Condensed tannins in traditional wet cooked and modern extrusion cooked sorghum porridges

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ABSTRACT

The profile and quantities of condensed tannins (CTs) in foods are affected by processing due to their highly reactive nature, which may affect their antioxidant activity and the nutritional value of the foods. The objective was to compare the quantity and profile of condensed tannins in traditional wet cooked and modern ready-to-eat extrusion cooked sorghum porridges. CTs were analyzed using normal-phase HPLC with fluorescence detection and their content was compared to CT and total phenols determined with standard colorimetric assays. Both the traditionally prepared and instant porridges had significantly reduced CT polymers (degree of polymerization (DP) >8)), with retentions of 38% and 9% respectively of the CTs present in the whole grain. Oligomer (DP 2-8) and monomer (DP 1) contents in traditional porridges were not significantly different from that of grain. In extruded porridges, the oligomers were reduced, and the monomer content was increased. The extractable CT oligomers and monomers in the extrusion cooked sorghum porridges may be more biologically available because extrusion appears to increase their availability.

Keywords: Sorghum porridge, condensed tannins, phenols, normal phase HPLC, extrusion

INTRODUCTION

Condensed tannins (CTs) occur in sorghum varieties that have a pigmented testa (Waniska and Rooney 2000). The levels of tannins in these sorghums range from 700 to 2 200 mg/100 g (Gu et al 2004; Awika et al 2003 a). Sorghums without the pigmented testa layer do not contain condensed tannins but often have high levels of flavanoids depending upon the variety (Dykes et al 2005).

The tannins in sorghum have potential health benefits, mainly as dietary antioxidants, and thus could be important in the protection of the body from damage induced by oxidative stress (Awika and Rooney 2004). The intake of CTs is high in parts of Africa where tannin sorghum varieties are grown and consumed (Salunkhe et al 1990). In Africa, sorghum is mostly consumed as traditional porridges (Murty and Kumar 1995). Today, as Africa urbanizes, there is an increasing demand for sorghum-based convenience foods, such as ready-to-eat breakfast porridges produced by extrusion cooking (Taylor and Emmambux 2008).

In previous studies, CTs and phenols were found to be good predictors of antioxidant activity of unprocessed grain (Awika et al 2003 b; Dykes et al 2005). Dlamini et al (2007) observed that processing the sorghum grain into traditional wet cooked and instant extrusion cooked porridges reduced phenols, CTs and antioxidant activity determined by the ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2'-diphenyl-Ipicrylhydrazyl) methods. Awika et al (2003 a) showed that processing sorghum into extruded snacks significantly reduced the CT polymer content (DP>10).

Since tannin sorghums are used widely in Africa to prepare the staple foods especially porridges, the objective of this study was to compare how traditional porridge cooking and extrusion cooking to produce instant porridges affect CT profile and quantities.

MATERIALS AND METHODS

Materials

Three condensed tannin sorghum varieties grown in 2003 were used. These were NS 5511 and Framida from Zimbabwe, obtained through ICRISAT, Matopos Research Station, Zimbabwe; and Early Sumac obtained from Texas A&M University, College Station, Texas. The characteristics of NS 5511 and Framida were described by Dlamini et al (2007).

The condensed tannin standard, with a known HPLC profile, was prepared from Sumac sorghum grown in 2003 (Awika et al 2003 a).

Sorghum processing

Preparation of traditional sorghum porridges

Whole sorghum grain was milled to pass through a 1 mm opening screen using a coffee grinder and then cooked into porridges. The milled grain (78.3 g) was mixed into 100 mL water. The slurry was added to 333 mL boiling water and cooked with constant

