CHAPTER 4

RESULTS

4.1 Total protein and polyphenol contents of sorghum and maize samples

Table 7. Total protein contents (g/100 g dry basis) of whole grain, endosperm and protein body-enriched samples of sorghum (NK 283, KAT 369), maize (PAN 6043) and protein body-enriched samples of P851171 and P850029 sorghum mutants and total polyphenol contents (% tannic acid equivalents dry basis) of whole grain, endosperm and protein body-enriched samples of sorghum (NK 283, KAT 369) and maize (PAN 6043).

	NK 283 sorghum (red)		KAT 369 sorghum (white)		PAN 6043 maize		P851171 sorghum Mutant		P850029 sorghum mutant	
	Total protein	Total polyphenol	Total Protein	Total polyphenol	Total protein	Total Polyphenol	Total Protein	Total polyphenol	Total protein	Total polyphenol
Whole Grain	10.8	0.28	9.0	0.21	10.0	0.19	ND[2]	ND	ND	ND
Endosperm	8.5	0.17	7.3	0.10	9.1	0.21	ND	ND	ND	ND
PB[1]	33.7	0.24	32.0	0.17	22.9	0.19	58.3	ND	53.9	ND

[1]Protein body-enriched sample

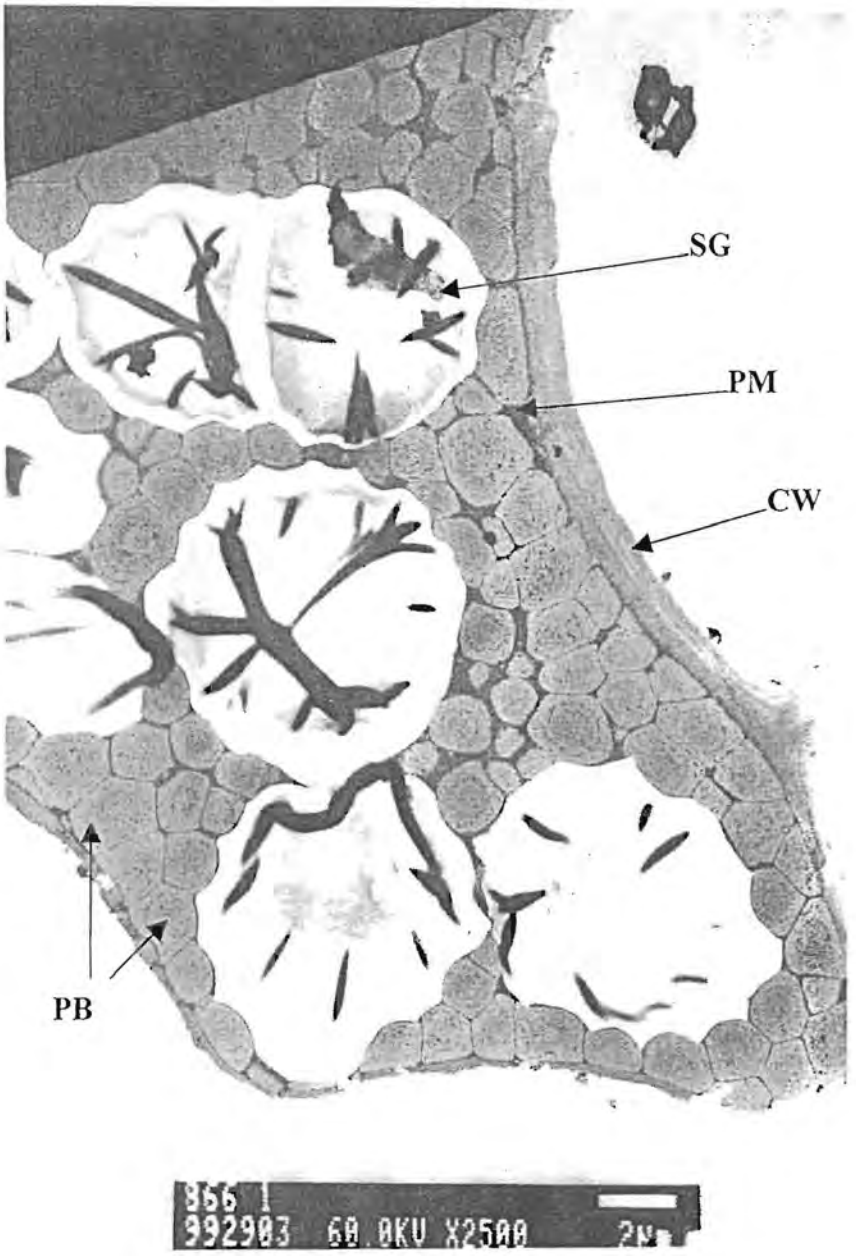
[2]Not determined

For whole grain, NK 283 sorghum had the highest protein content followed by PAN 6043 maize and then KAT 369 sorghum. They all had significantly higher protein content in the whole grain than endosperm. For the endosperm, PAN 6043 maize had the highest protein content followed by NK 283 sorghum and KAT 369 sorghum. The protein body-enriched samples of the two normal sorghums and the maize had much higher protein content (approximately 2-4 times greater) than their corresponding whole grain and endosperm. Of the protein body-enriched samples, PAN 6043 maize had the lowest protein content followed by the two normal sorghums (NK 283 and KAT 369) with the sorghum mutants (P851171 and P850029) having the highest protein contents.

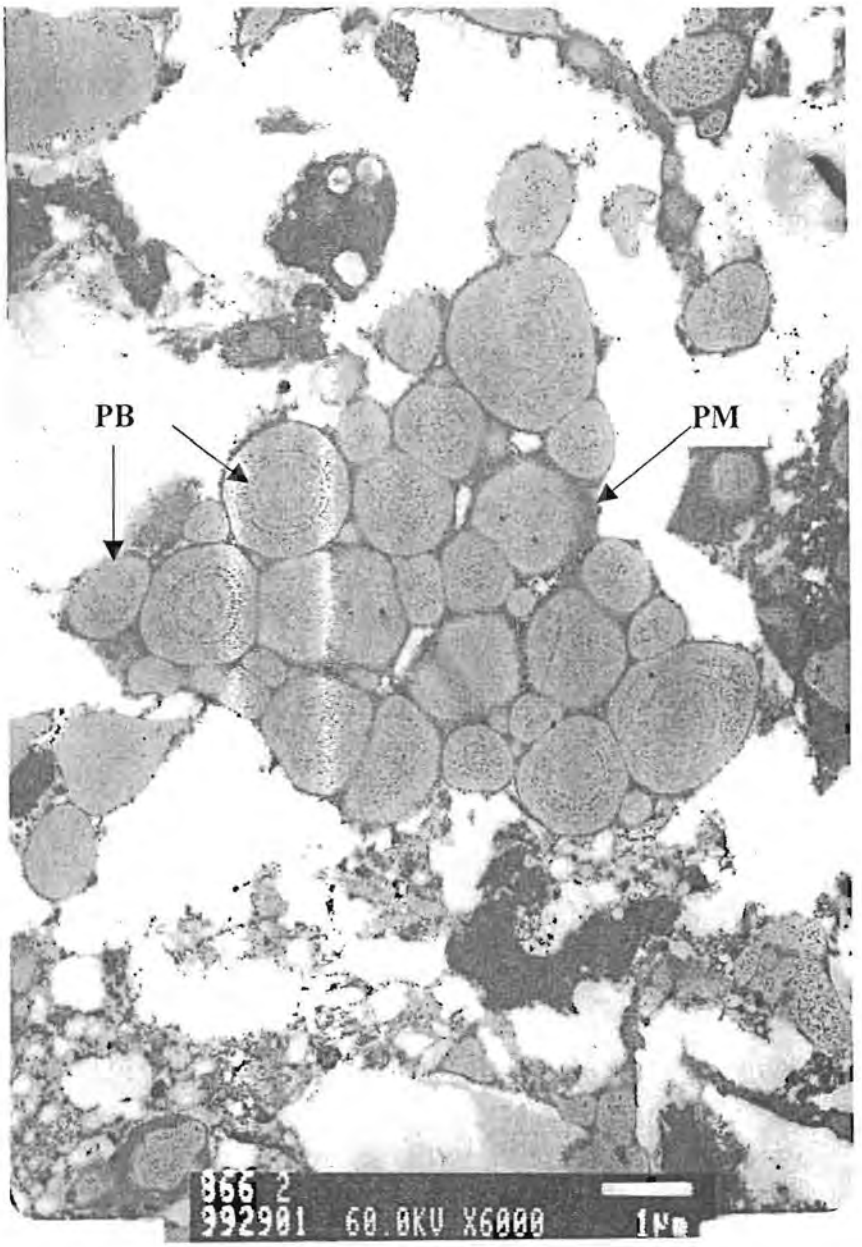
The NK 283 red sorghum grain contained more total polyphenols than the KAT 369 white sorghum and the white maize. For the two sorghums, the endosperm had lower total polyphenol content than whole grain. The protein body-enriched samples of the sorghums had similar total polyphenol content to whole grain. The PAN 6043 maize, in contrast to the sorghums, had similar total polyphenol contents at whole grain, endosperm and protein body levels.

4.2 Ultrastructure of protein body-enriched samples

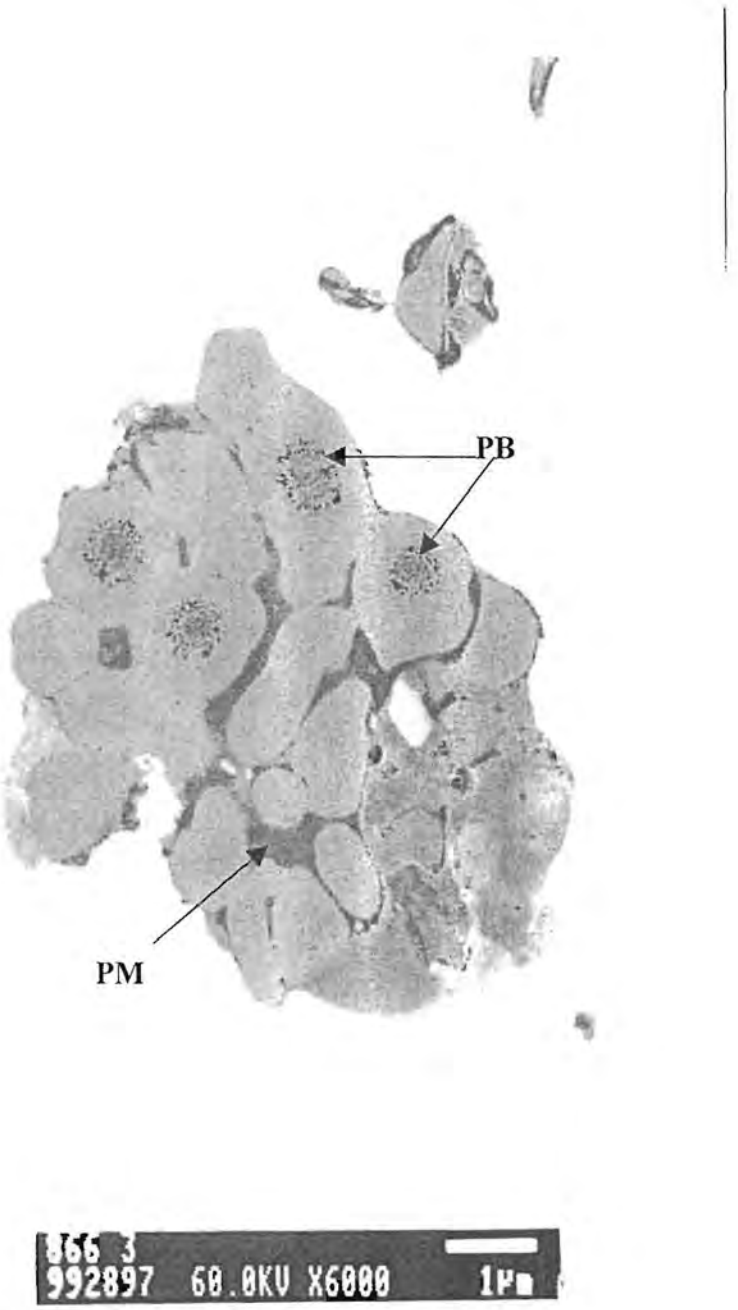
Figure 8. Transmission electron micrographs of uncooked protein body-enriched samples of sorghum (NK 283 and KAT 369), maize (PAN 6043) and sorghum mutants (P851171 and P850029).



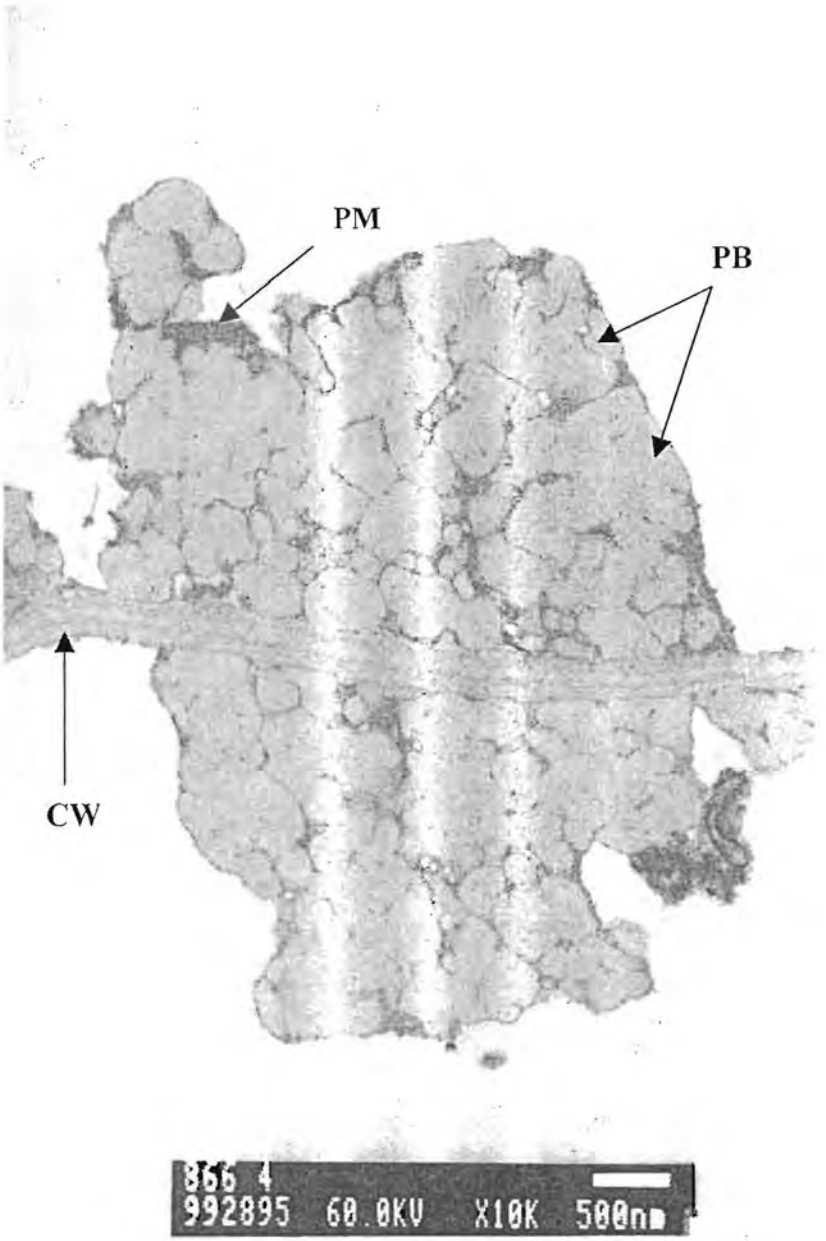
A) Transmission electron micrograph of uncooked NK 283 sorghum protein body-enriched sample. PB – protein body; PM – protein matrix; CW – cell wall; SG – starch granule.



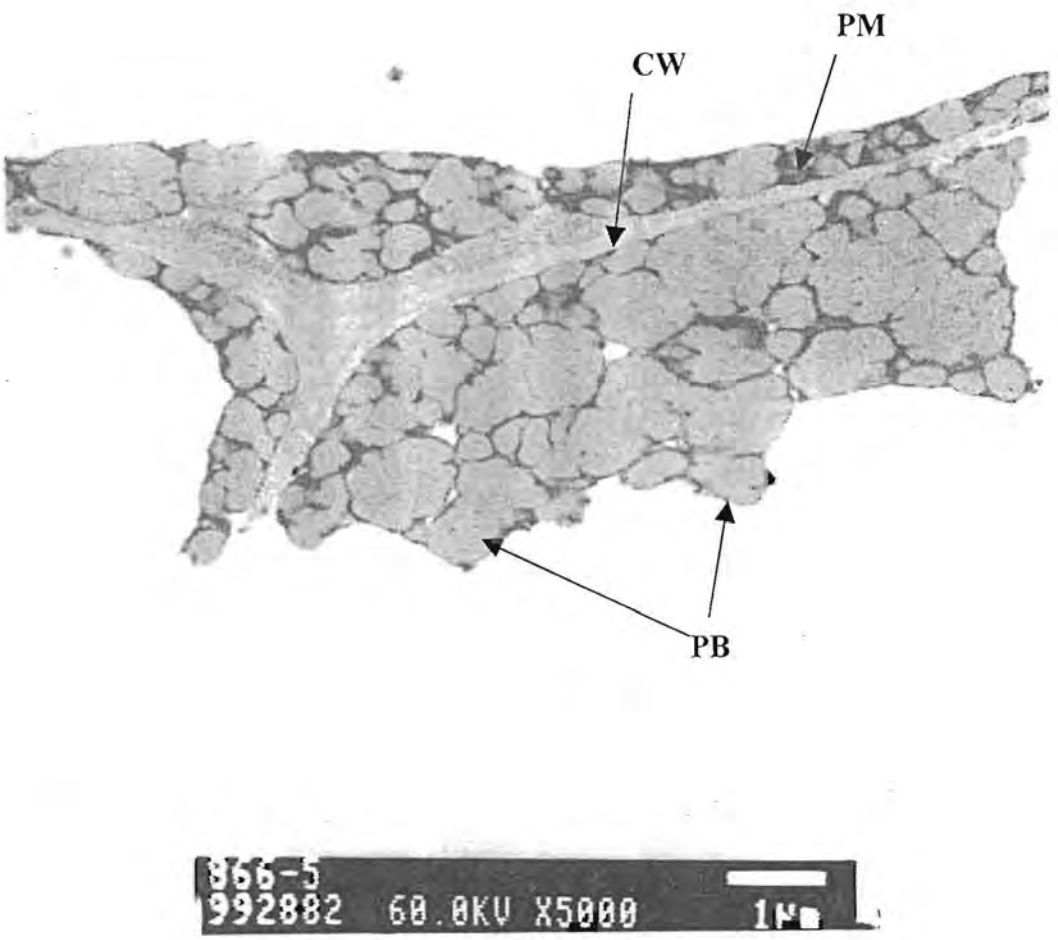
B) Transmission electron micrograph of uncooked KAT 369 sorghum protein body-enriched sample. PB – protein body; PM – protein matrix.



