4. CHAPTER IV

Effect of Steeping Treatment on Pasting and Thermal Properties of Starches from Sorghums of Different Tannin Content

ABSTRACT

Chemical treatments in wet milling could improve the physico-chemical properties of starch isolated from high-tannin sorghums. Sorghum varieties, Chirimaugute (medium-tannin), DC-75 (high-tannin) and SV2 (tannin-free) were steeped in water, dilute HCl (0.9%, v/v), formaldehyde (0.05%, v/v) and NaOH (0.3%, w/v) solutions prior to wet milling and starch separation. Pasting, textural and thermal properties of starch were determined. Steeping in NaOH resulted in starches with higher peak viscosity (PV), cool paste viscosity (CPV) and setback than when water, HCl and formaldehyde were used. The time to PV and PV temperature were markedly reduced by treatment with NaOH. NaOH could have caused some form of partial pre-gelatinisation. HCl treatment gave starches with higher PV temperature and took longer to reach PV presumably due to acid hydrolysis. The low hardness of DC-75 starch gels was slightly improved in NaOH-treated grains. Gelatinization temperatures of sorghum starches were generally low in comparison to maize starch regardless of steeping treatment. Surprisingly, chemical treatments during steeping of sorghum grains greatly affect starch properties. It is concluded that dilute alkali steeping during wet milling could be used to improve properties of starch isolated from tannin-containing sorghums.

INTRODUCTION

Tannin-containing sorghum grain poses problems to the food processor due to appearance and the anti-nutritional effects of condensed tannins (Butler 1982). Milling sorghum grains by decortication, as practised in southern Africa, removes most of the tannins located in the outer layers of the grain (Chibber et al 1978). However, most high-tannin sorghums have a soft, floury endosperm that makes decortication inefficient (Chibber et al 1980; Mwasaru et al 1988). Chemical treatments using water, NaOH, or HCl (Reichert et al 1980) and formaldehyde (Daiber1975) have been shown to reduce or deactivate tannins in sorghum grain. NaOH also improves water uptake of sorghum grains (Dewar et al 1997). Yang and Seib (1995) used NaOH to improve the brightness of sorghum starch. However, there is no information on the effect of chemical treatment on starch properties of high-tannin sorghums.

The possible adsorption and retention of tannins by starch if extracted from tannin-containing sorghum varieties has been indicated (Davis and Hoseney 1979). The pink colour of sorghum starch has been associated with pigments in the pericarp and endosperm of the grain (Freeman and Watson 1971, Norris 1971). Subramanian et al (1994) implicated alcohol-soluble components as causing the dullness of sorghum starch. Sorghum starches also exhibit high gelatinisation temperature ranges (71-81°C) (Akingbala et al 1982). The objective of this investigation was to determine the effect of chemical treatment during steeping on pasting, textural, and thermal properties of starch extracted from a tannin-free, medium-tannin and tannin-rich sorghum.

MATERIALS AND METHODS

Samples

Three sorghum varieties grown under uniform field conditions in the 1996/97 season at Matopos, Zimbabwe, were used: Chirimaugute (landrace, medium-tannin) and DC-75 (hybrid, high-tannin) and SV2 (cultivar, tannin-free).

Starch Extraction

Starch was extracted following a combination of the methods used by Watson et al (1955), Perez et al (1993) and Zhao and Whistler (1994). Sorghum grain (100 g) was steeped in 200 ml water, HCl (0.9 %, v/v), formaldehyde (HCHO) (0.05 %, v/v) or NaOH (0.3 %, w/v) at 5°C for 24 hr. The steeped grain was washed and ground with an equal volume of water using a Waring blender. The slurry was filtered through a 200-mesh sieve (75 μm opening screen). The material remaining on the sieve was rinsed with water. Grinding and filtering was repeated on this material. The material still remaining on the sieve was discarded. The filtrate was centrifuged at 760 x g for 10 min. The grey-coloured top protein layer was removed. Excess water was added to resuspend the sample and centrifugation was carried out for 3 min. Washing and re-centrifugation was repeated until the top starch layer was white. The starch was dried for 24 hr at 40°C.