63 stirring for 10 min. The porridge was cooled for 30-60 min, and frozen using liquid
64 nitrogen and freeze-dried. The freeze-dried samples were stored at -20^{o}C until analysis.
65 *Preparation of instant sorghum porridges by extrusion cooking*
66 The instant porridges were prepared by extrusion cooking of coarsely milled,
67 whole NS 5511 and Framida sorghum grains. The grain was milled using a hammer mill
68 to pass through a 1.58 mm opening screen, and then it was extruded in a Clextral BC92
69 twin-screw, co-rotating extruder (FIRMINY Cedex, France). The feed rate was 550
70 kg/hr; moisture content of feed was adjusted to 18%, by injecting 45 L water per hr. The
71 screw rotation speed was 230 revolutions per min (rpm), and barrel temperature was 150
72 and 160^{o}C, and residence time was 30-90 sec. The die diameter was 2 mm and the cutter
73 speed was 120 rpm. After extrusion cooking, the extrudates were cooled and equilibrated
74 for 4-5 hr. The final moisture content was 6-8%. The extrudates were milled and
75 analyzed without re-constitution.
76
77 **Sample preparation**
78 The sorghum samples and processed products, including the freeze-dried
79 products, were milled to pass through a 1 mm opening screen using a UDY cyclone mill
80 Model 3010-030 (Fort Collins, CO).
81
82 **Analyses**
83 *Expansion ratio (ER), water absorption (WAI) and solubility indexes (WSI)*

84 The expansion ratio of the sorghum extrudates was determined by dividing the
85 diameter of the extrudate (mean of ten measurements) by the diameter of the die
86 (Pelembe et al 2002).

87 The water absorption and solubility indexes of the raw grain and extrudates were
88 determined as described by Pelembe et al (2002). In brief, the grain and extrudates were
89 finely milled, and suspended in distilled water. WAI was the amount of water absorbed
90 after centrifuging the sample, while WSI was determined as soluble materials present in
91 the supernatant, after evaporation.

92 *Total phenols, tannin content and antioxidant activity*

93 Milled samples were extracted with 1% concentrated HCl in methanol and the
94 extracts analyzed. Total phenols were determined using the modified Folin Ciocalteu
95 method of Kaluza et al (1980); gallic acid was used as the standard. Tannin content was
96 determined using the Vanillin-HCl method described by Price et al (1978); catechin was
97 used as the standard. Antioxidant activity was determined using the ABTS method as
98 described by Awika et al (2003 b).

99 *Condensed tannin profile using normal phase HPLC*

100 The condensed tannins were extracted, purified and their profile determined as
101 described by Gu et al (2002). Samples (0.5-1.0 g) were extracted using a mixture of
102 acetone/ water/ acetic acid (70: 29.5: 0.5) for 2 hr at low speed in an Eberbach shaker
103 (Eberbach Corp., Ann Arbor, MI). The extracts were centrifuged, and the supernatant
104 evaporated to dryness at 25^{o}C in a Speed Vac SC201A (Thermo, Marietta, OH) under
105 vacuum. The dried residue (crude tannin extract) was dissolved in water and purified on

106 Sephadex LH-20 columns by washing with 30% aqueous methanol to remove the sugars
107 and other low molecular weight phenols, followed by 70% aqueous acetone to recover
108 the CTs. The eluted aqueous acetone was evaporated to dryness at 43^{o}C under vacuum,
109 and the residue dissolved with 70% aqueous acetone, filtered using a Whatman nylon
110 membrane filter (0.45 µm) and then injected into the HPLC.

111 The condensed tannin profile was determined using the Waters HPLC system
112 (Millford, MA). The mobile phase was (A) dichloromethane, (B) methanol, and (C)
113 acetic acid/water (1:1 v/v). The gradient was 0-30 min, 14.0-28.4% B; 30-45 min, 28.4-
114 39.6% B; 45-50 min, 39.6-86.0% B; 50-55 min, 86.0 B isocratic, 55-60 min, 86.0-14.0%
115 B; followed by 10 min re-equilibration of the column before the next run. A constant 4%
116 C was maintained throughout the gradient. Flow rate was 1 mL/min. Separation was on a
117 normal-phase 5-µm Luna silica column (250 x 46 mm) (Phenomenex, Torrance, CA).
118 Fluorescence detection was used; excitation – 276 nm, emission – 316 nm.

