4. CHAPTER I

Phenolic Compounds and Kernel Characteristics of Zimbabwean Sorghums

ABSTRACT

Sixteen sorghum varieties widely cultivated in Zimbabwe were examined for levels of phenolic compounds and kernel characteristics, to help identify those with desirable properties and develop suitable processing methods. Assays for polyphenols included the chlorox test, the vanillin-HCl, the ferric ammonium citrate and the butanol-HCl methods. Free phenolic acids were analysed using reverse-phase high performance liquid chromatography. Varieties DC-75, Mutode and Chirimaugute had the highest levels of condensed tannins. The polyphenols in Chibonda were mostly unextractable in methanol. No significant levels of polyphenols were found in 13 varieties. Phenolic acid content was related to pericarp colour. Endosperm texture and pericarp thickness were evaluated using video image analysis. Katandanzara and SV1 had relatively corneous endosperms (<30% floury). Mutode, Chibonda, and DC-75 had thick pericarps (>0.060 mm). Endosperm texture was not correlated with phenolic compounds. A positive correlation, however, was observed between pericarp thickness and polyphenol content ($r>0.64$). Zimbabwean sorghums lack ideal agronomic and processing physico-chemical characteristics defined in terms of high polyphenols, plus hard endosperm and thin pericarp. Research is required to develop effective methods to process the available polyphenol-rich sorghums.

INTRODUCTION

Sorghum remains an important crop in southern Africa and other semi-arid regions of the world due to its relatively drought-tolerant nature compared to other cereals. Thus it is critically important for food security. In Zimbabwe and elsewhere in southern Africa, sorghum is milled to produce flours for use in porridges. It is malted for the production of opaque beer and nonalcoholic beverages. Sorghum varieties vary widely in kernel characteristics such as pericarp colour and thickness, presence or absence of pigmented testa, endosperm texture and endosperm colour, all of which are genetically controlled (Rooney & Miller 1982). The structure of these grains has an important bearing on various processing and food quality parameters (Hoseney et al 1981). For example, endosperm texture is important in determining the quality of traditional sorghum foods (Rooney and Murty 1982), milling yield (Maxson et al 1971) and resistance to grain moulds (Waniska et al 1992). Similarly, pericarp thickness affects milling properties (Scheuring et al 1983) and mould susceptibility (Glueck and Rooney 1978). For commercial dry milling, white sorghums with thin pericarps and corneous endosperms have superior properties (Maxson et al 1971).

Certain sorghum varieties have a pigmented testa layer which contains condensed tannins. These tannins confer resistance to bird predation (McMillan et al 1972). Losses due to bird predation are a major problem in Africa especially in the western part of Zimbabwe. Tannin and polyphenols protect the grain against weathering (Harris and Burns 1973) and pre-harvest germination (Harris and Burns 1970). The cultivation of high-tannin sorghums reduces pre-harvest losses due to bird predation and post-harvest storage losses. Tannins bind proteins, however, reducing nutritional value (Hahn et al 1984). Hence, the sorghum food processor has the task of finding suitable processing

techniques for tannin removal or inactivation while at the same time producing quality products of high nutritional value.

The objective of this study was to classify and evaluate the physical and chemical attributes of the most important local Zimbabwean sorghums in terms of their levels of phenolic compounds and kernel properties. The information is needed to consider how to improve the acceptance and utilisation of sorghum for food in Zimbabwe and Southern Africa as well as overall rural food security.

MATERIALS AND METHODS

Materials

A total of 16 sorghum varieties grown during the 1995/96 season were obtained from the Crop Breeding Institute, Department of Research & Specialist Services, Zimbabwe. The selection included 13 local varieties, 2 improved cultivars (SV1 and SV2), and one international hybrid (DC-75). The sorghums were chosen to represent the most widely cultivated varieties in Zimbabwe. The grains were ground to pass through a 1 mm sieve for the quantitative determination of polyphenols.

Chlorox test

The chlorox test was used to detect sorghums with a pigmented testa (Waniska et al 1992). Sorghum grains were placed in a glass beaker and just covered with the chlorox reagent (50g litre^{-1} NaOH in commercial bleach, minimum 3.5% sodium hypochlorite). The beaker and contents were placed in an oven set at 70°C for 20 min. Sorghums with a testa had dark colour after bleaching which removed the pericarp and clearly showed

the testa. The number of kernels with complete testa, partial testa and no pigmented testa were counted after draining the bleach and results expressed as a percentage.

Ferric Ammonium Citrate method

A modified International Standardisation Organisation (ISO) (sorghum - determination of tannin content) was used to determine total polyphenols (ISO 1988). Milled grain (250 mg) was extracted with 5 ml of dimethyl formamide at room temperature for 1 h, with vortex mixing at 5-min intervals to stir the ground grain. Measures, 0, 10, 20, and 40 g kg^{-1}, of tannic acid (BDH Chemicals, England) were used to prepare a calibration curve. After centrifugation for 10 min at 1200 *x g*, the following were pipetted into a test tube in the sequence given and mixed carefully after each addition: 5 ml distilled water, 1 ml carboxymethylcellulose/EDTA, 0.2 ml DMF extract or working standard, 0.2 ml ferric reagent and 0.2 ml alkali reagent. Sample blanks were included in which the ferric reagent was replaced by distilled water. The results were expressed as mg tannic acid per 100-mg sample.