Pasting Properties

Pasting properties of sorghum starches were determined using the Rapid Visco Analyser model 3D (RVA) (Newport Scientific, Narrabeen, Australia). Sorghum starch (3 g, 14 % moisture basis) was mixed with 25 g of accurately weighed water (10.32 % dry solid

content) in the aluminium canister. Peak viscosity (PV), temperature at PV, time to PV, holding strength (hot paste viscosity) (HPV), cool paste viscosity (CPV), and setback (CPV-PV) were recorded. Two replicates per sample were analysed.

Textural Properties

The sample from RVA testing was allowed to stand for 24 hr at room temperature for gelation to take place (Bhattacharya et al 1997). Hardness or firmness of the starch gel was measured using an SMS Model TA-TX2 texture analyser (Stable Micro Systems, Godalming, England). A standard two-cycle programme was used to compress the gels for a distance of 10 mm at a crosshead speed of 30 mm/min using a 7-mm cylindrical probe with a flat end. Hardness was recorded as maximum force on cycle one in grams (g). Four repeat measurements were taken of each of the two gel replicates per sample.

Amylose / Amylopectin Ratio

An iodine-binding spectrophotometric method using 0.2 % iodine in 2.0 % potassium iodide was used (Juliano et al 1981). Samples of starch (100 mg) were weighed in volumetric flasks (100 ml). One millilitre of ethanol (95 %) was used to wash the sample down the flask. Nine millilitres of NaOH (1 M) was added to the starch sample before heating the flasks in a boiling water bath for 10 min. The samples were cooled and the volume made up to 100 ml with distilled water. The contents were mixed vigorously to disperse the starch. Amylose/amylopectin standard mixtures were prepared to represent from 0 to 60 % amylose content. For the iodine colour development, an aliquot (5 ml) of each solution was taken to which 1 M acetic acid (1 ml) was added. The contents were

mixed. The solutions were allowed to stand for 20 min after mixing with iodine solution (2 ml). Absorbance was read at 620 nm.

Thermal Properties

A Mettler DSC-20 differential scanning calorimeter (Mettler-Toledo AG Instruments, Naenikon-Uster, Switzerland) was used to measure thermal properties of the starch. Starch (2 mg, dwb) was weighed directly into a 40-µL aluminium standard pan and water added to give a final weight of 6.5 mg. The pan was covered with the lid and hermetically sealed. After equilibration at room temperature, the sample was heated from 30°C to 120°C at the rate of 10°C per min. The gelatinisation temperature (°C) parameters of onset temperature (To), peak (Tp), and conclusion (Tc) were determined. Gelatinisation enthalpy (ΔH) in J/g was also recorded. Two replicates per sample were analysed.

Statistical Analysis

Data was analysed using ANOVA procedures of the Statistical Analysis System version 6.10 (SAS Institute, Cary, NC). Means were compared at the 5 % significance level using Duncan's Multiple Range Tests.

RESULTS AND DISCUSSION

Starch colour

The colour of starch from NaOH-treated grain, was brighter than when water, HCl or HCHO were used during steeping. The effect of NaOH on brightness of sorghum starch

colour has been reported before (Yang and Seib 1995). SV2 gave a white starch with all treatments. Concerning SV2, it is a tannin-free variety with white pericarp and tan glumes. However, DC-75 and Chirimaugute gave pink-coloured starches presumably due to the polyphenols present in the pigmented testa layer.