119 The HPLC traces of the extracted CTs were interpreted using a calibration curve
120 that was based on the condensed tannin profile of Sumac grain (Fig 1), whose
121 purification was described by Awika et al (2003 a). The CTs of the exact Sumac grain
122 were characterized, quantified and reported as described by Gu et al (2002). The accuracy
123 of the data was confirmed using commercial standards for the monomers (DP 1) and
124 oligomers (DP 2-8), while a polymeric standard prepared from sorghum bran was used to
125 check the accuracy of the polymeric peak (DP>8). The DP group was integrated baseline
126 to baseline, and the polymeric peak included the trailing edges, thus it ranged from 57-73
127 min.

The CTs were resolved up to octamers (DP 8), with the low molecular weight CTs eluting first. Thus the CT profile was reported as monomers (DP 1), oligomers (DP 2-8), and polymers (DP>8). Total extractable CTs were obtained by adding the monomer, oligomer and polymer contents.

Experimental design and data analysis

A single batch of each type of porridge was prepared and then it was analyzed in triplicate. The total phenols, tannin and HPLC determinations were means of triplicate analyses. The means were analyzed with one way analysis of variance (ANOVA), and then separated using Fisher's least significant difference test.

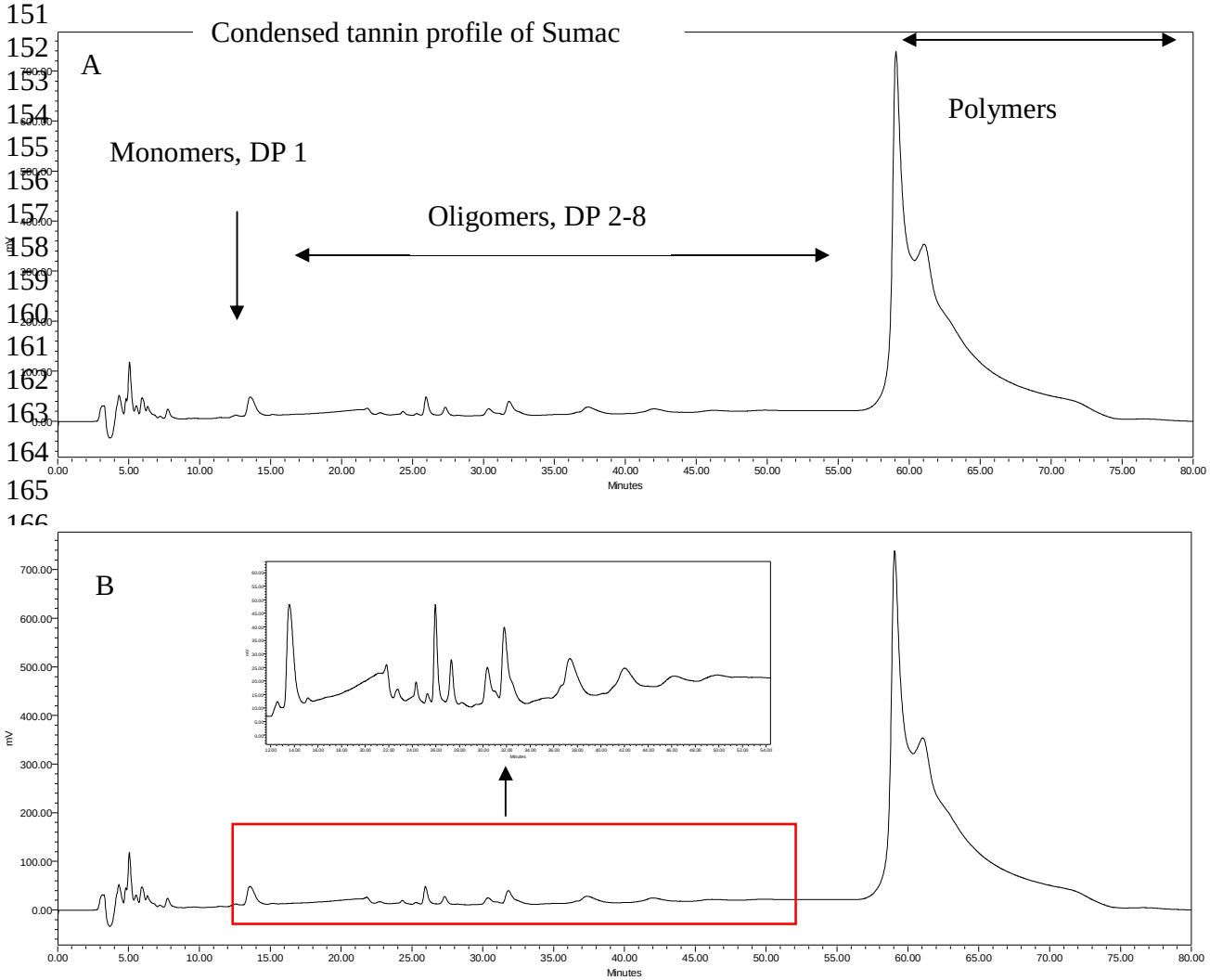

Fig. 1. HPLC chromatogram of sorghum condensed tannin profile (Sumac): A - overall appearance; B - monomer and oligomer portions after increasing the sensitivity of the detector. The condensed tannin standard was prepared as in Awika et al. (2003 a)

RESULTS AND DISCUSSION

Expansion ratio (ER), water absorption (WAI) and solubility indexes (WSI)

Extrusion cooking significantly increased WAI and WSI of milled sorghum grain (Table I). There was no significant effect of variety on these parameters, ($p<0.01$). A high ER and WAI is a desirable property in ready to eat porridges (Pelembe et al 2002). The WAI of cereal grain products generally increases with severity of processing, reaching a maximum at 180-200^{o}C (Fellows 2000). At high temperatures or shear, the starch is degraded or dextrinized to smaller soluble molecules, thus increasing WSI, and the WAI decreases (Ding et al 2005). These results show that a product of reasonable quality was obtained when whole grain was extruded.

Total phenols, condensed tannins and antioxidant activity

The unprocessed grains of Framida, NS 5511 and Early Sumac did not differ significantly in TP content, but the CT content measured by the Vanillin-HCl method was significantly lower for Early Sumac (Table II). The CT content measured by HPLC (CT-HPLC) was highest for Framida, followed by Early Sumac and then NS 5511 grain. Processing the sorghum into traditional and extrusion cooked porridges significantly reduced TP, CT and CT-HPLC contents (Table II). The greatest reductions were for CT content measured by the Vanillin-HCl method, while the TP content had the least reduction, followed by CT-HPLC. During processing CTs interact and bind with proteins and carbohydrates (Mehansho et al 1987), which affects extractability, and thus