Vanillin-HCl method

The vanillin-HCl method of Burns (1971) was used to measure condensed tannins. Milled grain (1 g) was extracted with 50 ml methanol at room temperature for 20 min, with vortex mixing at 5-min intervals. The supernatant was obtained by centrifuging for 10 min at 1200 *x g*. Sample blanks were included. Catechin (Sigma, St Louis, USA) was used as a standard. The results were expressed as mg CE per 100-mg sample.

Butanol-HCl method

A sample extract from the vanillin-HCl assay was used for the butanol-HCl assay of condensed tannins (Porter et al 1986). A measure, 6 ml, of acid butanol (50 ml HCL [32%] dm^{-3}) was added to 1 ml of sample extract in a test tube. Iron chloride was omitted. The test tubes were placed in a forced-air oven at 100°C for 50 min. Absorbance was read at 550 nm. No standards were used in the butanol-HCl method.

RP-HPLC Analysis of Free Phenolic Acids

Extraction

The procedure of Hahn et al (1983) was followed with modifications. First 5 g of ground samples were extracted twice in 20 ml of 100% methanol for 30 min with vigorous shaking at 5-min intervals. The solid material was removed by centrifugation for 10 min at 700 *x g*. The pooled extracts were evaporated under vacuum to near dryness at 35°C using a rotary evaporator. The extract was dissolved in 8 ml of the mobile phase consisting of 25 mM potassium hydrogen phosphate in water (and adjusted to pH 2.67 using phosphoric acid) and acetonitrile in a ratio of 75:25. This extract was applied to an isolute solid phase extraction (SPE) column (International Sorbent Technology, Mid-Glamorgan, UK) to retain the high molecular weight polyphenols. The column was washed with 2 ml of the mobile phase to elute the benzoic and cinnamic acids. The final volume was measured and the extract filtered through a 0.45-µm pore size filter (Millipore). A 100 µl sample was applied to the HPLC column.

Instrumentation

A Waters model 501 liquid chromatograph equipped with a model U6K injector (Waters Associates, Milford, MA) was used for the determination of free phenolic acids in sorghum grain. The column was a Supelcosil ABZ+ 15cm x 4.6 mm id, 5 µm particle cartridge with a guard column (Supelguard ABZ+Plus kit) obtained from Anatech Instruments (Johannesburg, South Africa). Detection was by ultraviolet absorption at 254 nm using a Waters Model 440 Absorbance Detector. Chromatograms were acquired and monitored from the Chromatography Signal Interface (CSI) using an Apex Chromatography Station (1988) on a computer. Retention times and peak areas were obtained using the process module to examine and integrate the chromatograms. Protocatechuic acid content in the grain was calculated using the standard. The total free phenolic acids were then calculated as protocatechuic acid equivalents.

Standards

Gallic, protocatechuic, vanillic, *p*-hydroxybenzoic, caffeic, ferulic, *p*-coumaric and cinnamic acids were obtained from Sigma. Each of the standards was dissolved in the mobile phase to give 5 µg 100 μl^{-1}. A mixture of these standards, excluding caffeic acid and cinnamic acids, containing 5 µg 100 μl^{-1} each was also prepared. Samples of 20 µl were chromatographed singly and as mixtures at a flow rate of 0.5 ml min^{-1} for 20 min. Optimum separation was obtained using acetonitrile and 25 mM $K_2H_2PO_4$ adjusted to pH 2.67 in the ratio 25:75 as eluent. Cinnamic acid was omitted as preliminary runs did not detect significant concentrations in the sorghum samples and its retention time was longer than 40 min. Caffeic acid eluted at the same time as *p*-hydroxybenzoic acid and was omitted from the standard mixture.

Video image analysis

Endosperm texture, endosperm colour and pericarp colour were evaluated visually as described by Rooney and Miller (1982). Endosperm texture and pericarp thickness were then determined using video image analysis of longitudinal sections of the kernels produced by hand-sectioning. The analysis was performed on an Apple Macintosh computer using the public domain NIH Image programme (developed at the US National Institute of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Sections were magnified 1.18 times for endosperm texture and 6.0 times for pericarp thickness with a scanning electron microscope (Nikon SMZ-2T, Japan). The images were captured using a Panasonic CCTV camera (model WV-BL 200/C) on an Apple Macintosh computer. The image was divided into square pixels, each of which had a grey scale value ranging from 0 to 256. The image was enhanced for contrast for proper measurement. The floury portion of the endosperm was measured as a density slice and compared to the total area of the sectioned kernel using the NIH images programme. Photographs of the pericarp were taken so as to avoid the tip area and its opposite end. Pericarp thickness was measured using the conversion 1 mm = 639 square pixels, obtained by measuring an equivalent length of a cm-ruler in square pixels. A total of eight kernel sections were used.

