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Effects of endosperm texture and cooking conditions on the in vitro starch digestibility of sorghum and maize flours

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Abstract

The effects of endosperm vitreousness, cooking time and temperature on sorghum and maize starch digestion in vitro were studied using floury and vitreous endosperm flours. Starch digestion was significantly higher in floury sorghum endosperm than vitreous endosperm, but similar floury and vitreous endosperm of maize. Cooking with 2-mercaptoethanol increased starch digestion in both sorghum and maize, but more with sorghum, and more with vitreous endosperm flours. Increasing cooking time progressively reduced starch digestion in vitreous sorghum endosperm but improved digestibility in the other flours. Pressure-cooking increased starch digestion in all flours, but markedly more in vitreous sorghum flour; probably through physical disruption of the protein matrix enveloping the starch. Irrespective of vitreousness or cooking condition, the *alpha*-amylase kinetic constant (k) for both sorghum and maize flours remained similar, indicating that differences in their starch digestion were due to factors extrinsic to the starches. SDS-PAGE indicated that the higher proportion of disulphide bond-cross-linked prolamin proteins and more extensive polymerisation of the prolamins on cooking, resulting in polymers of $M_r > 100$k, were responsible for the lower starch digestibility of the vitreous sorghum endosperm flour.
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Keywords: Vitreous endosperm; Floury endosperm; Starch digestibility; Sorghum; Maize; Cooking; Kafirin; Zein

1. Introduction

Grain sorghum (*Sorghum bicolor* L. Moench) is a very important source of dietary energy in Asia and Africa where it serves as principal staple for people in the semi-arid regions (ICRISAT/FAO, 1996). Sorghum, like other cereals, is rich in starch ($\geq 70\%$ with an approximately 75:25 amylopectin/amylose ratio) and should therefore be an optimal crop for industrial application in those parts of the world where it is grown (Horn et al., 1992; Zhan et al., 2003). Nevertheless, sorghum has remained industrially under-utilized. Attempts to optimise sorghum's use have resulted in an increase in the proportion of sorghum used as food, and in its exploitation as a cheap alternative source of fermentable extract in brewing (Goode and Arendt, 2003) and bioethanol processes (Zhan et al., 2003). Sorghum is also a potentially attractive energy source for the livestock industry (Rowe et al., 1999). While all sorghum starch is potentially digestible and technologically equivalent to maize starch, experience with livestock feeding (Rowe et al., 1999) and brewing (Goode and Arendt, 2003) suggests that starch in sorghum flour may be substantially less digestible. This constitutes a barrier to increased industrial utilization of sorghum.

The main protein constituents of sorghum grain, the kafirins (Taylor and Schüssler, 1986) exist in both monomeric and polymeric forms (El Nour et al., 1998; Oria et al., 1995). Polymeric kafirins are mainly formed by intermolecular disulphide cross-linking (Chandrashekar and Mazhar, 1999; Oria et al., 1995). Polymeric kafirins occur more in the vitreous endosperm fraction (Kumari and Chandrashekar, 1994), probably because the cysteine-rich γ- and β-kafirins abound in that part of the sorghum grain (Mazhar and Chandrashekar, 1995). Research suggests that they may be substantially less digestible than their

Abbreviations: 2-ME, 2-mercaptoethanol; R_{10}, initial velocity of hydrolysis; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SEM, scanning electron microscopy); HI, hydrolytic index; HMW, high molecular weight.

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monomeric counterparts (Duodu et al., 2002; Oria et al., 1995). Sorghum kafirins polymerize during cooking and the extent of polymerization appears to be greater than in maize (Duodu et al., 2003). There are indications that polymerized kafirin may impede sorghum starch granule gelatinisation and subsequent digestibility (Chandrashekar and Kirleis, 1988; Hamaker and Bugusu, 2003; Zhang and Hamaker, 1998). Additionally, the monomeric kafirin content and composition influences the extent of kafirin polymerization in sorghum (Oria et al., 1995). This suggests the possibility that differences in sorghum endosperm texture and protein composition may influence the starch digestion dynamics.

It is known that differences in moist heat processing methods can also cause variations in the digestion of starch in cereal flours (Abdelgadir et al., 1996; Mangala and Tharanathan, 1999; Kotarski et al., 1992). Thus, the aim of the present work was to determine and compare the influence of endosperm texture and cooking conditions and the possible role of the endosperm proteins on in vitro starch hydrolysis in sorghum and maize flours.

2. Experimental

2.1. Grains and preparation of materials

Grains of NK 283, a red tannin-free sorghum cultivar, were decorticated using a carborundum cone abrasive rice pearler (Miag, Braunschweig, Germany). Degermed pericarp-free grains were then used for preparing the vitreous and floury endosperm flours. The vitreous and floury sorghum endosperms were separated manually by carefully cracking the grains in a laboratory mortar with a pestle, then sieving to separate the coarse vitreous endosperm particles from the finer (powdery) floury endosperm. Floury endosperm particles were smaller than 200 μm, while the smallest vitreous endosperm particles were larger than 200 μm. This method of endosperm fractionation was effective and less laborious than manual dissection. Separation of floury and vitreous endosperm particles was confirmed using scanning electron microscopy (SEM) (data not shown). To enable comparison of rates of starch digestion, the vitreous and floury endosperm particles were milled separately to a common maximum particle size in a water-cooled coffee grinder to pass through a 175 μm sieve. SEM showed that after grinding, the cellular structure of the floury and vitreous endosperm particles remained essentially intact (data not shown).

The maize used in this study was a commercial sample of white maize purchased already degermed and decorticated. Separation of maize endosperms was as for sorghum. The floury and vitreous sorghum endosperm flours contained 5.13±0.04% and 9.56±0.03% crude protein (N×6.25) (dry basis) respectively, while the corresponding values for the two maize flours were 5.92±0.06% and 7.77±0.04% respectively. Protein content was determined by combustion analysis using a Leco FP-528 nitrogen/protein analyser (Leco Corporation, St Joseph, MI).

2.2. Starch digestion

Samples of flours equivalent to 100 mg starch were mixed vigorously in large test tubes (2.1×15 cm) with distilled water (5 ml) then boiled (10 min) in a water bath (96 °C). After cooling to 40 °C (in a water bath) the mixture was combined with diluted porcine pancreatic *alpha*-amylase (No A-3176, Sigma) solution (5 ml in 0.05 M tris–maleate buffer, pH 6.9) to give a final enzyme concentration of 2 U/ml. Contents of each test tube were mixed vigorously then incubated at 39 °C for 150 min, with mixing every 5 min. Samples (0.7 ml) were withdrawn at 10, 20, 30, 45, 60, 90 and 150 min. In experiments to study the effects of reducing agents, flour samples were boiled in distilled water containing 5 mM 2-mercaptoethanol (2-ME). All experiments were repeated three times.