Pasting and Swelling Properties

The pasting properties of sorghum starches are arranged in Table IV-1 according to treatment and variety. Starch pasting and swelling properties were affected by treatment and variety at $P < 0.05$ (Table IV-1). The peak viscosity (PV) was increased by the NaOH treatment. PV is an indication of the water binding capacity of the starch (Newport Scientific 1998). Treatment with NaOH apparently enhanced starch swelling causing an increase in viscosity. Molecules containing ionic groups have intensified water-binding ability at the ionic location, and, in addition, their repulsive charges cause the starch molecules to repel each other (Whistler and BeMiller 1997). Thus NaOH presumably hydrated the starch molecules and also caused some partial pre-gelatinisation. HCl and HCHO treatment gave relatively lower PV. HCl-treated sorghum could have undergone mild, acid-catalysed hydrolysis resulting in starch depolymerisation and hence lower-viscosity products (Whistler and BeMiller 1997). HCHO presumably cross-linked the polymers, particularly protein and polyphenols, present as minor constituents in the starch causing a reduction in swelling power. Starch from DC-75 had the highest PV regardless of treatment probably due to its relatively higher amylopectin content than the other starches (Table IV-2).

The ability of starch to withstand heating and shear stress was recorded as hot paste viscosity (HPV). Subjecting a starch sample to a constant high temperature (95°C) and mechanical shear causes more granules to disintegrate, and amylose molecules will generally leach out into the solution and undergo alignment, resulting in a greater decrease in viscosity (Whistler and BeMiller 1997). NaOH and HCl treatments gave markedly higher HPV than water and HCHO for SV2 and Chirimaugute starches. Starch from NaOH-treated grain, apparently underwent excessive shear thinning because the highly, swollen granules were broken up easily. Acid-modified starches also break up easily and dissolve when heated in water (Whistler and BeMiller 1997). The tannin-rich variety, DC-75 gave starch with the lowest HPV presumably due to the low starch amylose content. Researchers have surmised that the degree of shear thinning may also be related to the morphology and rigidity of the swollen granules (Williams and Bowler 1982, Steeneken 1987). Cool paste viscosity (CPV) and setback were generally higher in starch from NaOH-treated grain. Mistry and Eckhoff (1992) reported that an alkali extraction process results in maize starch with higher viscosity and higher hydration capacity than the commercial maize starch.

Starch from NaOH-treated grain took less time to reach PV presumably due to partial gelatinisation (Table IV-1). However, HCl treatment gave starches that took longer to reach PV probably due to acid hydrolysis. The time to reach PV was lowest for DC-75 due to its higher amylopectin content that accelerated granule swelling than in other starches. PV temperature was markedly lowered in starches from NaOH-treated grain as the partially gelatinised starch granules easily produced high viscosity pastes. Treatment

with HCl gave starches with higher PV temperature as more energy was required to produce viscous pastes from some of the relatively shortened starch chains. The PV temperature range (80.8-82.3°C) was lower for DC-75 starch compared to other starches that had higher amylose content.

Textural Properties

Hardness or firmness of the starch gels was significantly affected by both treatment and variety at $P < 0.05$ (Table IV-2). All treatments resulted in DC-75 starches with very soft gels. This was due to low amylose content of DC-75 as retrogradation of cooked starch involves both starch polymers, with amylose undergoing retrogradation at a much more rapid rate than does amylopectin (Whistler and BeMiller 1997). A firmer gel can also be prepared with starch from corneous rather than the floury endosperm of sorghum (Cagampang and Kirleis 1984). However, a firm starch gel was still obtained from Chirimaugute, a variety with a floury endosperm texture largely due to its relatively high amylose content. With the exception of Chirimaugute, NaOH generally gave starches with firmer gels than other treatments. Thus treatment with NaOH enhanced retrogradation as evidenced by the high setback values of the starch pastes (Table IV-1). The control (water) resulted in a gel that was less firm and significantly different from all the other treatments for SV2 starch. Gel firmness was largely determined by the amount of amylose present in the starches.