Statistical analysis

Statgraphics version 5.0 (Statistical Graphics Corporation, 1991) was used to analyse the data using multifactor analysis of variance and multiple range analysis. Pearson correlation was used to relate phenolic compounds and kernel properties of the grains.

RESULTS AND DISCUSSION

The sorghum varieties containing a pigmented testa were identified using the chlorox bleach test (Table I-1). Grains of DC-75, Chibonda, Chirimaugute and Mutode contained kernels with pigmented testa which showed up using the chlorox test for the presence of pigmented testa. None of the other varieties tested had a pigmented testa.

Condensed tannins impart bird and mould tolerance and reduce the nutritional value of the grain by 10-30% (Hahn et al 1984). Chemical assays which identify sorghums with high tannin levels are useful in predicting relative bird resistance. The vanillin assay is specific for flavanols (Sakar and Howarth 1976) in which an aromatic aldehyde condenses with certain flavonoids, mainly flavan-3-ols and their oligomers, to form soluble pigments. The use of methanol as solvent quenches the reaction of vanillin with monomeric flavanols, such as catechin, so that the reaction is more specific for oligomers (tannins). The acid butanol assay is specific for proanthocyanidins (condensed tannins) if the optimized reaction conditions described by Porter et al (1986) are used. In this assay, the flavonoid subunits of the condensed tannin polymer are oxidatively cleaved to yield the anthocyanidin (Porter et al 1986). The ISO method is a redox method which is non-specific, responding not only to tannins, but also to other polyphenols and even to other reducing agents such as ascorbate. According to Daiber (1975a), this ferric ammonium citrate procedure distinguishes between tannin-containing and tannin-free sorghums.

Table I-1 shows the amount of condensed tannins (proanthocyanidins) expressed as catechin equivalents or absorbance units and total polyphenols expressed as % tannic acid. The blank subtraction in both the vanillin and ferric ammonium citrate method reduced the polyphenols levels significantly. Cummings and Axtell (1973) classified

sorghum lines into three groups: type I sorghums do not have significant amounts of tannins; type II sorghums have tannins unextractable in methanol; and type III sorghums have tannins extractable in methanol. The three polyphenol analyses indicated varieties DC-75, Chirimaugute and Mutode are probably type III although this cannot be stated categorically as acidified methanol was not used as an extractant. These sorghums differed significantly in polyphenol content, but they all contained more than 1.1% (w/w) total polyphenols, and would therefore not be suitable for malting purposes (Daiber 1975a), as the tannins inactivate the malt amylase enzymes. Treatment of the grain to inactivate the tannins, however, for example, by steeping in dilute formaldehyde (Daiber, 1975b) may render the variety suitable for malting. In fact, in Zimbabwe, DC-75 is used after steeping in dilute formaldehyde for malting.

Concerning the variety Chibonda, the chlorox test indicated the presence of a pigmented testa, but the tannins could not be extracted with methanol (Table I-1). Thus Chibonda could possibly be a type II sorghum (Cummings and Axtell 1973). The other varieties can be classified as type I sorghums since they did not have a pigmented testa and were low in polyphenols as measured by the three different assays. Mukadzidzoka, Iganu, Brown Tsweta and Ntelwane, however, apparently contained some soluble tannins as assayed by the butanol method. Although this assay is said to be specific for proanthocyanidins (Porter et al 1986), other polyphenols are presumably responsible for the positive response in these condensed tannin-free sorghums. Varieties SV1, SV2 and NL330 were virtually polyphenol-free as assayed by the vanillin and butanol assays.

Phenolic acids inhibit growth of micro-organisms and may impart resistance to moulds on sorghum grain before and after grain maturity (Hahn et al 1983). Figure I-

1 shows chromatograms obtained using RP-HPLC of the free phenolic acids in the different sorghum varieties. The chromatograms are stacked in order of decreasing tannin content as assayed by the vanillin-HCl method. The retention times of the acid standards were obtained as: gallic 5.0 min; protocatechuic 6.7 min; vanillic 8.6 min; *p*-hydroxybenzoic 9.7 min; ferulic 13.8 min and *p*-coumaric 15.7 min. Protocatechuic acid levels were higher in DC-75, Mutode and Chirimaugute, those varieties containing a pigmented testa, levels of >50 μg g^{-1} being measured (Table I-1). Higher levels of protocatechuic acid also occurred in Chibonda, the presumed type II sorghum. Nyamidzi and Mukadzidzoka had high levels of vanillic acid, whereas most varieties contained low concentrations of vanillic and ferulic acids. The peak due to *p*-hydroxybenzoic acid was prominent in all the varieties and could have been enhanced by caffeic acid as these were not well resolved. *p*-Coumaric could only be detected in DC-75 and Mutode. Some unidentified phenolic acids appeared in significant quantities in most varieties.

