CHAPTER 5

RESULTS

5.1 Particle size distribution of maize meal made from different cultivars

Figure 12 shows the particle size distribution of the maize meal made from the endosperm of different maize cultivars. Within each particle size category the cultivars are numbered from A to F with A the cultivar with the softest endosperm (lowest % translucency) and F the cultivar with the hardest endosperm (highest % translucency).

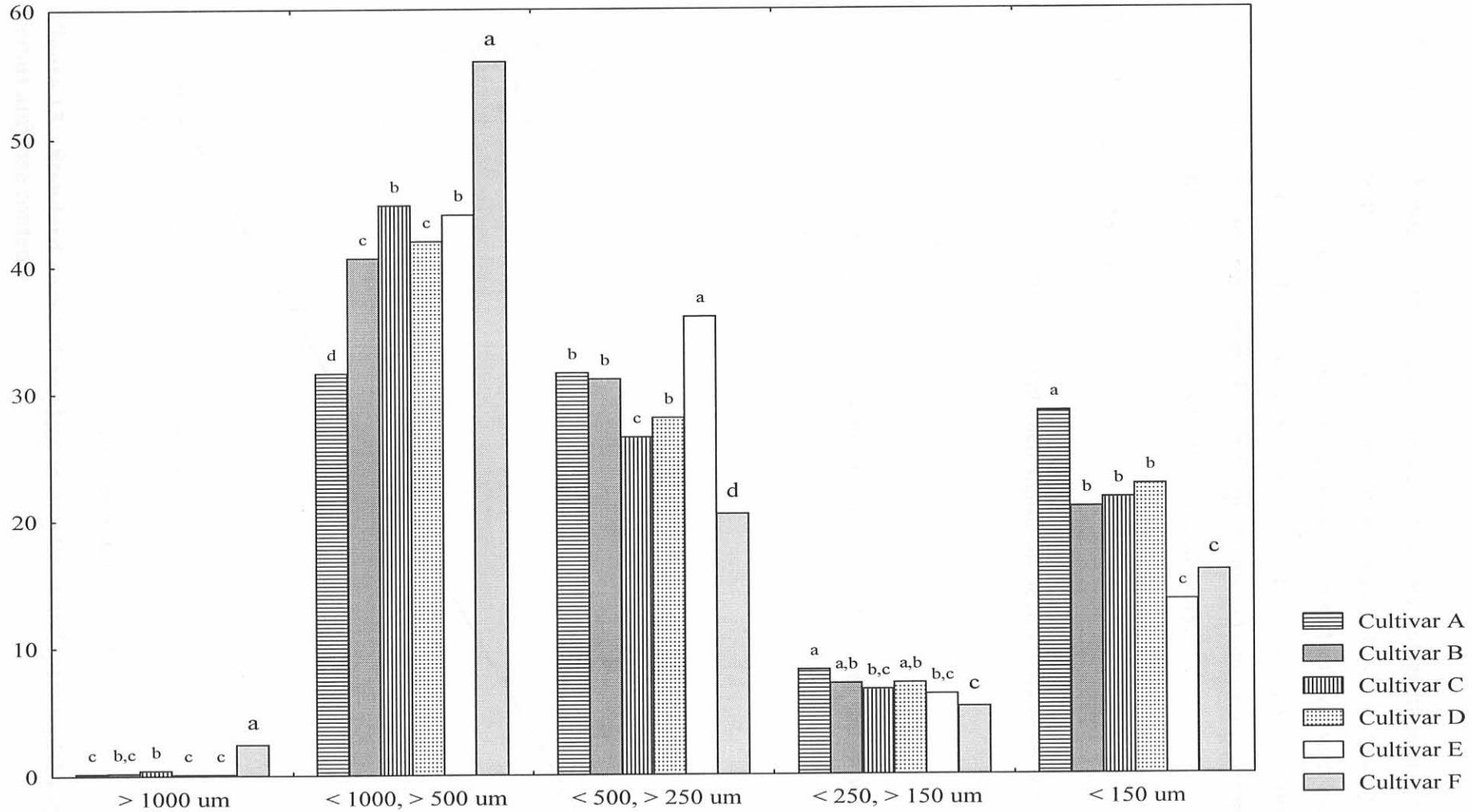


Figure 12: Particle size distribution of maize meal from cultivars with different endosperm vitreousness represented per particle size category to show significant differences[1] between cultivars within particle size categories

1 Columns with different letters in a particle size category are statistically significantly different ($p < 0.05$)

Cultivar F, which was a hard cultivar with the highest percentage translucency of the six samples, had significantly more particles in the two larger particle size categories than the other samples. On the contrary, cultivar A, which was a soft cultivar with the lowest percentage translucency of the six samples, had significantly less particles than the other samples in the second largest particle size category and had significantly more particles than the other samples in the smallest particle size category.

5.2 Damaged starch

None of the maize meal samples contained significant levels of damaged starch.

5.3 Amylose content

Figure 13 shows the relationship between absorbance (at 620 nm) and amylose content in mixtures of amylose and amylopectin.

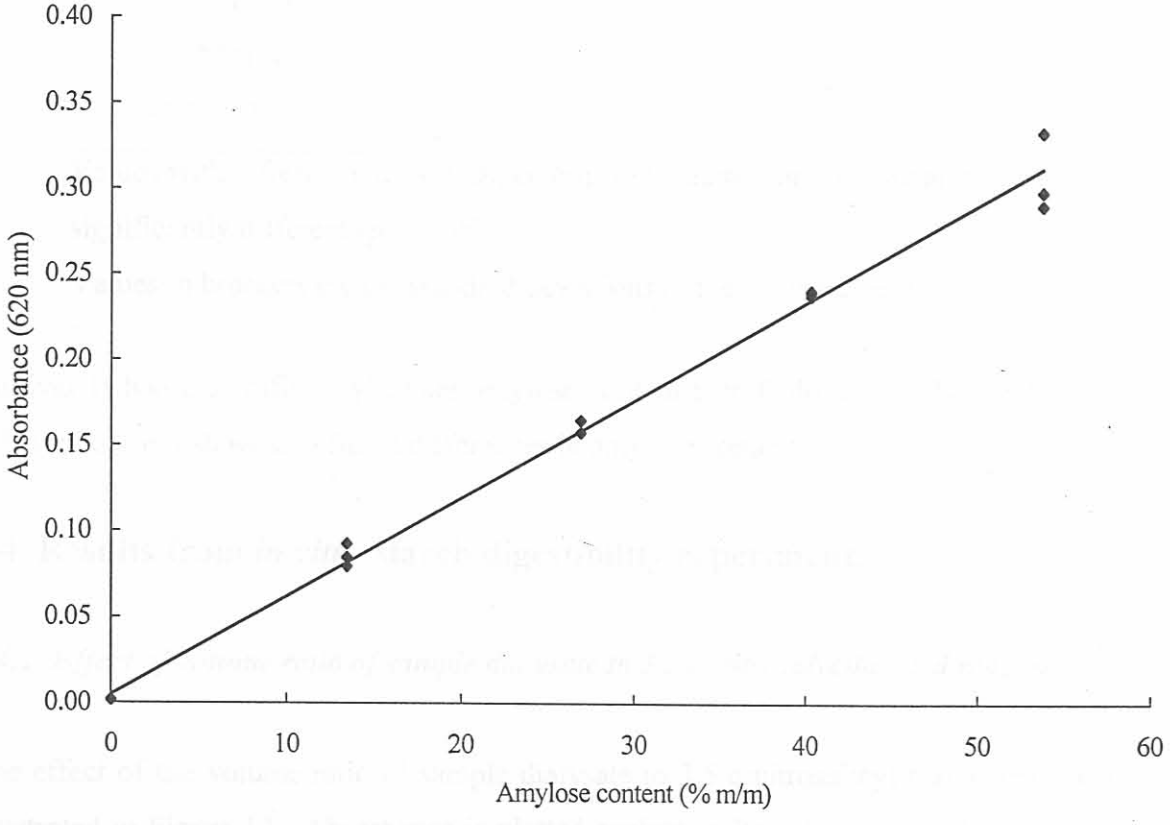


Figure 13: Standard curve of amylose in amylose:amylopectin plotting absorbance versus amylose content

A linear model was fitted to the data. The resulting regression equation was:

$y = 0.0057x + 0.0047$

where y = absorbance (620 nm) and x = amylose concentration (%)

An R^2 of 0.992 was obtained.

Table 9 shows the amylose content of the starch in the endosperm of the different maize cultivars.

Table 9: Amylose content of starch in maize meal from different cultivars

Cultivar	Amylose content (% of total starch)
A	$37.5^{1,a,b}$ $(1.6)^2$
B	39.9^{a} (1.5)
C	37.1^{b} (1.5)
D	$37.5^{a,b}$ (0.9)
E	$38.0^{a,b}$ (1.3)
F	$37.9^{a,b}$ (0.6)

1 Values with different letters in superscripts in columns are statistically significantly different ($p < 0.05$)

2 Values in brackets are the standard deviations of the measurements

Cultivar B had a significantly higher amylose content than Cultivar C. The rest of the cultivars did not show significant differences in amylose content.

5.4 Results from *in vitro* starch digestibility experiments

5.4.1 Effect of volume ratio of sample dialysate to 3,5-dinitrosalycilic acid reagent

The effect of the volume ratio of sample dialysate to 3,5-dinitrosalicylic acid reagent is illustrated in Figure 14. Absorbance is plotted against maltose (mg) and a linear model was fitted.