C) Transmission electron micrograph of uncooked PAN 6043 maize protein body-enriched sample. PB – protein body; PM – protein matrix.



D) Transmission electron micrograph of uncooked P851171 sorghum mutant protein body-enriched sample. PB – protein body; PM – protein matrix; CW – cell wall.



E) Transmission electron micrograph of uncooked P850029 sorghum mutant protein body-enriched sample. PB – protein body; PM – protein matrix; CW – cell wall.

The protein bodies of the normal sorghums (NK 283 and KAT 369) and the maize (PAN 6043) were mostly round-shaped compared to those of the sorghum mutants (P851171 and P850029) which appeared to be irregularly-shaped with many invaginations. For all five samples, the protein bodies appeared to be embedded in a dark staining matrix. The interior of the protein bodies had some dark-staining deposits which occurred in the form of concentric rings. The NK 283 sorghum and the mutant sorghum micrographs showed the presence of cell wall material. Starch granules were present in the NK 283 sorghum micrograph.

4.3 *In vitro* protein digestibility of whole grain, endosperm and protein body-enriched samples and enzyme inhibition by whole grain

Table 8 Effect of cooking and addition of alpha-amylase after cooking on percentage *in vitro* protein digestibility of whole grain, endosperm and protein body-enriched samples of NK 283 red sorghum

	Whole grain		Endosperm		Protein body-enriched sample		Mean treatment
Treatment	PD[1]	% of uncooked	PD	% of uncooked	PD	% of uncooked	
Uncooked	59.1 c[2] ± 3.7[3]	100	65.7 c ± 0.9	100	72.8 b ± 2.5	100	65.8 γ
Cooked	30.5 a ± 1.6	52	35.9 a ± 5.1	55	44.2 a ± 3.2	61	36.9 α
Cooked/ α-amylase	36.5 b ± 1.8	62	49.0 b ± 4.3	75	45.3 a ± 3.4	62	43.6 β
Mean OL[4]	42.0 x		50.2 y		54.1 z		

[1]Protein digestibility.

[2]Mean values with different letters in the same column or row are significantly different from each other ($p < 0.05$).

[3]Standard deviation.

[4]Organisational level.

Overall, there was progressive increase in protein digestibility with change in organisational level from whole grain, through endosperm to protein body-enriched samples. Cooking decreased protein digestibility whilst alpha-amylase treatment of cooked samples prior to pepsin digestion improved protein digestibility above the level of the cooked samples. There was progressive increase in protein digestibility of uncooked and cooked NK 283 red sorghum with change in organisational level from whole grain, through endosperm to protein body-enriched samples. Cooking reduced protein digestibility at all three levels of grain organisation. Treating cooked samples with alpha-amylase prior to pepsin digestion increased

protein digestibility above the level of cooked samples at the whole grain and endosperm levels but not at the protein body-enriched level.

Table 9 Effect of cooking and addition of alpha-amylase after cooking on percentage *in vitro* protein digestibility of whole grain, endosperm and protein body-enriched samples of KAT 369 white sorghum

	Whole grain		Endosperm		Protein body-enriched sample		Mean treatment
Treatment	PD[1]	% of uncooked	PD	% of uncooked	PD	% of uncooked	
Uncooked	55.8 c[2] ± 0.9[3]	100	67.4 c ± 1.2	100	74.3 b ± 4.7	100	65.9 γ
Cooked	36.6 a ± 2.8	66	39.4 a ± 4.4	58	63.5 a ± 1.7	85	46.5 α
Cooked/ α-amylase	42.2 b ± 2.0	76	43.7 b ± 2.9	65	62.7 a ± 3.9	84	49.6 β
Mean OL[4]	44.9 x		50.2 y		66.8 z		

[1]Protein digestibility.

[2]Mean values with different letters in the same column or row are significantly different from each other ($p < 0.05$).

[3]Standard deviation.

[4]Organisational level.

Overall, there was progressive increase in protein digestibility with change in organisational level from whole grain, through endosperm to protein body-enriched samples. Cooking decreased protein digestibility, whilst alpha-amylase treatment of cooked samples prior to pepsin digestion improved protein digestibility above the level of the cooked samples. There was progressive increase in protein digestibility of uncooked and cooked KAT 369 white sorghum with change in organisational level from whole grain, through endosperm to protein body-enriched samples. Cooking reduced protein digestibility at all three levels of grain organisation. Treating cooked samples with alpha-amylase prior to pepsin digestion increased protein digestibility above the level of cooked samples at the whole grain and endosperm levels but not at the protein body-enriched level. Uncooked and cooked KAT 369 white sorghum had similar protein digestibility to uncooked and cooked NK 283 red sorghum at the

whole grain and endosperm levels. However, the cooked protein body-enriched sample of KAT 369 was 19.3% more digestible than cooked protein body-enriched sample of NK 283 (see Table 8) and 17.4% more digestible when the cooked protein body-enriched sample was treated with alpha-amylase.

Table 10 Effect of cooking and addition of alpha-amylase after cooking on percentage *in vitro* protein digestibility of whole grain, endosperm and protein body-enriched samples of PAN 6043 maize

	Whole grain		Endosperm		Protein body-Enriched sample		Mean treatment
Treatment	PD[1]	% of uncooked	PD	% of uncooked	PD	% of uncooked	
Uncooked	66.6 b[2] ± 1.3[3]	100	67.4 a ± 1.2	100	68.8 a ± 2.3	100	67.6 β
Cooked	62.0 a ± 3.2	93	63.6 a ± 2.3	94	67.4 a ± 4.1	98	64.3 α
Cooked/ α-amylase	72.5 c ± 3.3	109	72.2 b ± 2.3	107	68.2 a ± 3.8	99	67.6 β
Mean OL[4]	67.0 x		67.7 x		68.1 x		

[1]Protein digestibility.

[2]Mean values with different letters in the same column or row are significantly different from each other ($p < 0.05$).

[3]Standard deviation.

[4]Organisational level.

Overall, PAN 6043 protein digestibility remained the same at the three organisational levels. Cooking reduced protein digestibility whilst alpha-amylase treatment of cooked samples improved protein digestibility back to the level of uncooked. The protein digestibility of uncooked PAN 6043 maize at the three organisational levels was essentially the same. Cooking reduced protein digestibility slightly at the whole grain level (4.6% reduction) but not at the endosperm and protein body-enriched levels. In contrast, cooking reduced protein digestibility of NK 283 red sorghum (see Table 8) and KAT 369 white sorghum (see Table 9) at the whole grain, endosperm and protein body-enriched levels. For NK 283 red sorghum, this reduction was by 28.6% at the whole grain level, 29.8% at the endosperm level and 28.6% at the protein body-enriched level, whilst for KAT 369 white sorghum, reduction on cooking was by 19.2% at the whole grain level, 28.0% at the endosperm level and 10.8% at

the protein body-enriched level. For cooked PAN 6043 maize samples, alpha-amylase treatment prior to pepsin digestion improved protein digestibility above the level of uncooked and cooked samples at the whole grain and endosperm levels but not at the protein body-enriched level.

Table 11 Effect of cooking and addition of alpha-amylase after cooking on percentage *in vitro* protein digestibility of protein body-enriched samples of P851171 and P850029 sorghum mutants in comparison with red sorghum NK 283, white sorghum KAT 369 and maize PAN 6043

	P851171		P850029		NK 283[4]		KAT 369[4]		PAN 6043[4]	
Treatment	PD[1]	% of uncooked	PD	% of uncooked	PD	% of uncooked	PD	% of uncooked	PD	% of uncooked
Uncooked	83.1 a[2] ± 2.0[3]	100	80.0 b ± 1.7	100	72.8	100	74.3	100	68.8	100
Cooked	80.3 a ± 1.5	97	74.3 a ± 3.0	93	44.2	61	63.5	85	67.4	98
Cooked/ α-amylase	78.8 a ± 1.9	95	78.3 b ± 2.1	98	45.3	62	62.7	84	68.2	99

[1]Protein digestibility.

[2]Mean values with different letters in the same column or row are significantly different from each other ($p < 0.05$).

[3]Standard deviation.

[4]Values from Tables 2, 3 and 4.

The protein body-enriched samples of the two sorghum mutants had higher protein digestibilities (uncooked) than the two normal sorghums and the maize. Cooking reduced the protein digestibility of P850029 sorghum mutant very slightly but not P851171. This reduction was low (5.7%) compared to the 10.8% reduction for white KAT 369 sorghum and 28.6% reduction for red NK 283 sorghum. The cooked protein body-enriched samples of the sorghum mutants had higher protein digestibility than the cooked normal sorghums and maize protein body-enriched samples. Treating cooked samples with alpha-amylase prior to pepsin digestion slightly increased protein digestibility of P850029 sorghum mutant by 4.0% but had no effect on the digestibility of P851171.

Table 12. Percentage of enzyme (amylase) inhibition caused in whole grain of NK 283 sorghum, KAT 369 sorghum and PAN 6043 maize in comparison with DC 75 sorghum (a high-tannin hybrid)

	NK 283 sorghum	KAT 369 Sorghum	PAN 6043 maize	DC 75 High-tannin sorghum
Enzyme inhibition (%)	5.7 b[1]	0.6 a	2.5 ab	64.9 c

[1]Mean values with different letters in the same row are statistically different from each other ($p < 0.05$).

The high-tannin sorghum very substantially inhibited amylase activity, while the condensed-tannin-free sorghums and the maize had a negligible effect on amylase activity.

4.4 *In vitro* protein digestibility of reduced/alkylated and reduced/non-alkylated kafirin and zein

Table 13 Effect of cooking and alkylation on *in vitro* protein digestibility (PD) of total kafirin (kafirin 1 and kafirin 2) and total zein (zein 1 and zein 2).

	Kafirin		Zein	
	% PD	% of uncooked	% PD	% of uncooked
Unalkylated, uncooked	53.2 c[1] ± 1.5[2]	100	54.5 b ± 2.7	100
Unalkylated, cooked	32.7 a ± 3.5	62	47.9 a ± 2.8	88
Alkylated, uncooked	60.6 d ± 1.2	100	58.9 b ± 1.9	100
Alkylated, cooked	43.1 b ± 3.2	71	54.3 b ± 1.8	92

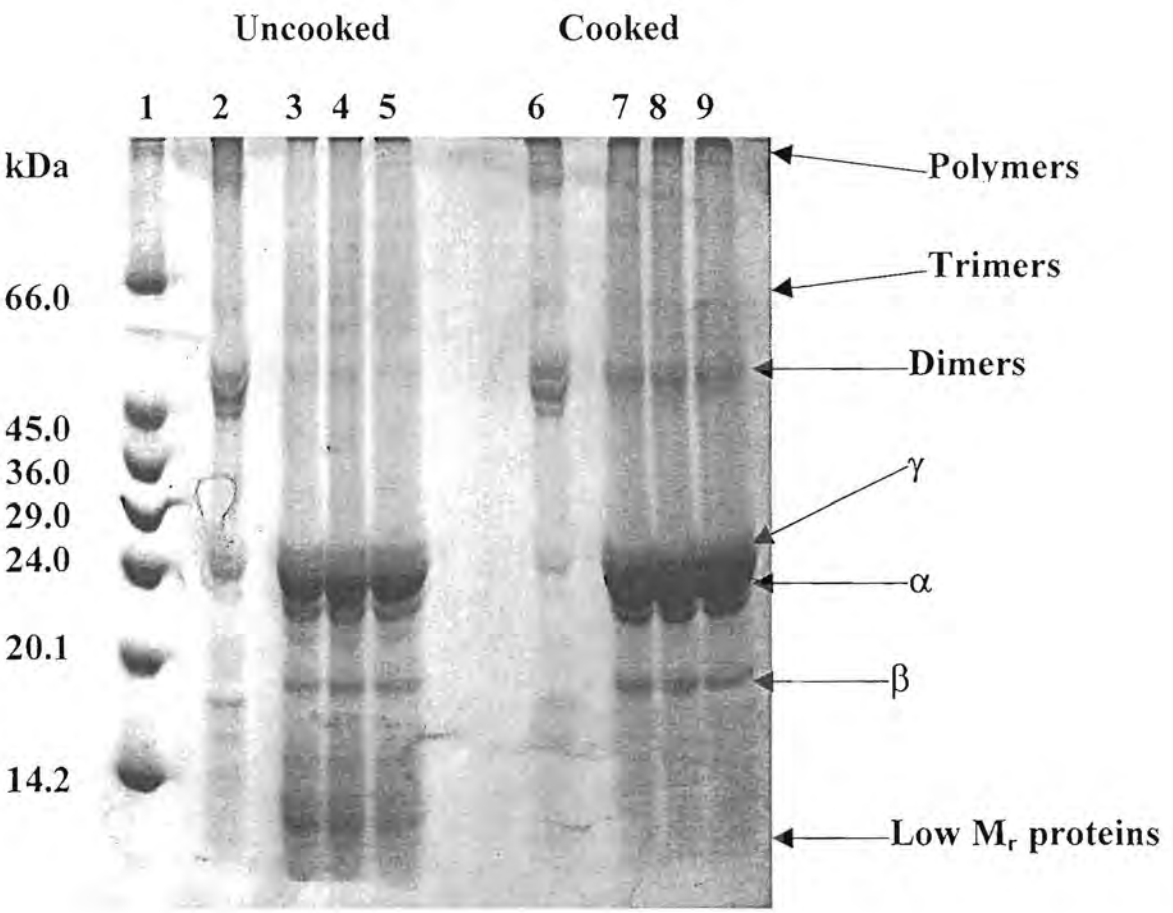
[1]Mean values with different letters in the same column are significantly different from each other ($p < 0.05$).

[2]Standard deviation.

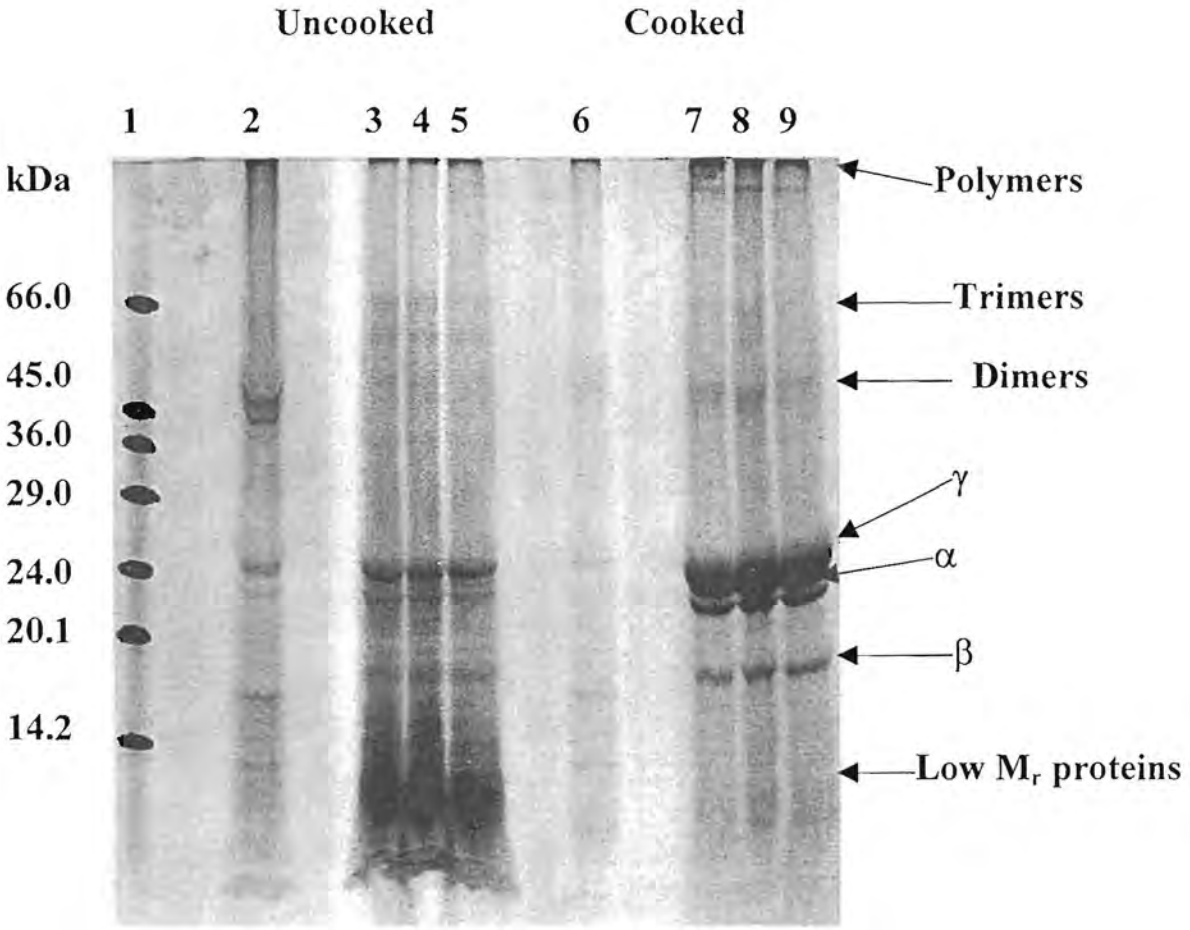
Cooking reduced protein digestibility of both unalkylated and alkylated kafirin and zein. However the drop in digestibility on cooking was more pronounced for the kafirins (by 20.5% for unalkylated kafirin and 17.5% for alkylated kafirin) than for the unalkylated zeins (by 6.6% for unalkylated zein). There was no significant drop in digestibility on cooking the alkylated zein. Unalkylated, uncooked kafirin and zein had the same digestibility as did alkylated, uncooked kafirin and zein Cooked zein (unalkylated and alkylated) was more digestible than cooked kafirin. Alkylated samples (uncooked and cooked) for both kafirin and zein were more digestible than the unalkylated.