Total free phenolic acid content, calculated as protocatechuic acid equivalents, was high (> 400 μg g^{-1}) in the condensed tannin-containing varieties and in type I sorghums Brown Tsweta, Iganu and Ntelwane (Table I-1). The presumed type II sorghum, Chibonda, however, was rather lower (291 μg g^{-1}). According to Hahn et al (1983) sorghums which are more resistant to fungal attack contain both a greater variety and larger amounts of phenolic acids in the free form. Katandanzara, Chihumani, SV1, SV2, and Kasvikisire, varieties with a white pericarp (Table I-2) had the lowest levels of both protocatechuic acid and total free phenolic acids. Waniska et al (1989) similarly observed that white cultivars without testa contained the lowest amount of phenolic acids.

Table I-2 shows pericarp colour and thickness and endosperm colour and texture. The colour of the pericarp is genetically controlled and can be red, lemon yellow or white (Rooney and Miller 1982). Varieties DC-75, Mutode, Mukadzidzoka, Iganu, Ntelwane, and Brown Tsweta had a red pericarp. Interestingly, Chibonda and Chirimaugute had a white pericarp with a pigmented testa underneath. In the case of the red type I sorghums, Mukadzidzoka, Iganu, Ntelwane and Brown Tsveta pericarp colour appeared to be related to a highish free phenolic acid content (Table I-1). It was not, however, related to polyphenol content as determined by any of the assays performed. According to Kambal & Bate-Smith (1976), flavonoid compounds are responsible for the pericarp colour of sorghum grains. For red and lemon yellow pericarp, luteoforol (an anthocyanidin) and erodictyol (a chalcone) were the major pigments (Kambal & Bate-Smith 1976), respectively. Nip and Burns (1969) also identified two yellow and one orange anthocyanin pigments whose pigmentation was controlled by light, hence the colour differences in sorghum pericarps. Generally a lighter pericarp colour is more desirable when traditionally milled products are to be produced for porridge making. The red pericarp colour is desirable in sorghum malting for the production of traditional opaque beer.

The endosperm colour was white in all the varieties investigated. Endosperm texture is defined as the relative proportions of the corneous to floury endosperm. Figure I-2 shows SEM photographs of sectioned kernels selected to represent varieties with corneous, intermediate and floury endosperm textures (Katandanzara, Mukadziusaenda, and Chibonda, respectively). Katandanzara and SV1 had relatively corneous endosperms (<30% floury). Katandanzara (meaning that it chases away hunger) might

have been given such a name on account of superior storage properties, since hard endosperm grain is more resistant to fungal attack (Kumari et al 1992). Chihumani and DC-75 were also corneous and similar in texture. Concerning DC-75, this is contrary to the general perception that high-tannin sorghums are almost always soft (Waniska et al 1989). Chibonda and Mukadzidzoka had floury endosperms. The other varieties had intermediate to floury endosperms and differed significantly between each other. Hardness, as determined visually by scoring on a scale of 1 (floury) to 5 (corneous), was comparable to the objective image analysis measurements (Table I-2). Rooney and Murty (1982) concluded that sorghum grains with 60-100% corneous endosperm (or <40% floury endosperm) are preferred for the preparation of stiff porridges and rice-like products. Varieties Katandanzara, SV2, SV1, and NL330 met this criterion. Although DC-75, a high-tannin variety, met the criterion, the reddish colour of the meal might not be desirable.

Pericarp thickness plays a role in mould susceptibility (Glueck and Rooney 1978), rendering sorghums with a very thick pericarp more susceptible to grain weathering. Three of the varieties (Mutode, Chibonda, and DC-75) had thick pericarps (>0.060 mm, Table I-2). The classification into thin, intermediate and thick pericarp, as illustrated in Figure 3, was based on visual determination. The thickness of the pericarp differed significantly among varieties with most ranging from thin to intermediate. Abrasive milling studies by Maxson et al (1971) led to the general conclusion that white sorghums with thin pericarps and corneous endosperms had superior dry-milling properties. On this basis, varieties Katandanzara, Chihumani, SV1, SV2, and NL330 are likely to have better milling properties, although none of these varieties is rich in polyphenols.

The three polyphenol assays (total polyphenols, butanol-HCl and vanillin-HCl), despite the fact that they supposedly are specific for different types of polyphenols (Porter et al 1986, Sakar and Howarth 1976), were all significantly correlated ($p<0.05$) with each other ($r\geq0.95$) (Table 3). Earp et al (1981) also observed high correlations for different polyphenol assays (vanillin-HCl, Prussian blue and alpha-amylase inhibition) when a range of sorghums was assayed. There was no correlation between the polyphenol content of the Zimbabwean sorghums and endosperm texture (Table I-3). A significant correlation, however, occurred between polyphenol content and pericarp thickness ($r>0.64$ at $p<0.05$). Protocatechuic acid content was also significantly correlated with pericarp thickness, although total free phenolic acids were not correlated.

