

## 4. CHAPTER II

### Effect of Chemical Treatments on Polyphenols and Malt Quality in Sorghum

#### ABSTRACT

Tannin-containing sorghums, Chirimaugute and DC-75, and a tannin-free sorghum, SV2, were steeped in water, HCl (0.25M), formaldehyde (0.017M) and NaOH (0.075M) for 8 and 24 h. Germination was carried out for 2 and 5 d. Steeping in NaOH enhanced water uptake of the grains compared to the other treatments. All treatments reduced the polyphenol content of the raw grain. Treatment with NaOH or formaldehyde (HCHO) was more effective than water or HCl. Malt quality was measured in terms of diastatic power (DP). Potential DP, determined after the peptone extraction, indicated higher amylase in DC-75 malt than in Chirimaugute and SV2 malt. Available DP, determined after water extraction, was low in malt from the tannin-containing varieties that had been treated with water or HCl. Malting alone, was not an effective method of reducing the enzyme inhibitory power of the sorghum tannins. Available DP was markedly improved by the NaOH and HCHO treatments of the tannin-containing varieties. It is concluded that steeping in dilute NaOH is effective in detoxifying high-tannin sorghums, reducing the steeping period and enhancing malt quality. Steeping in NaOH appears to be an alternative to HCHO for treatment of high-tannin sorghums in the malting industry and other food uses.

## INTRODUCTION

The phenolic compounds in sorghum grain play an important agronomic role by reducing grain damage and bird predation (Hahn et al 1984). They can be divided into phenolic acids, flavonoids and condensed tannins. However, the condensed tannins in sorghum grain bind and precipitate proteins causing a reduction in nutritional value (Butler 1982). Tannins also bind with amylase enzymes in the malt (Daiber 1975a), making them unavailable for starch degradation. It is therefore important that tannin-containing sorghum grains are properly treated before they can be used for food. Three major approaches have been reported in the literature: decortication, malting and chemical detoxification. Decortication by abrasive action can remove the outer pericarp and testa layers of the grain where most of the tannins are located (Rooney et al 1980). This technique can result in low milling yield and high protein loss due to softness of the endosperm, which is characteristic of some of the high tannin sorghums (Reichert et al 1988). Malting is a specially controlled form of germination, which mobilises enzymes and modifies the original grain structure and components. In Africa, sorghum grain is malted to produce opaque beers, weaning foods and other traditional dishes. Malting has been reported to result in a decrease in assayable tannins in some genotypes of sorghum (Butler 1982, Reichert et al 1980). Chemical detoxification has also been used to treat polyphenol-rich sorghums. Several alkalis, including ammonium hydroxide, sodium or potassium hydroxide, potassium or sodium carbonate, sodium bicarbonate and calcium oxide, have been used to detoxify high-tannin sorghum grains (Price et al 1979, Banda-Nyirenda and Vohra 1990). In southern Africa, high-tannin sorghums are treated with very dilute formaldehyde solution for malting (Daiber 1975b). However, there is concern

about the toxicity of formaldehyde in food applications (Cheftel et al 1985). High tannin sorghums have also been subjected to treatment with hydrochloric acid (Reichert 1980).

In most of these studies, detoxification treatments were carried out independently of each other. The purpose of this investigation was to compare the effect of various chemical treatments on the polyphenol content and final malt quality of tannin-containing sorghum varieties.

## MATERIALS AND METHODS

### Grains

Sorghum (*Sorghum bicolor* (L) Moench) grain of varieties, SV2 and Chirimaugute (Chiri), and a hybrid, DC-75, were used. Chiri and DC-75 are tannin-containing types. SV2 is a tannin-free improved variety. The grains were grown in the 1996/97 season at Matopos, Zimbabwe in field conditions under normal agronomic practices.

### Steeping

Samples of grain (100 g) were weighed in perforated nylon mesh bags (400 x 400 mm) and steeped for periods of 8 h and 24 h at 25°C. Initially, all grains were subjected to a static steep of 8 h during which individual bags were immersed in solutions of water (control), NaOH (0.3%, w/v), HCl (0.9%, v/v) and formaldehyde (HCHO) (0.05%, v/v) contained in plastic beakers. The solutions were discarded afterwards. The grains in nylon bags were rinsed five times with tap water. Excess water was removed using paper towels after the 8-h steep period. The steeped grains were dried in a forced air oven at

50°C for 48 h inside the nylon bags. The moisture content of the steeped grains was then determined.

After rinsing, steeping was continued for 16 h for those grains that were steeped for a total period of 24 h. The 16-h steep was carried out in running tap water at 25°C by use of two steeping tanks, one of which was thermostatically controlled so that the water temperature was 25°C before entering the other tank where the grains were immersed. Grains were steeped for 4 h followed by a 1-h air rest period outside the steeping tank. This cycle was repeated over 16 h. Excess water was removed and the grains dried as above.