The amount of starch digested was assayed as follows: samples (0.7 ml) collected in Eppendorf tubes were centrifuged at 7,200g (4 min). Aliquots (0.2 ml) of the supernatants were added to test tubes kept at 50 °C in a water bath. Soluble dextrins in supernatants were then digested to glucose using 10 U (0.3 ml) of *Aspergillus oryzae* glucoamylase (No A-9268, Sigma) solution in 0.2 M sodium acetate buffer (pH 4.5). Digestion was for 30 min at 50 °C. Glucose in digests was determined with the dinitrosalicylic acid reagent. Amounts of starch digested were calculated by multiplying the amount of glucose (in mg) by a factor of 0.9. Starch digestion was expressed in percentage of the amount of starch at the start of the reaction, as determined by the Megazyme Total Starch Assay Procedure (amyloglucosidase/*alpha*-amylase method) (Megazyme International Ireland, Bray, Ireland).

The non-linear model of Goni et al. (1997) was applied to describe the kinetics of starch hydrolysis. The model is described by the first order equation:

$$C = C_\infty(1 - e^{-kt})$$

where C corresponds to the percentage of starch hydrolysed at time t, C_∞ is the equilibrium percentage of starch hydrolysed after 150 min, k is the kinetic constant and t is the time (min). In these experiments t was chosen as 30 min, the time at which the starch hydrolysis curve approached steady state kinetics. Following Goni et al. (1997), the parameters C and k were estimated for each sample and each treatment based on data obtained during the in vitro hydrolysis procedure. Calculation of the samples' hydrolysis indices (HI%), the proportion of flour starch that is theoretically digestible, then followed as reported by both Goni et al. (1997) and Frei et al. (2003). First the area under the hydrolysis curve (AUC) was calculated using the equation (Goni et al., 1997; Frei et al., 2003):

$$\mathrm{AUC} = C_\infty(t_f - t_0) - (C_\infty/k)[1 - \exp[-k(t_f - t_0)]]$$

where C_∞ corresponds to the equilibrium percentage of starch hydrolysed after 150 min, t_f is the final time (150 min), t_0 is the initial time (0 min) and k is the kinetic constant. The hydrolysis index was then calculated by dividing the area under the hydrolysis curve of each sample by the corresponding area of a reference sample (in this case soluble starch).

The effects of cooking time and temperature on starch digestion in the flours were also studied. Experimental conditions for cooking time study were similar to those described above, except that flour samples were used at 200 mg starch equivalent and cooking times were 5, 10 or 30 min. For temperature studies, flour samples were cooked at 78, 96, and 110 °C (pressure-cooking). Except for differences in cooking temperatures, all conditions of study were the same as for cooking time. Cooking at 78 and 96 °C was for 30 min, while pressure-cooking was for 10 min.

2.3. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The discontinuous tris–HCl/glycine buffer system of Laemmli (1970) was employed, with 3.9 and 12% (w/v) polyacrylamide as stacking and resolving gels, respectively. Electrophoresis was conducted in a Protean II xi vertical cell and with a 1000 Powerpac (Bio-Rad Laboratories, Hercules, CA, USA) used at a constant current of 50 mA. Materials remaining after flour digestion with *alpha*-amylase were washed and freeze-dried. Protein extracted from the freeze-dried material was used for SDS-PAGE. Extraction of protein was with 4% (w/v) SDS in 0.3 M Tris–HCl buffer (pH 6.8) containing 20% glycerol. For SDS-PAGE under reducing conditions, the extraction buffer contained, in addition, 2-ME (10% v/v). Sample extraction was in a boiling water bath for 30 min. Sample loading was 60 μg protein. Gels were stained with Coomassie Blue R250 (0.1%). Gels were scanned using a GS-300 transmittance reflectance densitometer (Hoefer Scientific Instruments, San Francisco, CA) in the absorbance mode. The resolving gel of 12% (w/v) polyacrylamide used in this study allowed the separation of proteins with M_r of 14 to 100k.

2.4. Statistical analyses

Data were analyzed by two- and three-way analyses of variance (ANOVA) (Cohen, 1988). Means that differed significantly were identified by the least significant difference (LSD) test.

3. Results and discussion

The assay method for in vitro starch digestibility was developed to determine whether there were effects of endosperm type and cooking conditions on the rate of digestion by using a very low concentration of *alpha*-amylase. It was not intended to mimic animal digestion or conditions used in processes such as brewing.

3.1. Effect of endosperm vitreousness

Endosperm vitreousness considerably ($p<0.001$) influenced sorghum and maize flour starch digestion by alpha-amylase (Fig. 1). Mean starch digestion at 10, 20, and 30 min was 69.4, 79.5 and 81.5% respectively for the vitreous sorghum endosperm flour in contrast to 76.8, 86.2 and 88.2% for the floury endosperm flour (Fig. 1(A)). Mean starch hydrolysis, after 150 min, was 87.0 and 94.1% for the sorghum vitreous and floury endosperm flours respectively. The rates of starch hydrolysis (R_{10}) calculated for the first 10 min of assay when the rate of digestion was highest (Fig. 1(A)) were 1.42 and 1.50 mg/ml/min (Table 1), respectively for the sorghum vitreous and floury endosperm

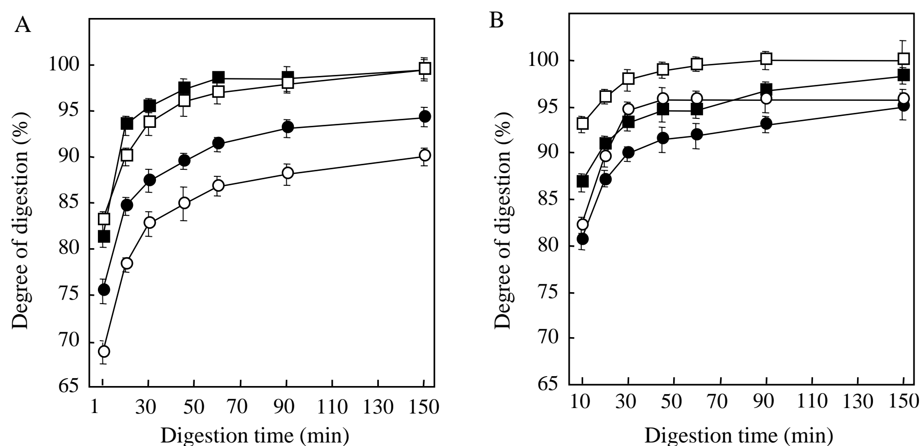

Fig. 1. Effect of 2-mercaptoethanol on starch digestion in vitreous and floury endosperms: (A), sorghum; (B), maize. Bars are standard deviations ($n=3$). ○, vitreous endosperm flour conventionally cooked; ●, floury endosperm flour conventionally cooked; □, vitreous endosperm flour cooked in the presence of 2-ME; ■, floury endosperm flour cooked in the presence of 2-ME.