CONCLUSIONS

A few of the widely grown sorghum varieties in Zimbabwe contain significant levels of polyphenols. There was a significant correlation between polyphenol content and pericarp thickness. No correlation existed between phenolic compounds and endosperm texture. None of the 16 sorghums met the criterion of high polyphenols, hard endosperm, and thin pericarp. Therefore, if polyphenol-rich Zimbabwean sorghum varieties are to be used for milling, there is a great need for suitable processing methods that will inactivate the tannins in high-tannin varieties and/or improve meal yields from varieties with a soft endosperm and thick pericarp, while giving products of high quality and acceptance.

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Table I-1. Polyphenol and phenolic acid content of 16 Zimbabwean sorghum varieties as measured by different methods

	Chlorox†			Van-HCl‡		Ferric ammonium§		Butanol¶	PTA∪	TFPA**
Variety*	CT%	PT%	NT%	WB††	BS††	WB††	BS††	A/g††	ug/g††	ug/g††
1. DC-75	91	7	2	6.29k	5.48f	1.43k	0.79h	45.88h	79.3j	635.7n
2. Chirimaugute	10	14	76	3.78j	3.07e	1.18I	0.63f	29.31g	57.0j	563.3m
3. Mutode	13	37	50	3.23i	2.79d	1.30j	0.73g	28.30f	56.0I	454.0k
4. Mukadzidzoka	0	0	100	0.66h	0.08c	0.32h	0.14e	1.62e	21.7f	277.7h
5. Chibonda	19	63	18	0.52g	0.03ab	0.20f	0.08cd	1.04cde	41.0h	291.3i
6. Iganu	1	1	98	0.36f	0.09c	0.19ef	0.05ab	1.21de	25.0g	490.7l
7. Ntelwane	0	0	100	0.36f	0.07c	0.29h	0.14e	1.05cde	21.3f	412.0j
8. Tsveta	1	1	98	0.32e	0.09c	0.25g	0.07bc	1.67e	23.0f	566.7m
9. Katandanzara	0	0	100	0.19d	0.03b	0.11b	0.05ab	0.34ab	7.0a	117.3a
10. Mukadziusaenda	0	0	100	0.18d	0.01ab	0.14bcd	0.07bc	0.42a	13.0d	257.3g
11. Nyamidzi	0	2	98	0.12c	0.00a	0.11bc	0.03a	0.87bcd	16.3e	223.0e
12. Kasvikisire	0	0	100	0.05b	0.01ab	0.14bcd	0.05ab	0.25ab	10.0c	205.7d
13. Chihumani	1	0	99	0.03b	0.00a	0.06a	0.02a	0.19a	8.0ab	136.7b
14. SV2	0	0	100	0.00a	0.00a	0.16de	0.14e	0.20a	9.0ab	192.7c
15. SV1	0	0	100	0.00a	0.00a	0.13bc	0.07bc	0.20a	7.7ab	210.3d
16. NL330	0	0	100	0.00a	0.00a	0.14cd	0.11de	0.14a	12.3d	241.0f
Mean	8	8	84	1.01	0.73	0.38	0.19	7.04	25.5	329.7

* Listed in order of decreasing catechins equivalents (CE) as measured by the vanillin-HCL assay.
† Grains with complete testa (CT), partial testa (PT), no testa (NT).
‡ mg CE per 100-mg sample without blank subtraction (WB) and with blank subtraction (BS).
§ mg tannic acid per 100-mg sample without blank subtraction (WB) and with blank subtraction (BS).
¶ Absorbance units at 550 nm per g sample.
∪ Protocatechuic acid in μg per g.
** Total free phenolic acids expressed as protocatechuic acid equivalents in μg per g.
†† Mean of three measurements. Values with different letters within the same column are significantly different at $p < 0.05$.

Table I-2. Pericarp colour, endosperm colour, endosperm texture and pericarp thickness of Zimbabwean sorghum varieties

			Endosperm				
Variety	Testa	Pericarp*	Colourψ	Texture†	Hard‡	Pericarp thickness§	
1. DC-75	yes	red	white	31.9b¶	3.5	6.6j¶	t
2. Chirimaugute	yes	white	white	63.0i	1.4	5.3h	m
3. Mutode	yes	red	white	45.1e	3.2	8.2k	t
4. Mukadzidzoka	no	red	white	71.9k	1.3	4.1g	m
5. Chibonda	yes	white	white	69.7j	1.1	7.0j	t
6. Iganu	no	red	white	56.0h	2.1	3.0cd	tn
7. Ntelwane	no	red	white	56.1h	1.7	3.4e	m
8. Brown Tsveta	no	red	white	51.4g	2.1	3.2de	tn
9. Katandanzara	no	white	white	28.4a	4.3	2.4b	tn
10. Mukadziusaenda	no	white	white	48.1f	3.3	2.8c	tn
11. Nyamidzi	no	white	white	37.8c	3.9	5.2h	m
12. Kasvikisire	no	white	white	38.1cd	3.5	3.7f	m
13. Chihumani	no	white	white	32.9b	3.7	2.3b	tn
14. SV2	no	white	white	39.9d	3.3	3.4e	m
15. SV1	no	white	white	29.8a	3.8	2.0a	tn
16. Ntelwane	no	white	white	39.4cd	2.4	3.9fg	m
Mean value				46.2		4.1	

*Genetics of pericarp colour.