### **Germination**

The grains were steeped for 24 h following the procedure described above. The steeped grains were centrifuged in a domestic spin-drier for 30 sec at 300 x g to remove the excess surface-held water. The grains were then weighed. Germination was carried out at 25°C and 100% relative humidity for 2 and 5 d inside a water-jacketed incubator (Forma Scientific, Marietta, U.S.A.). The grains were placed on racks with moist cloths placed on the surface and bottom of bags to ensure that the grains remained moist throughout the germination period. Twice a day, the germinating grains were weighed and steeped in tap water for 10 min, spin-dried for 30 sec, reweighed and returned to the incubator. After germination for 2 and 5 d, the grains were dried at 50°C for 48 h in a forced-air oven.

## **Milling**

The steeped, germinated and dried grains were ground to pass through an 800- $\mu\text{m}$  sieve, prior to analysis.

## **Chemical analyses**

*Polyphenols.* The polyphenols in sorghum were measured using a method based on anthocyanidin production (Swain and Hillis 1959) and the vanillin reaction (Burns 1971) assay. By using a blank subtraction with the latter, flavonoid components of the grain were eliminated and the reaction became more specific for condensed tannins (Price et al 1978). Sample extracts for the assays were obtained by shaking 0.2 g ground grain in 10 ml methanol at 5 min intervals for 20 min on a vortex mixer at room temperature. The supernatant was obtained by centrifuging for 10 min at 1200 x g. For the anthocyanidin production assay, 6 ml of 5 % HCl in n-butanol 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 against a reagent blank and no standard was used. The results were reported as absorbance units (A/g, dry basis). Catechin (Sigma Chemicals) was used as a standard in the vanillin assay and sample blanks were included. The results were expressed in catechin equivalents (g/100 g, dry basis).

*Diastatic Power (DP).* Sorghum diastatic power was determined following the South African Bureau of Standards method 235 (SABC 1970) with modifications. Water (to measure available DP) and peptone solution (to measure potential DP) were used as extractants. Malt (5 g) was weighed and the extraction volume was reduced proportionately.

The results were expressed as sorghum diastatic units (SDU/g, dry basis).

### **Statistical analyses**

The general linear model procedure of SAS version 6.12 (SAS Institute, Cary, NC) was used. Analysis of variance was used to determine the effect of variety, treatment, steeping, and germination period on polyphenol content and malt quality. Means were separated using the least significant difference at  $P < 0.05$ .

## **RESULTS AND DISCUSSION**

### **Effects of chemical treatment on steep out moisture**

Table II-1 shows the moisture content of the grains after steeping in water, HCl, HCHO and NaOH solutions for 8 and 24 h. The moisture content was significantly affected by variety, treatment, and steeping time ( $P < 0.001$ ). Chiri had higher moisture uptake than the other varieties. The variations in water uptake by the grains could be attributed to their differences in endosperm texture. Video image analysis showed that Chiri grains had a relatively soft endosperm texture compared to those of the other varieties (Chapter I). Grains that were steeped in NaOH solution had higher moisture contents compared to the other treatments, hence the alkali enhanced water uptake of the grain. As sorghum grains are required to have a moisture content of 33-35% (wet weight basis) after steeping (Hofmeyr 1970), the use of low concentrations of NaOH in steeping solutions could reduce the period required to reach such moisture levels. The treatment could also be useful in enhancing malt quality which has been shown to be related to steep-out moisture (Dewar et al 1997a). A shorter steep may also be advantageous, as longer steeping

periods may result in microbial proliferation (Dewar et al 1997b).

### **Effects of chemical treatment on polyphenols**

Table II-2 shows the condensed tannin content measured by the acid butanol assay. Variety, treatment, steeping and germination period significantly affected the tannin levels ( $P < 0.001$ ). The tannin content of the raw grains was higher in DC-75 than Chiri. All treatments resulted in a reduction in condensed tannins. Most of the reduction took place during the first 8 h steeping period. HCHO and NaOH were more effective than water and HCl. Further steeping in water for 16 h was done to bring the total steeping period to 24 h. With water and HCl treatments tannins decreased at 2 and 5 d germination. A marked increase at 5 d germination was observed with HCHO and NaOH. In the butanol assay, the anthocyanidin pigments that were measured resulted from the autoxidation of carbocations initially formed by cleavage of the interflavan bond of the condensed tannin polymer (Porter et al 1986). Given the problems with specificity of tannin assays (Mole 1986), the extracts were also assayed by the vanillin reaction. Its advantage is that vanillin reacts with the flavonoid A-ring at the C-6 position, forming a chromophore that is not influenced by the B-ring hydroxylation (Figure II-1a). The vanillin assay will thus detect any monomeric or polymeric flavanol (Sakar and Howarth 1976). A reduction in the condensed tannin content of the raw grain was observed with all treatments (Table II-3) when the vanillin method (without blank subtraction) was employed. Results closely resembled those obtained with the acid butanol assay with HCHO and NaOH being more effective than water and HCl. Polyphenols generally increased at 5 d germination with the increase being pronounced with HCHO and NaOH.

