leaching of these compounds into the cooking water. Lower levels of phenolic compounds in
374 cooked compared to raw formulations has previously been reported in pasta containing seaweed

(Prabhasankar et al., 2009), barley coarse fraction (Verardo et al., 2011b), buckwheat pasta (Verardo et el., 2011a) 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.

Both sorghum flours had higher ($p < 0.05$) antioxidant capacity as determined by the ABTS assay than DWS (Table 4A) and as expected all uncooked sorghum-containing formulations had significantly ($p < 0.05$) higher antioxidant capacity than the control pasta (Table 4B). Similar to total phenolic content the uncooked pastas had slightly lower antioxidant capacity than 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).

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. The results of the present study are in agreement with those of Prabhasankar et al. (2009), who reported lower antioxidant activity in cooked than uncooked seaweed-containing pasta a difference they attributed to the leaching of solids into the cooking water. However, in contrast to our results, Fares et al. (2010) observed a higher level of antioxidant activity in cooked wheat bran-containing pasta than uncooked, 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 (Table 4B) might explain the lower level of antioxidant capacity in the cooked compared to the uncooked pastas. However other antioxidant

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

3.5. Effect of sorghum addition on phenolic profile of pasta

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. Table 5A and B report the free phenolic acid content (PAC-free) and bound phenolic acid content (PAC-bound) 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 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. The amount and type of free and bound phenolic acids analysed were in fair agreement with that 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 ($p < 0.05$) total phenolic content and antioxidant capacity of RSF compared to WSF and DWS (Table 4A).

Anthocyanins (luteolinidin and apigeninidin) were observed only in RSF (Table 5C). These results are in agreement with the findings of Dykes, Seitz, Rooney, and Rooney (2009) that anthocyanins were present in red sorghum only with 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 (Table 4A).

Table 6 reports the phenolic acids (free and bound) and anthocyanin content of uncooked and cooked pasta formulations. The addition of RSF into uncooked pasta significantly ($p < 0.05$) increased the PAC-free at all incorporation levels compared to control pasta; a finding not unexpected given the higher PAC-free of RSF (Table 5A). 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 increased ($p < 0.05$) 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 4B).

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 (Table 6A). 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 (Table 5A), 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).

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 (Table 6A). Mean differences were higher in the sorghum-containing formulations than the control (eg. 12.2% reduction for control; 25.8% reduction for 40% RSF pasta). These results are in agreement with the data from the studies of Fares et al. (2010) and Verardo et al. (2011a), 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 (Table 6B). 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 with significantly ($p < 0.05$) higher concentration in the 40% RSF pasta compared to 20% and 30% RSF pastas (Table 6C). Pasta processing did not affect the anthocyanin content. However a significant ($p < 0.05$) 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).

4. 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 glycemic effect and antioxidant power of these sorghum-containing pasta formulations.

Acknowledgements

Financial support was provided by Deakin University and Curtin University internal research funding. The authors wish to thank Curtin University School of Public Health for providing durum semolina and sorghum flours. One of the authors (I. Khan) also acknowledges the scholarships from Deakin University and KPK-AUP/HEC Pakistan.

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646

Table 1

Proximate and dietary fiber composition 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 fiber (%)	4.61 ± 0.72[b]	9.00 ± 0.56[a]	6.46 ± 0.60[b]

Different letters in the same row indicate significant differences ($p < 0.05$, LSD test).

* Values are expressed in means ± SD (n =3).

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

Table 2

Optimal cooking time and cooking loss values of pasta samples*

Sample	Cooking time (min)	Cooking loss (%)
Control	15.2 ± 0.4	3.50 ± 0.34[b]
20% RSF	14.3 ± 0.4	4.99 ± 0.38[ac]
30% RSF	14.1 ± 0.3	5.66 ± 0.86[a]
40% RSF	14.3 ± 0.2	5.89 ± 0.20[a]
20% WSF	14.2 ± 0.4	4.48 ± 0.67[bc]
30% WSF	14.2 ± 0.3	4.86 ± 0.16[ac]
40% WSF	14.3 ± 0.4	5.93 ± 0.03[a]

Means with different letters are significantly different ($p < 0.05$, LSD test).

*Values are expressed in means ± SD (n =4).

Abbreviations: RSF = red sorghum flour; WSF = white sorghum flour.

Table 3

Starch fraction contents of flour and pasta samples* (% dry basis)

Sample		Total starch	Digestible starch	Resistant starch
(A) Flour samples				
DWS		73.62 ± 0.93^{b}	73.21 ± 0.81^{b}	0.42 ± 0.06^{c}
RSF		76.70 ± 1.21^{b}	73.75 ± 1.27^{b}	2.95 ± 0.06^{a}
WSF		80.96 ± 1.35^{a}	78.75 ± 1.20^{a}	2.21 ± 0.15^{b}
(B) Pasta samples				
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}

Means in the same column for either section (A) or section (B) with different letters are significantly different ($p < 0.05$, LSD test). * Values are expressed in means ± SD (n =4).

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

Table 4

Total phenolic content and antioxidant capacity of flour and pasta samples* (dry basis)

Sample		Total phenol (mg GAE/g)	Antioxidant capacity (μmol TE/g)
(A) Flour samples			
DWS		0.76 ± 0.07^{c}	9.20 ± 0.31^{c}
RSF		6.65 ± 0.12^{a}	71.20 ± 0.36^{a}
WSF		2.17 ± 0.05^{b}	23.80 ± 0.54^{b}
(B) Pasta samples			
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}

Means in the same column of either section (A) or section (B) with different letters are significantly different ($p < 0.05$, LSD test). * Values are expressed in means ± SD (n =4).

Abbreviations: GAE = gallic acid equivalents (Folin Ciocalteu method); TE = trolox equivalents; DWS = durum wheat semolina; RSF = red sorghum flour; WSF= white sorghum flour.

668 **Table 5**

669 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

670 Means in the same row with different letters are significantly different ($p < 0.05$, LSD test).

671 * Values are expressed in means ± SD (n =4).

672 Abbreviations: nd = not detected; PAC-free = phenolic acid content of free extract (is the result of the sum of free
673 phenolic acids); PAC-bound = phenolic acid content of bound extract (is the result of the sum of bound phenolic
674 acids); TPAC = total phenolic acid content (is the result of the sum of PAC-free and PAC-bound).

675 **Table 6**

676 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^{a}	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 ± 0.9^{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^{j}	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^{efg}	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^{c}	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

677 Means in the same row with different letters are significantly different ($p < 0.05$, LSD test). * Values are expressed in means ± SD (n = 4).

678 Abbreviations: nd = not detected; RSF = red sorghum flour; WSF= white sorghum flour; PAC-free: phenolic acid content of free extract; PAC-bound: phenolic acid content of bound extract

Highlights

- The effect of sorghum addition to pasta on starch and polyphenolic properties was studied
- Sorghum incorporation increased resistant starch and polyphenolic antioxidants in pasta
- Cooking decreased total phenolic content and antioxidant capacity of pasta
- Free phenolic acids decreased while bound phenolic acids increased in pasta during cooking