ψEndosperm colour determined subjectively on longitudinal sections of the grain.

†Measured as % floury endosperm.

‡ Visual hardness determined on a scale of 1 to 5: 1, completely floury; 5, completely corneous. Values are an average of 10 scores.

§ Pericarp thickness (m x 10^{-5})classification: t = thick, m = medium, tn = thin. Mean of eight measurements.

¶ Values within same column with different letters are significantly different at $p < 0.05$.

Table I-3. Correlation analysis between polyphenol content and kernel characteristics[a]

	Peri	Text	TP	TPB	BH	VH	PTA	TFPA
Text	0.29							
TP	0.72*	0.09						
TPB	0.71*	0.03	0.99*					
BH	0.67*	-0.04	0.98*	0.97*				
VH	0.66*	0.01	0.96*	0.95*	0.99*			
VHB	0.64*	-0.08	0.96*	0.96*	1.00*	1.00*		
PTA	0.80*	0.26	0.93*	0.90*	0.93*	0.94*	0.92*	
TFPA	0.46	0.36	0.73*	0.68*	0.70*	0.72*	0.69*	0.80*

[a]Pearson correlation coefficients. All values marked with * are significant at $p<0.05$. Text, endosperm texture; peri, pericarp thickness; TP, total polyphenols; TPB, total polyphenols with blank subtraction; BH, butanol-HCL; VH, vanillin-HCL; VHB: vanillin-HCL with blank subtraction; PTA, protocatechuic acid; TFPA, total free phenolic acids.

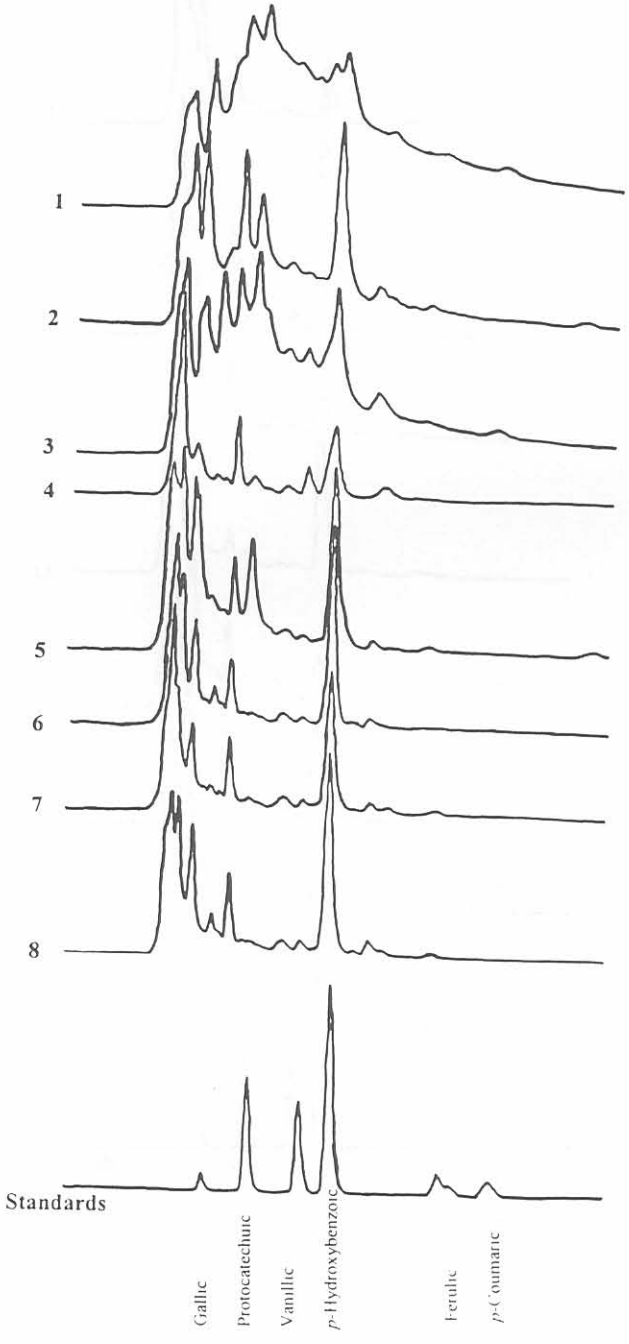

Figure I-1. (a) RP-HPLC chromatographic patterns of varieties: 1, DC-75; 2, Chirimaugute; 3, Mutode; 4, Mukadzidzoka; 5, Chibonda; 6, Iganu; 7, Ntelwane; 8, Brown Tsweta.