Reduction in tannins as germination proceeded did not appear to be the general trend, as had been anticipated. McGrath et al (1982) observed that during malting the properties of tannins change as a large complement of the non-inhibitory or non-tannin polyphenols are produced by roots and shoots. Stafford (1965) also reported formation of intensely red coloured cyanidins in the developing root, which cause an increase in the flavonoid content and colour of the malt (Glennie 1983). Thus the apparent increase in tannin content as measured by the butanol and vanillin (without blank subtraction) methods could be attributed to an increase in non-tannin polyphenols as germination proceeded. Modification of the vanillin assay to include a reagent blank that corrected for the colour of the seed extract was done to improve the specificity of condensed tannin measurement (Price et al 1978). The tannin levels with blank extraction (Table II-4) were lower than those without blank extraction (Table II-3). The contribution of the flavonoid components in the grain was thus eliminated. The HCHO and NaOH treatments reduced the level of condensed tannins essentially to zero. Additionally, when true condensed tannins were estimated (by the vanillin with blank extraction), and not polyphenols in general, malting did in fact substantially reduce their level, as has been stated (Butler 1982, Reichert et al 1980). The results confirmed the above observation that non-tannin polyphenols contributed to the tannin readings as the malting period increased.

Notwithstanding the limitations of the assays used, a significant decrease in polyphenols was found when the varieties were steeped in water, HCl, HCHO and NaOH solutions. All treated grains had to be dried prior to polyphenol analysis to eliminate the problem of diminished extractability of tannin from moist grain (Reichert et al 1980, Price et al

1979). Prior to the study, experiments were conducted to determine the lowest HCl concentration at which water uptake of the grain was enhanced with minimal changes in grain appearance. It was observed, however, that grains treated in HCl (0.25 M) were slow in germinating during malting. HCHO concentration chosen for this study was based on levels currently being used for tannin deactivation (Daiber 1975b) during brewing in southern Africa. The concentration of NaOH was chosen based on the finding that it improved the water uptake and apparently improved malt quality in condensed tannin-free sorghums (Dewar et al 1997a). Both the HCHO and NaOH treatments were found to be effective for the purpose of tannin reduction. Similar findings on the effectiveness of NaOH in tannin reduction were reported by Reichert et al (1980) when sorghum grain was allowed to imbibe 25% by weight of distilled water, 0.8 M HCl or 0.8 M NaOH prior to storage at 25 or 35°C for 2 d. However, the tannin values obtained were on freshly ground samples raising the question of diminished tannin extractability at higher moisture levels (Butler 1982). Our results confirm their findings, as the latter problem was eliminated by drying the samples prior to tannin analysis by the vanillin method with blank extraction.

Several authors have surmised that the mechanism of tannin deactivation involves formation of higher molecular weight polymers (Swain 1965, Gupta and Haslam 1978, Kennedy et al 1984, Porter 1992) that are highly cross-linked and insoluble. In the case of tannin deactivation by water or HCl, the mechanism may be similar to the reactions which takes place in the grain as it approaches maturity (Gupta and Haslam 1978). The acid-treated grains may form reactive carbocations from soluble polymers (n=5-6) during

storage (Reichert et al 1980). These carbocations then react to form higher oligomeric polymers which are not readily soluble in water and hence less likely to interfere with enzymes or proteins. Reichert et al (1980) postulated that, either storage of grains that had imbibed water (25% by weight) for 2 days allowed a continuation of the natural polymerization process that was taking place as the seed was drying out, or storage of the acid- or water-treated grains insolubilized the tannins by formation of covalent bonding between condensed tannins and both protein- and polysaccharide constituents of the grain.

The action of NaOH on tannins possibly involves oxidation of phenolic groups under the moist, alkaline conditions. Alkaline conditions promote oxidative polymerization of condensed tannins (Porter 1992), resulting in the formation of highly polymeric and probably nutritionally inactive compounds. In an alkaline solution, flavan-3-ols or condensed tannins can undergo C-ring opening and rearrangement via radical reactions involving traces of oxygen (Kennedy et al 1984). It has been shown that the radical anion intermediate formed in mild, alkaline solutions reacts further by a series of alternative annulation and ring migration reactions leading to a host of products (Figure II-1b). Such processes give phlobatannins, which are more rigid and less polar than the parent proanthocyanidins or condensed tannins (Porter 1992). Swain (1965) suggested additionally, a possible alteration in tannin structure, resulting in formation of insoluble phlobaphenes or the binding of tannin to a nearby component in the grain, rendering both substances insoluble and inert.