4.5 SDS-PAGE of protein body-enriched samples of sorghum and maize under non-reducing and reducing conditions

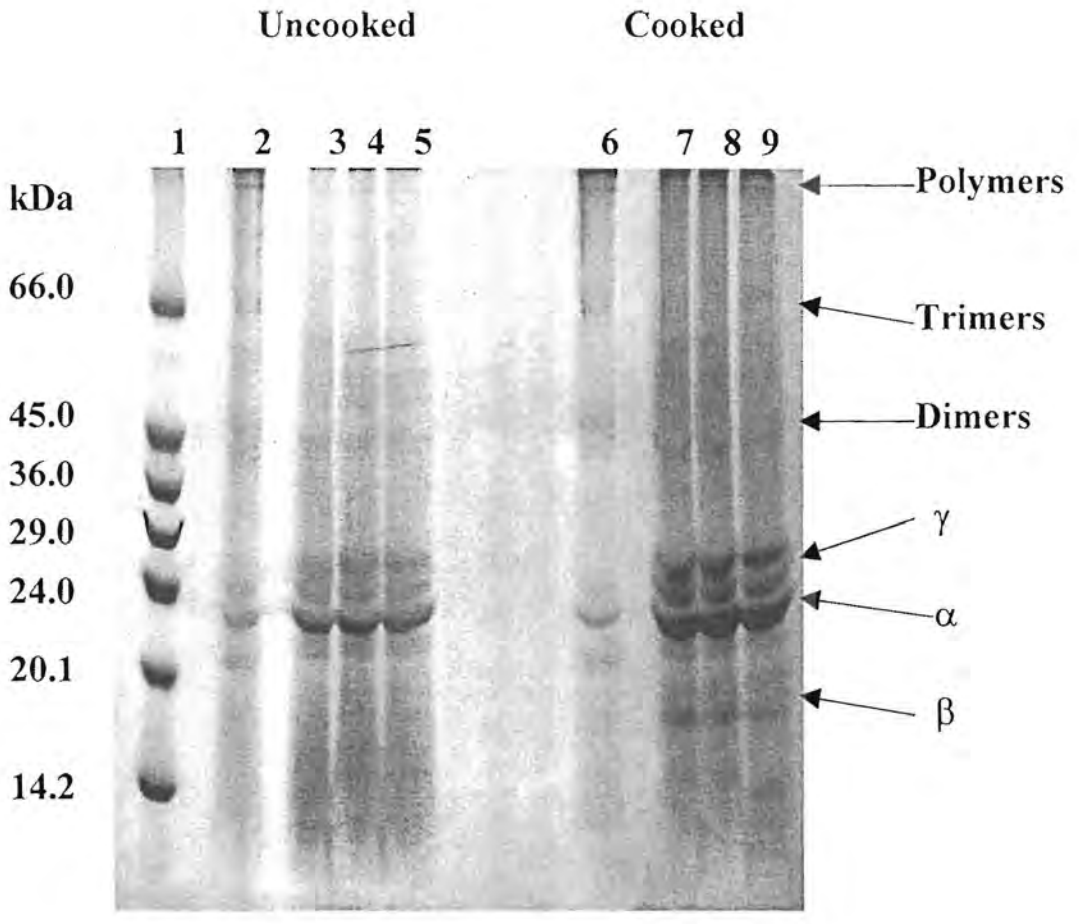
Figure 9: SDS-PAGE of uncooked and cooked protein body-enriched samples of sorghum (NK 283 and KAT 369), maize (PAN 6043) and sorghum mutant (P850029)



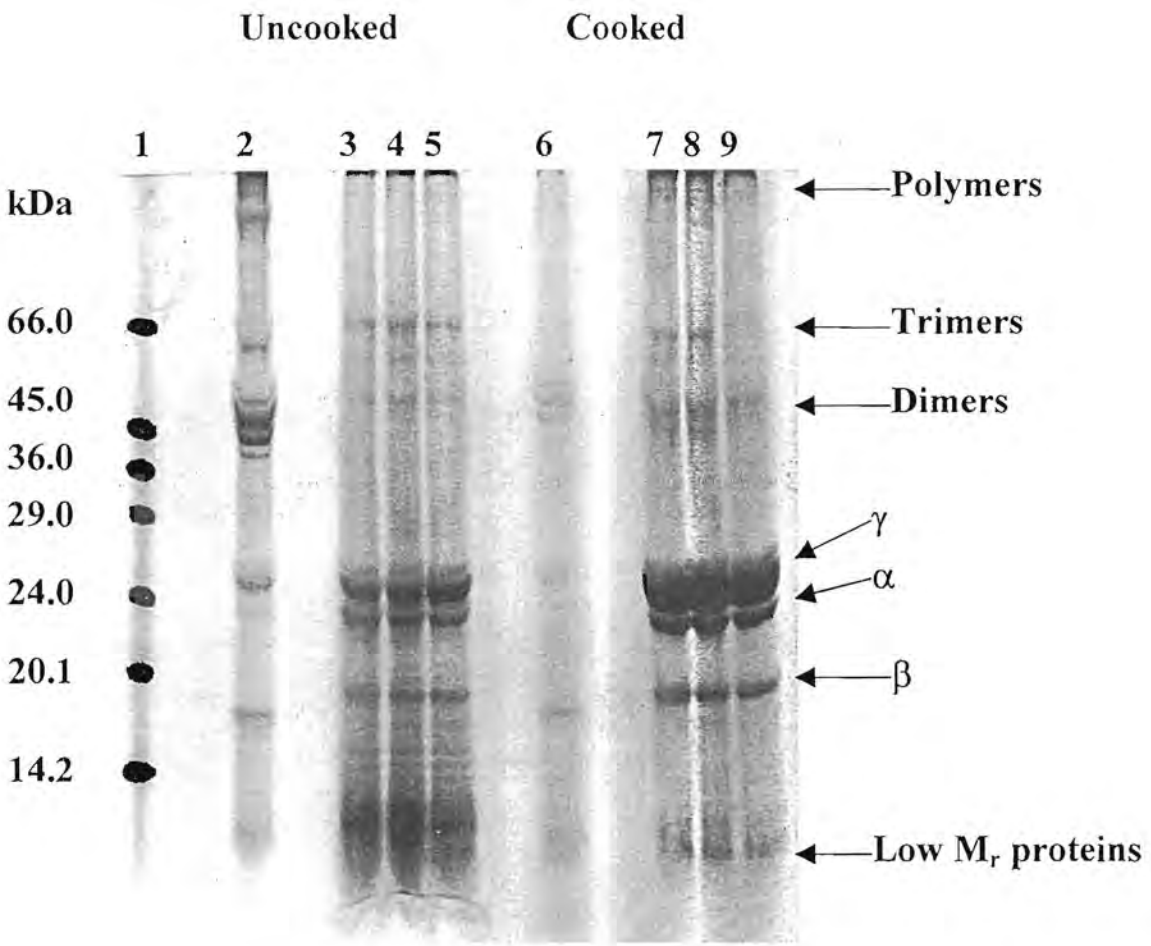
A) SDS-PAGE of uncooked and cooked protein body-enriched samples of sorghum (NK 283) under non-reducing and reducing conditions. Track 1, molecular weight standards; 2, uncooked, non-reduced; 3, uncooked, 50 mM DTT; 4, uncooked, 100 mM DTT; 5, uncooked, 200 mM DTT; 6, cooked, non-reduced; 7, cooked, 50 mM DTT; 8, cooked 100 mM DTT; 9, cooked, 200 mM DTT.



B) SDS-PAGE of uncooked and cooked protein body-enriched samples of sorghum (KAT 369) under non-reducing and reducing conditions. Track 1, molecular weight standards; 2, uncooked, non-reduced; 3, uncooked, 50 mM DTT; 4, uncooked, 100 mM DTT; 5, uncooked, 200 mM DTT; 6, cooked, non-reduced; 7, cooked, 50 mM DTT; 8, cooked 100 mM DTT; 9, cooked, 200 mM DTT.



C) SDS-PAGE of uncooked and cooked protein body-enriched samples of maize (PAN 6043) under non-reducing and reducing conditions. Track 1, molecular weight standards; 2, uncooked, non-reduced; 3, uncooked, 50 mM DTT; 4, uncooked, 100 mM DTT; 5, uncooked, 200 mM DTT; 6, cooked, non-reduced; 7, cooked, 50 mM DTT; 8, cooked 100 mM DTT; 9, cooked, 200 mM DTT.



D) SDS-PAGE of uncooked and cooked protein body-enriched samples of sorghum mutant (P850029) under non-reducing and reducing conditions. Track 1, molecular weight standards; 2, uncooked, non-reduced; 3, uncooked, 50 mM DTT; 4, uncooked, 100 mM DTT; 5, uncooked, 200 mM DTT; 6, cooked, non-reduced; 7, cooked, 50 mM DTT; 8, cooked 100 mM DTT; 9, cooked, 200 mM DTT.

The tracks of both uncooked and cooked samples under non-reducing and reducing conditions of the three sorghum varieties (NK 283, KAT 369 and P850029) were essentially identical. There was stained material at the origin of the gels of the three sorghums under both non-reducing and reducing conditions. Under non-reducing conditions, bands appeared in the 66 kDa and 45-50 kDa regions and at 24, 23, 22 and 18 kDa. Under reducing conditions, there were increases in the intensities of the bands at 24, 23, 22 and 18 kDa accompanied with decreases in intensities of the bands at 66 kDa and 45-50 kDa. For the three concentrations of reducing agent used, some bands in the region 45-96 kDa appeared to be resistant to reduction in both uncooked and cooked sorghum samples. However, there seemed to be more of these reduction-resistant bands in cooked than in uncooked sorghum samples especially in the 45-50 kDa region. Increasing the concentration of reducing agent (DTT) did not seem to decrease the intensity of persistent bands in the 45-50 kDa region. There were low molecular weight protein bands (≤14 kDa) under reducing conditions in uncooked and cooked protein-body-enriched samples of the three sorghums. However these bands were fainter for the cooked samples than the uncooked.

For uncooked and cooked maize (PAN 6043) under non-reducing conditions, bands appeared in the 45-50 kDa and 66 kDa regions and also above 66 kDa, towards the origin of the gel. There were also bands at 25, 22 and 19 kDa. No bands appeared in the 14-16 kDa region. After reduction, the intensities of the bands between 19 and 25 kDa increased and bands appeared at 14 and 16 kDa in both uncooked and cooked maize protein body-enriched samples. As observed for the sorghums, bands in the region between 45 and 96 kDa persisted following reduction of both uncooked and cooked samples of maize protein-body-enriched samples.

Under non-reducing conditions, it appears the proportion of bands in the 45-50 kDa region were much lower for the maize than the three sorghums.

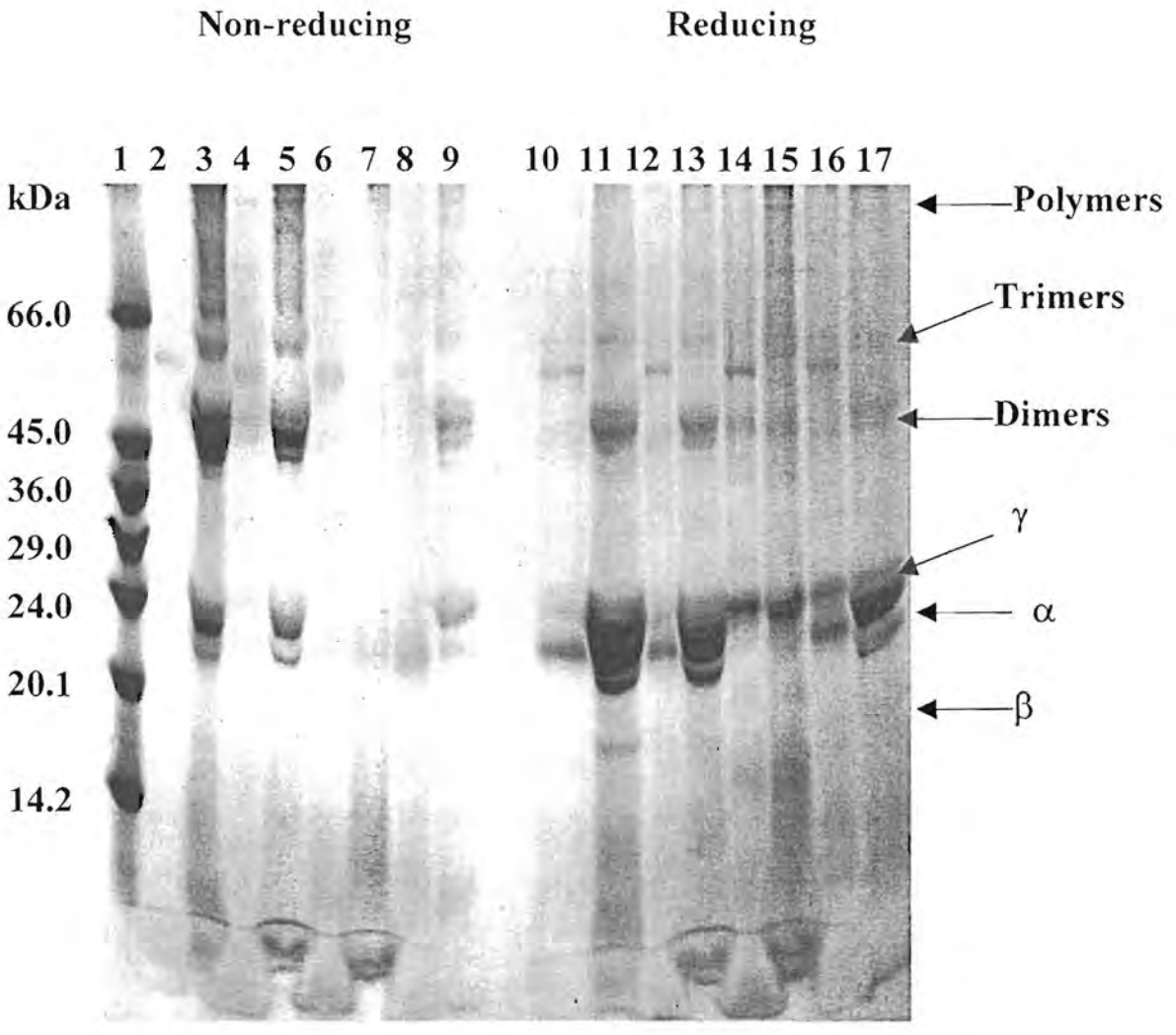


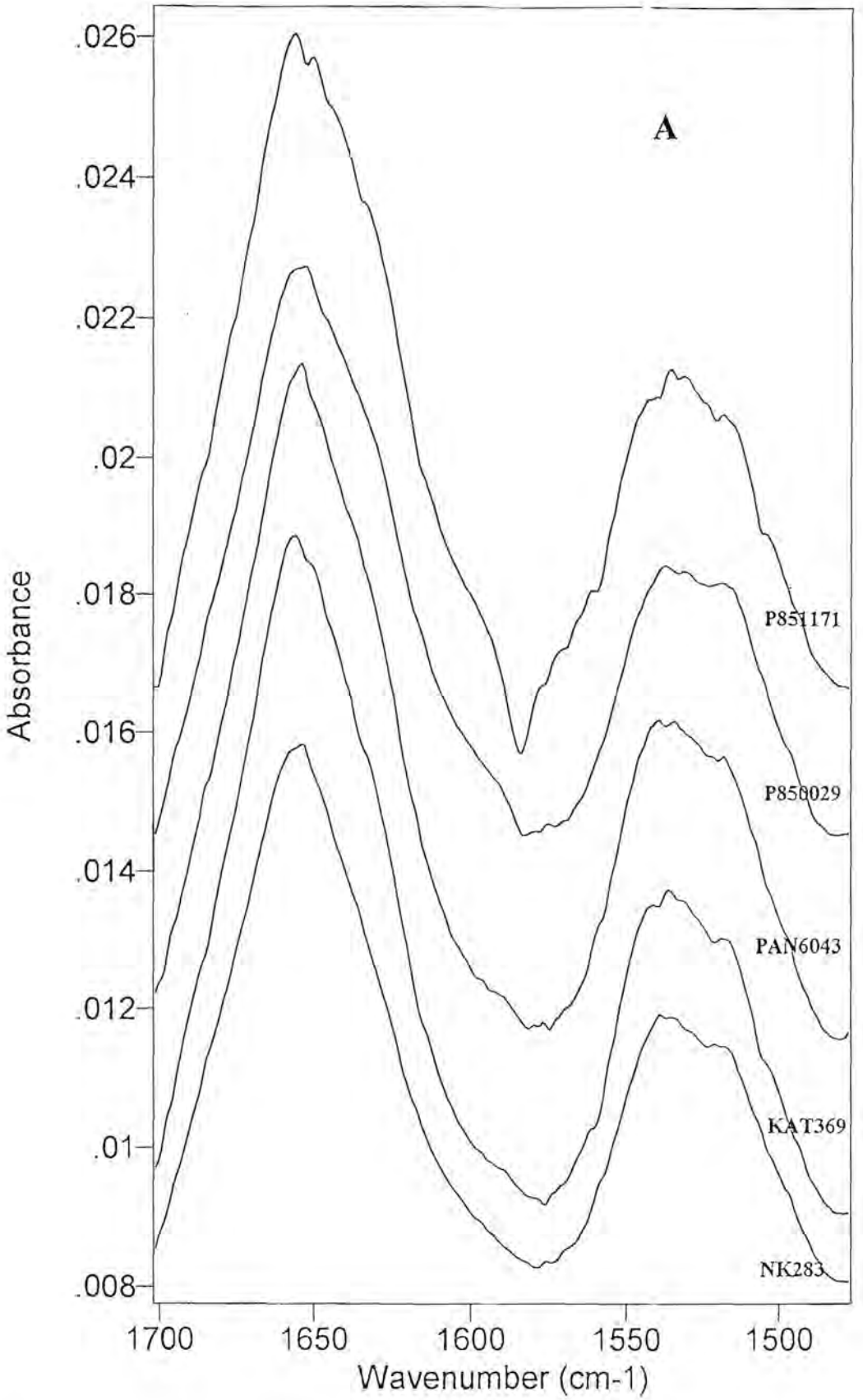
Figure 10. SDS-PAGE of pepsin-indigestible residues of sorghum (NK 283 and KAT 369), maize (PAN 6043) and sorghum mutant (P850029) protein body-enriched samples under non-reducing and reducing conditions (200 mM DTT).