Table 1
Effect of grain vitreousness and cooking with 2-ME on kinetic parameters of sorghum and maize vitreous and floury endosperm flours digestion by porcine pancreatic α-amylase

Grain type	Endosperm type	Heating condition	R_{10}[a] (mg/ml/min)	k[b]	Starch digestion (%)[c]	HI (%)[d]
Sorghum	Vitreous	Without 2-ME	1.42*a* ± 0.04	0.0190*a*	87.0*a* ± 0.9	90.6*a* ± 1.2
		With 2-ME	1.66*c* ± 0.03 (+16.9)[e]	0.0189*a*	99.5*c* ± 1.1	99.6*c* ± 0.8
	Floury	Without 2-ME	1.50*b* ± 0.02	0.0190*a*	94.1*b* ± 1.1	93.4 *a*, *b* ± 1.1
		With 2-ME	1.62*c* ± 0.01 (+8.0)	0.0190*a*	99.5*c* ± 1.2	99.6*c* ± 1.0
Maize	Vitreous	Without 2-ME	1.61*c* ± 0.02	0.0192*a*	95.6*b* ± 1.0	95.7*b* ± 0.8
		With 2-ME	1.86*e* ± 0.04 (+15.5)	0.0192*a*	100.0*c* ± 2.1	100.1*c* ± 1.3
	Floury	Without 2-ME	1.61*c* ± 0.04	0.0191*a*	94.9*b* ± 1.3	95.0*c* ± 1.2
		With 2-ME	1.73*d* ± 0.03 (+7.5)	0.0192*a*	98.3*c* ± 0.9	98.4*c* ± 0.8

All results are means of three replicate experiments; values in the same column followed by the same letter are not significantly different at $p = 0.05$.
[a] R_{10} = velocity of flour starch digestion calculated for the 1st 10 min of reaction. Values are presented in mg/ml/min (i.e. amount of starch (in mg) digested per ml of reaction mixture per minute of reaction time).
[b] k = flours' kinetic (digestibility) constants (i.e. intrinsic susceptibility of flours to digestion by α-amylase).
[c] Values are in vitro values of starch digestion obtained experimentally after 150 min of assay.
[d] Hydrolytic index, proportion of flour starch which, under the conditions of study, is theoretically digestible. Calculated from kinetic data as per Goni et al. (1997).
[e] Figures in brackets represent percentage increase in digestion velocities due to addition of 2-ME (5 mM) to cooking broth.

flours. Endosperm vitreousness also modulated maize flour starch digestion. However, unlike sorghum, the rate of maize flour starch digestion was higher with the vitreous endosperm (Fig. 1(B)). Mean starch digestion after 10, 20 and 30 min was 80.7, 89.4 and 94.6% respectively for the maize vitreous endosperm flour but 79.5, 86.1 and 89.9% for the maize floury endosperm flour (Table 1). However, after 150 min digestion, the vitreous and floury maize endosperm flours had statistically the same starch digestion values. This finding may appear to contradict reports showing that the rate of starch digestion in maize meal is inversely related to kernel vitreousness (Correa et al., 2002; Philippeau et al., 1999). However, these studies are not directly comparable, since digestion of raw starch in the coarsely ground meal from maize cultivars of different degrees of vitreousness were compared, i.e. the starch was not gelatinised and the endosperm from individual samples was not separated into vitreous and floury components and no attempt was made to obtain endosperm particles of a common size.

Cooking with 2-ME significantly altered amylase reaction kinetics (Table 1) and increased rates of flour digestion (Fig. 1) in both sorghum and maize. The R_{10} value of sorghum vitreous endosperm of 1.66 mg/ml/min was some 17% higher than that for flour cooked without 2-ME. Similarly, the initial velocity of starch digestion (R_{10}) in sorghum floury endosperm flour cooked with 2-ME was 1.62 mg/ml/min, or 8% higher than that for the flour cooked without 2-ME. Starch digestion after 150 min was 99.5% for both sorghum endosperm flours, very markedly ($p < 0.001$) increased compared to conventional cooking. The final starch digestion was 100 and 98.3%, respectively, for the maize vitreous and floury endosperm flours cooked with 2-ME also a significant increase ($p < 0.005$). Cooking with 2-ME increased maize vitreous and floury endosperm flours R_{10} values by 15.5 and 7.5%, respectively (Table 1). It is notable that 2-ME improved starch digestion (Fig. 1) and R_{10} values (Table 1) more in the vitreous endosperm flours of both grains compared with their individual floury endosperm flours. Cooking with 2-ME improved starch digestion more (Fig. 1 and Table 1) in both sorghum flours compared to the corresponding maize flours.

The steady state kinetic constant (k) of amylolysis has been proposed as a reliable index of the inherent susceptibility of flour starches to amylase hydrolysis (Frei et al., 2003; Goni et al., 1997). To ensure that variances in the amylase digestibilities of the flours were not due to differences in the innate properties of their starches, we studied the kinetic constants of their hydrolysis by *alpha*-amylase. The sorghum floury and vitreous endosperm flours gave identical kinetic constants (Table 1), as did the maize flours. Also the kinetic constants of the flours were not modified by the presence of 2-ME in the cooking medium. There have been suggestions that differences in physico-chemical properties may exist between starches found in the vitreous and floury endosperms of cereal grains (Palmer, 1989). However, the fact that the vitreous and floury endosperms of the grains gave similar kinetic constants suggests that differences in the digestibility of their flours were probably due to extrinsic factors.

Starch hydrolysis follows first order kinetics (Frei et al., 2003; Goni et al., 1997), whereby catalytic velocities increase with additional substrate until a maximum value is reached. Since 2-ME had no effect on the intrinsic *alpha*-amylase susceptibility of the sorghum flour starches, it appears that the reducing agent improved starch digestion by increasing substrate accessibility to *alpha*-amylase.

The roles of disulphide bond-mediated protein polymerization in the flours' starch digestibility behaviours were investigated using SDS-PAGE (Table 2). Non-reducing SDS-PAGE showed that protein in conventionally cooked (without 2-ME) sorghum floury endosperm flour contained more monomeric β-, α- and γ-kafirins than that from

Table 2
Densitometry data of SDS-PAGE of proteins in material remaining after sorghum and maize flours were subjected to cooking (with or without 2-ME) and then digestion by porcine pancreatic α-amylase

	Density peak values[a]															
	Non-reducing SDS-PAGE								Reducing SDS-PAGE							
	Floury endosperm				Vitreous endosperm				Floury endosperm				Vitreous endosperm			
	−2ME		+2ME		−2ME		+2ME		−2ME		+2ME		−2ME		+2ME	
Sorghum prolamins																
β-Kafirin	34		47 (+13)[c]		7		23 (+16)		134 [+100][d]		138 [+91]		111 [+104]		134 [+111]	
α-Kafirin	129	47.2%[b]	218 (+89)	47.0%	110	38.4%	261 (+151)	54.9%	337 [+208]	71.0%	349 [+131]	69.3%	390 [+280]	76.5%	359 [+98]	75.0%
γ-Kafirin	83		171 (+88)		65		185 (+120)		214 [+131]		188 [+17]		198 [+133]		198 [+13]	
Dimers	NB[e]		109 (+109)		NB		69 (+69)		82 [+82]		94 [−15]		62 [+62]		63 [−6]	
Trimers	149		211 (+62)		210		224 (+14)		93 [−56]		102 [−109]		82 [−128]		84 [−140]	
Tetramers	121		172 (+51)		82		93 (+11)		105 [−16]		103 [−69]		71 [−11]		83 [−10]	
Total Density	518		928 [+79.2][f]		474		855 [+80.4]		965 [+447] {+86.3}[g]		974 [+46] {+5.0}		914 [+440] {92.8}		921 [+66)] {7.7}	
Maize prolamins																
β-Zein	81		127 (+46)		24		64 (+40)		149 [+68]		175 [+48]		176 [+152]		177 [+113]	
α-Zein	145	49.7%	211 (+66)	49.1%	166	37.4%	211 (+45)	42.7%	366 [+221]	65.4%	361 [+150]	61.7%	375 [+209]	67.0%	379 [+168]	68.1%
γ-Zein	100		170 (+70)		96		163 (+67)		202 [+102]		209 [+39]		211 [+115]		182 [+19]	
Dimers	121		172 (+51)		121		199 (+78)		111 [−10]		114 [−58]		149 [+28]		128 [−71]	
Trimers	112		174 (+62)		147		181 (+34)		100 [−12]		179 [+5]		132 [−15]		128 [−53]	
Tetramers	97		180 (+83)		211		211 (+0)		169 [+72]		169 [−11]		94 [−117]		89 [−122]	
Total Density	656		1034 [57.6]		765		1029[34.5]		1097 [+441] {67.2}		1207[+173] {16.7}		1137[+372] {48.6}		1083 [+54] {5.2}	