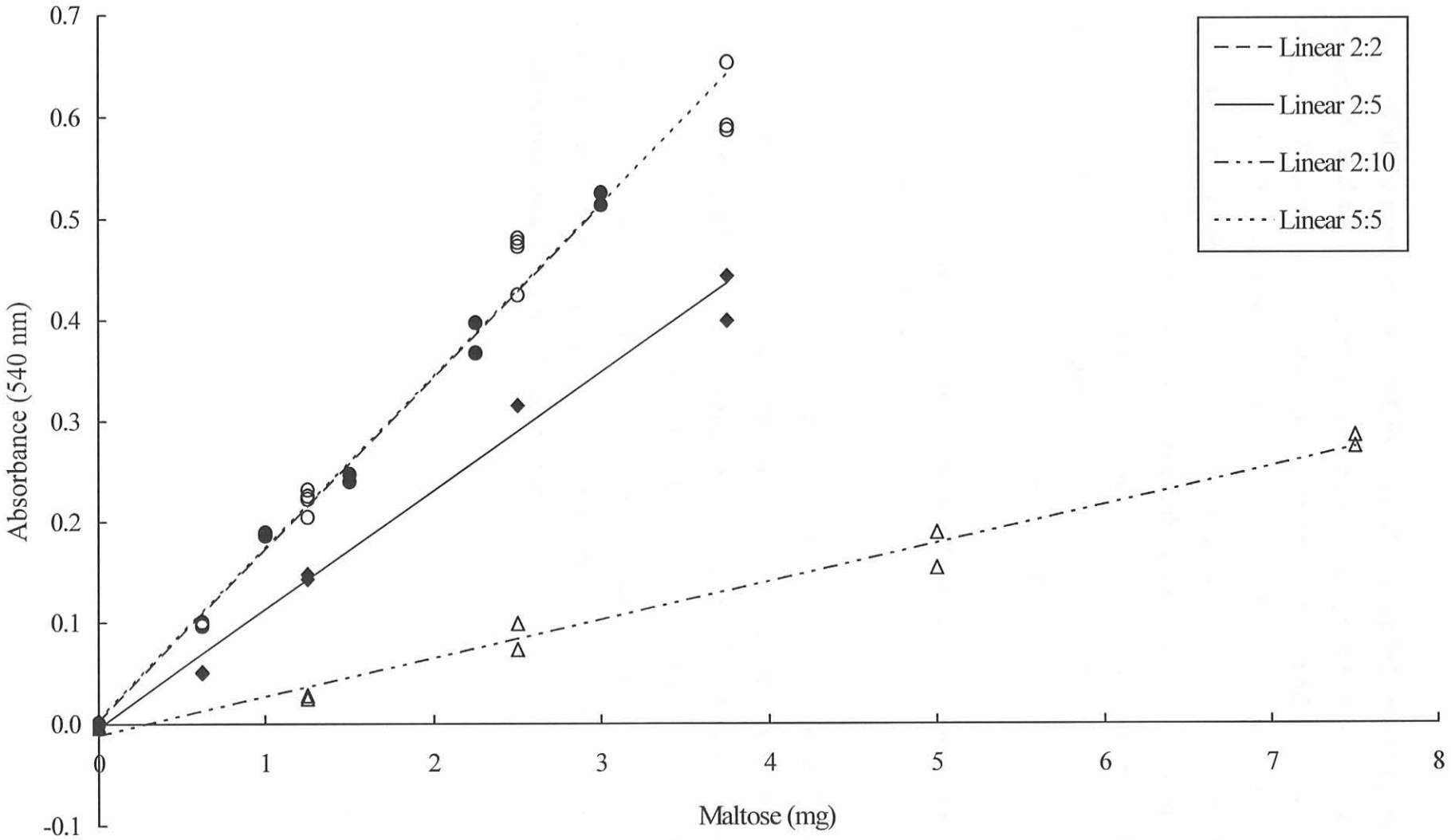


Figure 14: Effect of the ratio of dialysate to 3,5-dinitrosalycilic acid reagent (DNS) on the relationship between absorbancy at 540 nm and maltose (mg) DNS:dialysate 2:2 (●), 5:5 (○), 2:5 (◆) and 2:10 (Δ)

The R^2 of all the regressions were > 0.99. The relationship between absorbance and mg maltose changed significantly as the volume ratio of dialysate to 3,5-dinitrosalicylic acid changed. When the volume ratio was 1:1, there was no significant difference in the relationship between absorbance and mg maltose, even if the actual volumes were not the same (i.e. 2 ml dialysate and 2 ml reagent or 5 ml dialysate and 5 ml reagent). However, when the volume of dialysate was increased, the gradient of the relationship between absorbance and mg maltose decreased significantly ($p < 0.001$). For the 1:1 volume ratio, the slope was 0.17 for both 2 ml and 5 ml volumes. For the 2:5 ratio, the slope was 0.12 and for the 2:10 ratio the correlation coefficient was 0.04.

5.4.2 Starch digestibility of white bread and porridge made from maize cultivars with different endosperm hardness

After chewing, the bread was in the form of a dense lump, but expanded again into a porous structure when coming in contact with the liquid enzymes. With maize porridge, a part of the sample broke up into endosperm grit particles, while most of it remained in 1 to 3 lumps. After 180 min of incubation with α-amylase, the maize porridge sample had broken down into smaller lumps and more loose endosperm grit particles. Figure 15 compares the *in vitro* starch digestibility of white bread to that of porridge made from maize cultivars with different endosperm hardness.

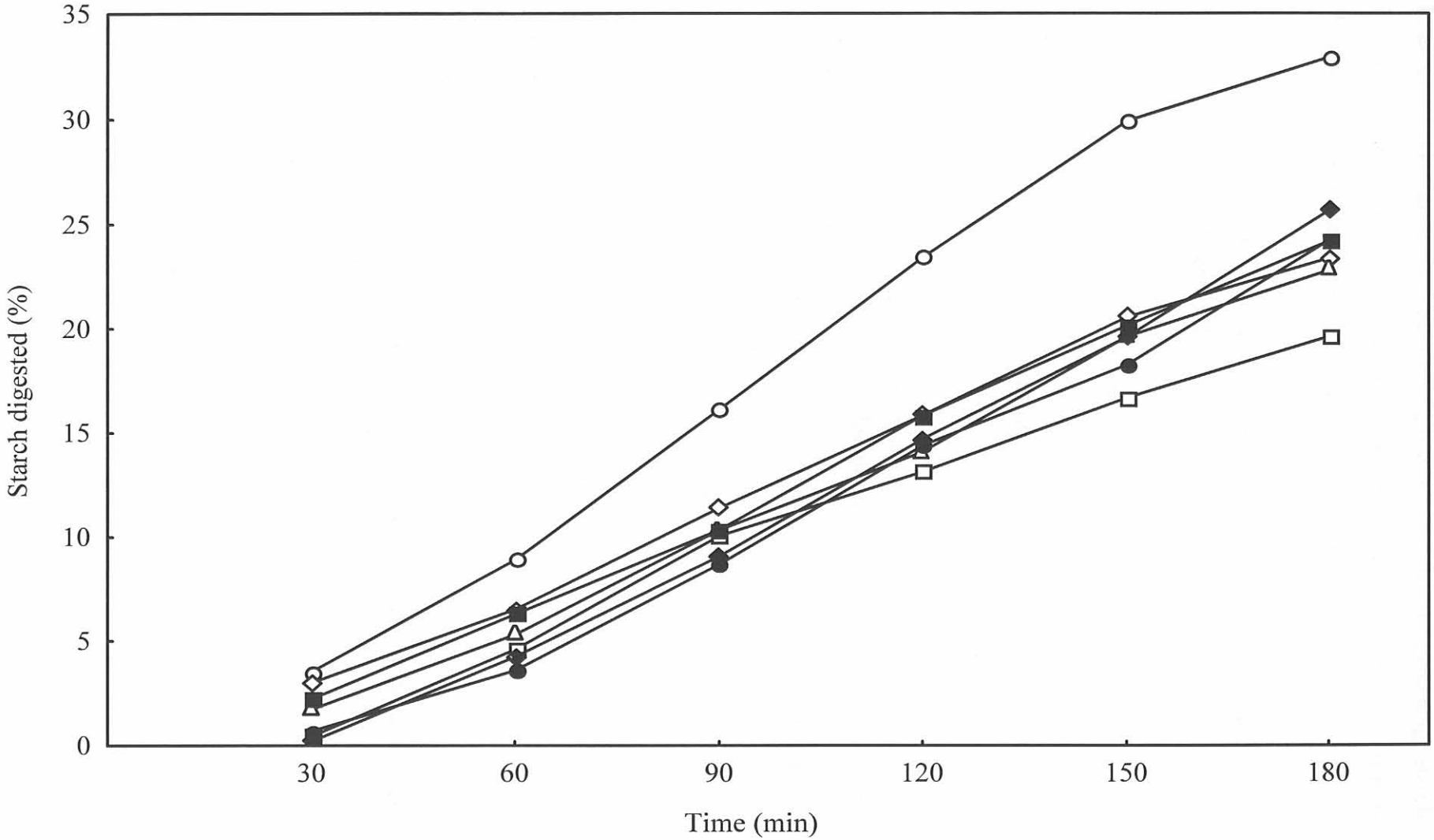


Figure 15: ***In vitro*** **starch digestibility of six maize cultivars with different endosperm hardness compared to white bread, cultivar A (□), B (◊), C (Δ), D (●), E (■), F (◆) and White bread (○)**

In all the starch digestibility experiments it was found that the percentage starch digested increased significantly ($p < 0.001$) from 30 to 180 min after incubation with α-amylase. White bread was statistically significantly ($p < 0.001$) more digestible than maize porridge. There were also small, but statistically significant ($p < 0.05$) differences between the cultivars at 30, 60 and 180 min after incubation with α-amylase. To aid in highlighting these differences, linear models were fitted on the data of the white bread and maize cultivars. The model was $y = mx + c$, where y is starch digested (%), x is time (min), m is the slope of the line and c the intercept. Table 10 gives the regression statistics of the fitted models and Figure 16 shows the fitted lines for starch digested over time of maize porridge made from cultivars with different endosperm hardness compared to white bread.