1:- molecular weight markers
2:- uncooked NK 283, non-reduced
3:- cooked NK 283, non-reduced
4:- uncooked KAT 369, non-reduced
5:- cooked KAT 369, non-reduced
6:- uncooked PAN 6043, non-reduced
7:- cooked PAN 6043, non-reduced
8:- uncooked P850029, non-reduced
9:- cooked P850029, non-reduced
10:- uncooked NK 283, reduced
11:- cooked NK 283, reduced
12:- uncooked KAT 369, reduced
13:- cooked KAT 369, reduced
14:- uncooked PAN 6043, reduced
15:- cooked PAN 6043, reduced
16:- uncooked P850029, reduced
17:- cooked P850029, reduced

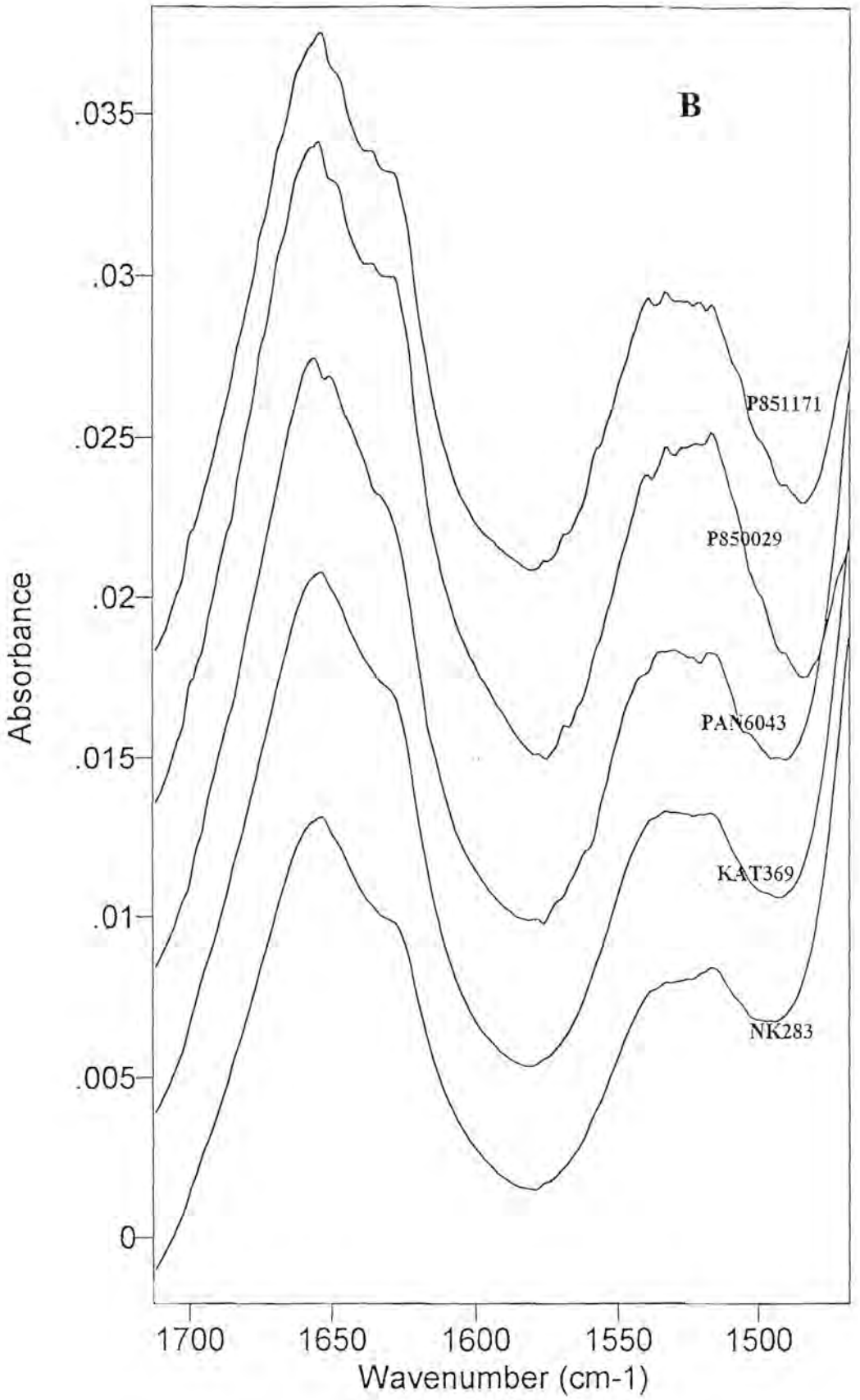
For the pepsin-indigestible sorghum and maize under non-reducing conditions, tracks for uncooked samples were very faint with bands barely visible at 60 kDa. The cooked, pepsin-indigestible sorghum samples under non-reducing conditions showed bands at the top of the gel (96 kDa), the 66 kDa region, 45-50 kDa and at 24, 23 and 22 kDa. These bands were much fainter for the sorghum mutant (P850029) compared to the normal sorghums. The cooked maize residue under non-reducing conditions showed a very faint band at 19 kDa. Under reducing conditions, there were increases in intensities of bands between 18 and 24 kDa for both uncooked and cooked sorghum and maize pepsin-indigestible residues. There were bands in the region 50-60 kDa which appeared to be resistant to reduction in uncooked and cooked sorghum and maize. These bands were fainter for uncooked samples, cooked maize and cooked sorghum mutant compared to the cooked normal sorghums.

4.6 FTIR and ^{13}C NMR spectroscopy of uncooked and cooked protein body-enriched samples of sorghum and maize

Figure 11: FTIR spectra of uncooked and cooked protein body-enriched samples of sorghum (NK 283 and KAT 369), maize (PAN 6043) and sorghum mutants (P851171 and P850029)



A) FTIR spectra of uncooked protein body-enriched samples of sorghum (NK 283 and KAT 369), maize (PAN 6043) and sorghum mutants (P851171 and P850029)



B) FTIR spectra of cooked protein body-enriched samples of sorghum (NK 283 and KAT 369), maize (PAN 6043) and sorghum mutants (P851171 and P850029)

In general, all samples (uncooked and cooked) gave similar spectra. There were two broad bands, one between 1675-1620 cm^{-1} with an absorption maximum at 1660 cm^{-1} and another between 1550-1500 cm^{-1}. For cooked samples, the 1675-1625 cm^{-1} band became broadened and had a shoulder at 1635 cm^{-1} in addition to the peak at 1660 cm^{-1} (Figure 11b). The shapes of the 1550-1500 cm^{-1} bands for all samples were altered on cooking with a peak forming at approximately 1520 cm^{-1} in all cases.

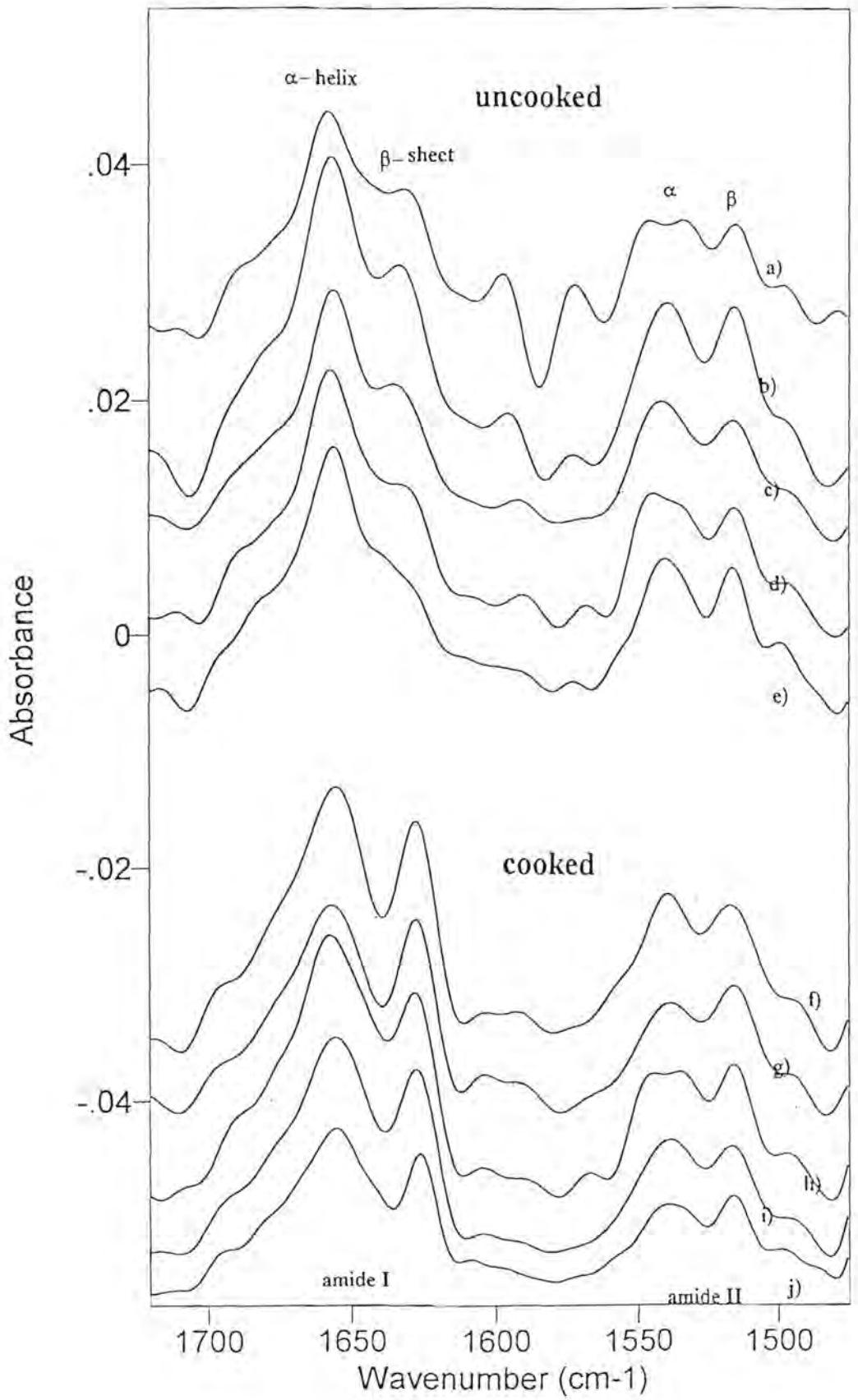
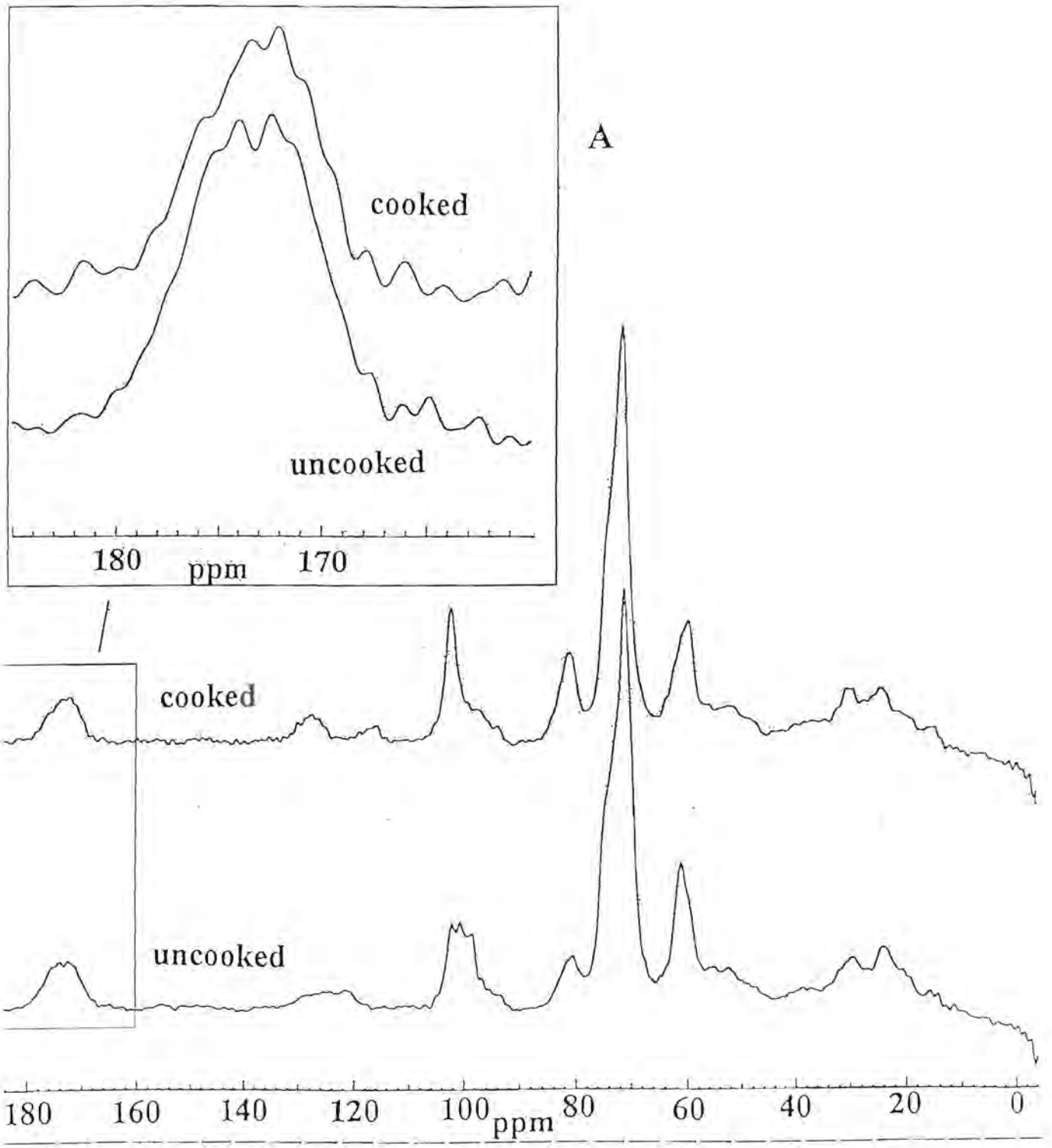


Figure 12: Fourier deconvoluted FTIR spectra of uncooked and cooked protein body-enriched samples of sorghum and maize varieties

a) uncooked P851171 sorghum mutant b) uncooked P850029 sorghum mutant c) uncooked PAN 6043 maize d) uncooked KAT 369 white sorghum e) uncooked NK 283 red sorghum f) wet cooked P851171 sorghum mutant g) wet cooked P850029 sorghum mutant h) wet cooked PAN 6043 maize i) wet cooked KAT 369 white sorghum j) wet cooked NK 283 red sorghum

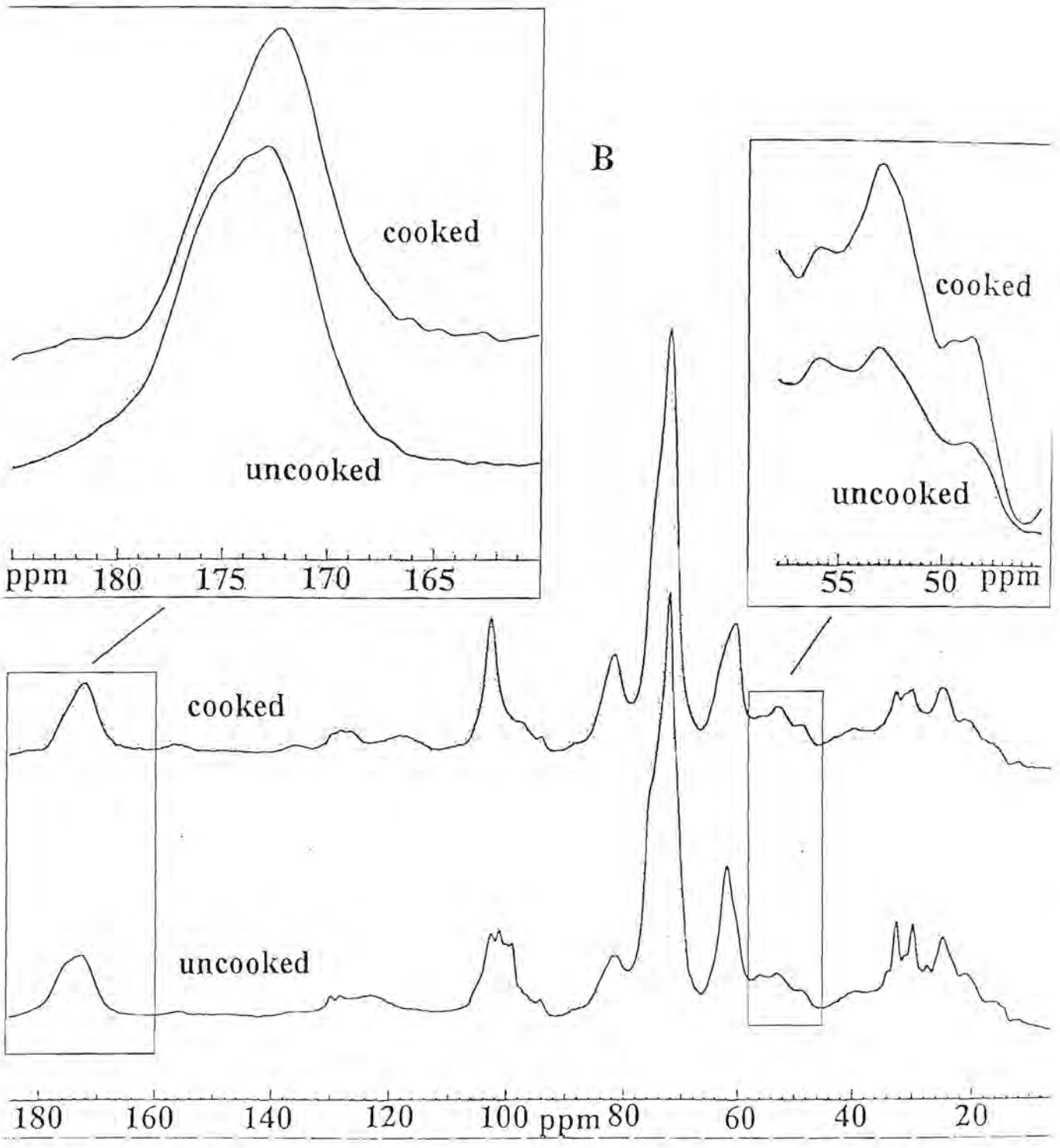
Fourier deconvolution brought about resolution enhancement of the bands at 1675-1625 cm^{-1} and 1550-1500 cm^{-1}. The spectra of all uncooked samples for all grain varieties were similar and so were the spectra of wet cooked samples. The band at 1675-1625 cm^{-1} had peaks at 1660 cm^{-1} and 1635 cm^{-1} for uncooked and wet cooked samples. Cooking increased the intensity of the 1635 cm^{-1} peak for all samples. The 1550-1500 cm^{-1} band produced peaks at 1540 cm^{-1} and 1520 cm^{-1} for both the uncooked and wet cooked samples. The 1540 cm^{-1} peak appeared to be split into two components at 1545 cm^{-1} and 1535 cm^{-1} for uncooked P851171 sorghum mutant, uncooked KAT 369 sorghum and cooked PAN 6043 maize. Wet cooked NK 283 sorghum, PAN 6043 maize and P850029 sorghum mutant appeared to have their absorption maxima for the 1550-1500 cm^{-1} band shifted towards the peak at 1520 cm^{-1}.