measurability. The reduction in CT extractability probably explains the low retentions in measured antioxidant activity of instant porridges (14%) and traditional porridges (34%), as both assays were conducted on extracts.

Tannin profile

HPLC revealed that the predominant CTs in the unprocessed sorghum grain were polymers (DP>8), which were 68-80% of the total CTs, followed by oligomers (DP 2-8) (20-31%), while monomer (DP 1) content was less than 1% (Table III). This agrees with similar observations by Awika et al (2003 a) for tannin sorghum grain, and for other foods (Gu et al 2003). Processing sorghum grain increased the proportions of oligomers (DP 2-8) and monomers; while that of polymers decreased, as is illustrated by the HPLC traces in Fig. 2. The oligomers increased to 41% and 52% in traditional and instant porridges respectively, while the polymers decreased to 58% in traditional porridges and 34% in instant porridges. The decrease in the proportion of CT polymers, and the increase in the CT oligomers support the idea that CT polymers interacted with proteins and carbohydrates as is well known (Mehansho et al 1987).

Processing the sorghum into traditional and extrusion cooked instant porridges significantly reduced total CT content (Table II). The CT polymers were reduced most, from an average 30 mg/g in the grain to 12 mg/g (38% retention) in traditional porridges, and 3 mg/g (9% retention) in instant porridges (Table III). The variety of sorghum significantly affected the retention of oligomers in traditional porridges. For example, Framida traditional porridges retained the least oligomers (58%) compared to NS 5511

and Early Sumac traditional porridges. The differences in the retention of oligomers could be due to structural differences in the monomer units of CTs, as observed by Krueger et al (2003). For example, an increase in the prodelphinidin / procyanidin ratio in condensed tannin increases the ability of that tannin to complex with proteins (Schofield et al 2001). Prodelphinidins differ from the procyanidins in that the monomer units have a hydroxyl group at position 5 of the B ring. In Framida and NS 5511 instant porridges, only 37% (4.0 mg/g) of the oligomers were retained. The monomer content of the traditional porridges did not change significantly from that in the grain, but that of instant porridges increased by two to almost eighty fold, for example in NS 5511 instant porridge, monomers increased from 0.01 mg/g to 0.8 mg/g.