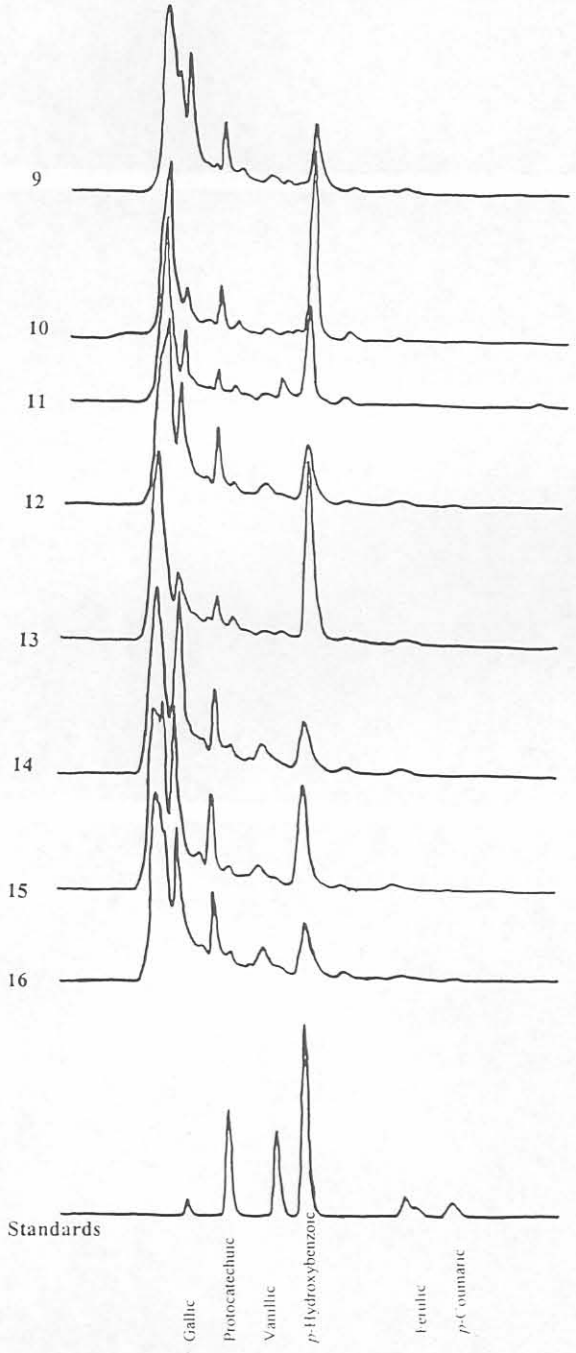

Figure I-1. (b) RP-HPLC chromatographic patterns of varieties: 9, Katandanzara; 10, Mukadziusaenda; 11, Nyamidzi; 12, Kasvikisire; 13, Chihumani; 14, SV1; 15, SV2; 16, NL330.