Thermal properties

Starch from NaOH-treated grain generally had slightly higher gelatinisation temperatures

than the water, HCl and HCHO treatments (Table IV-3). The effect of NaOH on lowering PV temperature during pasting could not be compared with starch thermal properties. Gelatinisation refers to the disruption of molecular order within starch granules while PV is reached when some of the highly swollen granules have ruptured and fragmented due to shearing forces (Whistler and BeMiller 1997). Steeping SV2 and DC-75 in HCHO or NaOH gave starches with similar Tp values. Tp values for starches isolated from Zimbabwean varieties were lower, regardless of treatment, than values reported by Akingbala et al (1982) that ranged from 74 to 76°C for normal sorghum starches. Tp values for sorghum starches from NaOH-treated grain were also lower than those reported for eight maize starches (72-75 °C) extracted in alkali (Mistry and Eckhoff 1992). Tc was lowest when water was used for steeping. Means of To, Tp and Tc for starches isolated from 35 tropical maize germplasm accessions were 63.0, 71.0, 77.8°C, respectively (Li et al 1994), values similar or slightly higher than the sorghum starches studied. These findings confirm theories by Taylor et al (1997) that southern African sorghums may have a relatively low gelatinisation temperature range. The enthalpy for gelatinisation (ΔH) was quite narrow and ranged from 9.0 to 10.8 J/g. ΔH was significantly higher in HCHO-treated DC-75 starch than when other steeping solutions were used. HCHO could have largely reacted with tannins (Daiber 1975) or proteins and had little effect on the molecular order of the starch granule. However, starches of SV2 and Chirimaugute, from water- and HCl-treated grain respectively, had lower ΔH than other treatments, an indication of less ordered granules.

CONCLUSIONS

Sorghum starch pastes had higher peak viscosity, cool paste viscosity and setback when grain was steeped in NaOH than water, HCl and formaldehyde. NaOH also reduced the time to reach peak viscosty and peak viscosity temperature. NaOH appears to improve sorghum starch properties for certain applications by causing partial pre-gelatinisation. The low hardness of DC-75 starch gels was slightly improved by the NaOH treatment of grain. Gelatinisation temperatures of Zimbabwean sorghum starches were generally low regardless of treatment. It is surprising how much soaking treatments effected sorghum starch properties. Dilute alkali solution, if used during steeping of sorghum grains, could enhance sorghum starch properties.

LITERATURE CITED

Akingbala, J. O., Rooney, L. W., Palacios, L. G., and Sweat, V. E. 1982. Thermal properties of sorghum starches. Pages 251-262 in: Proceedings of an International Symposium on Sorghum Grain Quality. L.W. Rooney and D.S. Murty, eds. Int. Crops Res. Inst. Semi-Arid Tropics (ICRISAT), Patancheru, A.P., India.

Bhattacharya, M., Jafari-Shabestari, J., Qualset, C. O., and Corke, H. 1997. Diversity of starch pasting properties in Iranian hexaploid wheat landraces. Cereal Chem. 74:417-423.

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.

Cagampang, G. B., and Kirleis, A. W. 1984. Relationship of sorghum hardness to selected physical and chemical measurements for grain quality. Cereal Chem. 61:100-105.

Chibber, B. A. K., Mertz, E. T., and Axtell, J. D. 1978. Effects of dehulling on tannin content, protein distribution and quality of high and low tannin sorghum. J. Agric. Food Chem. 26:679-683.

Chibber, B. A. K., Mertz, E. T., and Axtell, J. D. 1980. *In vitro* digestibility of high tannin sorghum at different stages of dehulling. J. Agric. Food Chem. 28:160-161.

Daiber, K. H. 1975. Treatment of cereal grains. South African patent 75/4975.

Davis, A. B., and Hoseney, R. C. 1979. Grain sorghum condensed tannins. I. Isolation, estimation, and selective adsorption by starch. Cereal Chem. 56:310-314.

Dewar, J., Orovan, E., and Taylor, J. R. N. 1997. Effect of alkaline steeping on water uptake and malt quality in sorghum. J. Inst. Brew. 103:283-285.

Freeman, J. E., and Watson, S. A. 1971. Influence of sorghum endosperm pigments on starch quality. Cereal Sci. Today 16:378-381.