Table 10: Regression statistics of the linear models fitted to the data of digestibility over time for white bread and porridge made from maize cultivars with different endosperm hardness

Sample	Coefficient of determination (R^2)	Slope	Intercept
Cultivar A	0.932	$0.129^{1,a}$	-2.78
Cultivar B	0.973	0.141^{b}	-1.41
Cultivar C	0.947	0.145^{b}	-2.90
Cultivar D	0.979	0.158^{c}	-5.02
Cultivar E	0.917	$0.151^{b,c}$	-2.64
Cultivar F	0.974	0.170^{d}	-5.65
White wheat bread	0.966	0.207^{e}	-2.59

1 Slopes with different letters in the superscript are statistically significantly different ($p < 0.05$)

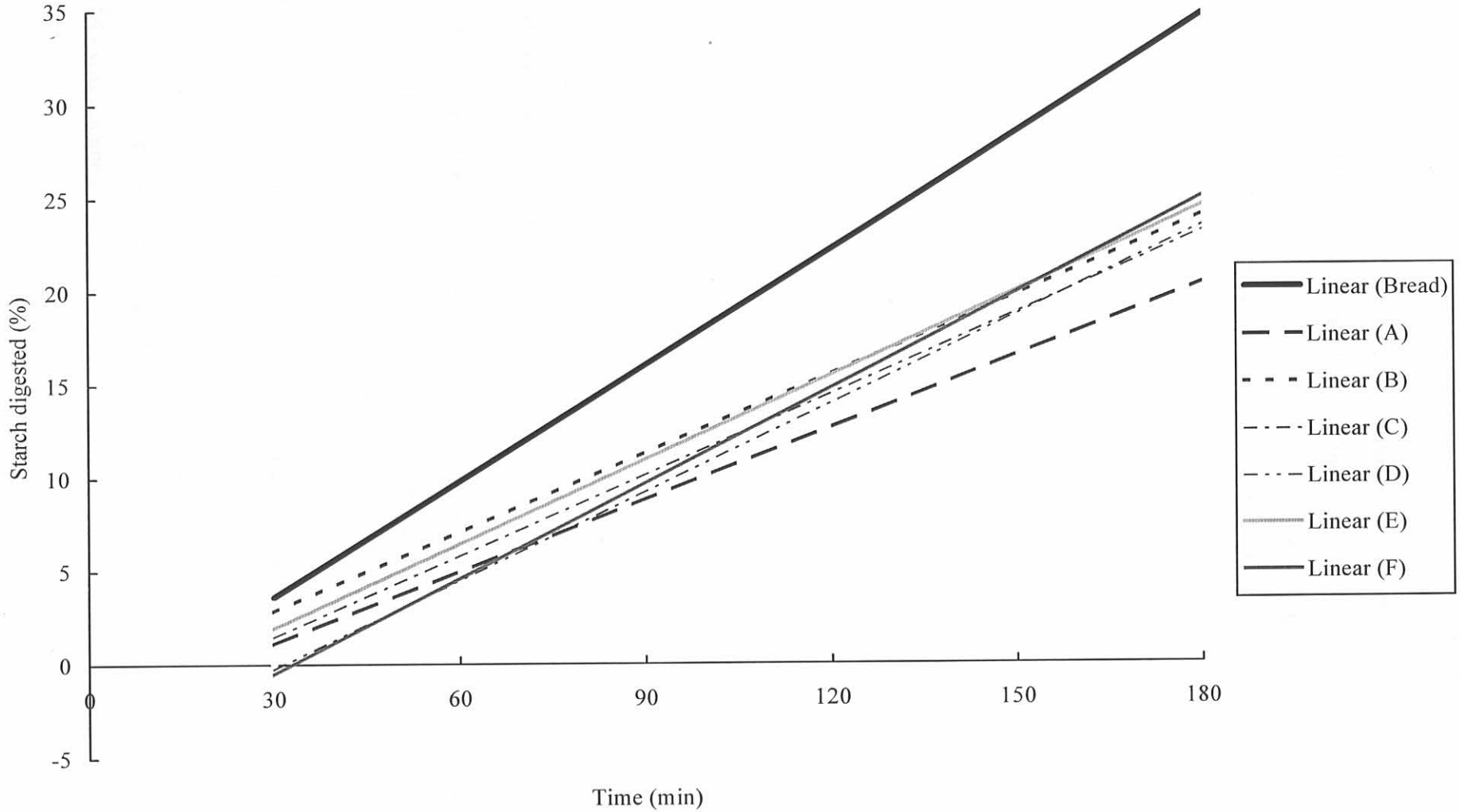


Figure 16: Fitted linear models of percentage starch digested over time in maize porridge made from cultivars with different endosperm hardness compared to white bread

White bread was digested significantly faster than the maize porridge from all the cultivars ($p < 0.001$). The digestibility rate of the maize porridge increased in the order A, B, C, E, D, F. A correlation was done between % starch digested in porridge after 180 min and maize kernel endosperm hardness. The correlation is shown in Figure 17.

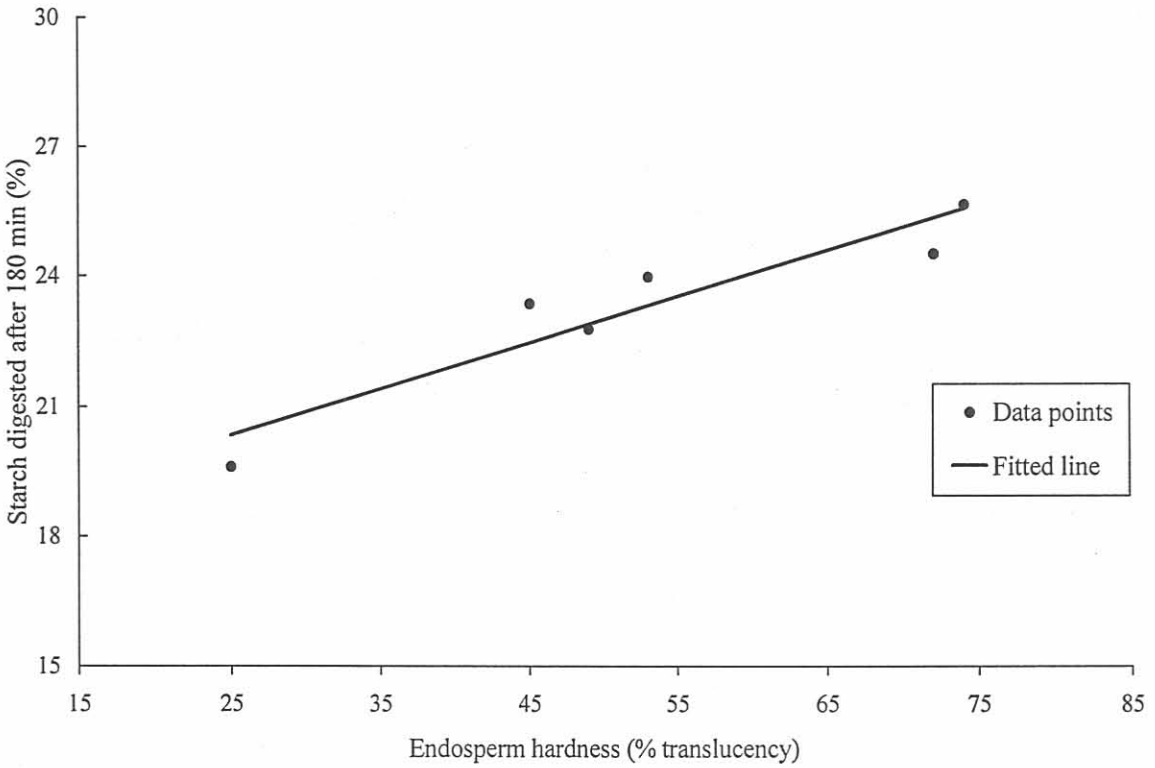


Figure 17: Correlation between % starch digested in maize porridge after 180 min and maize kernel endosperm hardness

With r (correlation coefficient) = 0.94, the correlation was found to be statistically significant ($p < 0.01$). There was also a significant correlation ($p = 0.05$) between the rate of starch digestibility (slopes of fitted lines in Figure 16) and maize endosperm hardness.

5.4.3 Hydrolysis index and predicted GI of maize porridge

The average hydrolysis index of maize porridge was 64 and the predicted GI value 63. (with white bread as reference). Converting this to glucose as a reference, a predicted GI of 44 was obtained.

5.4.4 Effect of particle size

The starch digestibility of maize meal compared to maize flour of cultivar C is shown in Figure 18.