[a] Densitometer readings.
[b] Bold figures represent the total prolamin monomers as a proportion of total density.
[c] Figures in parentheses (()) represent increase in density values of specific protein band and total bank density as a result of cooking with for 30 min.
[d] Figures in brackets ([]) represent increases in the density values of specific protein bands due to reducing SDS-PAGE.
[e] NB = no band.
[f] Bracketed figures on a shaded background represent the percentage increase in the total protein band density values of the flour samples resulting from cooking under reducing conditions (with 2-ME).
[g] The figures in brackets ({}) represent percentage increase in total protein band density values in reducing SDS-PAGE when compared to corresponding values in non-reducing SDS-PAGE.

the vitreous endosperm flour, while protein in the vitreous endosperm contained slightly more in total of the oligomers than the floury endosperm. More of the individual kafirin monomers are said to be found in vitreous endosperm fractions of uncooked sorghum (Chandrashekar and Mazhar, 1999; Mazhar and Chandrashekar, 1995), as was also found to be the case in the materials used in this study (data not shown). The fact that the proteins of the cooked sorghum floury endosperm fraction contained a higher proportion of α-, β-, and γ-kafirin monomers than the vitreous endosperm protein and the vitreous endosperm contained a slightly higher proportion of oligomers than the floury endosperm suggests that polymerization as a result of cooking was more intensive in the vitreous endosperm fraction. Bound β-kafirin occurs almost exclusively in polymers as a cross-linker of kafirin oligomers (El Nour et al., 1998).

SDS-PAGE under non-reducing conditions also showed that prolamin monomer levels increased substantially in all protein fractions of sorghum and maize endosperm flours when 2-ME was added to the cooking medium (Table 2). In addition, differences in total band densities between reducing and non-reducing SDS-PAGE were far lower (relative to corresponding values for the conventionally cooked flours), indicating that the extent of disulphide-cross-linking reduced appreciably when flours were cooked in the presence of 2-ME. This indicates that 2-ME improved flour starch digestion and HI by preventing or reducing disulphide-bonded polymerization of the prolamin proteins, thus increasing flour starch accessibility to amylase. More prolamin monomers and oligomers (dimers, trimers and tetramers) occurred in maize flours than in sorghum flours, irrespective of vitreousness or cooking condition. Similarly, the total band density values show that the amount of prolamin, which was detectable by SDS-PAGE, both under non-reducing and reducing conditions, was always higher for the maize flours compared to the corresponding sorghum flours. Since the separating gel could only allow the detection of protein bands with M_r 100k or less, it can be assumed that the lower total band densities of the sorghum flours (compared to their individual maize counterparts) was because they contained more high molecular weight (HMW) protein polymers (i.e. proteins with $M_r > 100k$, too large to penetrate the gel). The significance of this finding is that there was probably less HMW protein polymer formation on cooking in the maize flours when compared to the sorghum flours. The results additionally indicate that the lower prolamin monomer and oligomer content of the sorghum flours probably resulted from a possibly more extensive or rigorous disulphide bonded-polymerization reaction in wet cooked sorghum compared to maize. The above strengthens our earlier suggestion that the higher starch digestibility of the maize flours was due to higher substrate availability rather than to higher innate digestibility.

3.2. *Effects of cooking time*

Cooking time markedly ($p < 0.001$) influenced sorghum and maize flour starch digestion (Fig. 2). For floury and vitreous sorghum endosperm, starch digestion after the first 10 min in the cooking time was in the order 30 min < 10 min < 5 min (Table 3), with hydrolysis rates increasing as cooking time reduced. This order persisted over the 150 min digestion period for the sorghum vitreous endosperm, but changed after 30 min of digestion for the floury endosperm flour (Fig. 2). Mean starch digestion after 150 min was 96.8, 94.4, and 93.9% respectively for floury endosperm flours cooked for 30, 10 and 5 min but only 82.5, 84.9, and 87.4% for the corresponding vitreous endosperm samples. In contrast, for the maize flours, starch digestion rates generally increased with cooking time.

HI of the flours followed the same trends as their starch digestion values (Table 3), while cooking time had no

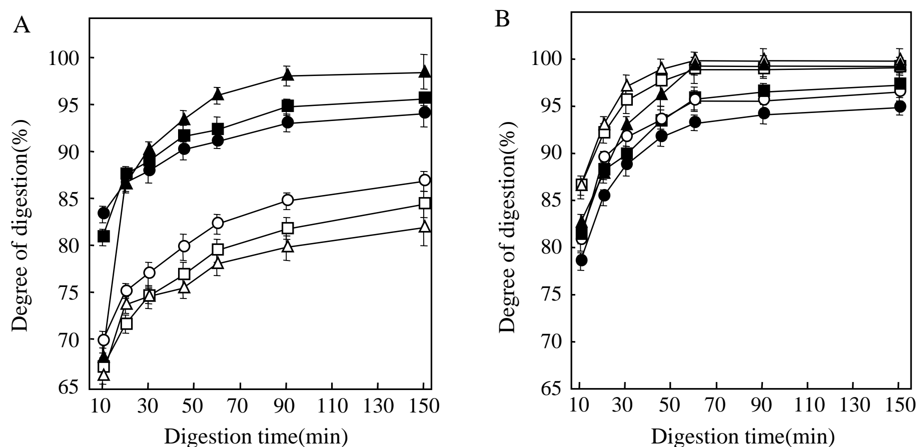

Fig. 2. Effect of cooking time on starch digestion in vitreous and floury endosperms: (A), sorghum; (B), maize. Bars are standard deviations ($n = 3$). ○, vitreous endosperm flour cooked for 5 min; ●, floury endosperm flour cooked for 5 min; □, vitreous endosperm flour cooked for 10 min; ■, floury endosperm flour cooked for 10 min; △, vitreous endosperm flour cooked for 30 min; ▲, floury endosperm flour cooked for 30 min.