Figure 13: ^{13}C CPMAS NMR spectra of uncooked and wet cooked protein body-enriched samples of sorghum and maize

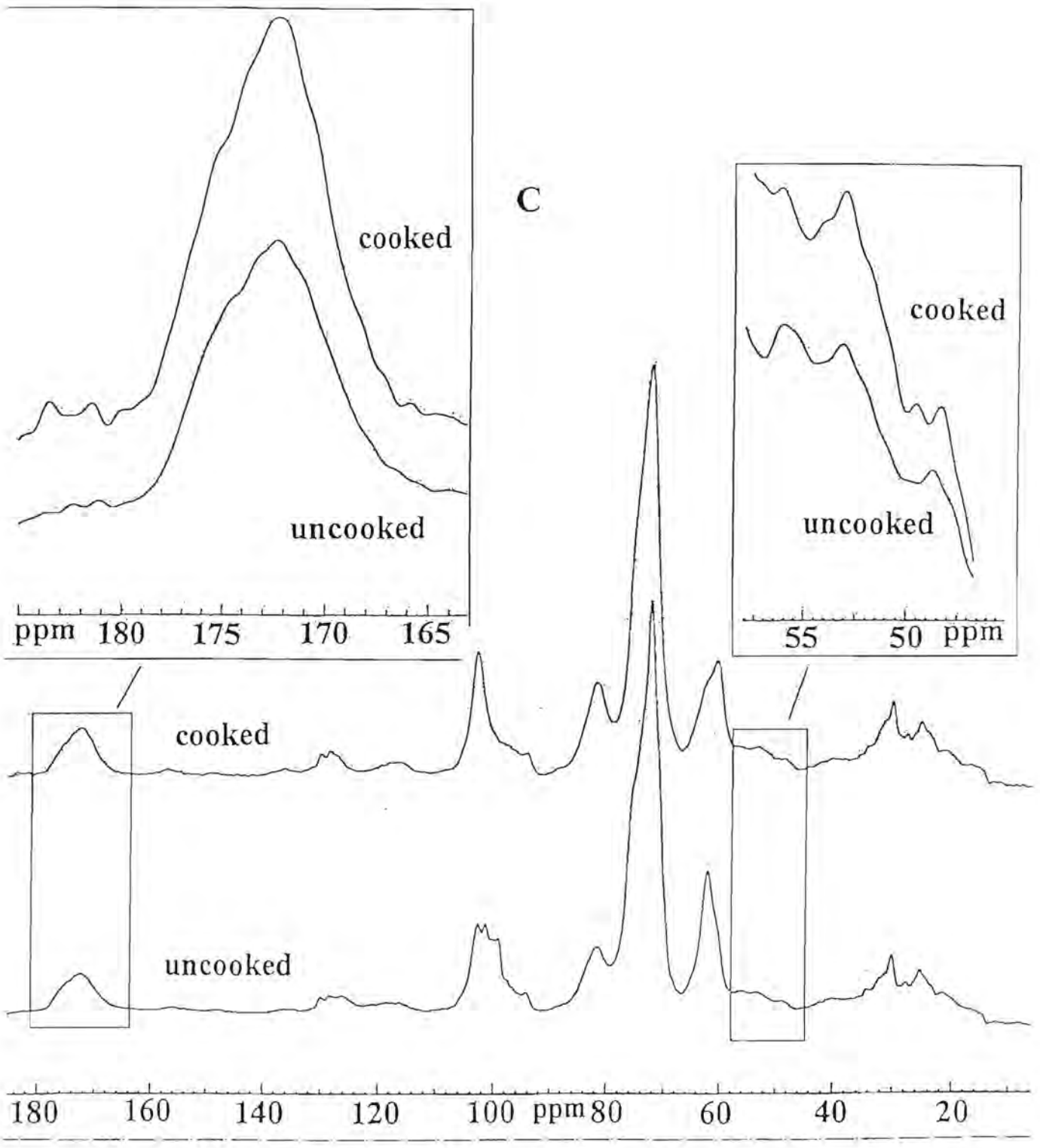


A) ^{13}C CPMAS NMR spectra of uncooked and wet cooked protein body-enriched samples of NK 283 sorghum

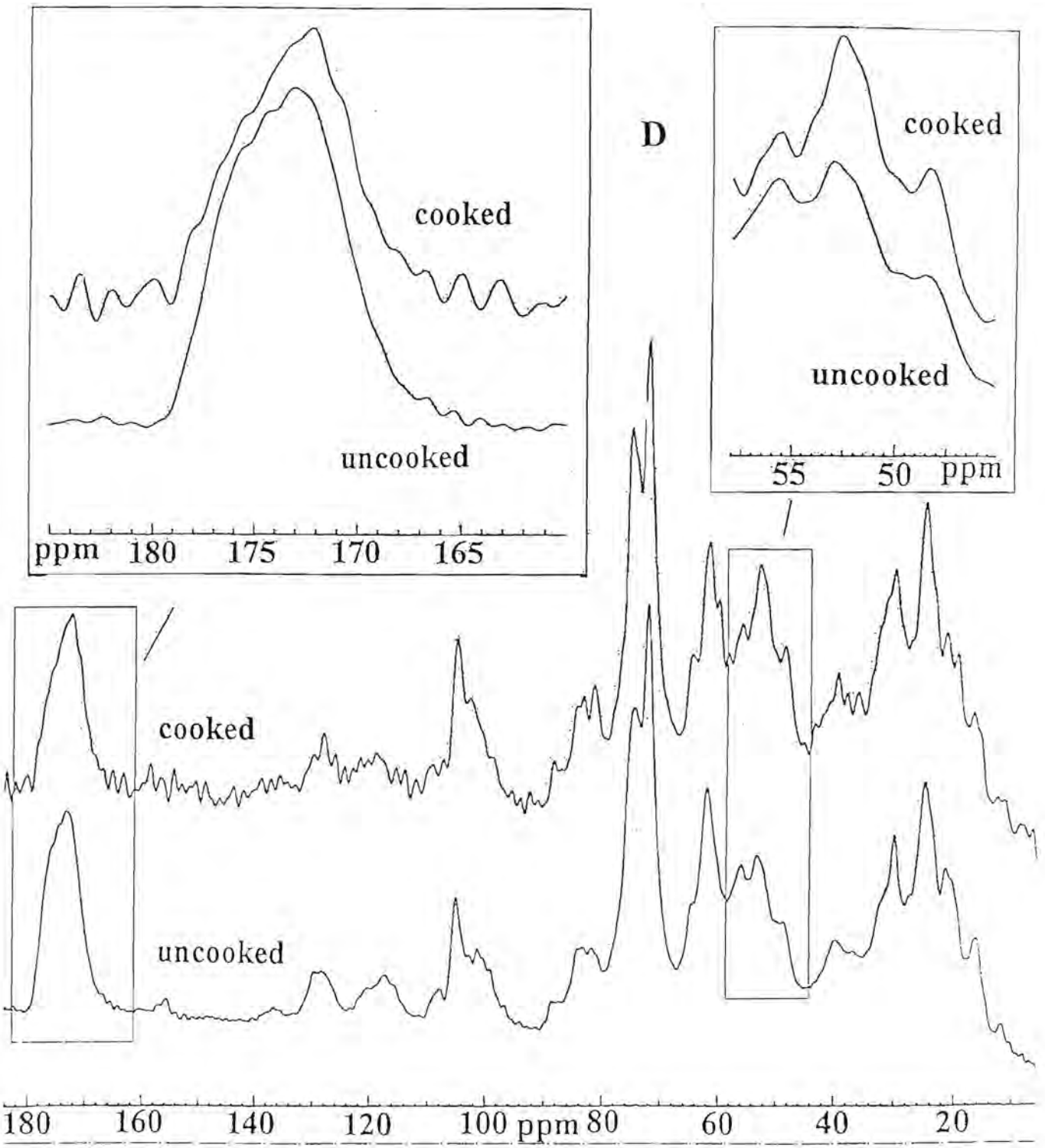
(NB: The 45-58 ppm region is not shown inset due to high signal-to-noise ratio)



B) ^{13}C CPMAS NMR spectra of uncooked and wet cooked protein body-enriched samples of KAT 369 sorghum



C) ^{13}C CPMAS NMR spectra of uncooked and wet cooked protein body-enriched samples of PAN 6043 maize



D) ^{13}C CPMAS NMR spectra of uncooked and wet cooked protein body-enriched samples of P850029 sorghum mutant

Peaks were obtained in the 20-58 ppm, 58-110 ppm, 120-130 ppm and 170-180 ppm regions of the spectra of all samples, uncooked and cooked. The effect of wet cooking on the spectra of the normal sorghums, maize and the highly digestible sorghum mutant appeared to be the same for all samples. There were alterations in shapes and intensities of the peaks in the above-named regions. In addition to changes in shape and intensity, peaks in the 45-58 ppm and 170-180 ppm regions were shifted upfield (towards the right or lower ppm) in all samples on wet cooking. This is illustrated in the insets of the spectra of the four samples.

4.7 *In vitro* protein digestibility and FTIR spectroscopy of popped sorghum and maize

Table 14. *In vitro* protein digestibility of popped NK 283 sorghum and PAN 6043 maize in comparison with uncooked and wet cooked whole grain

	NK 283	PAN 6043
Popped	41.3 a[1]	53.3 b
	±0.5[2]	±2.1
Uncooked	(59.1)[3]	(66.6)
Wet cooked	(30.5)	(62.0)

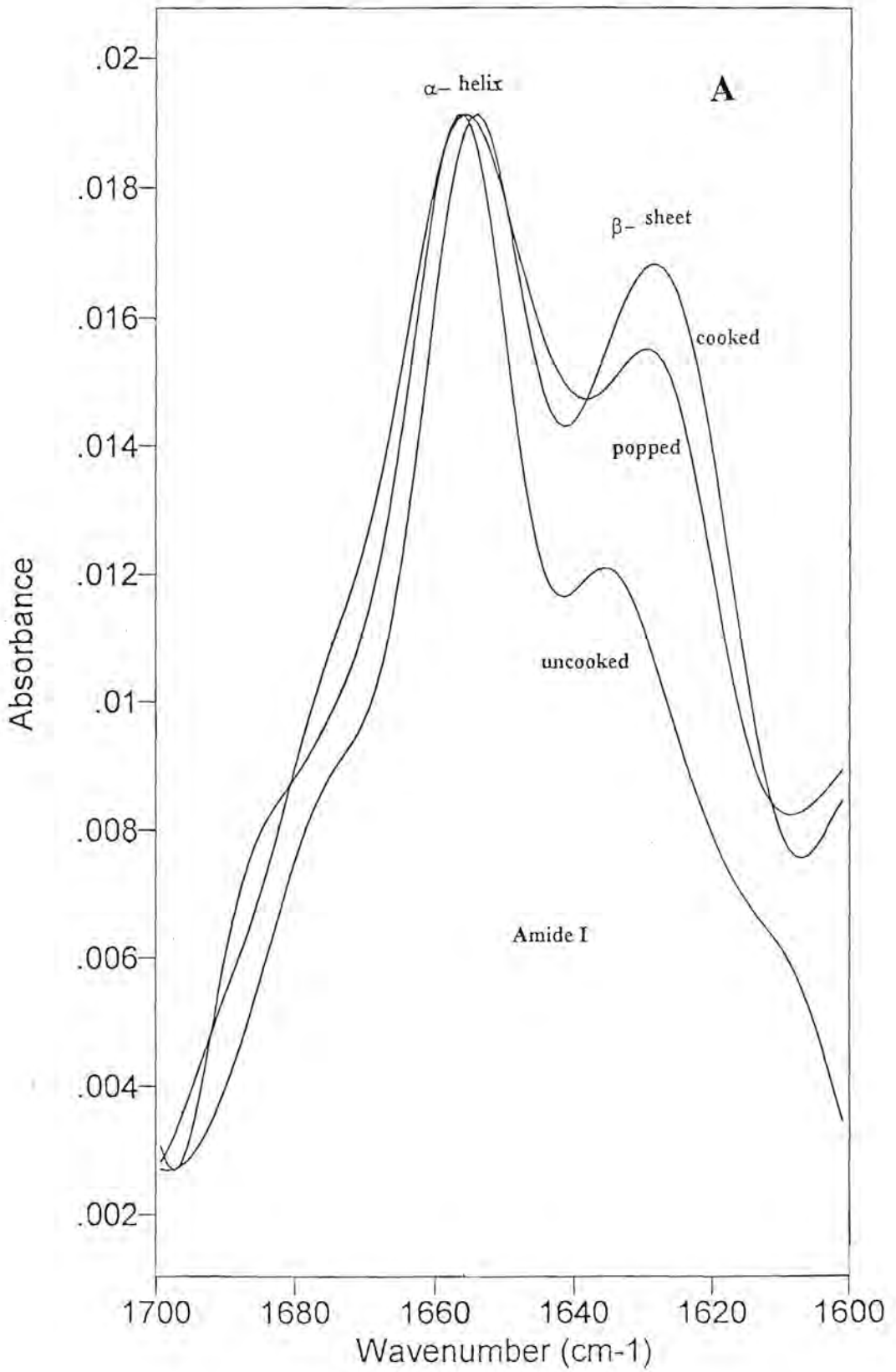
[1]Mean values with different letters in the same row are significantly different from each other ($p < 0.05$).

[2]Standard deviation.

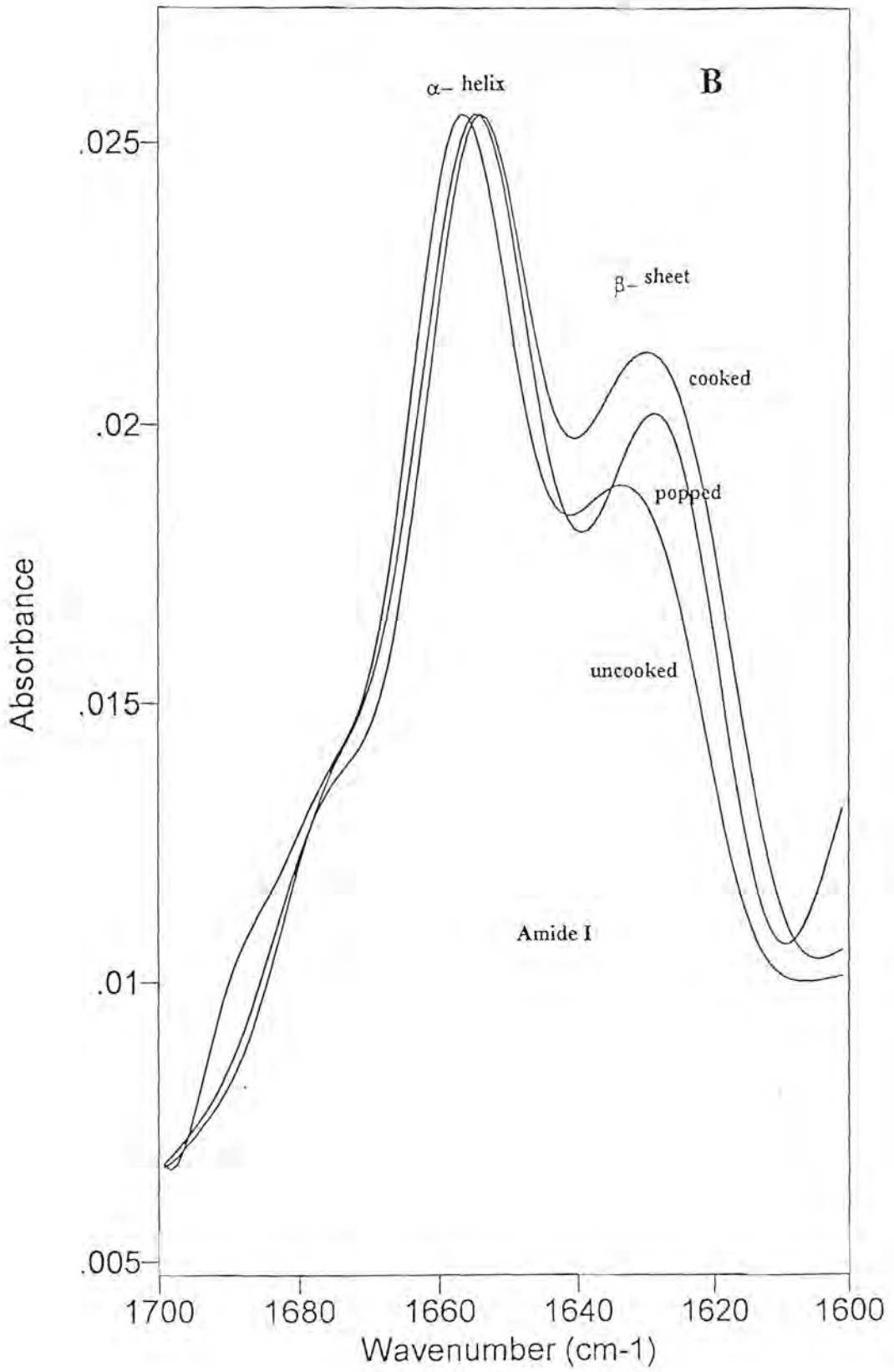
[3]Values in parenthesis from Table 3

Popped maize had higher protein digestibility compared to popped sorghum. Uncooked whole grain of both sorghum and maize were more digestible than the popped whole grain. Wet cooked sorghum was less digestible than popped. However, wet cooked maize was more digestible than popped.

Figure 14: Fourier deconvoluted FTIR spectra of uncooked, wet cooked and popped whole grain of sorghum and maize



A) Fourier deconvoluted FTIR spectra of uncooked, wet cooked and popped whole grain of NK 283 sorghum



B) Fourier deconvoluted FTIR spectra of uncooked, wet cooked and popped whole grain of PAN 6043 maize

Uncooked sorghum and maize samples produced two peaks at approximately 1655 cm^{-1} and 1635 cm^{-1}. Popping and wet cooking brought about shifts of these peaks towards lower wavenumbers in addition to increase in intensity of the peak at 1635 cm^{-1} for both sorghum and maize. There was a greater increase in intensity of the 1635 cm^{-1} peak of wet cooked sorghum and maize compared to popped sorghum and maize. The increase in intensity of the 1635 cm^{-1} peak on popping and on wet cooking was greater in sorghum than in maize

CHAPTER 5

DISCUSSION

It has been suggested that sorghum in the uncooked state has lower protein digestibility than uncooked maize (Hamaker *et al.*, 1986; Hamaker *et al.*, 1987; Oria *et al.*, 1995a). The slightly lower digestibility of the uncooked sorghums compared to uncooked maize at the whole grain level agrees with this suggestion. However, the fact that this investigation showed that at the endosperm level, protein digestibility of the uncooked sorghums was essentially the same as uncooked maize, whilst at the protein-body-enriched level, the two sorghums (uncooked), had higher protein digestibility than the maize shows that this is not always the case. Protein digestibility values of uncooked sorghum in the literature show a lot of variation. Marginally lower protein digestibilities for uncooked sorghum than uncooked maize have been reported by Hamaker *et al.* (1986) (80.7% for sorghum and 81.5% for maize) and Hamaker *et al.* (1987) (80.8% for sorghum and 83.4% for maize). Values as low as 65.8% for uncooked, decorticated sorghum (Weaver *et al.*, 1998) have been reported. Axtell *et al.* (1981) reported 92.9% protein digestibility for uncooked sorghum. One of the reasons for such variations is that different sorghum cultivars or varieties may show different protein digestibilities. Equally importantly, uncooked sorghum protein digestibility also depends on the nature of the material being assayed or the form in which it is. Protein digestibility assays have been conducted on either whole grain (Axtell *et al.*, 1981; Hamaker *et al.*, 1986; Hamaker *et al.*, 1987; Rom *et al.*, 1992; Oria *et al.*, 1995a), decorticated grain (Axtell *et al.*, 1981; Mertz *et al.*, 1984; Oria *et al.*, 1995b; Weaver *et al.*, 1998) or some undefined commercial grain fraction (Bookwalter, Kirleis & Mertz, 1987). These different types of grain material have differing proportions of pericarp, endosperm and germ. This investigation shows that protein digestibility of uncooked sorghum is improved as the proportion of pericarp and germ material becomes less. Similar results have been reported by Bradbury, Collins and Pyliotis (1984) who observed an improvement in uncooked rice protein digestibility from whole grain to endosperm.