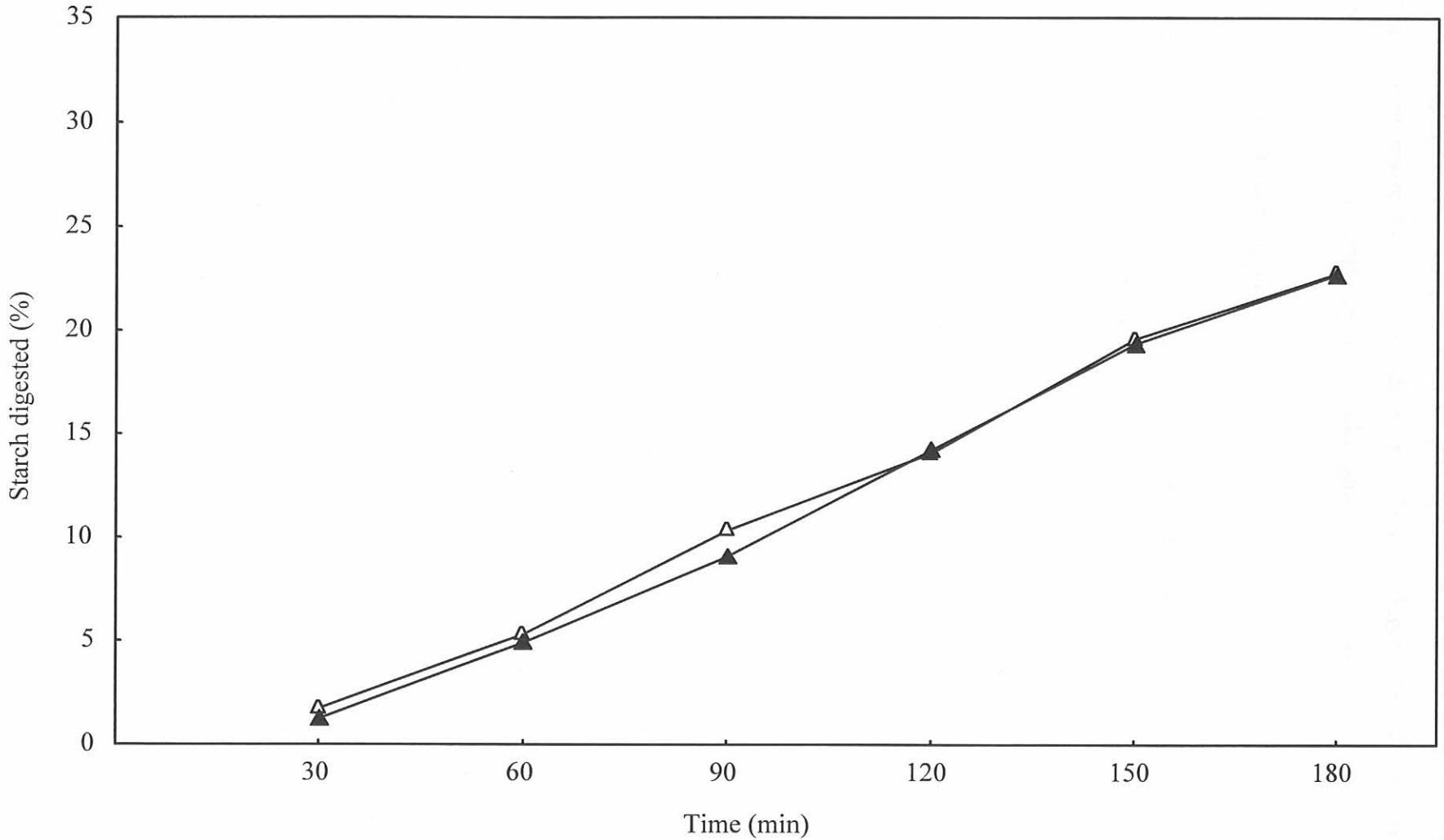


Figure 18: ***In vitro*** **starch digestibility of maize meal (Δ) and maize flour (▲) hotplate cooked maize porridge made from cultivar C maize meal**

Surprisingly, the starch digestibility of porridge made from maize meal and porridge made from maize flour did not differ significantly (Figure 18). Unlike maize meal porridge, maize flour porridge had a sticky, glue-like consistency.

5.4.5 Effect of cooking time

Figure 19 compares the starch digestibility of short, standard and long cooked porridge made from cultivar C maize meal.

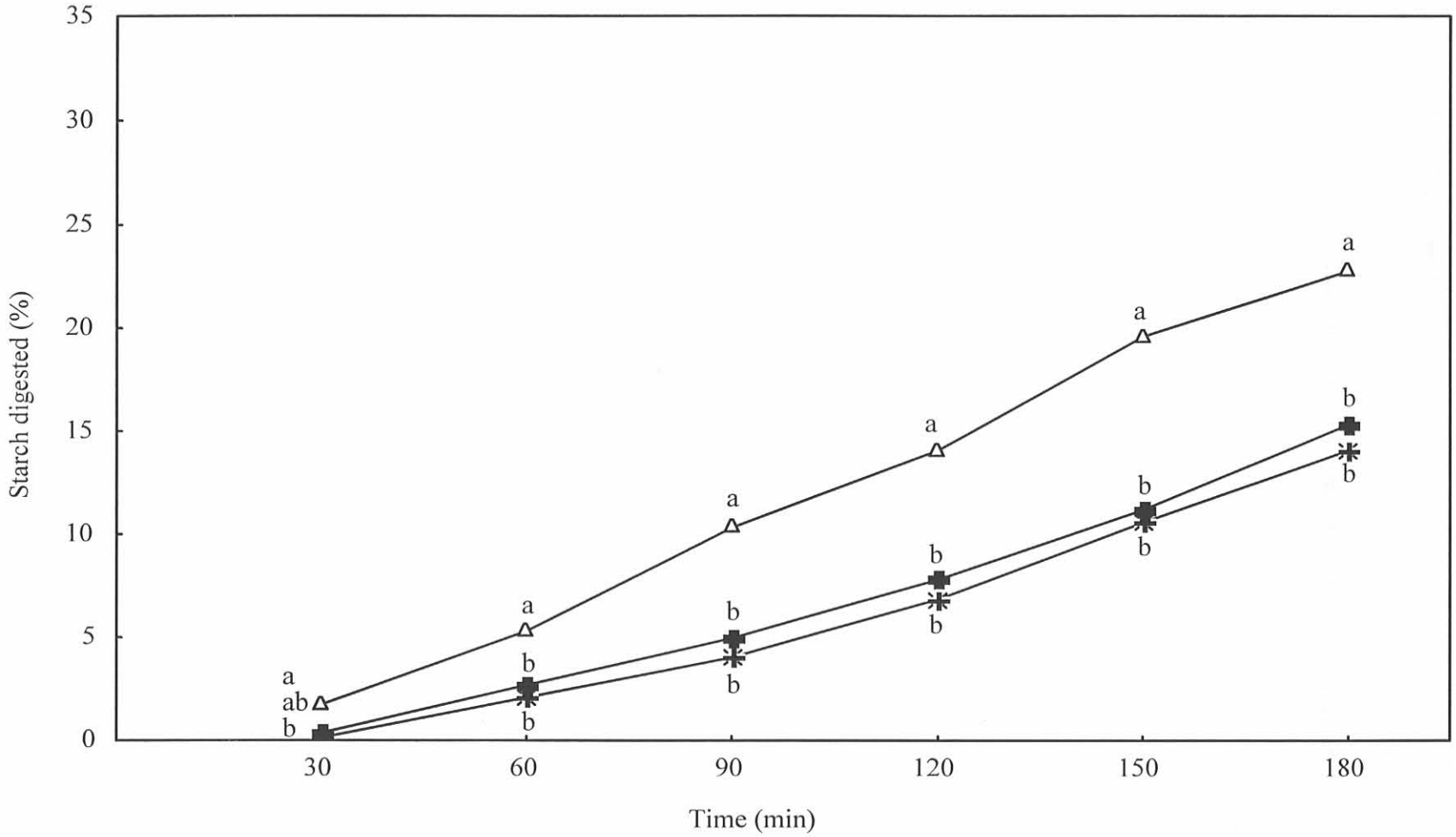


Figure 19: ***In vitro*** **starch digestibility of short (✚), standard (Δ) and long (✳) hotplate cooked maize porridge made from cultivar C maize meal (at each time, means not sharing the same letter are significantly ($p < 0.05$) different)**

Both increasing and decreasing the cooking time decreased the starch digestibility significantly. There was no significant difference between the digestibility of the long and short cooked porridge.

5.4.6 Starch digestibilities of maize, wheat and oat flour porridges

Figure 20 compares the *in vitro* starch digestibility of maize, wheat and oat flour with that of white bread.

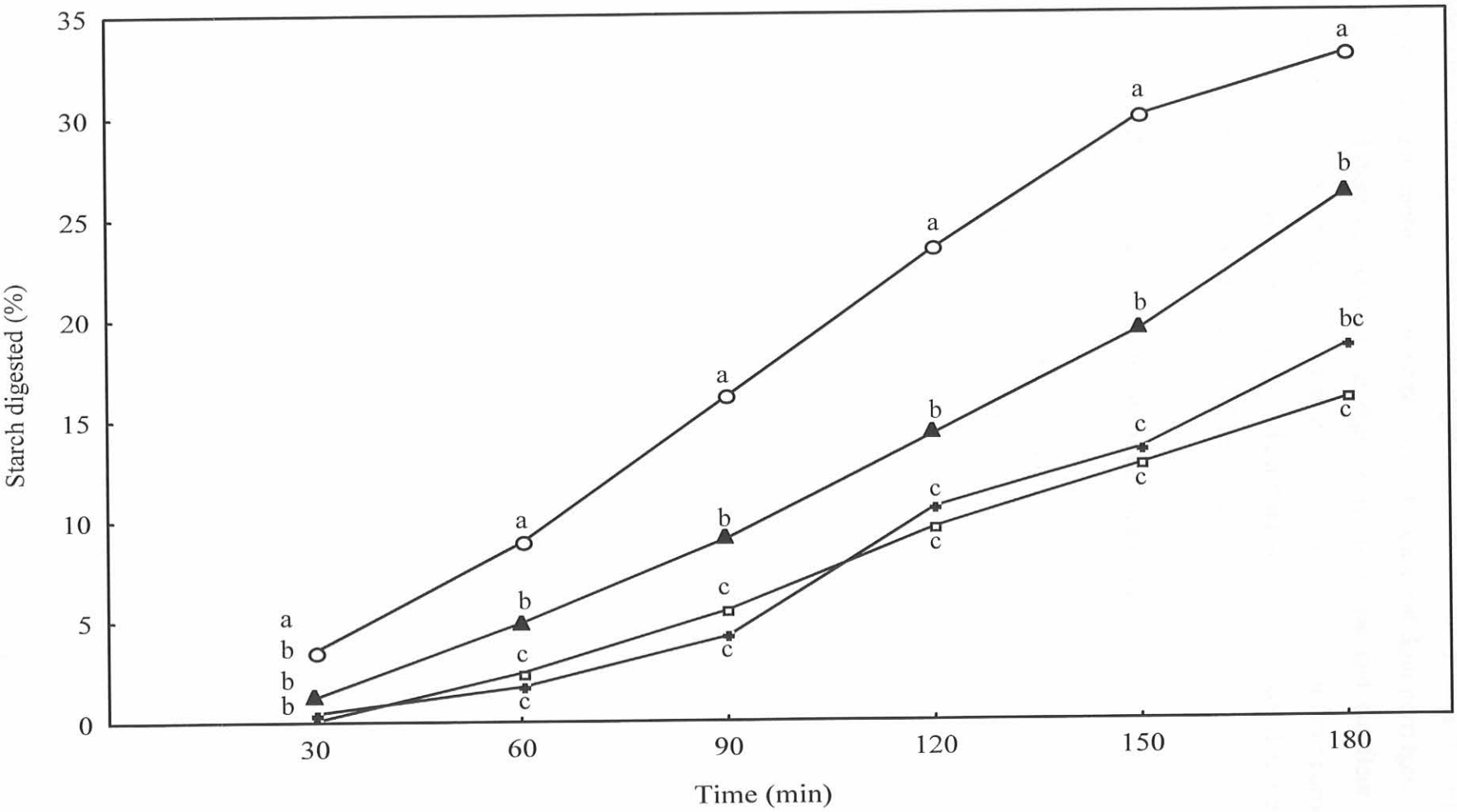


Figure 20: ***In vitro*** **starch digestibility of standard hotplate cooked porridge made from cultivar C maize flour (▲), wheat flour (□) and oat flour (+) compared to white bread (○) (at each time, means not sharing the same letter are significantly ($p < 0.05$) different)**

White bread was significantly more digestible than all the porridges. Maize flour porridge was significantly more digestible than wheat and oat flour porridges. There was no significant difference between the digestibility of wheat and oat flour porridges. During the experiment it was observed that both wheat flour and oat flour porridges were more viscous than maize flour porridge. Wheat flour porridge, and oat flour porridge to a lesser extent, had an almost elastic and rubbery consistency.