Juliano, B. O., Perez, C. M., Blakeney, A. B., Castillo, T., Kongseree, N., Laignelet, B., Lapis, E. T., Murty, V. V. S., Paule, C. M., and Webb, B. D. 1981. International cooperative testing on the amylose content of milled rice. Starch/Staerke 33:157-162.

Li, J., Berke, T. G., and Glover, D. V. 1994. Variation in thermal properties of starch in tropical maize germ plasm. Cereal Chem. 71:87-90.

Mistry, A. H., and Eckhoff, S. R. 1992. Characteristics of alkali-extracted starch obtained from corn flour. Cereal Chem. 69:296-303.

Mwasaru, M. A., Reichert, R. D., and Mukuru, S. Z. 1988. Factors affecting the abrasive dehulling efficiency of high-tannin sorghum. Cereal Chem. 65:171-174.

Newport Scientific 1998. Interpretation. Pages 13-15 in: Applications Manual for the Rapid Visco^{TM} Analyzer. Newport Scientific Pty. Ltd. Warriewood NSW 2102 Australia.

Norris, J. K. 1971. Chemical, physical and histological characteristics of sorghum grain as related to wet milling properties. PhD dissertation. Texas A&M University: College Station, TX.

Perez, E., Bahnassey, Y. A., and Breene, W. M. 1993. A simple laboratory scale method for isolation of *Amaranthus* starch. Starch/Staerke 45:215-220.

Reichert, R. D., Fleming, S. E., and Schwab, D. J. 1980. Tannin deactivation and

nutritional improvement of sorghum by anaerobic storage of H_2O-, HCl-, or NaOH-treated grain. J. Agric. Food Chem. 28:824-829.

Steeneken, P. A. M. 1987. Rheology of suspensions of swollen starch granules. Food Hydrocolloids 1:589-590.

Subramanian, V., Hoseney, R. C., and Brannel-Cox, P. 1994. Factors affecting the color and appearance of sorghum starch. Cereal Chem. 71:272-278.

Taylor, J. R. N., Dewar, J., Taylor, J., and von Ascheraden, R. F. 1997. Factors affecting the porridge-making quality of South African sorghums. J. Sci. Food Agric. 73:464-470.

Watson, S. A., Sanders, E. H., Wakely, R. D., and Williams, C. B. 1955. Peripheral cells of the endosperms of grain sorghum and corn and their influence on starch purification. Cereal Chem. 32:165-182.

Whistler, R. L. and BeMiller, J. N. 1997. Starch. Pages 117-151 in: Carbohydrate Chemistry for Food Scientists. American Association of Cereal Chemists, St Paul, MN.

Williams, M. R., and Bowler, P. 1982. Starch gelatinization: A morphological study of triticeae and other starches. Starch/Staerke 34:221-223.

Yang, P., and Seib, P. A. 1995. Low-input wet milling of grain sorghum for readily accessible starch and animal feed. Cereal Chem. 72:498-503.

Zhao, J., and Whistler, R. L. 1994. Isolation and characterization of starch from amaranth flour. Cereal Chem. 71:392-393.

Table IV-1. Effect of steeping sorghum varieties, SV2, Chirimaugute, and DC-75, in HCl (0.9%, v/v), HCHO (0.05%, v/v), NaOH (0.3%, w/v), on the pasting properties of starch[1].