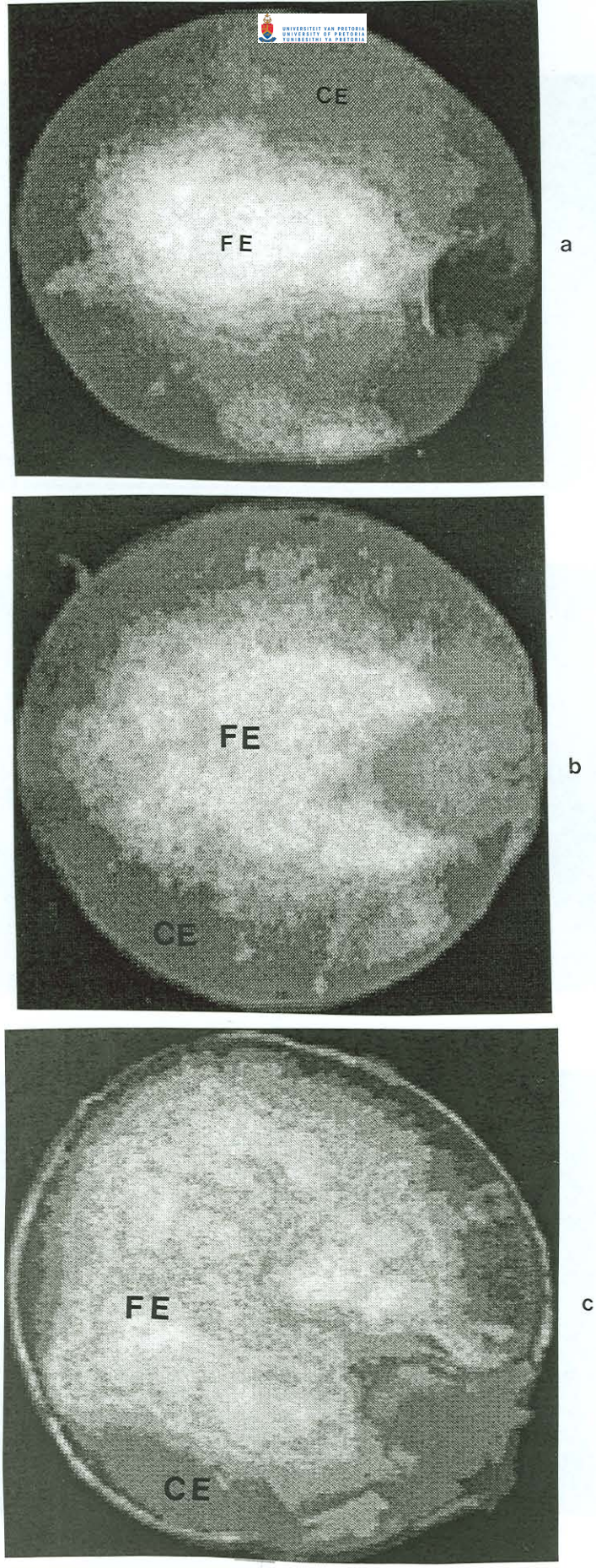

Figure I-2. SEM photographs of varieties Katandanzara, Mukadziusaenda, and Chibonda depicting: (a) corneous endosperm; (b) intermediate endosperm; and (c) floury endosperm, respectively. [FE, floury portion of the endosperm, CE, corneous portion of the endosperm, bar = 1 mm (22 x magnification)]

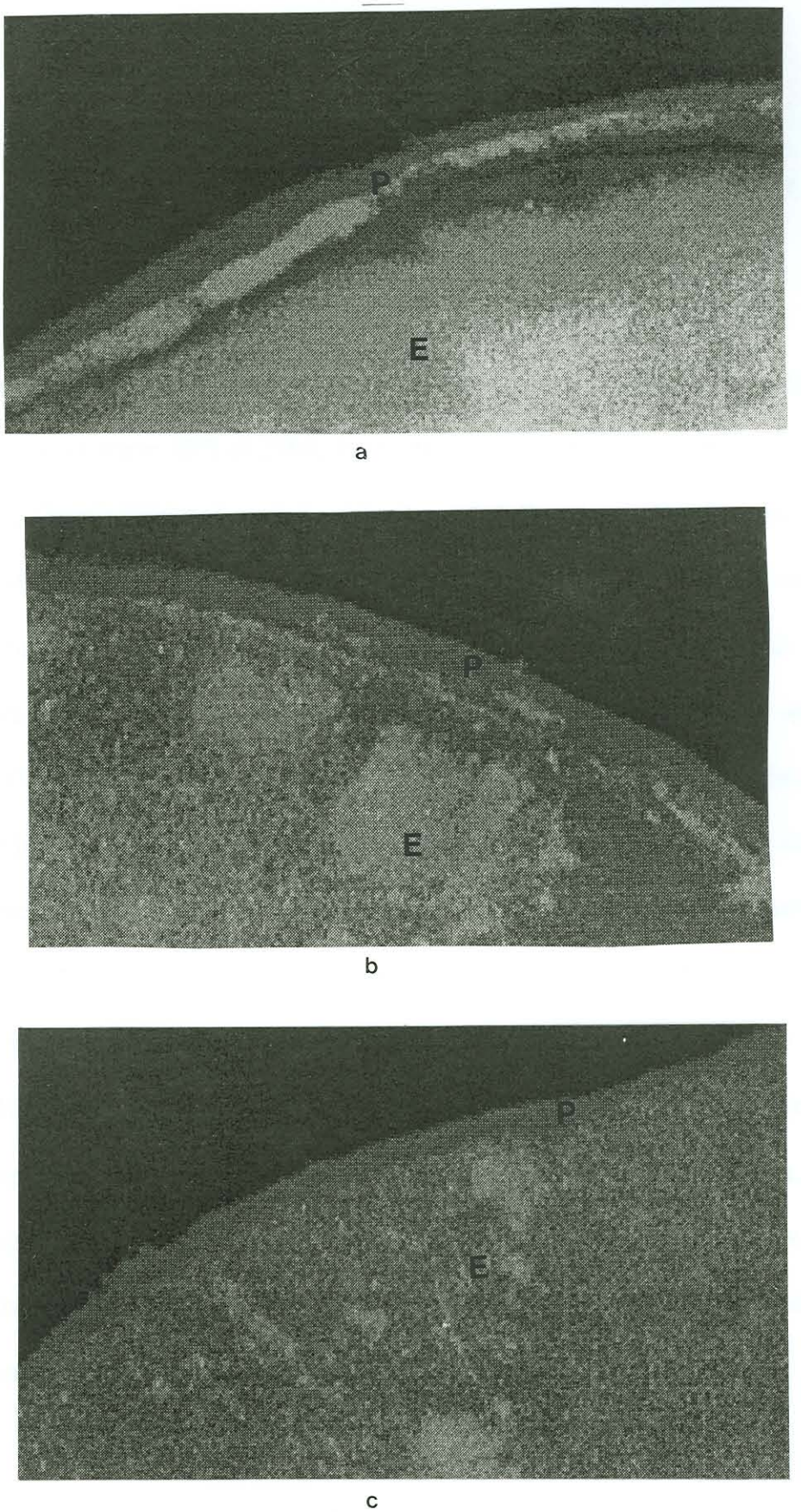

Figure I-3. SEM photographs of varieties Chibonda, SV2, and SV1 depicting: (a) thick pericarp, (b) intermediate pericarp, and (c) thin pericarp. [P, pericarp; E, endosperm; bar = 0.1 mm (140 x magnification)]