5.4.7 Starch digestibility of microwave cooked maize porridge

The digestibility of microwave cooked porridge from maize of cultivars with different endosperm hardness is shown in Figure 21.

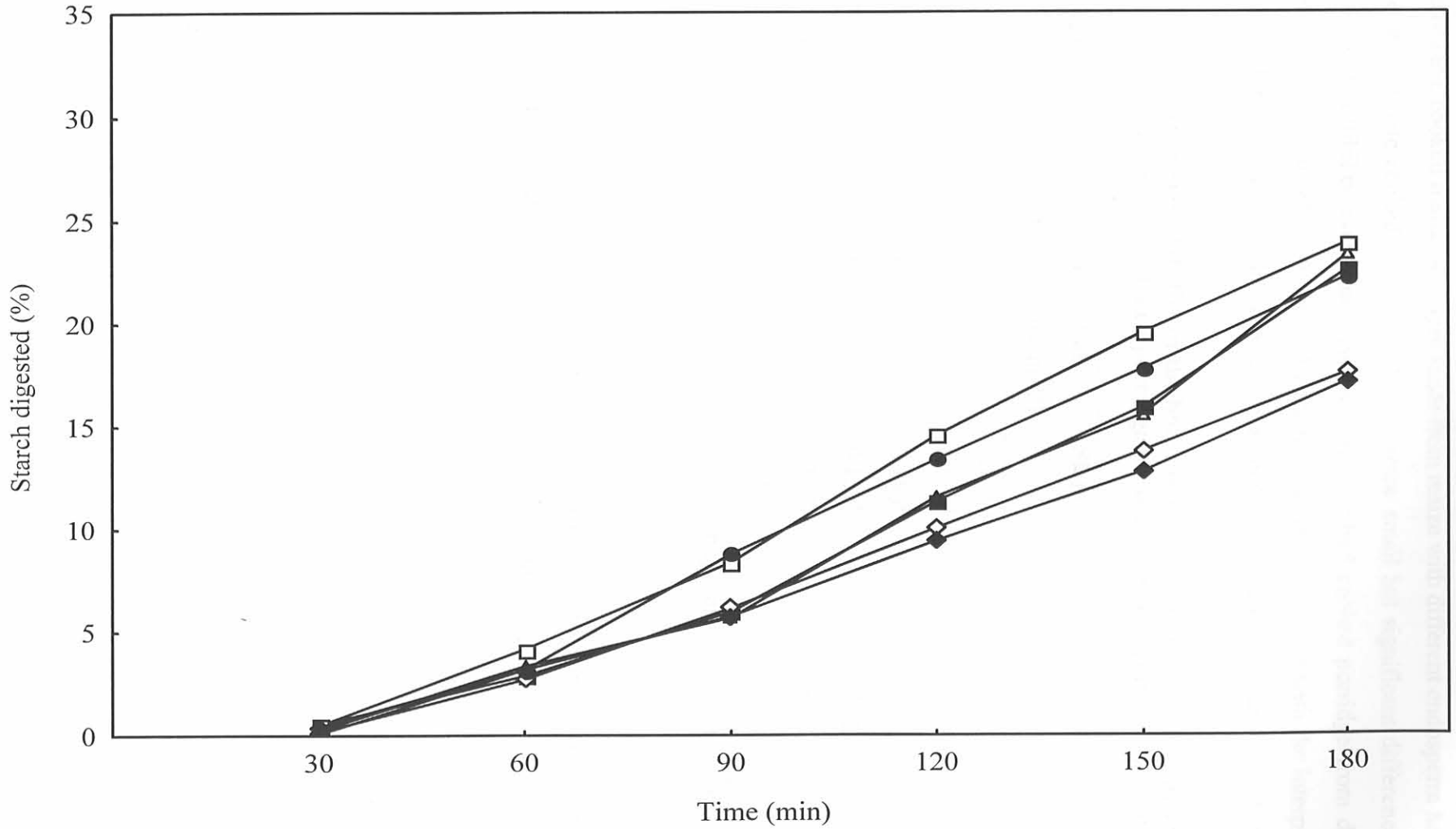


Figure 21: The *in vitro* starch digestibility of microwave cooked porridge made from meal of different maize cultivars. Cultivar A (□), B (◊), C (Δ), D (●), E (■) and F (◆)

Microwave cooked maize porridges made from maize with different endosperm hardness, like the hotplate cooked porridges, showed some small but significant differences ($p < 0.05$) in digestibility over time. As with the standard cooked porridge from different cultivars, linear models ($y = mx + c$) were fitted to the data to aid with the interpretation of the differences between the cultivars. The regression statistics are shown in Table 11.

Table 11: Regression statistics of the linear models fitted to the data of digestibility over time for microwave cooked porridge made from maize cultivars with different endosperm hardness

Sample	Coefficient of determination(R^2)	Slope	Intercept
Cultivar A	0.936	$0.161^{1,a}$	-5.11
Cultivar B	0.948	0.118^{b}	-4.02
Cultivar C	0.924	0.150^{a}	-5.82
Cultivar D	0.936	0.152^{a}	-5.02
Cultivar E	0.940	0.147^{a}	-5.62
Cultivar F	0.921	0.111^{b}	-3.50

1 Slopes with different letters in the superscript are statistically significantly different ($p < 0.05$)

Figure 22 shows the fitted lines for starch digested over time in microwave cooked maize porridge made from cultivars with different endosperm hardness.