The general reaction between formaldehyde and phenols could be used to explain the action of HCHO on tannins. Formaldehyde reacts with phenols to form phenol-formaldehyde resins or Bakelite and related polymers (Morrison and Boyd 1984). The resulting substances are of high molecular weight in which phenol rings are held together by  $-CH_2-$  groups. McGrath et al (1982) postulated that either the formaldehyde simply inactivates the phenolic group in the tannin molecules or that it cross-links the tannin to form large polymers.

### **Effects of chemical treatment on diastatic power of sorghum malt**

Potential DP was assayed after extracting the enzymes from the malt using peptone solution (Table II-5). The reason for peptone use in DP determination is that the high concentration of peptone swamps out the reaction between tannins and the diastatic enzymes, as the peptone reacts preferentially with the tannins (Taylor 1989). Variety, chemical treatment and germination period significantly affected ( $P < 0.001$ ) the DP of sorghum malt and hence the brewing quality of the malt. Malt from DC-75 had the highest potential DP. The HCl treatment gave the lowest DP in all malts. Germination was reduced in HCl-treated grains raising the question of whether the treatment adversely affected the synthesis of the  $\alpha$ - and  $\beta$ -amylases which the DP assay measures. The HCHO treatment gave the highest DP in malts from tannin-containing varieties. The potential DP of malts from water and NaOH-treated grain was in general slightly lower than in malts from HCHO-treated grains.

Available DP was measured after extracting the enzymes with water (Table II-6). SV2, the

tannin-free variety, gave similar DP when water or peptone was used as the extractant. High DP was also found using both assays when the grain from tannin-containing types (DC-75 and Chiri) was treated with HCHO and NaOH. However, malts from DC-75 and Chiri grain that had been treated with HCl and water gave very low water extract DP, as the tannins inhibited the malt amylases (Daiber 1975). Table II-4 shows that malting alone reduced the levels of assayable tannins in Chiri to virtually zero and almost halved the levels in DC-75. However, if one examines the situation with regard to available DP (Table II-6) then DP in Chiri in the 5 day malt control is half that in the peptone (potential) DP and in the case of DC-75 the water DP is only about 15 % of that of peptone DP. The bottom line is that although malting only very substantially reduces assayable tannins (Table II-4), it does not actually greatly reduce the inhibitory power of the tannins in the malt. Malting was not an effective method of reducing the enzyme inhibitory power of the sorghum tannins. This can only be done in combination with treatment with NaOH or HCHO. Thus the NaOH and HCHO treatments were effective in tannin detoxification, such that tannins did not inhibit the malt amylases, whereas the water and HCl treatments were not effective.

## CONCLUSIONS

Steeping high tannin sorghum grains in water, HCl, NaOH and HCHO solutions results in lower tannin content, with the latter two being most effective in reducing the tannins. Malting is not an effective method of reducing the enzyme inhibitory power of the sorghum tannins. This can only be done in combination with treatment with NaOH and HCHO. NaOH also enhances the water uptake of the grain. Of importance is the similar

effectiveness of NaOH in tannin inactivation compared to formaldehyde, since the latter, despite its associated health risk, is currently being used to deactivate the tannins by the sorghum malting industry in southern Africa. Optimum conditions still need to be established, however, it appears that NaOH treatment is potentially a simple, relatively safe alternative for use in sorghum malting and other sorghum food applications. There is need for additional chemical work on sorghum tannins.