Table 3
Effect of grain vitreousness and cooking time on the kinetic parameters of sorghum and maize vitreous and floury endosperm flours digestion by porcine pancreatic α-amylase

Grain type	Endosperm type	Cooking time (min)	R_{10}[a] (mg/ml/min)	k[b]	Starch digestion (%)[c]	HI (%)[d]
Sorghum	Vitreous	5	1.37*a* ± 0.03 (−1.5)[e]	0.0187*a*	87.4*b* ± 0.4	87.3*b* ± 1.0
		10	1.35*a* ± 0.01	0.0188*a*	84.9*a*,*b* ± 1.0	84.9*a*,*b* ± 1.4
		30	1.33*a* ± 0.02 (+1.5)	0.0188*a*	82.5*a* ± 1.2	82.4*a* ± 2.0
	Floury	5	1.69*c*,*d* ± 0.02 (+4.3)	0.0189*a*	93.9*c* ± 0.9	94.7*c* ± 1.5
		10	1.62*b*,*c* ± 0.04	0.0188*a*	94.4*c* ± 1.1	95.8*c* ± 0.7
		30	1.37*a* ± 0.02 (−15.4)	0.0189*a*	96.8*c*,*d*,*e* ± 2.1	99.6*d* ± 1.9
Maize	Vitreous	5	1.62*b*,*c* ± 0.03 (−6.4)	0.0192*a*	96.4*c*,*d*,*e* ± 1.3	95.7*c* ± 0.9
		10	1.73*d* ± 0.02	0.0191*a*	99.0*e* ± 1.8	98.8*d* ± 1.1
		30	1.73*d* ± 0.04 (+0.0)	0.0191*a*	99.9*e* ± 0.9	100*d* ± 1.3
	Floury	5	1.57*b* ± 0.02 (−3.6)	0.0191*a*	94.8*c* ± 1.2	94.3*c* ± 0.8
		10	1.63*b*,*c* ± 0.03	0.0191*a*	97.2*d*,*e* ± 0.8	96.6*c*,*d* ± 1.2
		30	1.65*c*,*d* ± 0.02 (+1.2)	0.0191*a*	99.3*e* ± 1.0	99.6*d* ± 0.9

All results are means of three replicate experiments; values in the same column followed by the same letter are not significantly different at p = 0.05.
[a] R_{10} = velocity of flour starch digestion calculated for the 1st 10 min of reaction. Values are presented in mg/ml/min (i.e. amount of starch (in mg) digested per ml of reaction mixture per minute of reaction time).
[b] k = flours' kinetic (digestibility) constants (i.e. intrinsic susceptibility of flours to digestion by α-amylase).
[c] Values are in vitro values of starch digestion obtained experimentally after 150 min of assay.
[d] Hydrolytic index, proportion of flour starch which, under the conditions of study, is theoretically digestible. Calculated from kinetic data as per Goni et al. (1997).
[e] Figures in brackets represent percentage increase or decrease in digestion velocities due to cooking for 5 or 30 min.

detectable influence on the kinetic constants (k) of the flours, indicating that the intrinsic nature of the starch in the flours was not markedly altered by varying the cooking time.

SDS-PAGE under non-reducing conditions showed that free kafirin monomer levels decreased as cooking time increased from 5 to 30 min irrespective of the sorghum flour type (Table 4). Increasing cooking time from 5 to 30 min caused substantial increases in kafirin trimer and tetramer levels for the floury endosperm flour but produced a decrease in the sorghum vitreous endosperm flour, indicating that polymerization reactions to produce HMW polymers were probably more extensive in the sorghum vitreous endosperm flour. The total amounts of kafirin detectable after SDS-PAGE (total band density of $M_r < 100$k) fell by approx. 22 and 32% for the floury and vitreous endosperm flour, respectively, as cooking time was increased to 30 min.

Under non-reducing SDS-PAGE, the patterns of zein distribution, for the two maize flour types, were similar to the sorghum flours, except that the amount of prolamin with $M_r \leq 100$k was substantially higher for the maize floury endosperm flour. Further, the total protein band density values were always higher with the maize vitreous endosperm flour compared to the maize floury endosperm flour. With SDS-PAGE under reducing conditions all the protein bands increased in density for both sorghum and maize flours. Nevertheless, total density figures followed the same patterns as was observed under non-reducing conditions, whereby the cumulative densities of all protein bands were higher for all 5 min-cooked flours of both sorghum and maize endosperm.

The fact that flour prolamin monomer contents were lower in the samples cooked for 30 min compared with those cooked for 5 min suggests that these proteins were converted to higher molecular weight forms through disulphide-bonding. This is supported by the substantial increase in these proteins when the flours were subjected to reducing conditions. On the other hand, the total band densities of the proteins, under reducing SDS-PAGE, were substantially higher suggesting that high proportions of cooked flour protein occurred in the form of HMW polymers, which, because of their large sizes, could not enter the separating gel and thus were not detected. The reduction of disulphide bonds in those proteins would thus be responsible for the additional protein detected under reducing conditions. Both under non-reducing and reducing SDS-PAGE, all sorghum and maize flours cooked for 5 min gave higher total band densities than their longer-cooked counterparts. This suggests that the flours cooked for 30 min contained more prolamin polymers with reduction-resistant bonds. Reduction-resistant bonds also appeared to occur more in the vitreous sorghum endosperm flour residue as deduced from the lower cumulative density values of its proteins after reducing SDS-PAGE.

3.3. Effects of cooking temperature

Cooking temperature influenced ($p < 0.001$) the *alpha*-amylase digestibility profiles of the cereal flours (Fig. 3). Endosperm vitreousness also considerably influenced ($p < 0.001$) the manner in which flour digestion kinetics responded to cooking temperature. Pressure-cooking made flours of both cereals more susceptible to *alpha*-amylase. For sorghum, R_{10} was 1.40 and 1.43 mg/ml/min, respectively for the 78 and 96 °C-cooked vitreous endosperm flour but 1.67 mg/ml/min with pressure-cooking (Table 5). Similar improvements in the initial rates of flour amylolysis

Table 4
Densitometry data of SDS-PAGE of proteins remaining after sorghum and maize flours were subjected to cooking for different durations of time and digestion by porcine pancreatic α-amylase

