EFFECT OF ENZYMIC HYDROLYSIS ON ANTIOXIDANT ACTIVITY AND POTENTIAL HEALTH BENEFITS OF TRADITIONAL AFRICAN SORGHUM-BASED FOODS

N.R. Dlamini[1,2,3], L.W. Rooney[1] , R.D. Waniska[1] and J.R.N. Taylor[2]

[1]Cereal Quality Laboratory, Soil & Crop Sciences Department, Texas A&M University, College Station, TX 77843-2474, USA.
[2]Department of Food Science, University of Pretoria, Pretoria 0002, South Africa.
[3]Department of Applied Biology and Biochemistry, National University of Science and Technology, Bulawayo, Zimbabwe
nomusand@gmail.com

INTRODUCTION

Sorghum is an important crop in semi-arid regions of the world. Sorghum varieties that contain condensed tannins have the highest level of antioxidants of any cereals analyzed (Gu et al., 2004), and are thus recognized as important sources of dietary antioxidants (Awika and Rooney 2004, Dykes and Rooney 2006). Measurable phenolic compounds, including tannins and total phenols in unprocessed sorghum grain are strongly correlated with antioxidant activity measured by the ABTS, DPPH and ORAC methods (Dykes et al., 2005). The processing of sorghum by fermentation, extrusion cooking and porridge cooking has been found to reduce measurable phenols and tannins, which lowers measurable antioxidant activity of the processed foods (Dlamini et al., 2007). The objective of the study was to determine how antioxidant activity of extrudates and porridges was affected by amylase and pepsin hydrolysis of these sorghum products. The Goni et al. (1997) method for estimating glycemic index of foods was used to hydrolyze the foods. Thus the study simulated digestion.

MATERIALS AND METHODS

Sorghum varieties and products

Two types of sorghums were used: condensed tannin containing NS 5511l; and white tan plant, non tannin sorghum Macia. The porridges and extrudates were prepared from whole milled grain according to the procedures described by Dlamini et al. (2007).

Enzyme hydrolysis of sorghum products

The sorghum extrudates and freeze dried porridges were milled to pass through a 1 mm screen using the UDY cyclone mill (Model 3010-030, UDY Corporation, Fort Collins, CO). The samples were hydrolyzed with amylase, pepsin and pepsin followed by amylase using the modified method of Goni et al. (1997). The enzyme blanks were prepared by incubating the samples in respective enzyme buffers without the enzyme. The hydrolysates were centrifuged and the supernatants retained for analysis, while the residues were dried at room temperature. All the samples were kept in air-tight packages at -20^{o}C until analysis.

Chemical analyses

The untreated grain, extrudates and porridges, and residues from the enzyme treatments were milled to pass through a 1 mm screen using UDY cyclone mill and then extracted for 2 hours

using acidified methanol (1 % conc. HCl in methanol) with constant shaking. The clarified extracts and supernatants (from enzyme hydrolysis) were analysed for total phenols and antioxidant activity. Total phenols were determined using the modified Folin-Ciocalteu method (Kaluza et al., 1980) using catechin as standard, while ABTS antioxidant activity was determined as described by Awika et al. (2003) with Trolox as standard.

Statistical Analyses

The experiments were done in triplicate, one-way analysis of variance (ANOVA) was determined and the means were separated using Fisher's least significant difference at $P<0.05$.

RESULTS AND DISCUSSION

The measurable phenols were significantly affected by sorghum type, processing and enzyme treatment. The tannin sorghum and its food products had higher levels of phenols than the non-tannin Macia (Table 1). Processing the sorghum into porridges and extrudates reduced phenol content. The observed decrease in measurable phenols is consistent with previous work (Dlamini et al., 2007). In tannin sorghums, the condensed tannins (procyanidins) bind to food components such as protein and carbohydrates, thus reducing their extractability. Prolamins of sorghum have high affinity for tannins (Emmambux and Taylor 2003). The major phenol interactions are reported to be non-covalent and comprise mainly of hydrogen bonding and hydrophobic interactions (Baxter et al., 1997). Thus theoretically, disruption of the food matrix using hydrolytic enzymes may release some of the phenols.

Pepsin followed by amylase treatments of extrudates and porridges significantly increased total phenols, compared to untreated, pepsin only and amylase only treated samples (Table 1). Total phenols (**TP**) for the enzyme treated samples were obtained by combining phenols solubilized by enzyme treatment [enzyme solubilized phenols (**ESP**)] and those extracted by acidified methanol from the residues [residue phenols (**RP**)]. For the untreated or control samples and grain, **TP** was determined in samples extracted with acidified methanol. The increase in phenol content of pepsin treated sorghum products indicated that phenols were released when protein was hydrolyzed. Although pepsin has broad specificity, studies show that it preferentially cleaves peptide bonds with aromatic amino acids (Schnaith 1989), thus effectively disrupting hydrophobic regions of the protein. Thus it can be inferred that hydrophobic associations of proteins and phenols are also weakened, and tannins may be released into solutions. In addition peptides and aromatic amino acids such as tyrosine and tryptophan are also released, and these react with Folin Ciocalteu reagent, thus increase measurable phenols (Schnaith 1989).