## LITERATURE CITED

- Banda-Nyirenda, D. B. C., and Vohra, P. 1990. Nutritional improvement of tannin-containing sorghums (*Sorghum bicolor*) by sodium bicarbonate. *Cereal Chem.* 67:533-537.
- Burns, R. E. 1971. Method for estimation of tannin in grain sorghum. *Agron. J.* 63:511-512.
- Butler, L. G. 1982. Polyphenols and their effects on sorghum quality. Pages 294-311 in: *Proc. Int. Symp. Sorghum Nutritional Quality*. L. W. Rooney and D. S. Murty, eds. Int. Crops Res. Inst. Semi-Arid Tropics (ICRISAT), Patancheru, A. P., India.
- Cheftel, J. C., Cuq, J. L., and Lorient, D. 1985. Amino acids, peptides and proteins. Pages 246-369 in: *Food Chemistry*, 2nd edn. O. R. Fennema, ed. Marcel Dekker, New York.
- Daiber, K. H. 1975a. Enzyme inhibition by polyphenols of sorghum grain and malt. *J. Sci. Food Agric.* 26:1399-1411.
- Daiber, K. H. 1975b. Treatment of cereal grains. South African patent 75/4975.
- Dewar, J., Orovan, E., and Taylor, J. R. N. 1997a. Effect of alkaline steeping on water uptake and malt quality in sorghum. *J. Inst. Brew.* 103:283-285.
- Dewar, J., Taylor, J. R. N., and Berjak, P. 1997b. Determination of improved steeping conditions for sorghum malting. *J. Cereal Sci.* 26:129-136.
- Glennie, C.W. 1983. Polyphenol changes in sorghum grain during malting. *J. Agric. Food Chem.* 31:1295-1299.
- Gupta, R. K., and Haslam, E. J. 1978. Plant proanthocyanidins. Part 5. Sorghum polyphenols. *J. Chem. Soc., Perkin Trans. 1*, 8:892-896.

- Hahn, D. H., Rooney, L. W., and Earp, C. F. 1984. Tannins and phenols of sorghum. *Cereal Foods World* 29:776-779.
- Hofmeyr, J. F. 1970. Moisture uptake by sorghum grain during the steeping stage of malting. M.Sc. Thesis, University of the Witwatersrand, Johannesburg.
- Kennedy, J. A., Munro, M. H. G., Powell, H. K. J., and Porter, L. J. 1984. The protonation reactions of catechin, epicatechin and related compounds. *Austr. J. Chem.* 37:885-892.
- McGrath, R. M., Kaluza, W. Z., Daiber, K. H., Van der Riet, W. B., and Glennie, C. W. 1982. Polyphenols of sorghum grain, their changes during malting and their inhibitory nature. *J. Agric. Food Chem.* 30:450-456.
- Mole, S. 1986. Tannins, a biochemical re-analysis of their importance as anti-feedants. Ph.D. Thesis, University of Strathclyde, Glasgow.
- Morrison, R. T., and Boyd, R. N. 1984. *Organic Chemistry*. Pages 978-979, 4th edn. Allyn and Bacon, Newton, Massachusetts.
- Porter, L. J. 1992. Structure and chemical properties of the condensed tannins. Pages 245-258 in: *Plant Polyphenols*. R. W. Hemingway and P. E. Laks, eds. Plenum Press, New York.
- Porter, L. J., Hirstich L. N., and Chan B. C. 1986. The conversion of procyanidins and prodelfhins to cyanidin and delphinidin. *Phytochem.* 25:223-230.
- Price, M. L., Van Scoyoc, S., and Butler, L. G. 1978. A critical examination of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agric. Food Chem.* 26:1214-1218.
- Price, M. L., Butler, L. G., Rogler, J. C., and Featherston, W. R. 1979. Overcoming the

- nutritionally harmful effects of tannin in sorghum grain by treatment with inexpensive chemicals. *J. Agric. Food Chem.* 27:441-445.
- Reichert, R. D., Fleming, S. E., and Schwab, D. J. 1980. Tannin deactivation and nutritional improvement of sorghum by anaerobic storage of H<sub>2</sub>O-, HCl-, or NaOH-treated grain. *J. Agric. Food Chem.* 28:824-829.
- Reichert, R. D., Mwasaru, M. A., and Mukuru, S. Z. 1988. Characterization of coloured grain sorghum lines and identification of high tannin lines with good dehulling characteristics. *Cereal Chem.* 65:165-170.
- Rooney, L. W., Blakely, M. E., Miller, F. R., and Rosenow, D. T. 1980. Factors affecting the polyphenols of sorghum and their development and location in the sorghum kernel. Pages 25-35 in: *Polyphenols in Cereals and Legumes*. J. H. Hulse, ed. International Development Research Centre (IDRC), Ottawa.
- Sakar, S. K., and Howarth, R. E. 1976. Specificity of the vanillin test for flavanols. *J. Food Chem.* 24:317-320.
- South African Bureau of Standards. 1970. Standard test method for the determination of diastatic power of malts prepared from kaffircorn (sorghum) including bird-proof varieties, and from millet. South African Bureau of Standards, Pretoria, South Africa.
- Stafford, H. A. 1965. Flavonoids and related phenolic compounds produced in the first internode of *Sorghum vulgare* in darkness and in light. *Plant Physiol.* 40:130-138.
- Swain, T. 1965. The tannins. Pages 552-582 in: *Plant Biochemistry*. J. Bonner and J. F. Varner, eds. Academic Press, New York.
- Swain, T., and Hillis, W. E. 1959. Phenolic constituents of *Prunus domestica* I: the

quantitative analysis of phenolic constituents. *J. Agric. Food Chem.* 10:63-68.