The observed improvement of protein digestibility of uncooked and cooked sorghum with change in organisational level from whole grain to endosperm but not with maize suggests that certain factors that interfered with protein digestibility in sorghum were either not present in maize or present in lower quantities in maize compared to sorghum. Phenolic compounds

are a possible candidate. The pericarp colour of sorghum has been attributed to flavonoid-type phenolic compounds (Hahn *et al.*, 1984) like the anthocyanin and anthocyanidin pigments which have been found in the pericarp of red sorghum (Nip & Burns, 1969) and white sorghum (Nip & Burns, 1971). The antinutritional effect of tannins in sorghum by formation of indigestible protein-tannin complexes *in vivo* (Armstrong *et al.*, 1973; Rostagno *et al.*, 1973; Armstrong *et al.*, 1974a) and *in vitro* (Armstrong *et al.*, 1974b; Schaffert *et al.*, 1974; Butler *et al.*, 1984) is well known. Though the red NK 283 sorghum hybrid and the white KAT 369 sorghum variety used in this investigation are condensed-tannin-free, a possible involvement of polyphenols (flavonoids and phenolic acids in this case) in lowering protein digestibility of uncooked and cooked whole grain sorghum may be inferred from the above results. If polyphenols do play a role in lowering protein digestibility, it would be expected that a reduction in polyphenol content would bring about an improvement in protein digestibility.

The maize cultivar PAN 6043 in contrast to the sorghums, had similar total polyphenol contents at whole grain flour, endosperm flour and protein-body-enriched levels. The protein digestibility of the uncooked maize at these three levels was essentially the same. The higher total polyphenol content of the red sorghum NK 283 whole grain compared to the white sorghum KAT 369 whole grain and the white maize whole grain was expected. Total polyphenol contents reported for sorghum and maize are 0.17-10.23% dry basis (sorghum) and 0.03% dry basis (maize) (reviewed by Bravo, 1998). Since grain pericarp colour is attributable to flavonoid-type phenolic compounds, the red sorghum variety would be expected to have a higher total polyphenol content. The decrease in total polyphenol content from whole grain to endosperm for the two sorghums is due to removal of the pericarp which is rich in phenolic compounds (Nip & Burns, 1969; Nip & Burns, 1971). The accompanying increase in protein digestibility of the uncooked and cooked sorghums from whole grain to endosperm may suggest possible involvement of polyphenols in lowering protein digestibility. This observation appears to be at odds with the current view regarding the absence of effect of the smaller molecular weight polyphenols, namely, phenolic acids and flavonoids on protein digestibility. Though these polyphenols have been reported to hinder iron absorption in the gastro-intestinal lumen (Brune, Rossander & Hallberg, 1989), they are not known to have any adverse effects on protein digestibility (reviewed by Serna-Saldivar & Rooney, 1995).

The sorghum protein-body-enriched samples had similar total polyphenol content to whole grain. These sorghum protein-body-enriched samples were coloured and this is a reflection of the ability of non-tannin polyphenols to bind to protein. In addition, the protein-body-enriched samples contained endosperm cell wall material (see Figure 8) which is a source of phenolic compounds such as ferulic acid (Hahn *et al.*, 1984). This increase in total polyphenols going from endosperm to protein-body-enriched samples did not bring about a decrease in protein digestibility of the uncooked and cooked sorghums at the protein-body-enriched level; an observation which does not support the hypothesis that the polyphenols of the sorghum varieties used here may be involved in reducing protein digestibility. From the observation that there is improvement in uncooked and cooked sorghum protein digestibility in going from whole grain to endosperm, a possible role of polyphenols of the condensed-tannin-free sorghums reducing protein digestibility at the whole grain level may not be discounted totally. According to Damodaran (1996), polyphenols in several plant proteins may be oxidised to quinones by molecular oxygen at neutral to alkaline pH. The quinones may go on to form peroxides which are highly reactive oxidising agents and could bring about oxidation of several amino acid residues and polymerisation of proteins. Polyphenols may influence sorghum protein digestibility at the endosperm level but not as phenolic acids or flavonoids. An alternative mechanism may be through protein crosslinking with ferulic acid in endosperm cell walls. This may explain why the uncooked and cooked sorghum protein body-enriched samples, which have a lower proportion of cell wall material, have better digestibility than the endosperm samples.

Condensed tannins inhibit enzymes (Daiber, 1975). However, the antinutritional effect of sorghum condensed tannins is believed to lie in their ability to form complexes with dietary protein rather than inhibition of digestive enzymes (Butler *et al.*, 1984). These authors argued that grinding, cooking or other processing of high-tannin sorghum enhances the opportunity for interaction of tannin with dietary protein before it encounters digestive enzymes *in vivo*. Nevertheless, the likelihood of enzyme inhibition in the *in vitro* system used in this investigation contributing to reduction in sorghum protein digestibility was investigated. The high-tannin sorghum very substantially inhibited amylase activity while the condensed-tannin-free sorghums and the maize had a negligible effect on amylase activity. It may be concluded therefore, that the polyphenols of the condensed-tannin-free sorghums and the maize are not enzyme inhibitory. Therefore if polyphenols are involved in reducing protein

digestibility, the mechanism would likely be through interaction of these polyphenols with substrate protein and not with the pepsin enzyme.

The reduction in protein digestibility on cooking for both sorghum varieties but not to any great extent with maize (and other cereals) is an effect well documented in literature (Axtell *et al.*, 1981; Mertz *et al.*, 1984; Hamaker *et al.*, 1986; Hamaker *et al.*, 1987). This has been shown to occur for whole grain (Axtell *et al.*, 1981; Hamaker *et al.*, 1986; Hamaker *et al.*, 1987; Rom *et al.*, 1992; Oria *et al.*, 1995a) and decorticated grain (Axtell *et al.*, 1981; Mertz *et al.*, 1984; Bookwalter *et al.*, 1987; Oria *et al.*, 1995b; Weaver *et al.*, 1998). This investigation shows that cooking reduces sorghum protein digestibility at the endosperm and protein-body-enriched levels and at the level of the extracted proteins (Table 13). Considering uncooked and cooked samples together, the overall improvement in protein digestibility of the two sorghums from whole grain, through endosperm to protein-body-enriched samples is an indication that grain organisational structure has an effect on protein digestibility. Removal of the outer layers of the grain, namely the pericarp and germ improved protein digestibility in the sorghum grain but not the maize. This indicates interaction between sorghum protein and pericarp and germ components which leads to a reduction in protein digestibility.

Dietary fibre refers to the polysaccharide fraction of plant foods, particularly cereals, that are not digested by the human alimentary tract (Johnson & Southgate, 1994). In cereals, this fraction is derived from the pericarp and endosperm cell walls with the major constituents being cellulose and non-cellulosic polysaccharides mainly, heteroxylans and variable amounts of β-glucans (Johnson & Southgate, 1994; Verbruggen, 1996). The binding of protein to these non-starch polysaccharides is believed to be one of the factors which impairs protein digestion (Cheftel *et al*, 1985). Various workers have reported results that show an association between protein and pericarp or endosperm cell walls (Gram, 1982; Glennie, 1984; Bach Knudsen & Munck, 1985), the latter two with sorghum. It is possible that such an association could lower protein digestibility either by reduction of access to enzymes or formation of indigestible complexes. Studies conducted by Gram (1982) on germinating barley seeds showed that some endosperm cells of ungerminated and germinated seeds appeared to be identical. They both contained intact cell walls and storage protein, indicating that the cell walls constituted a barrier to proteases. Within sorghum (Shull *et al.*, 1990) and maize (Khoo & Wolf, 1970) endosperm, starch granules and protein bodies are surrounded by cell walls. Glennie (1984)

observed that isolated sorghum endosperm cell walls had 46% protein associated with them. Bach Knudsen and Munck (1985) found significant amounts of protein associated with total dietary fibre and acid detergent fibre fractions in uncooked and cooked sorghum. The amino acid composition of the sorghum proteins associated with acid detergent fibre resembled that of kafirins. Choct and Annison (1992) have reported that addition of wheat pentosans (arabinoxylans) to the diets of chicken broilers decreased protein digestibility by 18.7% in the ileum. In legumes, it has been shown that cell walls represent a physical barrier to protein digestion, thus limiting protein digestibility (Melito & Tovar, 1995).

The nature of this protein-cell wall adhesion is not very clear. The cell wall itself has been described as a biphasic structure, consisting of a rigid skeleton of cellulose microfibrils held together by a gel-like matrix (reviewed by Fry, 1986). The matrix is very complex in chemical terms, and is built up of noncellulosic polysaccharides (like the arabinoxylans), glycoproteins and phenolic compounds (Brett & Waldron, 1990). Saulnier and Thibault (1999) have proposed a model for the cell walls of maize bran in which highly crosslinked heteroxylans (of the cell wall matrix) constitute a network in which cellulose microfibrils may be embedded (Figure 15). Endosperm cell walls would be composed of the heteroxylans crosslinked with ferulic acid and glycoproteins. Crosslinks within the cell wall are important in maintaining its integrity. After extraction from the wall, most of the matrix polysaccharides are soluble, being polyhydroxy, hydrophilic molecules. However, within the cell wall, they are water-insoluble and it makes the wall matrix very coherent, a characteristic feature of the cell wall, which may be attributed to the existence of crosslinks between the cell wall polymers (reviewed by Fry, 1986; Brett & Waldron, 1990). These crosslinks may be of a non-covalent or covalent nature. Hydrogen bonds between heteroxylans and cellulose microfibrils and ionic bonds between positively charged glycoproteins and negatively charged wall polysaccharides are two examples of non-covalent crosslinks within the cell wall (Fry, 1988).

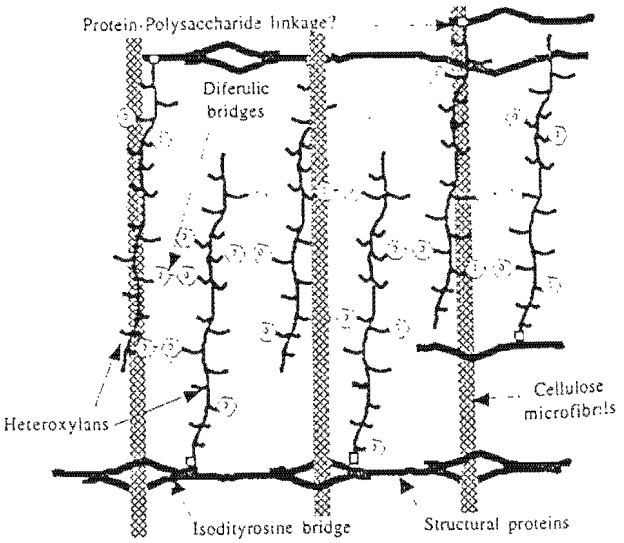
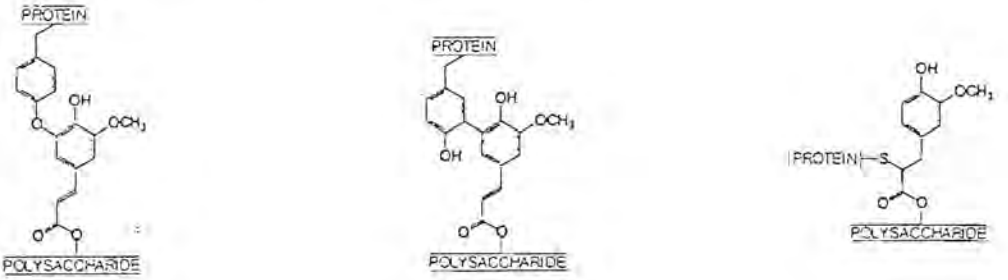


Figure 15: Proposed model for maize bran cell walls. (Saulnier & Thibault, 1999).

Phenolic acids have been identified as being involved in covalent crosslinks within the cell wall. Cell wall arabinoxylans of the Poaceae may be esterified with phenolic acids such as *p*-coumaric and ferulic acid (Nishitani & Nevins, 1989; Saulnier, Vigouroux & Thibault, 1995; Ng, Greenshields & Waldron, 1997). Ferulic acid has the ability to couple oxidatively to another ferulic acid of an arabinoxylan to form a diferulate crosslink. The formation of phenolic crosslinks is dependent on the synthesis of the phenol-bearing arabinoxylan, the presence of peroxidase enzyme and a supply of hydrogen peroxide or an equivalent oxidising agent (Fry, 1988). Therefore it may be assumed that in general, oxidising conditions could promote formation of phenolic or specifically, ferulic crosslinks. Hypothetically, phenolics-mediated crosslinking of proteins within the cell wall is considered possible. It has been suggested that dimerisation may occur between tyrosine residues in proteins and ferulic acid residues on arabinoxylans (Bacic, Harris & Stone, 1988). From a study of oxidative gelation of wheat flour pentosans, Hoseney and Faubion (1981) suggested that the esterified ferulic acid may be crosslinked to the sulphydryl group of cysteine residues in proteins (see Figure 16).

It could be hypothesised therefore, that the cooking process, which is conducted in the presence of oxygen, could lead to the formation of such ferulic acid crosslinks between proteins and arabinoxylans and in so doing, bring about adhesion between proteins and the cell wall. Removing the outer layers of the grain would reduce the amount of cell wall

material and hence, less protein-cell wall adhesion and improved protein digestibility. Van Sumere, De Pooter, Ali and Degrauw-Van Bussel (1973) have suggested an alternative mode of ferulic acid crosslinking in which the carboxylic acid group of the phenolic is attached to the amino group of the N-terminal amino acid of a polypeptide forming a so-called pseudopeptide bond.



A B C

Figure 16: Structures of proposed covalent crosslinks between polysaccharides and proteins in cell walls. A) and B) Tyrosyl-feruloyl crosslinks (Bacic *et al.*, 1988); C) Feruloyl-sulphydryl crosslink (Hoseney & Faubion, 1981).

Another mode of protein-cell wall adhesion could be by direct attachment of the protein to carbohydrate moieties. Cell walls are known to contain a variety of different proteins and most of these are glycosylated (Brett & Waldron, 1990). The best characterised are the structural cell wall glycoproteins known as hydroxyproline-rich glycoproteins (HRGPs) or extensins. Their presence in sorghum (Raz, Crétin, Puigdomènech & Martínez-Izquierdo, 1991) and maize (Kieliszewski & Lamport, 1987; Hood, Shen & Varner, 1988; Hood, Hood & Fritz, 1991) have been reported. The amino acid compositions of sorghum and maize HRGPs are similar; rich in hydroxyproline, proline, lysine, tyrosine and threonine (Kieliszewski, Leykam & Lamport, 1990; Raz *et al.*, 1991). The hydroxyproline residues normally serve as attachment points for arabinose oligosaccharides (Figure 17) (Kieliszewski & Lamport, 1987; Kieliszewski, Kamyab, Leykam & Lamport, 1992). The polypeptide-carbohydrate linkage is thought to be an O-glycosidic bond in which the reducing terminus of the carbohydrate is attached to an –OH group on the polypeptide (Fry, 1988). These structural glycoproteins are highly resistant to most proteases especially when the oligoarabinose side chains are still attached. By analogy, it may be suggested that such enzyme-resistant protein-carbohydrate linkages may be formed in sorghum and maize on cooking through formation of O-glycosidic bonds between proline residues of sorghum and maize proteins and the

arabinose residues of the cell wall. Such an event is made more likely by the fact that the kafirins of sorghum and the zeins of maize are rich in proline (Evans, Schüssler & Taylor, 1987).

Though normal sorghum and maize both contain pericarp and endosperm cell walls, it appears that the protein-cell wall adhesion is stronger and occurs to a greater extent in sorghum than in maize. Bach Knudsen and Munck (1985) observed that higher amounts of protein were associated with dietary fibre fractions of cooked sorghum than other cereals like wheat, rye, barley and maize. This might explain the superior protein digestibility of cooked maize compared to cooked sorghum.

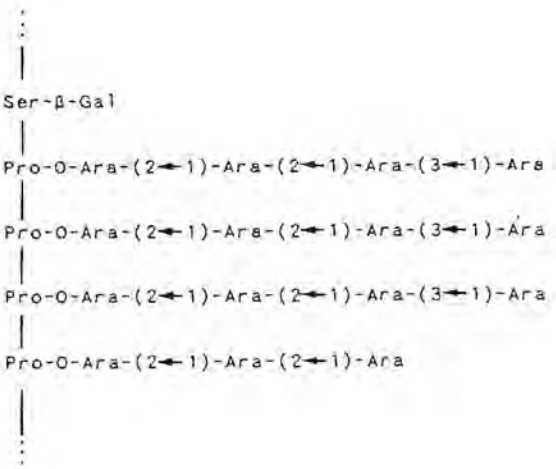


Figure 17: Structure of the hydroxyproline-rich region of plant cell wall glycoproteins. (Brett & Waldron, 1990).

Treatment of the cooked sorghum and maize whole grain and endosperm flours with *alpha*-amylase improved protein digestibility. This indicates that gelatinised starch reduces protein digestibility, a finding supported by the fact that in the protein-body-enriched samples, where the proportion of starch was much lower, there were no differences in protein digestibility between cooked and cooked plus *alpha*-amylase treatments. *Alpha*-amylase breaks α-1,4 glycosidic bonds of starch at random, forming glucose and dextrins leading to a reduction in viscosity (Belitz & Grosch, 1999). This would allow the pepsin greater access to the protein in the cooked grain. However, Oria *et al.*, (1995b) found that the protein digestibility of decorticated sorghum flour cooked with heat-stable *alpha*-amylase was approximately the

same as that cooked without, which appears to contradict the findings of this investigation. The mode of treatment with *alpha*-amylase was different in the present work as samples were treated with *alpha*-amylase after cooking, before pepsin digestion. Protein appears to have an effect on starch gelatinisation and digestibility in sorghum. Chandrashekar and Kirleis (1988) observed that sorghum grains with lower capacities for starch gelatinisation had more kafirin-containing protein bodies. Addition of 2-mercaptoethanol to cooking media increased degree of rice starch gelatinisation (Hamaker & Griffin, 1993). Furthermore, Zhang and Hamaker (1998) reported that treating sorghum flour with pepsin before cooking led to an increase in starch digestibility. It would be expected therefore, that the presence of starch could in turn influence protein digestibility. Improvement of protein digestibility of sorghum and maize porridge after treatment with *alpha*-amylase has important nutritional implications. This scenario is similar to the order of events in the *in vivo* situation where food is first attacked by salivary amylase in the mouth before digestion with pepsin in the stomach and with other proteases in the duodenum.