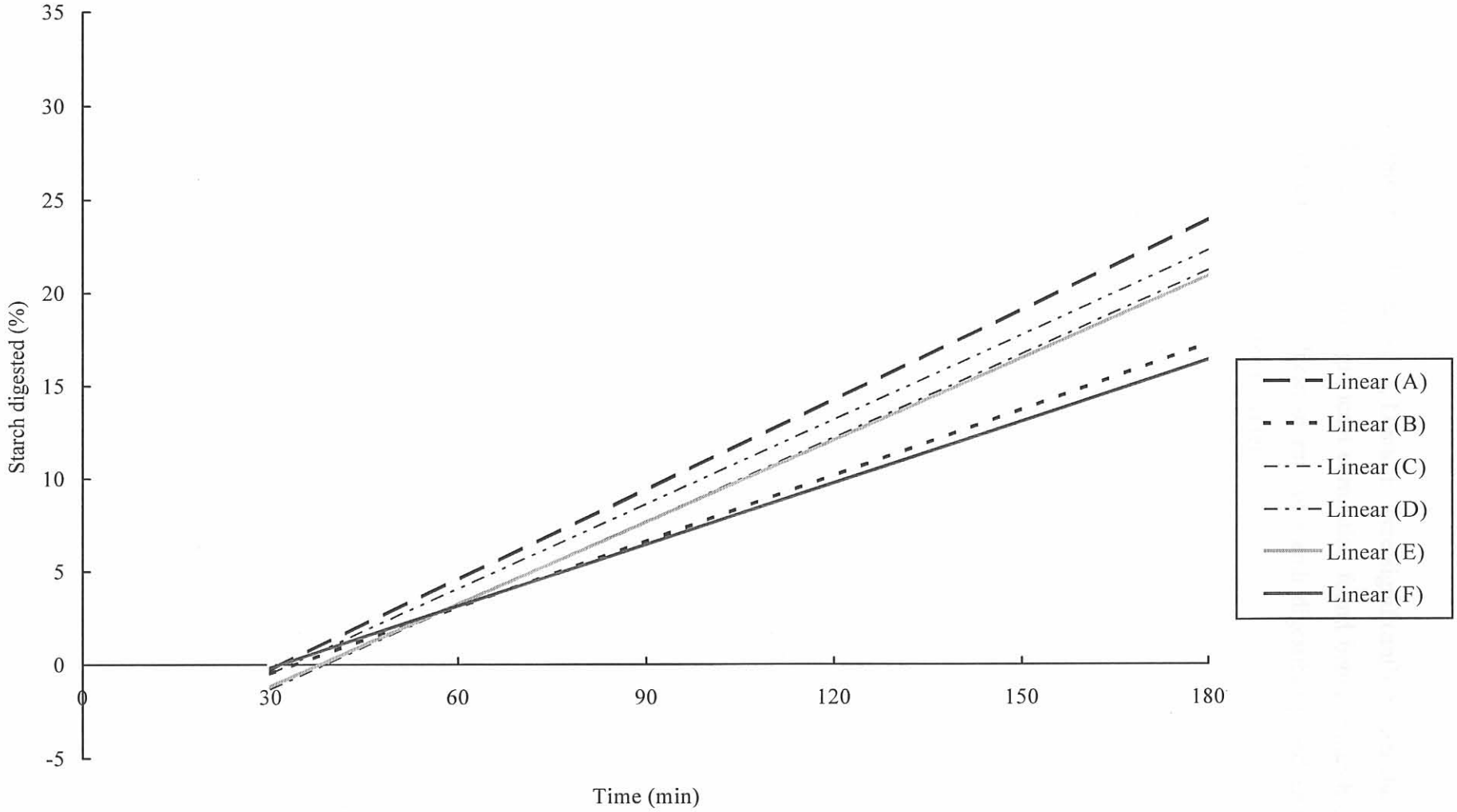


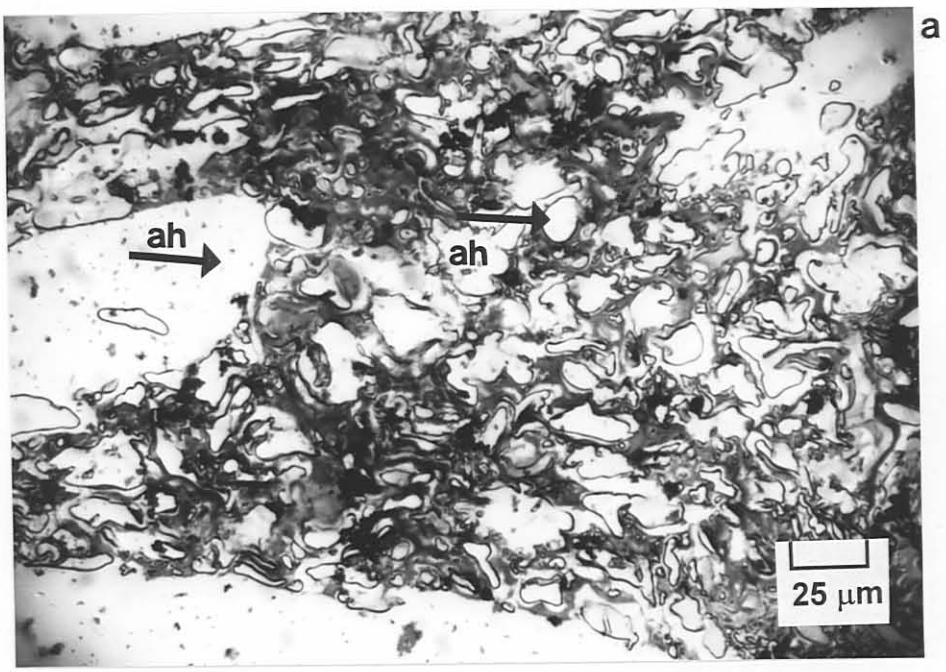
Figure 22: Fitted linear models of percentage starch digested over time in microwave cooked maize porridge made from cultivars with different endosperm hardness

The digestibility rates of cultivars A, C, D and E were significantly higher than that of cultivars B and F. There was no significant correlation found between starch digested after 180 min and endosperm hardness or rate of starch digestibility and endosperm hardness for microwave cooked maize porridge.

5.5 Microscopy

5.5.1 White bread

Figures 23a-c are light micrographs of white wheat bread before and after digestion with pepsin and α-amylase.



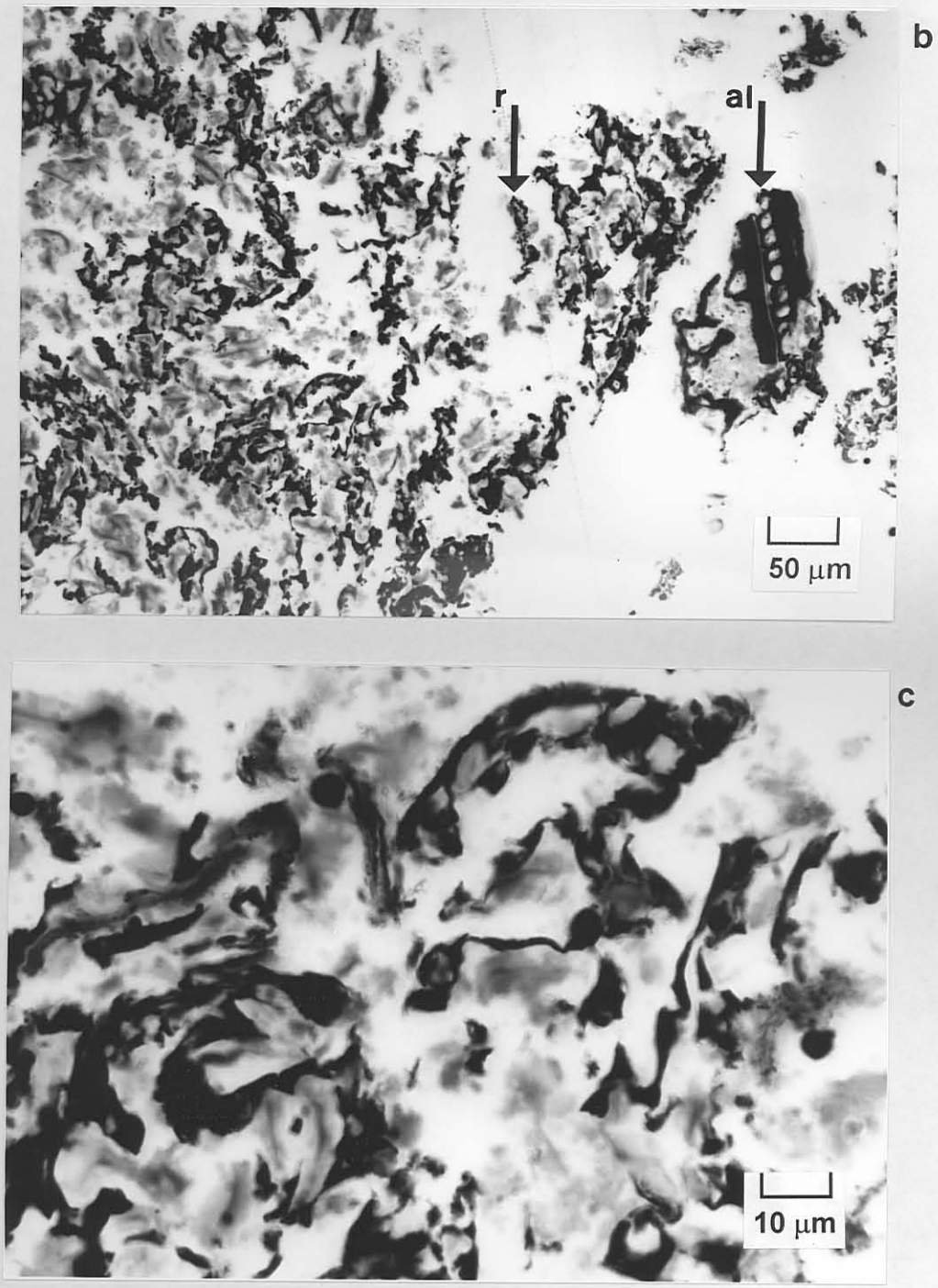
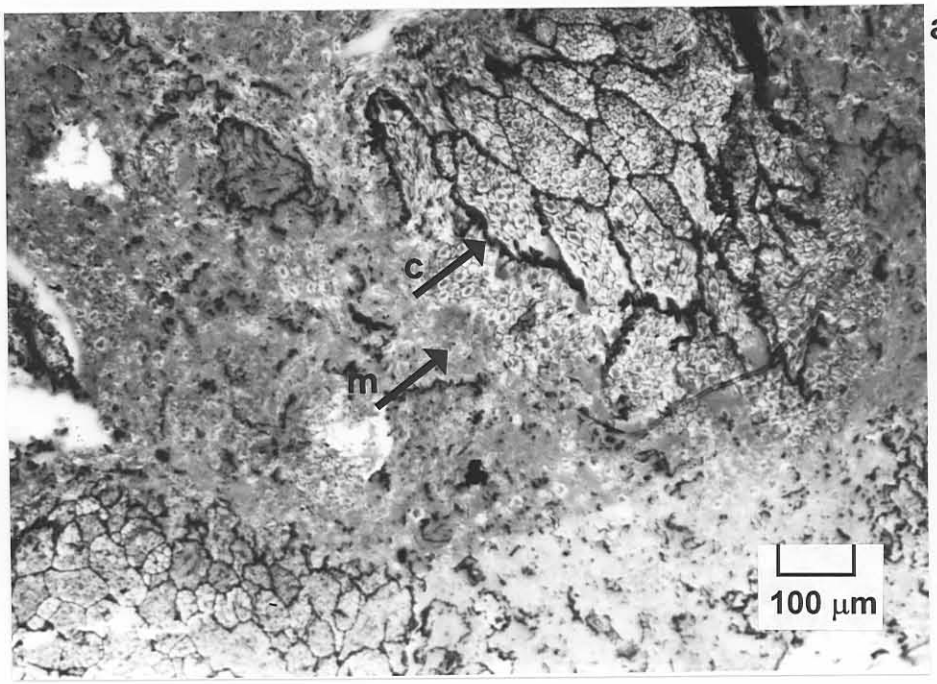


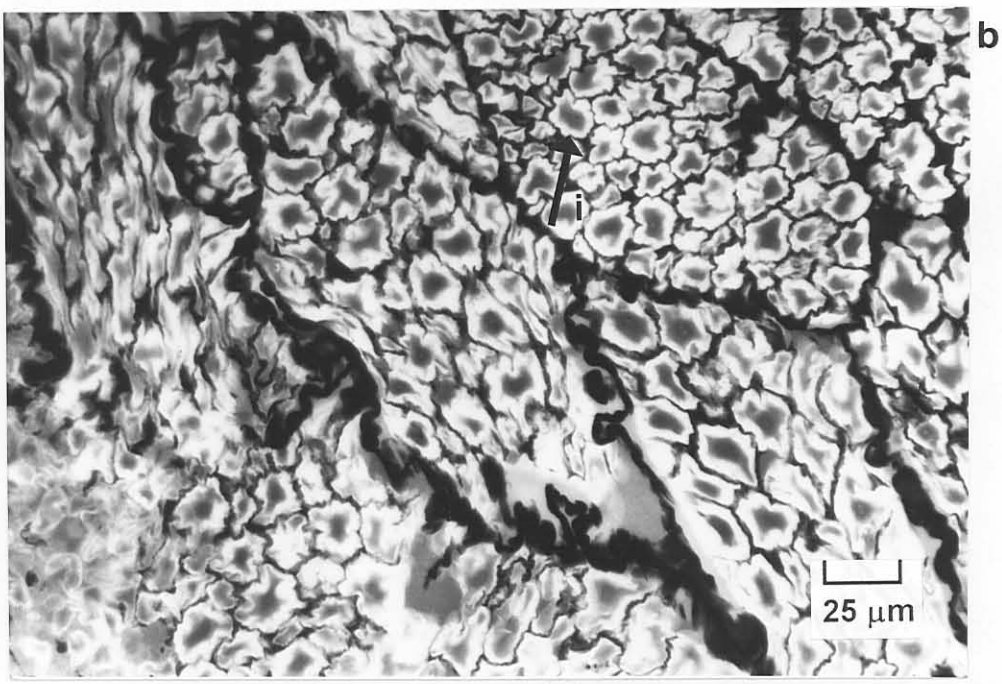
Figure 23: Light micrographs of white bread before (a) and after (b,c) digestion with pepsin and α-amylase (c is a higher magnification of one of the non-cellular areas in b)

Before digestion, the structure of bread was open and there were a large number of air holes (ah). No endosperm cell structures or intact starch granules were visible. After digestion with pepsin and α-amylase, the structure was basically amorphous consisting of cell wall remnants (r), except for a piece of aleurone layer (al) here and there.