Table I
Effects of Extrusion Cooking of Whole Sorghum on Water Absorption Index (WAI), Water Solubility Index (WSI) and Extrudate Expansion Ratio

Variety	Treatment	Water absorption index (g/g)	Water solubility index (g/100 g)	Expansion Ratio[a]
NS5511	Whole grain	2.62 b	3.45 b	
	Whole extruded	4.43 a	34.20 a	5.95 a
Framida	Whole grain	2.71 b	3.94 b	
	Whole extruded	4.65 a	30.98 a	5.50 a

[a]Expansion ratio is expressed for extruded grain only
Values within the same column with different letters are significantly different at $P<0.01$

Table II

Effects of Traditional Wet Cooking and Extrusion Cooking on the Total Phenol and Condensed Tannin Contents of Sorghum Porridges

Treatment	Sorghum variety	Total phenols[a]	CT content[b] (Vanillin-HCl)	CT content[c] (HPLC)	Antioxidant activity[d]
Grain	Framida	20.7 a	47.8 a	59.1 a	427 a
	NS 5511	18.1 b	49.0 a	26.4 c	384 b
	Early Sumac	19.4 ab	19.6 b	36.6 b	262 c
Traditional, wet cooked porridge	Framida	13.4 c (65)[e]	13.7 c (29)	26.2 c **(**44)	118 e (28)
	NS 5511	8.7 e (48)	6.6 d (13)	12.5 d (47)	70 f (18)
	Early Sumac	11.1 d (57	4.5 de (23)	20.8 c (57)	145 d (55)
Instant, extrusion cooked porridge	Framida	5.3 g (26)	0.4 e (1)	11.2 e (19)	53 g (12)
	NS 5511	6.7 f (37)	1.9 e (4)	4.4 e (17)	58 g (15)

[a]Total phenols expressed as mg gallic acid equivalents /g sample (mg GAE/g), dry weight basis (Folin-Ciocalteu method).

[b]Condensed tannin content determined using the vanillin-HCl method and expressed as mg catechin equivalents/g (mg CE/g), dry basis (Price et al 1978)

[c]Condensed tannin content determined using normal phase HPLC and expressed as mg/g sample, dry weight basis (Gu et al 2002)

[d]Antioxidant activity (ABTS assay) data is from Dlamini et al (2007), and is expressed as µmol trolox equivalents/g (µmol TE/g).

[e]Figures in parentheses are % retentions in the porridges, of the TPs, CTs, HPLC-CT and antioxidant activity originally present in grain.

As mentioned, CT polymers (DP>8) interact more than oligomers with protein and carbohydrates (Mehansho et al 1987), which reduces their extractability. CTs have strong affinity for proteins high in proline content like the prolamins (Emmambux and Taylor 2003), and probably interact by hydrophobic associations further stabilized by hydrogen bonding (Verge et al 2002).