Taylor, J. R. N. 1989. Sorghum: the African quest. Pages 164-176 in: Proceedings of the SAAFoST Tenth Biennial Congress and a Cereal Science Symposium. South African Association for Food Science and Technology, Durban, South Africa.

**Table II-1.** Effect of steeping sorghum varieties, Chiri and DC-75, for 8 h in HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), on grain moisture content (% wet weight basis).

Variety	SV2		Chiri		DC-75	
	8 h	24 h	8 h	24 h	8 h	24 h
<b>Treatment</b>						
Control (water)	28.0 <sup>1,2</sup>	33.1 <sup>a</sup>	33.8	38.8 <sup>a</sup>	32.0	37.0 <sup>a</sup>
	(0.3)	(0.6)	(0.3)	(0.2)	(0.4)	(0.1)
HCl	27.3	32.9 <sup>a</sup>	32.1	37.1 <sup>a</sup>	30.3	36.7 <sup>a</sup>
	(0.2)	(0.3)	(0.3)	(0.5)	(0.2)	(0.6)
HCHO	28.0	33.1 <sup>a</sup>	33.0	38.0 <sup>a</sup>	31.1	36.3 <sup>a</sup>
	(0)	(0.3)	(0)	(0)	(0.7)	(0.1)
NaOH	34.0	36.2 <sup>b</sup>	39.4	41.5 <sup>b</sup>	38.4	39.6 <sup>b</sup>
	(0.5)	(0.6)	(0.7)	(0.4)	(0.4)	(0.5)

<sup>1</sup>Mean and standard deviation

<sup>2</sup>Treatments with different letters in the same column are statistically different ( $P < 0.05$ )

**Table II-2.** Effect of steeping sorghum varieties, Chiri and DC-75, for 8 h in HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), followed by germination, on the condensed tannin content as measured by the butanol-HCl assay (A/g, dry basis).

Variety	Chiri					DC-75				
	Malting period		2 d		5 d	Malting period		2 d		5 d
	8 h	24 h								
Raw grain			29.31					45.88		
<b>Treatment</b>										
Control (water)	13.28 <sup>a1,2</sup>	10.53 <sup>b</sup>	7.34 <sup>b</sup>	8.45 <sup>a</sup>	33.00 <sup>b</sup>	36.64 <sup>b</sup>	25.70 <sup>a</sup>	27.89 <sup>a</sup>		
	(0.55)	(0.13)	(0.30)	(0.14)	(0.40)	(1.13)	(1.31)	(1.89)		
HCl	13.08 <sup>a</sup>	16.11 <sup>a</sup>	8.64 <sup>a</sup>	6.82 <sup>b</sup>	34.35 <sup>a</sup>	38.69 <sup>a</sup>	23.77 <sup>b</sup>	20.16 <sup>b</sup>		
	(0.13)	(0.72)	(0.21)	(0.05)	(0.48)	(0.97)	(1.26)	(0.51)		
HCHO	1.59 <sup>b</sup>	1.68 <sup>c</sup>	2.30 <sup>c</sup>	5.28 <sup>c</sup>	3.24 <sup>c</sup>	3.20 <sup>c</sup>	2.46 <sup>d</sup>	5.60 <sup>c</sup>		
	(0.03)	(0.14)	(0.08)	(0.06)	(0.23)	(0.08)	(0.09)	(0.22)		
NaOH	1.16 <sup>b</sup>	1.48 <sup>c</sup>	2.02 <sup>c</sup>	6.64 <sup>b</sup>	1.66 <sup>d</sup>	1.63 <sup>d</sup>	1.26 <sup>d</sup>	6.12 <sup>c</sup>		
	(0)	(0.06)	(0.05)	(0.16)	(0.08)	(0.13)	(0.08)	(0.06)		

<sup>1</sup>Mean and standard deviation

<sup>2</sup>Values with different letters in the same column are statistically different ( $P < 0.05$ )

**Table II-3.** Effect of steeping sorghum varieties, Chiri and DC-75, for 8 h in HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), followed by germination, on the condensed tannin content as measured by the vanillin-HCl assay in catechin equivalents (g/100 g, dry basis).