	Density peak values[a]							
	Non-reducing SDS-PAGE				Reducing SDS-PAGE			
	Floury endosperm		Vitreous endosperm		Floury endosperm		Vitreous endosperm	
	5 min	30 min	5 min	30 min	5 min	30 min	5 min	30 min
Sorghum prolamins								
β-Kafirin	109	33 (−76)[c]	11	7 (−4)	128 [+19][d]	91 [+68]	100 [+89]	111 [+104]
α-Kafirin	191 } 71.2%[b]	135 (−56) } 43.9%	137 } 40.9%	75 (−62) } 36.4%	356 [+165] } 59.8%	347 [+212] } 65.7%	360 [+223] } 72.8%	358 [+183] } 75.1%
γ-Kafirin	137	43 (−84)	57	43 (−14)	174 [+37]	155 [+112]	173 [+116]	161 [+118]
Dimers	NB[e]	NB	NB	NB	135 [+135]	98 [+98]	66 [+66]	62 [+62]
Trimers	132	149 (+17)	176	145 (−31)	152 [+20]	115 [−34]	95 [−81]	80 [−65]
Tetramers	45	121 (+76)	120	73 (−47)	155 [+110]	97 [−2]	76 [−44]	67 [−6]
Total density	614	481 (−133)	501	343(−158)	1100 [+486]	903 [+422]	870 [+369]	839 [+496]
		[–21.7][f]		[–31.5]	{79.2}[g]	{87.7}	{73.7}	{144.6}
Maize prolamins								
β-Zein	33	18 (−15)	28	17 (−1)	173 [+140]	140 [+122]	154 [+126]	148 [+137]
α-Zein	239 } 50.1%	141 (−98) } 27.0%	263 } 38.4%	221 (−42) } 37.3%	366 [+127] } 58.6%	361 [+220] } 59.9%	373 [+110] } 61.5%	370 [+159] } 65.1%
γ-Zein	113	16 (−97)	41	26 (−15)	190 [+77]	190 [+174]	190 [+49]	182 [+156]
Dimers	120	68 (−52)	171	133 (−38)	165 [+45]	142 [+74]	153 [−18]	128 [−5]
Trimers	137	235 (+98)	171	151 (−20)	168 [+31]	131 [−104]	132 [−39]	128 [−23]
Tetramers	127	170 (+43)	190	159 (−31)	181 [+54]	190 [+20]	163 [−57]	172 [+11]
Total density	769	648 (−121)	864	707 (−157)	1243 [+474]	1154[+506]	1165 [+30]	1128 [+42]
		[–18.7]		[–18.2]	{61.6}	{78.1}	{34.8}	{59.4}

[a] Densitometer readings.
[b] Bold figures represent the total prolamin monomers as a proportion of total density.
[c] Figures in parentheses (()) represent increases or decreases in density values of specific protein band and total band density as a result of cooking with for 30 min.
[d] Figures in brackets ([]) represent increases or decreases in the density values of specific protein bands due to reducing SDS-PAGE.
[e] NB = no band.
[f] Bracketed figures on a shaded background represent the percentage decrease in the total protein band density values of the flour samples resulting from cooking for 30 min compared to 5 min.
[g] The figures in brackets ({}) represent percentage increase in total protein band density values in reducing SDS-PAGE when compared to corresponding values in non-reducing SDS-PAGE.

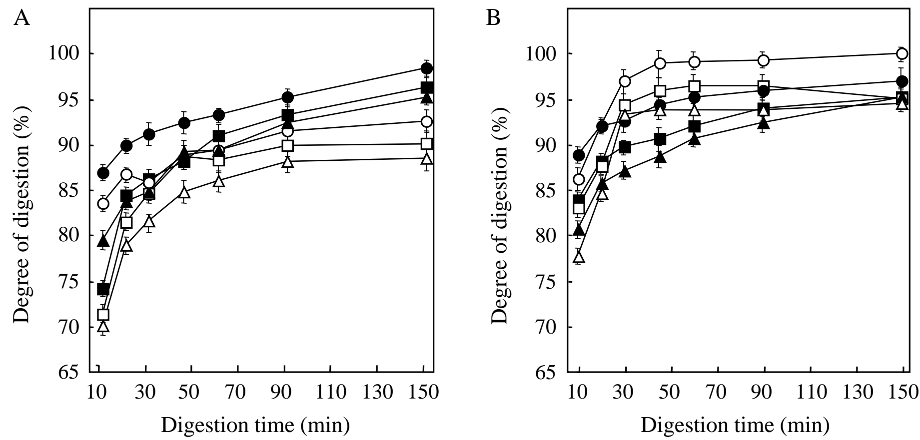

Fig. 3. Effect of cooking temperature on starch digestion in vitreous and floury endosperms: (A), sorghum; (B), maize. Bars are standard deviations ($n=3$). ○, pressure-cooked vitreous endosperm flour; ●, pressure-cooked floury endosperm flour; □, vitreous endosperm flour cooked at 96 °C; ■, floury endosperm flour cooked at 96 °C; △, vitreous endosperm flour cooked at 78 °C; ▲, floury endosperm flour cooked at 78 °C.

were observed with the sorghum floury endosperm as well as both maize flours.

The *alpha*-amylase kinetic constants (k) of individual sorghum and maize samples were identical (Table 5), suggesting that the physico-chemical states of their starches were not influenced by the differences in cooking temperature. Intrinsic *alpha*-amylase susceptibility and substrate availability are the most important factors that determine the potential rates of cereal starch hydrolysis by *alpha*-amylase (Abdelgadir et al., 1996). Thus the significantly ($p<0.001$) higher starch digestibilities of pressure-cooked samples may be due to enhanced substrate accessibility. It is probable that this was through physical disruption of the structural protein network. This is supported by reports on the use of disruptive processing methods to improve the amylase digestibilities of both sorghum and maize for animal feeding (Abdelgadir et al., 1996; Kotarski et al., 1992; Twomey et al., 2003). Pressure-cooking probably generated strong internal pressures within starch granules, causing them to expand abnormally and, in the process, disrupted the integrity of the enveloping protein barrier. The fragmentation/disruption of sorghum matrix protein by starch granules has been reported for popped sorghum (Parker et al., 1999).

With SDS-PAGE under non-reducing conditions, protein bands were more prominent in the order 78 °C > 96 °C > pressure-cooking for both cereals (Table 6). The α-, β- and γ-prolamin monomer levels in all the 78 °C-cooked flours were higher than at 96 °C, while levels for the corresponding pressure-cooked flours were lower. There was also a decrease in the densities of all prolamin oligomers in all the pressure-cooked flours compared to values at 96 °C. Under non-reducing SDS-PAGE, dimers could not be detected in

Table 5
Effect of cooking temperature and endosperm vitreousness on kinetic parameters of sorghum and maize vitreous and floury endosperm flours digestion by porcine pancreatic α-amylase

Grain type	Endosperm type	Heating temp. (°C)	R_{10}[a] (mg/ml/min)	k[b]	Starch digestion (%)[c]	HI (%)[d]
Sorghum	Vitreous	78	1.40*a* ± 0.02 (−2.1)[e]	0.0189*a*	88.6*a* ± 1.8	88.4*a* ± 1.6
		96	1.43*a* ± 0.01	0.0190*a*	90.2*a* ± 0.4	89.6*a* ± 1.4
		Press.	1.67*d* ± 0.03 (16.8)	0.0191*a*	92.6*a*,*b* ± 0.8	91.6*a* ± 1.2
	Floury	78	1.59*c* ± 0.02 (+7.4)	0.0187*a*	95.2*b*,*c* ± 1.0	95.9*b*,*c* ± 0.9
		96	1.49*b* ± 0.03	0.0188*a*	96.4*c* ± 1.2	95.6*b*,*c* ± 1.1
		Press.	1.74*e* ± 0.02 (17.6)	0.0188*a*	98.5*c*,*d* ± 0.4	98.5*c*,*d* ± 1.0
Maize	Vitreous	78	1.56*c* ± 0.03 (−6.0)	0.0191*a*	94.6*b*,*c* ± 1.3	94.7*b* ± 0.9
		96	1.66*d* ± 0.02	0.0192*a*	95.1*b*,*c* ± 1.4	95.2*b*,*c* ± 1.0
		Press.	1.72*e* ± 0.01 (+3.6)	0.0192*a*	100.0*d* ± 1.0	101.0*d* ± 0.8
	Floury	78	1.61*c* ± 0.03 (−4.2)	0.0188*a*	95.3*b*,*c* ± 0.8	95.4*b*,*c* ± 1.0
		96	1.68*d* ± 0.02	0.0189*a*	95.3*b*,*c* ± 1.4	95.4*b*,*c* ± 1.2
		Press.	1.78*f* ± 0.04 (+6.0)	0.0190*a*	97.1*c*,*d* ± 1.2	97.2*c*,*d* ± 1.4