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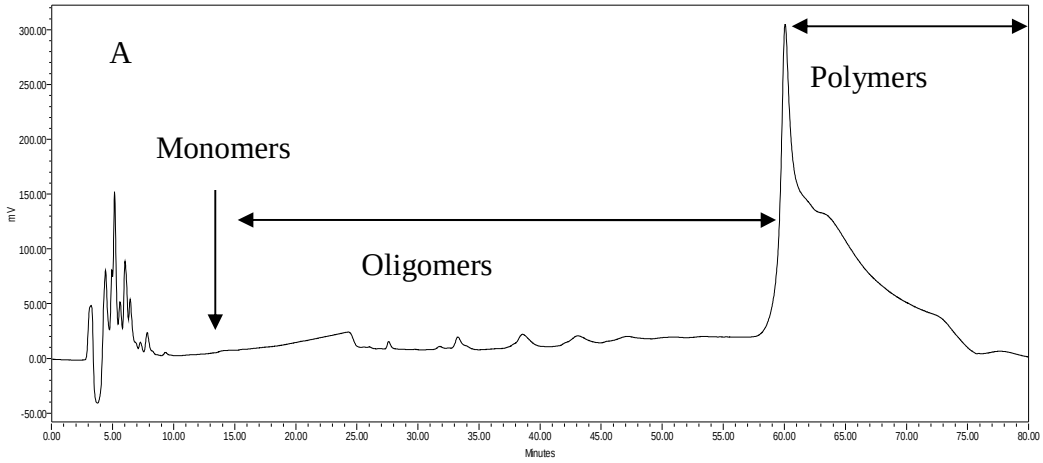
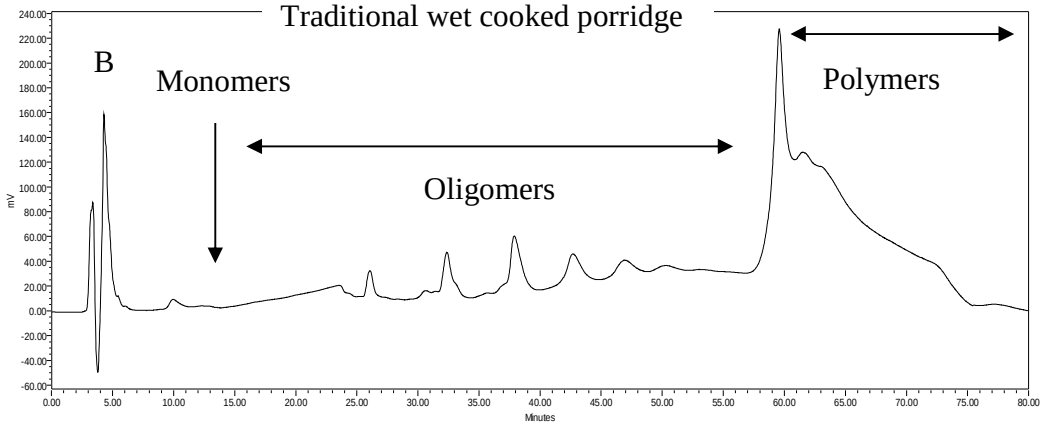


273 Traditional wet cooked porridge



274 Instant extrusion cooked porridge

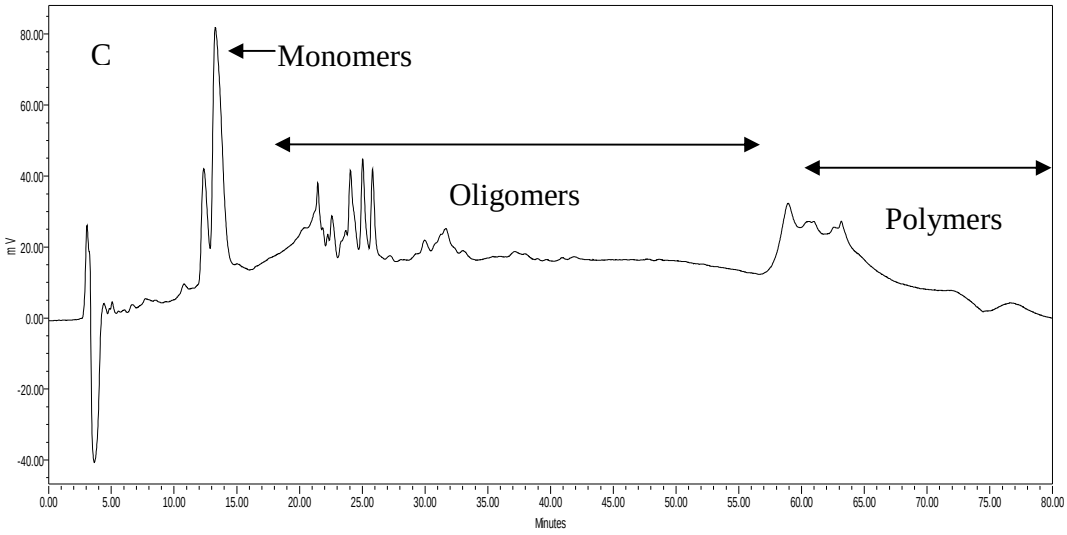


Fig. 2. HPLC chromatograms of condensed tannins of A: NS 5511 grain, B: traditional wet cooked and, C: instant extrusion cooked porridges. Changes in the proportions of monomers and oligomers relative to polymers is illustrated