From the foregoing, it appears that polyphenols may affect protein digestibility of uncooked and cooked sorghum in going from whole grain to endosperm. Protein-cell wall adhesion affects protein digestibility of cooked sorghum, whilst gelatinised starch reduces accessibility of enzymes to protein in sorghum and maize thus limiting protein digestibility. However, unlike maize, the protein digestibility of cooked sorghum remained far lower than uncooked at the protein-body-enriched level where other components like cell walls and starch occurred in much lower proportions. This suggests that changes occur in the proteins themselves on cooking which makes them less digestible. It has been proposed that higher molecular weight, disulphide-bonded protein polymers are formed in sorghum (Hamaker *et al.*, 1986) and maize (Batterman-Azcona & Hamaker, 1998) on wet cooking. Such an event would indicate an alteration in protein secondary structure as a result of the wet cooking process.

The disulphide crosslinking hypothesis is supported by the observations in this investigation from SDS-PAGE. For uncooked and cooked protein-body-enriched samples of sorghum and maize, large amounts of stained material with extremely low mobility at the origin of gels run under non-reducing conditions indicate the existence of substantial amounts of high molecular weight oligomers (Figure 9). For the sorghums, (uncooked and cooked under non-reducing and reducing conditions; Figure 9A, B and D), bands appearing at approximately 24, 23, 22 and 18 kDa are identified as the monomeric γ-, α1-, α2- and β-kafirins respectively (Shull *et*

al., 1991). For maize (Figure 9C), the band at 25 kDa is identified as γ-zein and those at 22 and 19 kDa as α-zein (Esen, 1987). The β-zeins (14-16 kDa) appeared only under reducing conditions, an indication that they are involved in disulphide crosslinking in maize. The appearance of monomeric kafirins and zeins in uncooked sorghum and maize under non-reducing conditions indicates that some of the prolamins exist as monomers in the sorghum and maize seed, a similar finding to that observed by El Nour *et al.* (1998) in sorghum. Under reducing conditions, the increases in monomer band intensities for both uncooked and cooked protein-body-enriched samples of sorghum and maize accompanied with decreases in oligomer bands (≥ 45 kDa) was expected. This is as a result of oligomers comprising of disulphide-linked monomeric units of the α-, β- and γ-kafirins or zeins being separated into their constituent monomers (El Nour *et al.*, 1998).

The appearance of bands in the 45-50 kDa and 66 kDa regions and also above 66 kDa, towards the origin of the gels indicate the presence of high molecular weight protein oligomers in uncooked and cooked sorghum and maize protein-body-enriched samples. Such oligomeric proteins in the 44 kDa to 97 kDa region have been found in maize (Landry, Paulis & Wall, 1987) and sorghum (El Nour *et al.*, 1998) and have been designated dimers (45 kDa), trimers (66 kDa) and polymers (97 kDa) (Landry *et al.*, 1987; El Nour *et al.*, 1998).

The observation that for SDS-PAGE of the pepsin-indigestible residues (Figure 10) under reducing conditions, uncooked tracks were much fainter than cooked is an indication that though some disulphide-bonded oligomers are present in the uncooked samples, more of such oligomeric protein species are formed on cooking in both sorghum and maize. This is in agreement with the proposal that on cooking or thermal processing, higher molecular weight, disulphide-bonded protein polymers are formed in sorghum (Hamaker *et al.*, 1986), wheat (Ummadi *et al.*, 1995), rice (Mujoo *et al.*, 1998) and maize (Batterman-Azcona & Hamaker, 1998). On electrophoretic examination of proteins in Landry-Moureaux fractions 4 (extracted with pH 10 buffer and 2-mercaptoethanol) and 5 (extracted with pH 10 buffer, 2-mercaptoethanol and SDS) of pepsin-indigestible residue of cooked sorghum, Hamaker *et al.* (1986) reported the presence of monomeric kafirins in these fractions. This is confirmed by the observation in this investigation that the cooked pepsin-indigestible residues consisted of monomeric kafirins and zeins on reduction.

Concerning α-, β- and γ-kafirins and sorghum protein bodies, Oria *et al.*, (1995b) have proposed that during cooking of sorghum, enzymatically-resistant protein polymers are formed by disulphide crosslinking of the β- and γ-kafirins which are located at the periphery of the protein body. Such crosslinking would restrict digestion of the more centrally located α-kafirin. The absence of β-kafirin from the uncooked sorghum pepsin-indigestible residue (Figure 10, track 10) and its appearance in the cooked sample after reduction (Figure 10, track 11) indicates that it was involved in disulphide cross-linking during the cooking process. The reduction of rice protein digestibility on cooking has also been attributed to formation of enzyme-resistant, disulphide-bonded protein polymers which in contrast to sorghum, are believed to occupy the core of rice protein bodies (Resurreccion, Li, Okita & Juliano, 1993). However this hypothesis has been challenged by Barber, Lott and Yang (1998) who found no apparent concentration of sulphated prolamin polypeptides in the central region of rice protein bodies in faecal protein particles.

The observation that sorghum (uncooked and cooked) contains more oligomers in the 45-50 kDa region than maize might indicate that the extent of oligomer formation differs in the two cereals in both the uncooked and cooked states. It appears that in uncooked sorghum, the degree of crosslinking is much more than in maize. Various workers have reported results which indicate that in uncooked sorghum, the more crosslinked proteins of Landry-Moureaux fraction 3 (extracted with alcohol and reducing agent) occur in higher quantities than fraction 2 (extracted with alcohol) (Jambunathan & Mertz, 1973; Guiragossian *et al.*, 1978; Hamaker *et al.*, 1986; Vivas *et al.*, 1992; Hamaker *et al.*, 1994), whilst the opposite is the case for maize (Landry & Moureaux, 1980; Hamaker *et al.*, 1986; Vivas *et al.*, 1992; Hamaker *et al.*, 1994) (see Table 5). It is possible that whilst cooking may lead to oligomer formation involving the monomeric prolamins, this might be more extensive in sorghum than in maize. As a result, more enzyme-resistant protein oligomers would be formed in sorghum than in maize which may explain the superior digestibility of cooked maize proteins compared to cooked sorghum.

The occurrence of protein oligomers around 45-50 kDa resistant to reduction in pepsin-indigestible residues of cooked sorghum protein-body-enriched samples in higher quantities compared to maize lends weight to this hypothesis and is supported by the work of Hamaker *et al.* (1986). Landry-Moureaux fractionation of uncooked and cooked sorghum and maize

conducted by Hamaker *et al.* (1986) showed that non-extractable proteins were 25.8% for cooked sorghum compared to 14.2% for cooked maize. Their results showed a more pronounced shift in alcohol-soluble proteins (towards the more crosslinked fractions) in sorghum than in maize. They also reported that cooked sorghum had a higher amount of indigestible protein (35.2%) compared to uncooked sorghum (19.3%) while there was essentially no difference between cooked (18.1%) and uncooked maize (18.5%).

Oria *et al.* (1995b) reported that cooking sorghum flour with a reducing agent improved protein digestibility but not to the level of uncooked flour. These authors attributed this to the possible presence of inaccessible disulphide bonds. In this light therefore, occurrence of reduction-resistant protein oligomers in pepsin-indigestible residues of cooked sorghum (Figure 10) is not surprising. Perhaps these oligomers are in such a conformation which does not allow easy access of reducing agent to disulphide bonds. The likelihood that cooking may also bring about protein crosslinking not involving disulphide bonding could be considered. Initially, the hydroxyproline-rich glycoproteins (HRGPs) within the cell wall are secreted in soluble form and bind ionically to acidic wall polysaccharides. Later they become more firmly bound in the wall and this is thought to be as a result of oxidative coupling of tyrosine residues to form a crosslinking dimer known as isodityrosine (IDT, made up of two tyrosine units linked by a diphenyl ether bridge) (Fry, 1982). It is believed that IDT may form both intra-polypeptide loops (Epstein & Lamport, 1984) and inter-polypeptide crosslinks (Biggs & Fry, 1990) and such crosslinks may contribute to the insolubility and indigestibility of HRGPs within the cell wall (Fry, 1988). Brady, Sadler and Fry (1996) showed the existence of a tetramer of tyrosine (di-isodityrosine) in plant cell wall proteins. They suggested that due to steric factors, this tetramer is incapable of forming an intra-polypeptide loop in which all four tyrosine units are neighbour residues within a single polypeptide chain. They therefore concluded that this tetramer may participate in inter-polypeptide crosslinking. In similar vein, it could be proposed that the oxidising conditions of the cooking process could lead to formation of more of such non-disulphide crosslinks in sorghum proteins than in maize proteins and this may account for the existence of the reduction-resistant oligomers. Perhaps other non-disulphide crosslinks may be formed by esterification of amino acid residues between different polypeptide chains.

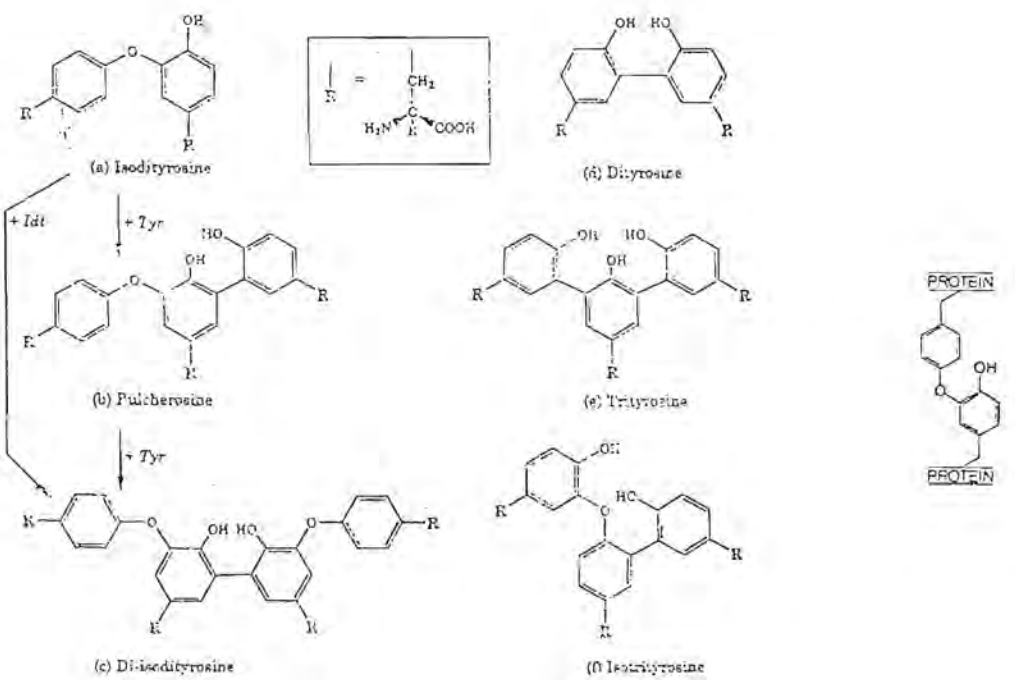


Figure 18: Proposed structures of oxidatively coupled products of tyrosine which may lead to formation of non-disulphide crosslinks in proteins (Brady, Sadler & Fry, 1998) and possible structure of a tyrosyl-tyrosyl crosslink between proteins (Bacic *et al.*, 1988).

Formation of non-disulphide crosslinks is made more likely by the finding that cooked, alkylated sorghum kafirin did not have as high protein digestibility as uncooked kafirin (Table 13). This also confirms the finding by Oria *et al.*, (1995b) that cooking sorghum flour with reducing agent did not improve digestibility to the level of uncooked flour. The alkylation process involves using a reducing agent to cleave disulphide bonds and then free thiols thus generated are trapped with an alkylating agent to prevent their re-oxidation (Hollecker, 1997). If protein crosslinking on cooking is exclusively through disulphide bond formation, it would be expected that alkylation would very significantly improve the protein digestibility of cooked samples. The observation that alkylated and cooked kafirin still had much lower protein digestibility than uncooked suggests that there could be crosslinking not involving disulphide bonds.

However, there is no reason why non-disulphide crosslinking should not occur in maize zein as well. The better digestibility of the alkylated and cooked zein as compared to alkylated, cooked kafirin is a reflection of the possibility that the extent of crosslinking may be lower in maize compared to sorghum. This observation also agrees with those from SDS-PAGE in this present investigation, where pepsin-indigestible residues of cooked sorghum appeared to have protein oligomers in higher quantities compared to maize. A possible role played by polyphenols is also brought in focus by this observation. Like the sorghum protein body-enriched samples, the kafirin samples were coloured compared to the zein samples; a reflection of polyphenols binding to protein. Such polyphenol-protein interaction which may not be occurring to the same extent in zein may also explain the better digestibility of the zein samples.

In FTIR spectroscopy, absorption bands due to the N-substituted amide groups in the polypeptide backbone dominate protein spectra (Fraser, 1956). The amide I (1650 cm^{-1}) and amide II (1550 cm^{-1}) are two of such characteristic amide absorption bands in protein FTIR spectra. The amide I vibration mode is primarily a C=O stretching vibration coupled to contributions from the CN stretch, CCN deformation and in-plane NH bending modes whilst the amide II vibration mode is considered to be an out-of-phase combination of CN stretch and in-plane NH deformation modes of the peptide group (Lavialle *et al.*, 1982; Bandekar, 1992). The absorption bands in the FTIR spectra may be considered to arise predominantly from the kafirins and zeins of sorghum and maize respectively, since these are the major storage proteins in the mature seed. Further, Taylor *et al.* (1984c) reported that in sorghum, the kafirins make up about 85% of the proteins in the type of protein body preparation used in this present investigation. For the normal sorghums, maize and the sorghum mutants (Figures 11 and 12), the band appearing in the 1670-1620 cm^{-1} region is identified as the amide I and that in the 1550-1500 cm^{-1} region as amide II.

The amide bands of proteins are sensitive to the conformation in which the protein is (Surewicz & Mantsch, 1988). Bands in the amide I region centred between 1650 and 1658 cm^{-1} show the presence of proteins in α-helical conformation (Lavialle *et al.*, 1982; Jakobsen *et al.*, 1983; Surewicz & Mantsch, 1988; Bandekar, 1992), whilst bands between 1620 and 1640 cm^{-1} (Jakobsen *et al.*, 1983; Surewicz & Mantsch, 1988; Bandekar, 1992), and sometimes at 1675 cm^{-1} (Lavialle *et al.*, 1982), are due to antiparallel, intermolecular β-sheet

structure. In the amide II region, bands at 1545 cm^{-1} and 1547 cm^{-1} arise from α-helical proteins and bands at 1524 cm^{-1} from β-sheet components (Surewicz & Mantsch, 1988; Bandekar, 1992). Consequently, the amide I peak located at 1660 cm^{-1} is assigned to α-helical components and that at 1625 cm^{-1} to antiparallel intermolecular β-sheet. The amide II peak at 1540 cm^{-1} is assigned to α-helical components and that at 1520 cm^{-1} to β-sheet (Figure 12). These peaks were common to the normal sorghums, maize and the sorghum mutants.

It is clear from the absorption maxima in the amide I band, that there was a predominance of α-helical structures in the uncooked samples. This is in agreement with earlier observations from circular dichroism and optical rotatory dispersion experiments which indicated that the zeins contained an average α-helical content of 50-60% (Argos, Pedersen, Marks & Larkins, 1982). The kafirins of sorghum may have similar α-helical content as they have been shown to have extensive homology with the zeins of maize (DeRose *et al.*, 1989). Estimated α-helical contents of the uncooked samples from peak heights in the spectra were 54.0% for P851171 mutant sorghum, 54.5% for P850029 mutant sorghum, 58.5% for KAT 369 sorghum, 57.5% for NK 283 sorghum and 57.5% for PAN 6043 maize. The amide I band shapes of uncooked and cooked sorghum and maize whole grain confirm the spectral observations from the protein-body-enriched samples. The effect of cooking was to bring about increases in intensity of bands attributable to antiparallel intermolecular β-sheet structures and this effect was observable in the normal sorghums, maize and the sorghum mutants. In the amide II region, the apparent shift of absorption maxima towards the β-sheet peaks at 1520 cm^{-1} for NK 283 sorghum, PAN 6043 maize and P850029 sorghum, might indicate that this increase in β-sheet character may have been accompanied by a loss in some α-helical conformation.

The transition from the α-helical conformation to antiparallel intermolecular β-sheet on cooking is supported by the observations from the ^{13}C NMR spectra (Figure 13). Peaks in the 20-58 ppm region as observed in the normal sorghums, maize and the sorghum mutant arise from resonances due to carbons of aliphatic amino acid side chains (Schofield & Baianu, 1982) such as leucine, glutamine, alanine and proline. Peaks at 20-40 ppm may be assigned to β-, γ- and δ-carbons (Kricheldorf *et al.*, 1983; Kricheldorf & Muller, 1984) and peaks at 45-58 ppm to α-carbons of the aliphatic amino acids (Kricheldorf *et al.*, 1983; Kricheldorf & Muller, 1984). Resonances due to aromatic amino acids like phenylalanine, histidine and

tyrosine are identified within the 120-130 ppm region (Schofield & Baianu, 1982). The resonances in the region 170-180 ppm are due to the carbonyl carbon (C=O) in the peptide bond whilst those in the 58-110 ppm are due to carbohydrate carbons (starch or non-starch polysaccharides) (Chinachoti, White, Lo & Stengle, 1991). The chemical shifts of these signals are sensitive to protein secondary structure (Saito, Tabeta, Shoji, Ozaki & Ando, 1983; Shoji, Ozaki, Saito, Tabeta & Ando, 1984). In general, the peaks at 56 and 53 ppm (shown inset for all samples except NK 283 sorghum) are associated respectively with proteins in α-helical and β-sheet conformations (Kricheldorf *et al.*, 1983; Kricheldorf & Muller, 1984; Wishart *et al.*, 1991; Wishart & Sykes, 1994), as are signals at 176 and 172 ppm. Signals at 174 ppm correspond to carbons in disordered structures (Wishart & Sykes, 1994).

The alterations in shapes and intensities of the peaks attributable to carbohydrates (58-110 ppm) is likely due to starch gelatinisation (Chinachoti *et al.*, 1991). The upfield shift for both carbonyl and α-carbon signals observed on wet cooking is related to secondary structure changes from α-helical to β-sheet conformation (Kricheldorf *et al.*, 1983; Kricheldorf & Muller, 1984; Wishart *et al.*, 1991; Wishart & Sykes, 1994). The carbonyl group forms part of the peptide bond and hence, an intrinsic part of the protein backbone. The proximity of the α-carbons to the protein backbone also indicates that they would have an influence on protein secondary structure.

The FTIR and ^{13}C NMR spectra show that similar secondary structural changes occur on wet cooking in the normal sorghums, maize and the highly digestible sorghum mutants. The protein assumed more intermolecular β-sheet structure, perhaps at the expense of some α-helical conformation. Such changes have been reported to be characteristic of heat- or solvent-denatured and aggregated proteins (Kretschmer, 1957; Surewicz & Mantsch, 1988) and have previously been observed in zein (Kretschmer, 1957).