Table 1. Total phenols[a] solubilized by pepsin and amylase treatments and in residues extracted by acidified methanol in sorghum grain, extrudates and porridges

	Macia			NS 5511		
Sample/Treatment	**ESP**	**RP**	**TP**	**ESP**	**RP**	**TP**
Grain (Control)			**2.7 e**			**22.4 a**
Extrudate (Control)			**1.8 e**			**6.7 g**
Extrud-Pepsin	5.7 b	1.6 b	7.3 bc (78)[b]	7.5 b	4.2 c	11.7 d (64)
Extrud-Amylase	2.4 d	2.2 a	4.6 d (52)	4.5 d	5.5 b	10.0 e (45)
Extrud- Pep+Amyl	7.1 a	2.3 a	9.4 a (75)	9.2 a	5.2 b	14.4 c (64)
Porridge (Control)			**2.0 e**			**8.7 f**
Porr-Pepsin	3.5 c	1.6 b	5.1 d (69)	5.6 c	4.1 c	9.7 e (58)
Porr-Amylase	1.7 e	2.2 a	3.1 e (55)	3.2 e	8.3 a	11.5 d (28)
Porr-Pep+Amyl	5.1 b	1.6 b	6.8 c (75)	6.9 b	8.7 a	15.6 b (44)

ESP-(enzyme solubilized phenols)-phenols solubilized by enzymes (phenols in the supernatants); RP-residue phenols that were extracted with acidified methanol; TP-total phenols = ESP+RP; for controls, TP is for phenols extracted from the untreated samples using acidified methanol.

[a]Phenols expressed as mg catechin equivalents/g (mg CE/g), dry weight basis (Folin-Ciocalteu method).

[b]Data in parentheses after TP is per cent solubilized phenols.

Values within the same column with different letters are significantly different, $p<0.05$.

Abbreviations: Porr – porridge; Pep-Pespin; Amyl- amylase; Pep+Amyl-Pepsin followed by amylase treatment

Table 2. Antioxidant activity[a] solubilized by pepsin and amylase treatments and in residues extracted by acidified methanol in sorghum grain, extrudates and porridges

	Macia			NS 5511		
Sample/Treatment	**ESAO**	**RAO**	**TAO**	**ESAO**	**RAO**	**TAO**
Grain-Control			**22 e**			**384 a**
Extrudate-Control			**4 f**			**58 f**
Extrud-Pepsin	226 a	1 b	227 a (99)	212 a	31 d	243 c (87)
Extrud-Amyl	16 d	1 b	17 e (94)	52 c	64 c	116 d (45)
Extrud Pep+Amyl	128 b	4 a	132 b (97)	216 a	58 c	274 c (79)
Porridge-Control			**1 f**			**70 e**
Porr-Pepsin	96 b	8 a	105 c (91)	197 a	46 c	243 c (81)
Porr-Amyl	3 e	3 a	6 f (50)	10 d	101 b	111 d (9)
Porr-Pep+Amyl	79 c	0 b	79 d (100)	181 b	127 a	308 b (59)

ESAO-(Enzyme solubilized antioxidant activity): antioxidant activity solubilized by pepsin and amylase treatments (antioxidant activity of supernatants); RAO-(Residue antioxidant activity)-antioxidant activity of acidified methanol-extracts of residues; TAO- total antioxidant activity = ESAO+RAO; TAO of controls (untreated samples) were extracted with acidified methanol.

[a]Antioxidant activity (ABTS method) expressed as μmol Trolox Equivalents per g (μmol TE/g), dry basis (Awika et al 2003a). Data in parentheses after TAO is per cent solubilized antioxidant activity

Values within the same column with different letters are significantly different, $p<0.05$

Abbreviations: Porr –porridges; extrud-extrudates; Amyl- amylase; Pep+Amyl-Pepsin, followed by amylase

As expected, tannin sorghum and food products had high antioxidant activity than non tannin sorghum (Table 2). Processing sorghum reduced measurable antioxidant activity by 55-78% in tannin sorghum extrudates, while in porridges it was reduced by 45-78%. These observations are consistent with previous work (Dlamini et al., 2007), and are as a result of reduced extractability of phenols and tannins, the major antioxidants.

Pepsin alone and pepsin followed by amylase hydrolysis significantly increased antioxidant activity of porridges and extrudates (Table 2). In pepsin treated food products the highest antioxidant activity was in the supernatant [enzyme solubilized antioxidant activity (**ESAO**)], while in amylase only treated foods the antioxidant activity was located mainly in the residues and extracted with acidified methanol [residue antioxidant activity (**RAO**)]. Total antioxidant activity (**TAO**) for enzyme treated samples was obtained by combining **ESAO** and **RAO**; while in controls (untreated products) and grain **TAO** was obtained by extracting the samples with acidified methanol. As mentioned, pepsin hydrolysis may release bound phenols and aromatic amino acids such as tyrosine and tryptophan. Tyrosine and tryptophan have been observed to present high ABTS antioxidant activities at very low concentrations (Perez-Jimenez and Saura-Calixto 2006). The antioxidant activity of tyrosine and tryptophan (Meucci and Mele 1997, Gulcini 2007) overshadowed that of phenols; and thus probably played a significant role in the high antioxidant activity of pepsin treated Macia extrudates and porridges.

CONCLUSIONS

Hydrolysis of food by pepsin in the stomach has potential to improve antioxidant bioavailability of processed foods through freeing up of bound phenols and the release of antioxidant amino acids; thus protecting the alimentary system from free radicals generated during digestion.

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