Table III
Effects of Traditional Wet Cooking and Extrusion Cooking on the Condensed Tannin Profile of Sorghum Porridges

Treatment	Sorghum variety	Monomers (DP 1)[a]	Oligomers (DP 2-8)	Polymers (DP>8)	Total CT content[b]
Grain	Framida	0.5 c	18.5 a	40.1 a	59.1 a
	NS 5511	0.01 g	5.5 d	20.9 c	26.4 c
	Early Sumac	0.2 f	7.2 cd	29.3 b	36.6 b
Traditional, wet cooked porridge	Framida	0.4 d (80)[c]	10.8 b (58)	15.0 d (37)	26.2 c (44)
	NS 5511	0.02 g (200)	5.6 d (102)	6.9 f (33)	12.5 d (47)
	Early Sumac	0.3 e (150)	7.7 c (107)	12.8 e (44)	20.8 c (57)
Instant, extrusion cooked porridge	Framida	1.1 a (220)	5.2 d (28)	4.8 f (12)	11.2 e (19)
	NS 5511	0.8 b (8 000)	2.5 e (45)	1.1 f (5)	4.4 e (17)

285 [a]DP- Degree of polymerization
286 [b]Condensed tannin content (mg/g dry basis), obtained by normal phase HPLC method
287 (Gu et al., 2002).
288 [c]Figures in parentheses are % retentions in the porridges, of CT-HPLC monomers,
289 oligomers and polymers of those originally present in the grain
290 Values within the same column with different letters are significantly different at $P<0.05$.
291

292 The oligomers in extruded porridges interacted more than those in traditional
293 porridges because of the rigorous mechanical action that occurs in the extruder barrel
294 which cause the components to interact more strongly during processing. Extrusion
295 cooking reduces protein-protein and starch-protein associations (Fellows 2000), thus
296 leaving the protein to interact with CT polymers. During traditional porridge making
297 there is low shear, and hence the sorghum kafirin storage protein can crosslink via
298 disulphide bonds (Duodu et al 2003), which may reduce the opportunities for protein
299 interaction with the CTs. The increase in levels of monomers in the instant porridges
300 could be due to molecular fragmentation under high temperatures and shear in the
301 extruder, and possibly increased extractability of monomers as the food polymers unfold
302 (Alonso et al 2000), and preferentially interact with CT polymers).

303 The oligomers of condensed tannins are probably more bioavailable than the
304 polymers. In an *in vitro* study, Deprez et al (2001) showed that CTs up to trimers were
305 absorbed through intestinal cell mono layers. Furthermore, in an *in vivo* study with
306 weanling pigs, Gu et al (2008) showed that extrusion improved the bioavailability of
307 catechins in sorghum. In the present study, the CT oligomers and monomers were more
308 extractable than the polymers, which is an indication of potential bioavailability.

The reduction in CT polymers which occurs when sorghum is processed into porridges is probably the cause of the decreased *in vitro* antioxidant activity observed by Dlamini et al (2007). This agrees with the fact that high molecular weight CTs (with higher degree of polymerization) have been shown to have higher antioxidant capacity than low molecular weight CTs (Hagerman et al 1998).

CONCLUSIONS

Extruded sorghum porridges have increased CT monomers, and decreased CT oligomers and polymers compared to traditional cooked porridges and the grain. Hence, the antioxidant activity of phenolics in extruded sorghum porridges may be more readily available than in the conventionally cooked porridges.

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ABBREVIATIONS

HPLC: High performance liquid chromatography

DP: degree of polymerization

CTs: condensed tannins

ABTS: 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)

µmol TE/g: µmol Trolox Equivalents /g

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