All results are means of three replicate experiments; values in the same column followed by the same letter are not significantly different at $p=0.05$.
[a] R_{10} = velocity of flour starch digestion calculated for the 1st 10 min of reaction.
[b] k = flours' kinetic (digestibility) constants (i.e. intrinsic susceptibility of flours to digestion by α-amylase).
[c] Values are in vitro values of starch digestion obtained experimentally after 150 min of assay.
[d] Hydrolytic index, proportion of flour starch which, under the conditions of study, is theoretically digestible.
[e] Figures in brackets represent percentage increase or decrease in digestion velocities due to cooking at 78 °C or under pressure. Calculated from kinetic data as per Goni et al. (1997).

Table 6
Densitometry data of SDS-PAGE of proteins in material remaining after sorghum and maize flours were subjected to cooking at different temperature and then digestion by porcine pancreatic α-amylase

	Density Peak values[a]											
	Non reducing SDS-PAGE						Reducing SDS-PAGE					
	Floury endosperm			Vitreous endosperm			Floury endosperm			Vitreous endosperm		
	78 °C	96 °C[‡]	Press	78 °C	96 °C	Press	78 °C	96 °C	Press	78 °C	96 °C	Press
Sorghum prolamins												
β-Kafirin	59 (+27)[b]	32	10 (−22)	9 (+2)	7	3 (−4)	140 [+81][c]	86 [+54]	81 [+71]	141 [+132]	123 [+116]	75 [+72]
α-Kafirin	135 (+30)	105	23 (−82)	78 (+5)	73	20 (−53)	325 [+190]	324 [+219]	218 [+195]	337 [+259]	339 [+266]	208 [+188]
γ-Kafirin	102 (+57)	45	15 (−30)	42 (+4)	38	14 (−24)	225 [+123]	160 [+115]	156 [+141]	245 [+203]	207 [+169]	195 [+181]
Di-	NB[d]	NB	NB	NB	NB	NB	118 [+118]	81 [+81]	54 [+54]	139 [+139]	70 [+70]	83 [+83]
Tri-	150 (+10)	140	32 (−108)	165 (+38)	127	18 (−109)	137 [−13]	100 [−40]	76 [+44]	85 [−80]	94 [−33]	24 [+6]
Tetra-	58 (−72)	130	93 (−37)	66 (−3)	96	16 (−80)	121 [+63]	92 [−38]	64 [−29]	56 [−10]	55 [−41]	28 [+12]
Total	507 (+55) [12.2][e]	452	173 (−279) [−61.7]	360 (+19) [+5.6]	341	71 (−270) [−79.2]	1066 [+559] {+110.3}[f]	843 [+391] {+86.5}	649 [+476] {+275.1}	1003 [+643] {178.6}	939 [+598] {175.4}	613 [+542] {763.4}
Maize prolamins												
β-Zein	61 (+34)	27	10 (−17)	50 (+32)	18	3 (−15)	127 [+66]	142 [+115]	125 [+115]	149 [+99]	140 [+122]	108 [+105]
α-Zein	260 (+115)	145	109 (−36)	277 (+19)	258	93 (−165)	432 [+172]	366 [+221]	325 [+180]	455 [+178]	371 [+113]	327 [+234]
γ-Zein	125 (+62)	63	32 (−31)	98 (+10)	88	24 (−64)	226 [+101]	98 [+35]	196 [+164]	228 [+130]	181 [+93]	151 [+127]
Di-	140 (+80)	60	37 (−23)	128 (+20)	108	47 (−61)	140 [+0]	142 [+82]	71 [+34]	126 [−2]	112 [+4]	41 [−6]
Tri-	172 (+63)	109	66 (−43)	185 (+50)	135	95 (−40)	142 [−30]	153 [+44]	158 [+92]	112 [−73]	124 [−11]	85 [−10]
Tetra-	152 (+31)	121	88 (−33)	177 (+0)	177	100 (−77)	122 [−30]	188 [+67]	107 [−70]	132 [−45]	130 [−47]	75 [−25]
Total	910 (+385) [+73.3]	525	342 (−183) [−34.9]	915 (+131) [+14.3]	784	362 (−422) [−53.8]	1189 [+279] {+30.7}	1089 [+564] {107.4}	982 [+640] {187.1}	1202 [+287] {31.4}	1058 [+274] {34.9}	787 [+425] {117.4}

[a] Densitometer readings.
[b] Figures in parentheses (()) represent increases or decreases in density values of specific protein bands and total band density as a result of cooking at 78 °C or under pressure.
[c] Figures in brackets ([]) represent increases or decreases in the density values of specific protein bands due to reducing SDS-PAGE.
[d] NB = no band.
[e] Bracketed figures on a shaded background represent the percentage increase or decrease in the total protein band density values of the flour samples resulting from cooking at 78 °C or under pressure.
[f] The figures in brackets ({}) represent percentage increase in total protein band density values in reducing SDS-PAGE when compared to corresponding values in non-reducing SDS-PAGE.

any of the sorghum residues but appeared in all maize residues.

Although SDS-PAGE under reducing conditions increased individual protein band densities, total band density values of the samples followed essentially the same trends as were observed with SDS-PAGE under non-reducing conditions. Total band density values were always higher in the order 78 °C > 96 °C > pressure-cooking for sorghum and maize, indicating that more reduction-resistant bonds were formed at the higher cooking temperatures. Compared to the 78 and 96 °C-cooked samples, disulphide-polymerised proteins (protein released by 2-ME) accounted for considerably higher proportions of all proteins identified in the reducing SDS-PAGE of pressure-cooked floury and vitreous sorghum flours (Table 6). This shows that disulphide bonding was more prominent under pressure-cooking. This trend also occurred in the maize samples, although the proportions of maize flour prolamins, which were polymerized through disulphide bonds, were lower than in the corresponding sorghum samples. Additionally, sorghum samples displayed lower total band densities in reducing SDS-PAGE than the corresponding maize endosperm fractions, again indicating that they contained more protein polymers with reduction-resistant bonds.

It has been suggested that the intermolecular bonds of polymeric sorghum prolamins may become reduction-resistant if they are of non-disulphide (e.g. di-tyrosyl) type (Duodu et al., 2003), or if the protein polymers are folded so as to render some of their intermolecular disulphide bonds unavailable for reduction (Duodu et al., 2002; 2003; El Nour et al., 1998). Thus, the fact that total protein densities (under reducing SDS-PAGE) were always lowest with pressure-cooking indicates that pressure-cooking modified the structure of sorghum and maize endosperm proteins more drastically than cooking at either 78 or 96 °C.