Variety	Chiri				DC-75			
	8 h	24 h	2 d	5 d	8 h	24 h	2 d	5 d
Raw grain			3.78				6.29	
<b>Treatment</b>								
Control (water)	2.45 <sup>a 1,2</sup> (0.02)	2.06 <sup>a</sup> (0.02)	2.01 <sup>a</sup> (0.01)	3.74 <sup>a</sup> (0.04)	5.49 <sup>a</sup> (0.14)	5.62 <sup>a</sup> (0.02)	4.12 <sup>a</sup> (0.09)	5.90 <sup>a</sup> (0.14)
HCl	2.24 <sup>b</sup> (0.03)	1.93 <sup>b</sup> (0.04)	1.44 <sup>b</sup> (0.03)	2.15 <sup>a</sup> (0.02)	5.32 <sup>b</sup> (0.02)	4.93 <sup>b</sup> (0.04)	3.57 <sup>b</sup> (0.07)	4.63 <sup>b</sup> (0.06)
HCHO	0.45 <sup>c</sup> (0.03)	0.31 <sup>c</sup> (0)	0.92 <sup>c</sup> (0.01)	2.72 <sup>d</sup> (0.01)	0.16 <sup>c</sup> (0.01)	0.18 <sup>c</sup> (0)	0.15 <sup>c</sup> (0.01)	2.12 <sup>d</sup> (0.03)
NaOH	0.31 <sup>d</sup> (0.01)	0.33 <sup>c</sup> (0)	0.83 <sup>d</sup> (0)	3.56 <sup>b</sup> (0.06)	0.01 <sup>d</sup> (0.01)	0.00 <sup>d</sup> (0)	0.13 <sup>c</sup> (0.01)	2.90 <sup>c</sup> (0.04)

<sup>1</sup>Mean and standard deviation

<sup>2</sup>Values with different letters in the same column are statistically different ( $P < 0.05$ )

**Table II-4.** Effect of steeping sorghum varieties, Chiri and DC-75, for 8 h in HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), followed by germination, on the condensed tannin levels as measured by the vanillin-HCl assay with blank subtraction in catechin equivalents (g/100 g, dry basis).

Variety	Chiri				DC-75			
	8 h	24 h	2 d	5 d	8 h	24 h	2 d	5 d
Raw grain			3.07				5.48	
<b>Treatment</b>								
Control (water)	1.77 <sup>a 1,2</sup> (0.02)	1.31 <sup>a</sup> (0.02)	0.51 <sup>a</sup> (0.01)	0.11 <sup>b</sup> (0.04)	4.77 <sup>a</sup> (0.14)	5.36 <sup>a</sup> (0.02)	3.59 <sup>a</sup> (0.09)	2.88 <sup>a</sup> (0.15)
HCl	1.09 <sup>b</sup> (0.03)	1.04 <sup>b</sup> (0.04)	0.57 <sup>a</sup> (0.03)	0.27 <sup>a</sup> (0.02)	4.78 <sup>a</sup> (0.02)	4.02 <sup>b</sup> (0.04)	3.07 <sup>b</sup> (0.07)	2.10 <sup>b</sup> (0.06)
HCHO	0.02 <sup>c</sup> (0.03)	0.00 <sup>c</sup> (0)	0.01 <sup>b</sup> (0.01)	0.07 <sup>b</sup> (0.01)	0.15 <sup>b</sup> (0.01)	0.00 <sup>c</sup> (0)	0.08 <sup>c</sup> (0.01)	0.18 <sup>c</sup> (0.03)
NaOH	0.31 <sup>c</sup> (0.01)	0.33 <sup>c</sup> (0)	0.02 <sup>b</sup> (0)	0 <sup>b</sup> (0.06)	0.01 <sup>c</sup> (0.01)	0.00 <sup>c</sup> (0)	0.02 <sup>c</sup> (0.01)	0.09 <sup>d</sup> (0.04)

<sup>1</sup>Mean and standard deviation

<sup>2</sup>Values with different letters in the same column are statistically different ( $P < 0.05$ )

**Table II-5.** Effect of steeping sorghum varieties, Chiri and DC-75, for 8 h in HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), followed by germination, on the diastatic power (peptone extraction) of sorghum malt (SDU/g, dry basis).

Variety	SV2		Chiri		DC-75	
	2 d	5 d	2 d	5 d	2 d	5 d
<b>Treatment</b>						
Control (water)	19.3 <sup>a 1,2</sup> (0.7)	30.8 <sup>a</sup> (0.6)	17.8 <sup>c</sup> (0.3)	30.6 <sup>c</sup> (0.7)	28.3 <sup>b</sup> (0.2)	47.7 <sup>b</sup> (0.3)
HCl	7.3 <sup>c</sup> (0.4)	18.4 <sup>c</sup> (0.4)	6.8 <sup>d</sup> (0.4)	12.4 <sup>d</sup> (0.4)	15.2 <sup>c</sup> (0.4)	33.9 <sup>c</sup> (0.2)
HCHO	16.4 <sup>b</sup> (0.7)	30.0 <sup>a</sup> (0.6)	20.6 <sup>a</sup> (1.4)	38.5 <sup>a</sup> (0.7)	32.5 <sup>a</sup> (0.7)	49.3 <sup>a</sup> (0.4)
NaOH	17.1 <sup>b</sup> (0.4)	26.6 <sup>b</sup> (0.7)	19.6 <sup>b</sup> (0.4)	32.2 <sup>b</sup> (0.5)	32.1 <sup>a</sup> (0)	46.9 <sup>b</sup> (0.3)