Concerning the mutant sorghums of known high protein digestibility (Weaver *et al.*, 1998), the observations from spectroscopy that their proteins underwent the same type of secondary structural change as in the normal sorghums on wet cooking supports those from SDS-PAGE where tracks for the cooked sorghum mutant under non-reducing and reducing conditions though fainter, were identical to those for the normal sorghums. This suggests that alternative factors other than change in protein secondary structure may be at play in the sorghum

mutants. These mutant sorghums have been reported to have highly invaginated protein bodies (Weaver *et al.*, 1998) compared to the spheroid-shaped protein bodies of normal sorghum and maize. Additionally, highly disulphide-bound γ-kafirins at the periphery of normal sorghum protein bodies, are found at the base of folds in the mutant protein bodies (Oria *et al.*, 2000). The invaginated form and changed location of γ-kafirin should allow greater accessibility of proteases to the protein bodies (in particular, the highly digestible α-kafirin) of the sorghum mutants and hence their very high digestibilities as observed in this investigation and reported earlier by Weaver *et al.*, (1998). These sorghum mutants contain cell wall material and protein-cell wall adhesion may well occur. A consequence of the invaginated structure of their protein bodies is that the α-kafirins will be much closer to the cell wall than the β- and γ-kafirins which are more likely to form crosslinks because of their high content of cysteine residues. This, in addition to the better accessibility of proteases to α-kafirins may account for the high digestibilities of the mutants in spite of possible protein-cell wall adhesion.

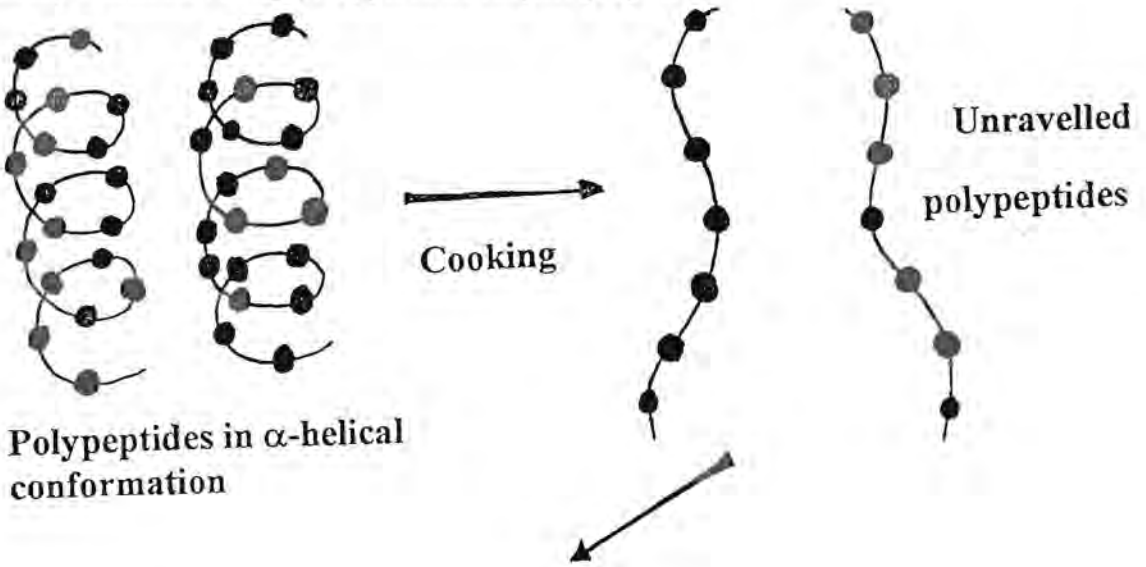
The observed shifts in the α-helix and β-sheet peaks of popped sorghum and maize towards lower wavenumbers and increase in intensity of the β-sheet peak at 1630 cm^{-1}, signify an increase in β-sheet character of the protein when the grains are popped. This indicates that popping brings about the same type of change in the spectra (for sorghum and maize) as observed for wet cooking. It is clear from the spectra that even though the same type of secondary structural change occurred in the protein by either wet cooking or popping, the increase in β-sheet components on thermal processing occurred to a greater extent by wet cooking than by popping in both sorghum and maize. This secondary structural change which may contribute to lowered protein digestibility on thermal processing, occurs to a greater extent in wet cooked than in popped samples and may explain the better protein digestibility of popped grain compared to wet cooked as observed with sorghum in this investigation and also by Parker *et al.*, (1999). The effect of popping on microstructure of the grain is also believed to contribute to the better protein digestibility of popped grain. During the popping process, the pericarp acts as a pressure vessel which allows the moisture in the kernel to turn into superheated steam (Hoseney, Zeleznak & Abdelrahman, 1983). Rupture occurs eventually when the hull can no longer withstand the internal pressure. Hoseney *et al.*, (1983) suggest that in the horny endosperm the superheated steam vaporises into the hilum of the starch granule and then gelatinise and expand the starch into a thin film. According to Parker

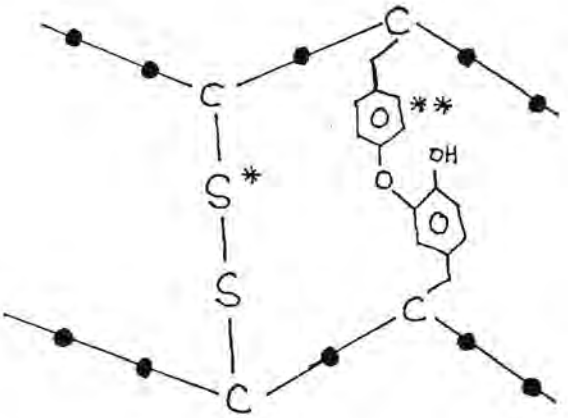
et al., (1999), the explosive popping process leads to fragmentation of the cell walls of the vitreous endosperm. This appears to improve accessibility of protein components within the endosperm to enzymes hence better protein digestibility of popped compared to wet cooked sorghum.

However, the observation that popped PAN 6043 maize had lower protein digestibility than the wet cooked PAN 6043 maize whole grain was an anomaly. This appears to contradict the observations from the FTIR spectra where the extent of protein secondary structural change in popped maize was less than wet cooked. The PAN 6043 maize is a dent maize variety and therefore does not pop as effectively as normal popcorn would. Popcorn is a flint-type maize which consists predominantly of horny endosperm with tightly-packed starch granules (Pordesimo, Anantheswaran & Mattern, 1991). This contrasts with dent maize varieties which contain a higher proportion of floury endosperm with many intergranular spaces. The floury endosperm with its intergranular spaces, provide channels of escape for the superheated steam generated during the popping process. As a result the starch granules are not expanded and retain their birefringence. It may be that less effective popping of the PAN 6043 dent maize variety could lead to less cell wall fragmentation, hence less accessibility of protein components within the endosperm to enzymes. In addition, popped grains were selected visually for the *in vitro* protein digestibility assay. It is likely that the popped maize grains selected for FTIR spectroscopy and for the digestibility assay were not representative of each other.

The better digestibility of popped compared to wet cooked sorghum may be related to findings from studies on the effect of extrusion on solubility and digestibility of sorghum kafirin from the perspective of improved accessibility of enzymes to proteins. Hamaker *et al.*, (1994) reported that extrusion improved cooked sorghum protein digestibility to the level of uncooked flour and prevented the decrease normally observed. They proposed that the extrusion process disrupted the structure of the protein bodies due to the heat and shearing action involved, thus permitting easy access to α-kafirin by digestive enzymes. The same concept of improved accessibility is applicable in the popping situation where the explosive process fragments cell walls and brings about greater exposure of protein components within the endosperm to digestive enzymes.

Whilst the SDS-PAGE results provide information about protein secondary structural change on cooking through disulphide and other forms of crosslinking, the spectra (FTIR and ^{13}C NMR) provide such information from the perspective of α-helical to antiparallel intermolecular β-sheet conformational transitions. In order to relate these two forms of protein secondary structural change, a hypothesis may be proposed.





Polypeptides in β-sheet conformation with crosslinks

Figure 19: Representation of α-helix to β-sheet conformational change on cooking followed by crosslink formation between polypeptide chains. -●-●-, amino acid residues.
* disulphide crosslink; ** tyrosyl crosslink.

A scenario whereby the application of energy during the wet cooking process breaks intra-chain hydrogen bonds that would otherwise stabilise the α-helices could be imagined. Polypeptides (most likely β- and γ-kafirins or zeins) forming these helices may thus become unravelled and aligned next to each other to form the intermolecular β-sheet conformation. It

is then not inconceivable that disulphide and other forms of crosslinking could occur between polypeptides closely aligned to each other in this manner (Figure 19). This would result in a compact structure in which accessibility of enzymes to the protein is restricted, thus leading to a reduction in protein digestibility. The SDS-PAGE results show that protein crosslinking on cooking (which brings about aggregation and change in secondary structure) occurs to a greater extent in the normal sorghums than in maize. This indicates that though changes in protein secondary structure may be similar qualitatively between sorghum and maize, they may differ quantitatively, being more extensive in the normal sorghums.

It is not clear from this investigation the reasons why thermal processing leads to more extensive protein crosslinking in sorghum than in maize. Sorghum kafirins and maize zeins are believed to bear extensive homology to each other (DeRose *et al.*, 1989). However, this may not mean that they are exactly identical. It is possible that subtle differences in tertiary structure between sorghum kafirins and maize zeins may be responsible for the observed different extents of protein crosslinking on thermal processing between sorghum and maize.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

The protein digestibility of sorghum is influenced by a number of factors. Some factors are more important than others depending on whether one is dealing with uncooked or cooked grain or the nature of the grain, that is, whole grain, endosperm, protein bodies or the extracted proteins.

The present study showed that whilst uncooked sorghum whole grain has lower protein digestibility than uncooked maize whole grain, at the endosperm level, uncooked sorghum protein digestibility is essentially the same as maize and higher than maize at the protein body-enriched level and the same as maize at the level of extracted kafirins and zeins. This is an indication that in contrast to what has been suggested by other workers, uncooked sorghum may not always have lower protein digestibility than uncooked maize. The protein digestibility of uncooked sorghum depends on the nature of the material being assayed.

The protein digestibility of uncooked and cooked sorghum is improved with change in organisational level from whole grain to endosperm and this is accompanied with a decrease in total polyphenol content. This suggests that polyphenols (phenolic acids and flavonoids) may influence protein digestibility of uncooked sorghum at the whole grain level, which is at odds with the current view that the smaller molecular weight polyphenols are not known to have any adverse effects on protein digestibility. However, in going from the endosperm to the protein body-enriched level, an increase in total polyphenol content does not bring about a decrease in uncooked and cooked sorghum protein digestibility which is in agreement with the current view regarding the effect of smaller molecular weight polyphenols on protein digestibility. This is an indication that at the endosperm and protein body-enriched levels, polyphenol involvement in influencing protein digestibility is more likely through an alternative mechanism such as protein crosslinking with ferulic acid in cell walls rather than with phenolic acids and flavonoids. Thus the lower proportion of cell wall material in the protein body-enriched samples explains the better uncooked and cooked protein digestibility of the protein body-enriched samples compared to the endosperm.

The condensed-tannin-free sorghums and the maize used in this study have a negligible effect on amylase activity in contrast with the high-tannin sorghum variety which substantially inhibits amylase activity. It is therefore proposed that if polyphenols are involved in reducing protein digestibility, the mechanism is likely to be through interaction with substrate protein rather than with the pepsin enzyme.

Cooking reduces sorghum protein digestibility at the whole grain level but not maize, which is in agreement with what is documented in literature. This study shows that reduction of sorghum protein digestibility on cooking also occurs at the endosperm, protein body-enriched levels and at the level of extracted kafirins. In contrast, the protein digestibilities of uncooked and cooked maize remain essentially the same at these levels of organisation.

It is suggested that a possible interaction between sorghum protein and cell walls leads to reduction in protein digestibility on cooking. This is in agreement with the observations of other workers who have reported an association of cereal and legume proteins with dietary fibre or cell wall components.

Some mechanisms are proposed to explain the nature of this protein-cell wall adhesion which may bring about reduction in protein digestibility on cooking.

- The oxidising conditions of the cooking process could promote phenolics-mediated crosslinking of proteins within the cell wall. Dimerisation may occur between tyrosine residues in proteins and ferulic acid residues esterified to arabinoxylans of the cell walls. Ferulic acid may also be crosslinked to the sulphydryl group of cysteine residues in proteins.
- There may be direct attachment of protein to carbohydrate moieties of the cell wall. In an analogous fashion to the structure of plant cell wall glycoproteins, O-glycosidic bonds may be formed between proline residues of sorghum and maize proteins (if hydroxylated) and the arabinose residues of the cell wall on cooking. This could result in enzyme-resistant protein-carbohydrate linkages.

It is suggested that the superior protein digestibility of cooked maize compared to sorghum may be because the protein-cell wall adhesion is stronger and occurs to a greater extent in sorghum than in maize.

Treatment of cooked sorghum and maize whole grain and endosperm flours with alpha-amylase improves protein digestibility, which is an indication that gelatinised starch, probably by reducing accessibility of pepsin enzyme to protein substrate, reduces protein digestibility. In support of this observation, in the protein body-enriched samples where the proportion of starch is much lower, there are no differences in protein digestibility between cooked and cooked plus alpha-amylase treatments in both sorghum and maize.

Reduction of sorghum protein digestibility as a result of cooking may also be through an alteration in protein secondary structure. Disulphide crosslinking on cooking (a form of protein secondary structural change) has been proposed by other workers as responsible for the lowered protein digestibility of sorghum. This hypothesis is confirmed by observations in this investigation from SDS-PAGE. Sorghum (uncooked and cooked) contains more oligomers than maize and therefore, it is proposed that formation of disulphide-bonded protein oligomers may be more extensive in sorghum than in maize possibly due to subtle differences in tertiary structure between sorghum kafirins and maize zeins. More enzyme-resistant oligomers would then be formed in sorghum than in maize and hence the superior digestibility of cooked maize proteins compared to cooked sorghum.

Some protein oligomers in cooked sorghum and maize are resistant to reduction. More of such reduction-resistant oligomers occur in sorghum than in maize. It is suggested that these oligomers may be in such a conformation which does not allow easy access of reducing agent to disulphide bonds. It is also proposed that these reduction-resistant oligomers may not have been formed through disulphide crosslinking. This proposal is made more likely by the finding that alkylation of cooked sorghum kafirin does not improve protein digestibility to the level of uncooked kafirin. A hypothesis is that such non-disulphide-bonded protein oligomers may be formed by

- oxidative coupling of tyrosine residues between polypeptide chains to form the crosslinking dimer known as isodityrosine or tetramer known as di-isodityrosine,
- esterification of amino acid residues between polypeptide chains.

The FTIR and ^{13}C NMR spectra provide information about protein secondary structural change on cooking from the perspective of α-helical to antiparallel intermolecular β-sheet conformational transitions. Cooking appears to bring about a change in protein secondary

structure from the α-helical conformation to antiparallel intermolecular β-sheet in both sorghum and maize. The extent of this structural change appears to be more in sorghum compared to maize which explains the higher digestibility of maize proteins. Proteins of sorghum mutants of known high protein digestibility undergo the same kind of secondary structural change on cooking as the normal sorghums namely, α-helix to antiparallel intermolecular β-sheet.

In agreement with recent published results, popped sorghum has higher protein digestibility than wet cooked. Similar to wet cooking, popping also brings about secondary structural changes from α-helical to antiparallel intermolecular β-sheet conformation. This structural change which is associated with reduction in protein digestibility, occurs to a greater extent in wet cooked than in popped samples and may explain the better protein digestibility of popped grain compared to wet cooked. Improved accessibility of protein components in the endosperm to enzymes as a result of endosperm cell wall fragmentation due to the explosive popping process may also explain the better digestibility of popped grain.

From a broad perspective, this investigation provides evidence that grain organisational structure does affect protein digestibility of sorghum. Emanating from this, four main factors namely, polyphenols (phenolic acids and flavonoids), cell walls, gelatinised starch and protein crosslinking may be identified as affecting protein digestibility. In Table 15 below, these proposed factors and their levels of importance at the three levels of organisation of sorghum and maize are summarised.

Table 15. Proposed factors affecting protein digestibility of uncooked and cooked sorghum and maize and their levels of importance at the whole grain, endosperm, protein body and extracted protein levels.

	Sorghum				Maize			
Factors	Whole grain	Endosperm	Protein bodies	Extracted proteins	Whole grain	Endosperm	Protein bodies	Extracted proteins
Uncooked								
Polyphenols	♦♦	♦	♦	♦	♦	♦	♦	♦
Cell walls	♦♦♦	♦♦	♦	♦	♦♦	♦♦	♦	♦
Gelatinised starch	♦	♦	♦	♦	♦	♦	♦	♦
Disulphide crosslinking	♦♦	♦♦	♦♦	♦♦	♦♦	♦♦	♦♦	♦♦
Non-disulphide crosslinking	♦♦	♦♦	♦♦	♦♦	♦♦	♦♦	♦♦	♦♦
Cooked								
Polyphenols	♦♦	♦	♦	♦	♦	♦	♦	♦
Cell walls	♦♦♦	♦♦♦	♦	♦	♦♦	♦♦	♦	♦
Gelatinised starch	♦♦	♦♦	♦	♦	♦♦	♦♦	♦	♦
Disulphide crosslinking	♦♦♦♦	♦♦♦♦	♦♦♦♦	♦♦♦♦	♦♦	♦♦	♦♦	♦♦
Non-disulphide crosslinking	♦♦	♦♦	♦♦	♦♦	♦♦	♦♦	♦♦	♦♦

♦ Not involved; ♦♦ Involved; ♦♦♦ More important; ♦♦♦♦ Most important

It appears that for uncooked sorghum, polyphenols (phenolic acids and flavonoids) and cell walls appear to be important factors affecting protein digestibility whilst cell walls and not polyphenols are important for uncooked maize. Cell walls, protein crosslinking and gelatinised starch are the main factors affecting protein digestibility in cooked sorghum and maize. Association of protein with cell walls and protein crosslinking appear to occur to a greater extent in sorghum than in maize and may explain the worse digestibility of cooked sorghum compared to cooked maize.

Further research is required to investigate the possible formation of non-disulphide crosslinks on cooking. A possible line of investigation would be to conduct a more detailed structural characterisation of the pepsin-indigestible residues of both sorghum and maize. These may be purified and subjected to spectroscopic analysis. Protein oligomers which are resistant to reduction may also be examined in this way to gain an understanding of their structure and conformation. X-ray crystallography and spectroscopy may also be used to investigate possible differences in tertiary structure between kafirins and zeins. This would provide a better understanding of the reasons why sorghum proteins have the tendency to form more oligomers on cooking compared to maize. The possibility of polyphenols influencing protein digestibility of cooked condensed-tannin-free sorghum could be investigated by extraction of polyphenols from the isolated proteins (kafirins and zeins) and determining protein digestibility after adding them back.