Throughout this study, it was observed that sorghum flours contained more reduction-resistant protein polymers than maize flours, as did the vitreous sorghum endosperm flour compared to its floury counterpart. It therefore seems that sorghum prolamins formed more intermolecular linkages and probably more complex structures than their maize counterparts under all cooking conditions studied.

Differences in total protein band densities (between reducing and non-reducing SDS-PAGE) were always higher in sorghum samples than in their maize counterparts. This indicates that the sorghum flours contained more HMW (M_r > 100k) disulphide bonded proteins and is consistent with view that disulphide bond cross-linking is more extensive in sorghum than maize (Batterman-Azcona and Hamaker, 1998; Duodu et al., 2002; 2003). Unlike Duodu et al. (2002), no kafirin dimer was detected in this study, suggesting that the cooking conditions brought about more rigorous kafirin polymerization.

4. Conclusions

The digestibility of starch in sorghum and maize flours is influenced by endosperm vitreousness, the effects being more pronounced in sorghum. Depression of starch digestion, in vitreous endosperm sorghum flour, appears to result from reduced access by *alpha*-amylase to the starch, resulting from the formation of a more complex and more restrictive prolamin protein network. The effects of cooking temperature and time on flour starch digestibility also appear to be related to differences in the amounts of disulphide bonding and polymerization of the prolamin proteins. Higher starch digestibility with pressure-cooking could be due to the physical disruption of starch-enveloping protein structures resulting in enhanced substrate accessibility. The results presented above are consistent with the findings of Zhang and Hamaker (1998) and have implications for the processing of sorghum for industrial and food uses.

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References

Abdelgadir, I.E.O., Morrill, J.L., Higgins, J.J., 1996. Effect of roasted soybeans and corn on performance and ruminal and blood metabolites of dairy calves. Journal of Dairy Science 79, 465–474.

Batterman-Azcona, S.J., Hamaker, B.R., 1998. Changes occurring in protein body structure and α-zein during cornflake processing. Cereal Chemistry 75, 217–221.

Chandrashekar, A., Kirleis, A.W., 1988. Influence of protein on starch gelatinisation in sorghum. Cereal Chemistry 65, 457–462.

Chandrashekar, A., Mazhar, H., 1999. The biochemical basis and implications of grain strength in sorghum and maize. Journal of Cereal Science 30, 193–207.

Cohen, S.S., 1988. Practical Statistics. Edward Arnold, London.

Correa, C.E.S., Shaver, R.D., Pereira, M.N., Lauer, J.G., Kohn, K., 2002. Relationship between corn vitreousness and in situ starch degradability. Journal of Dairy Science 85, 3008–3012.

Duodu, K.G., Nunes, A., Delgadillo, I., Parker, M.L., Mills, E.N.C., Belton, P.S., Taylor, J.R.N., 2002. Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. Journal of Cereal Science 35, 161–174.

Duodu, K.G., Taylor, J.R.N., Belton, P.S., Hamaker, B.R., 2003. Factors affecting sorghum protein digestibility. Journal of Cereal Science 38, 117–131.

El Nour, I.N.A., Peruffo, A.D.B., Curioni, A., 1998. Characterisation of sorghum kafirins in relation to their cross-linking behaviour. Journal of Cereal Science 28, 197–207.

Frei, M., Siddhuraju, P., Becker, K., 2003. Studies on the in vitro starch digestibility and the glycemic index of six different indigenous rice cultivars from the Philippines. Food Chemistry 83, 395–402.

Goni, I., Garcia-Alonso, A., Saura-Calixto, F., 1997. A starch hydrolysis procedure to estimate glycemic index. Nutrition Research 17, 427–437.

Goode, D.L., Arendt, E.K., 2003. Pilot scale production of a lager from grist containing 50% unmalted sorghum. Journal of the Institute of Brewing 109, 208–217.

Hamaker B.R., Bugusu, B.A., 2003. Sorghum proteins and food quality. In *Afripro Conference Proceedings*, Belton, P.S., Taylor, J.R.N. (eds). www.afripro.org.uk. Paper 08.

Horn, C.H., du Preez, J.C., Kilian, S.G., 1992. Fermentation of grain sorghum starch by co-cultivation of Schwanniomyces occidentalis and Saccharomyces cerevisiae. Bioresource Tehnology 42, 27–31.

ICRISAT/FAO. The World Sorghum and Millet Economies: Factors, Trends and Outlook. Patancheru, India/Rome. ICRISAT/FAO. (1996) pp 1 and 2.

Kotarski, S.F., Waniska, R.D., Thurn, K.K., 1992. Starch hydrolysis by ruminal microflora. Journal of Nutrition 122, 178–190.

Kumari, S.R., Chandrashekar, A., 1994. Relationship between grain vitreousness and the contents of prolamins and three anti-fungal proteins in sorghum. Journal of Cereal Science 20, 93–99.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 277, 680–685.

Mangala, S.L., Tharanathan, R.N., 1999. Structural studies of resistant starch derived from processed (autoclaved) rice. European Food Research Technology 209, 38–42.

Mazhar, H., Chandrashekar, A., 1995. Quantitation and distribution of kafirins in the kernels of sorghum cultivars varying in endosperm vitreousness. Journal of Cereal Science 21, 155–162.

Oria, M.P., Hamaker, B.R., Schull, J.M., 1995. In vitro protein digestibility of developing and mature grain in relation to α-, β-, and γ-kafirin disulphide cross-linking. Journal of Cereal Science 22, 85–93.

Palmer, G.H., 1989. Date required Cereals in malting and brewing. In: Palmer, G.H. (Ed.), Cereal Science and Technology. Aberdeen University Press, Aberdeen, U.K., pp. 170–263.

Parker, M.L., Grant, A., Rigby, N.M., Belton, P.S., Taylor, J.R.N., 1999. Effects of popping on the endosperm cell walls of sorghum and maize. Journal of Cereal Science 30, 209–216.

Philippeau, C., Le Deschault de Monredon, F., Michalet-Doreau, B., 1999. Relationship between ruminal starch degradation and the physical characteristics of corn grain. Journal of Animal Science 77, 238–243.

Rowe, J.B., Choct, M., Pethick, D.W., 1999. Processing cereal grains for animal feeding. Australian Journal of Agricultural Research 50, 721–736.

Taylor, J.R.N., Schüssler, L., 1986. The protein compositions of different anatomical parts of sorghum grain. Journal of Cereal Science 4, 361–369.

Twomey, L.N., Pluske, J.R., Rowe, J.B., Choct, M., Brown, W., Pethick, D.W., 2003. The replacement value of sorghum and maize with or without supplemental enzymes for rice in extruded dog foods. Animal Feed Science and Technology 108, 61–69.

Zhan, X., Wang, D., Tuinstra, M.R., Bean, S., Seib, P.A., Sun, X.S., 2003. Ethanol and lactic acid production as affected by sorghum genotype and location. Industrial Crops and Products 18, 45–255.

Zhang, G., Hamaker, B.R., 1998. Low α-amylase starch digestibility of cooked sorghum flours and the effect of protein. Cereal Chemistry 75, 710–713.