<sup>1</sup>Mean and standard deviation

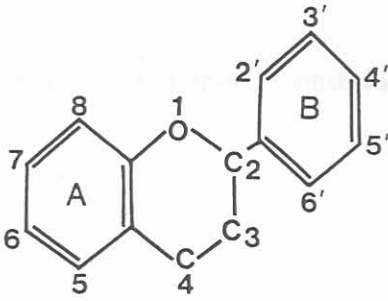
<sup>2</sup>Values with different letters in the same column are statistically different ( $P < 0.05$ )

**Table II-6.** Effect of steeping sorghum varieties, Chiri and DC-75, for 8 h in HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), followed by germination, on the diastatic power (water extraction) of sorghum malt (SDU/g, dry basis).

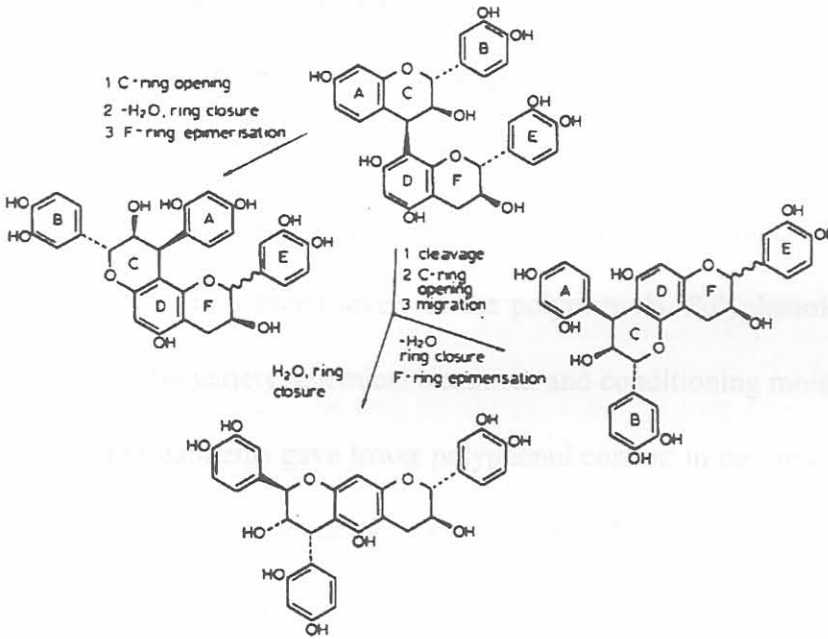
Variety	SV2		Chiri		DC-75	
	2 d	5 d	2 d	5 d	2 d	5 d
<b>Treatment</b>						
Control (water)	17.7 <sup>a1,2</sup> (0.8)	29.4 <sup>b</sup> (0.6)	1.9 <sup>c</sup> (0)	13.3 <sup>c</sup> (0.4)	0.3 <sup>c</sup> (0.2)	6.0 <sup>c</sup> (0.4)
HCl	7.4 <sup>b</sup> (0.6)	17.7 <sup>c</sup> (0.5)	0.38 <sup>d</sup> (0.1)	3.7 <sup>d</sup> (0.2)	0.0 <sup>c</sup> (0)	0.5 <sup>d</sup> (0.4)
HCHO	17.3 <sup>a</sup> (0.2)	33.0 <sup>a</sup> (0.5)	17.5 <sup>a</sup> (0.5)	32.1 <sup>a</sup> (0.9)	25.9 <sup>b</sup> (0.2)	43.5 <sup>a</sup> (0.3)
NaOH	17.4 <sup>b</sup> (1.0)	29.5 <sup>b</sup> (0.9)	15.6 <sup>b</sup> (0.4)	25.2 <sup>b</sup> (0.9)	27.6 <sup>a</sup> (0.4)	38.5 <sup>b</sup> (0.3)

<sup>1</sup>Mean and standard deviation

<sup>2</sup>Values with different letters in the same column are statistically different ( $P < 0.05$ )



(a)



(b)

**Figure II-1.** (a) Basic structure of a flavonoid ring; and (b) rearrangement reactions for proanthocyanidins to form 'phlobatannins' in mildly basic solutions. From Porter (1992).