5.5.2 Maize porridge

Figure 24a-c are light micrographs of maize porridge before digestion.





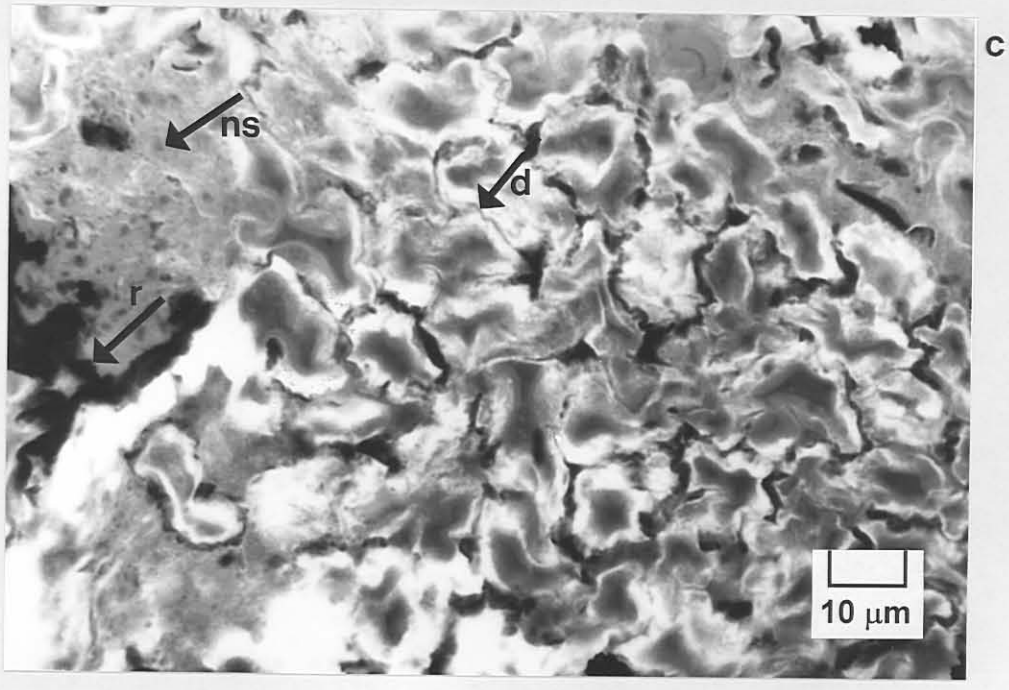
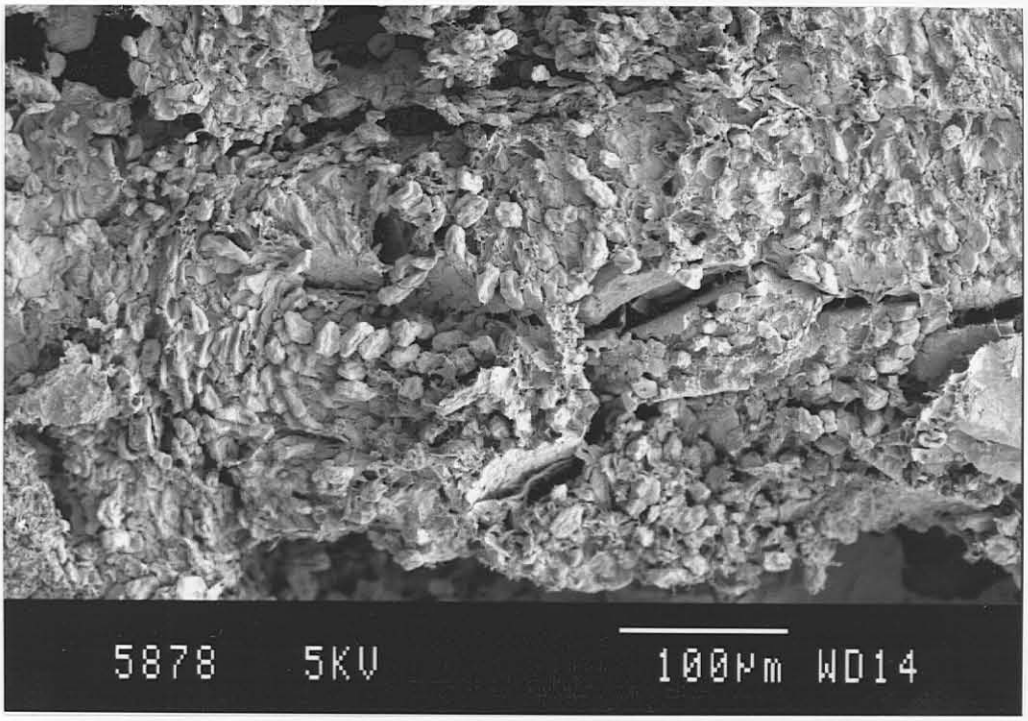


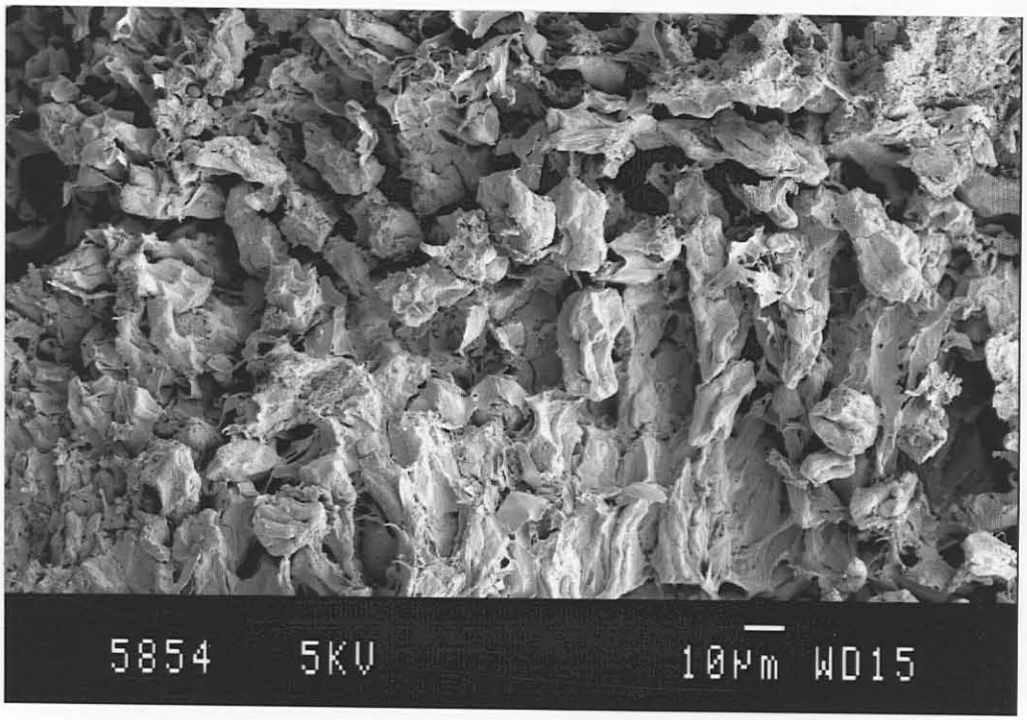
Figure 24: Light micrographs of maize porridge before digestion (a, low magnification; b, higher magnification of cellular area; c, higher magnification of amorphous area)

The structure of maize porridge was very dense, there were no air holes. The maize porridge consisted of amorphous (m) and cellular (c) areas. The cellular areas will be called endosperm grit particles and the amorphous areas surrounding the porridge particles will be called the porridge matrix. Magnification of a cellular area (23b) revealed that the endosperm grit particles consisted of swollen, but in many cases still intact (i) starch granules in the cells. When the starch granules were viewed under polarised light, almost all the granules showed a lack of birefringence. The porridge matrix (Figure 24c) consisted of swollen, distorted starch granules (d), cell wall remnants (r) and areas that stained the same as starch, but showed no structure (ns) under 1000 times magnification. The shown light micrographs are all of cultivar C maize porridge. No difference could be observed between the microstructure of maize meal porridge made from cultivars with different kernel endosperm hardness.

Figure 25a-d are SEM micrographs of maize porridge before digestion.



a



b

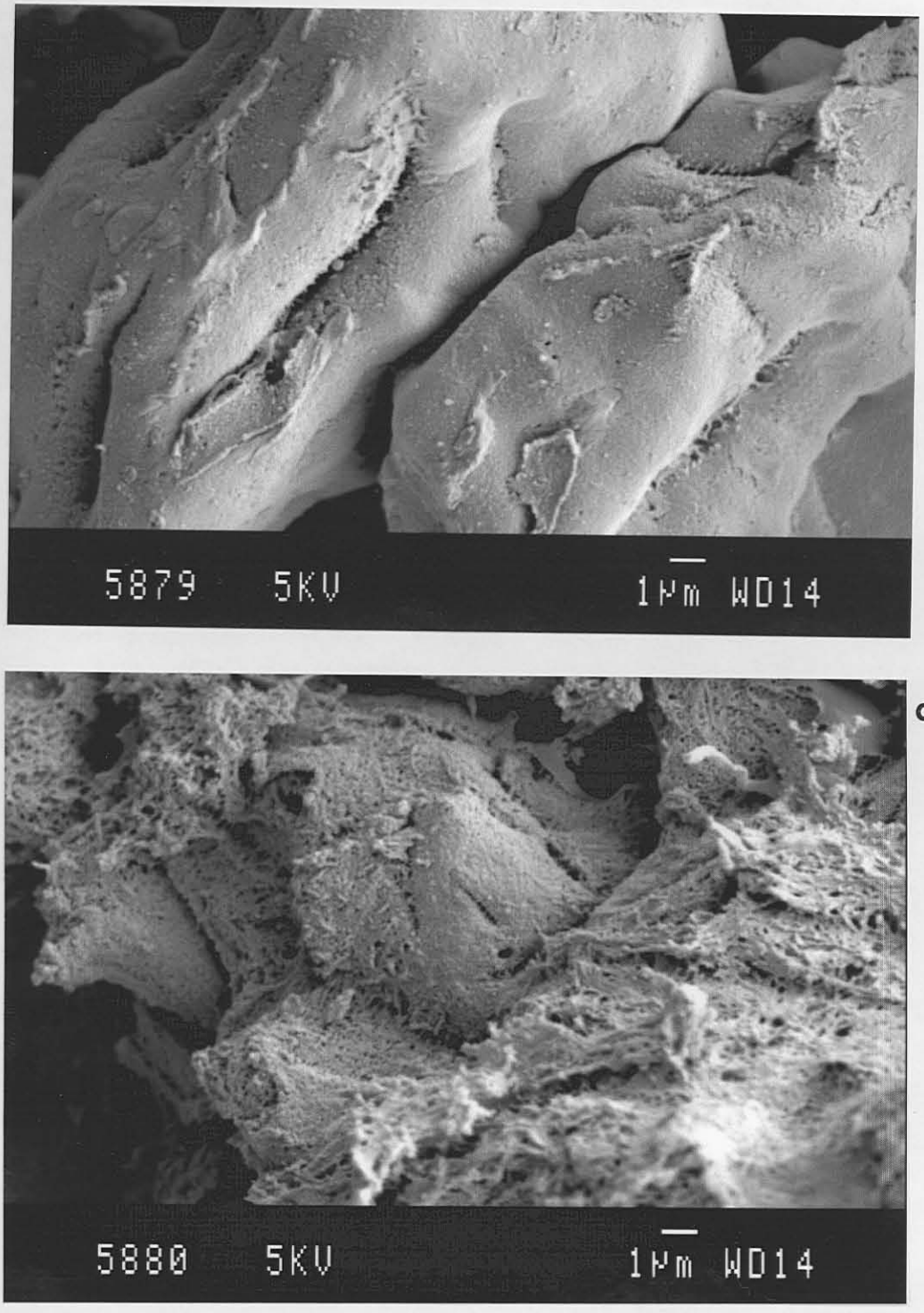
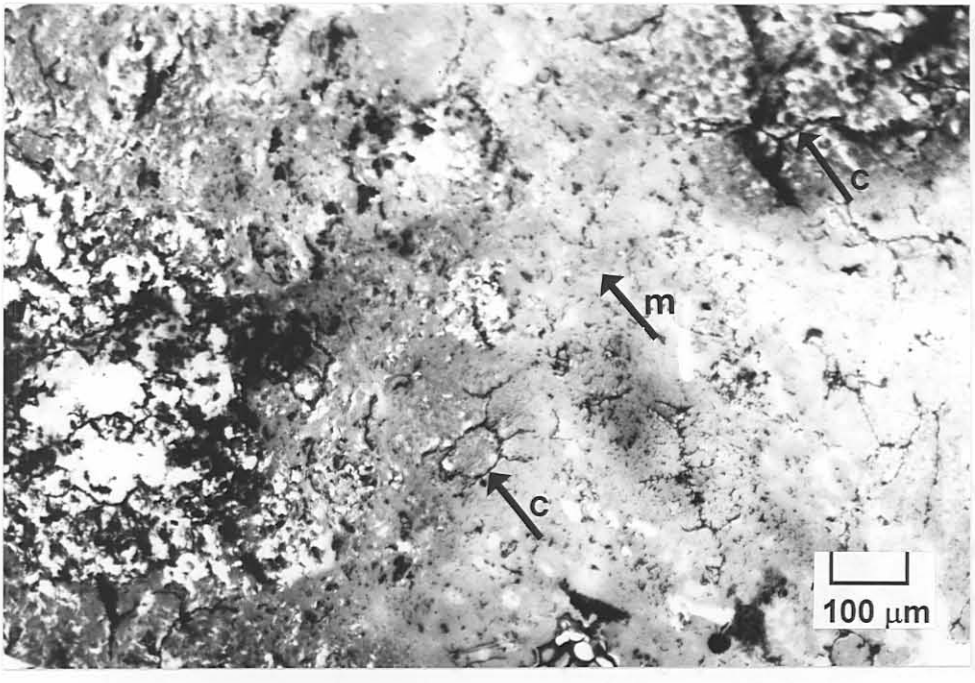


Figure 25: SEM micrographs of maize porridge before digestion with pepsin and α-amylase (a, showing starch granules in cells; b, showing loose starch granules in the porridge matrix; c, showing two intact starch granules on the surface of the porridge particle; d, showing disrupted starch granules on the surface of the porridge matrix)

Observing the surface of maize porridge by SEM showed that some starch granules were still partially or completely contained in cells (Figure 25a). These starch granules were located on the surface of porridge particles. The starch granules on the surface of the porridge matrix were not contained in cells (Figure 25b, 4 times higher magnification than 25a). At higher magnification (25c and d) it could be seen that many starch granules on the surface of the porridge particles were still intact, but on the surface of the porridge matrix many starch granules were disrupted and had a spongy appearance.

Figure 26a-c are light micrographs of maize porridge after digestion with pepsin and α-amylase.



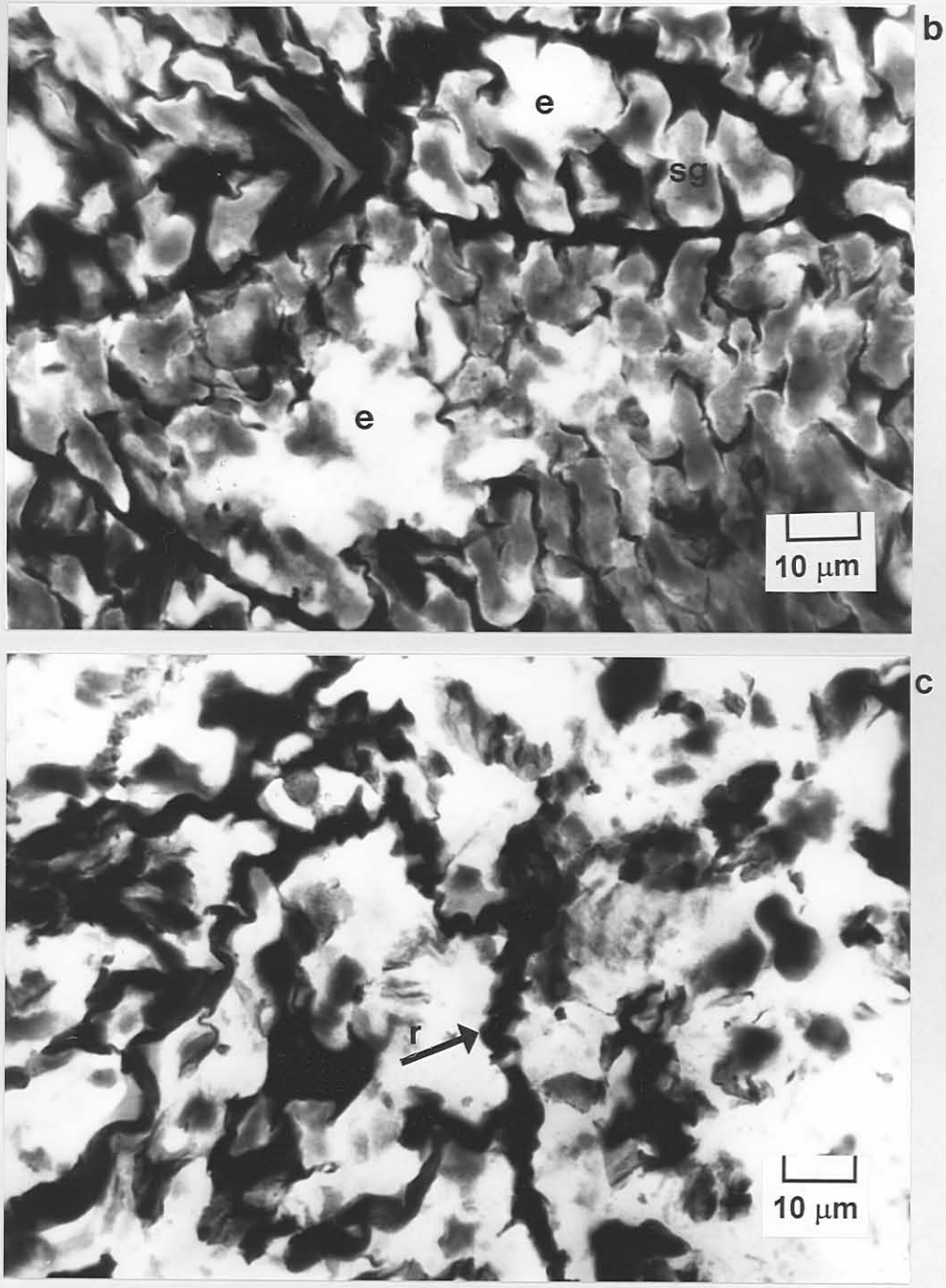


Figure 26: Light micrographs of maize porridge after digestion with pepsin and α-amylase (a, low magnification; b, magnification of cellular area; c, magnification of non-cellular area)

After digestion with pepsin and α-amylase, there were still amorphous (m) and cellular (c) areas in the residue (26a). There were also areas consisting of cell wall remnants (r). At higher magnification it could be seen that the cellular areas (26b) consisted of cells with starch granules (sg) inside. There were also empty spaces (e) in the cells. The amorphous areas (26c) consisted mainly of cell wall remnants