Variety	SV2						Chirimaugute						DC-75					
Treatment	PV (RVU)	HPV (RVU)	CPV (RVU)	STB (RVU)	Time (min)	PVT (°C)	PV (RVU)	HPV (RVU)	CPV (RVU)	STB (RVU)	Time (min)	PVT (°C)	PV (RVU)	HPV (RVU)	CPV (RVU)	STB (RVU)	Time (min)	PVT (°C)
Control	$373^{b,2}$	112^{c}	257^{d}	145^{bc}	7.27^{b}	87.6^{b}	373^{b}	124^{c}	270^{d}	146^{c}	7.10^{b}	86.5^{c}	463^{b}	116^{bc}	239^{b}	124^{b}	6.27^{ab}	81.5^{b}
HCl	360^{d}	151^{a}	299^{b}	149^{b}	7.93^{a}	91.6^{a}	368^{bc}	162^{b}	306^{b}	145^{c}	7.90^{a}	91.3^{a}	443^{c}	126^{bc}	245^{b}	119^{b}	6.37^{a}	82.1^{a}
HCHO	368^{bc}	125^{b}	272^{c}	148^{b}	7.34^{b}	87.9^{b}	361^{cd}	127^{c}	281^{c}	153^{b}	7.18^{b}	87.1^{b}	424^{d}	116^{a}	239^{b}	123^{b}	6.40^{a}	82.3^{a}
NaOH	396^{a}	151^{a}	319^{a}	168^{a}	6.44^{c}	82.6^{c}	410^{a}	177^{a}	356^{a}	179^{a}	6.27^{c}	81.5^{d}	495^{a}	118^{b}	255^{a}	136^{a}	6.14^{b}	80.7^{c}

[1]PV = peak viscosity, HPV = hot peak viscosity, CPV = cool paste viscosity, STB = setback (CPV-HPV), Time = time to PV, PVT = temperature at PV, RVU = Rapid Visco Analyser Units.
[2]Means with the same letter in each column are not significantly different ($\alpha = 0.05$) according to Duncan's Multiple Range test.

F-values from analysis of variance of starch pasting and textural properties[1].

Source	Degrees of Freedom	Time	PVT	PV	HPV	CPV
Variety	2	622.8[2]	682.0	1833.0	235.2	409.2
Treatment	3	373.0	401.1	309.5	223.6	195.2
Variety*Treatment	6	58.3	62.4	20.3	44.0	29.6

[1]Time = time to peak viscosity (PV), PVT = temperature at peak viscosity (PV), PV = peak viscosity, HPV = hot peak viscosity, CPV = cool paste viscosity, STB = setback (CPV-HPV).
[2]All means significant at $P < 0.05$.

Table IV-2. Effect of steeping sorghum varieties, SV2, Chirimaugute, and DC-75, in HCl (0.9%, v/v), HCHO (0.05%, v/v), NaOH (0.3%, w/v), on the hardness of starch gels.

Variety	SV2	Chirimaugute	DC-75
Amylose content (%)*	27.4	28.7	21.5
	Hardness	Hardness	Hardness
Treatment	(g)	(g)	(g)
Control	73.6[b,1]	82.9[a]	31.0[c]
HCl	81.5[a]	82.9[a]	31.5[c]
HCHO	81.5[a]	79.2[b]	33.9[b]
NaOH	80.6[a]	77.4[c]	36.8[a]

*Determined on starch from NaOH-treated grain.

[1]Means with the same letter in each column are not significantly different ($\alpha = 0.05$) according to Duncan's Multiple Range test.

F-values from analysis of variance of starch gel hardness.

Source	Degrees of Freedom	Hardness
Variety	2	2942.6[1]
Treatment	3	5.0
Variety*Treatment	6	12.4

[1]All means significant at $P < 0.05$.

Table IV-3. Effect of steeping sorghum varieties, SV2, Chirimaugute, and DC-75, in HCl (0.9%, v/v), HCHO (0.05%, v/v), NaOH (0.3%, w/v), on the thermal properties of starch[1].

Variety	SV2				Chirimaugute				DC-75			
Treat-ment	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Control	59.7^{c,2}	67.0^{c}	75.9^{b}	9.4^{c}	60.6^{a}	66.8^{b}	75.8^{b}	9.1^{ab}	61.2^{a}	67.5^{a}	77.9^{b}	9.7^{b}
HCl	60.6^{b}	68.3^{b}	78.5^{a}	10.8^{a}	60.3^{a}	66.5^{b}	76.1^{ab}	9.0^{b}	60.6^{b}	67.0^{b}	78.3^{b}	9.7^{b}
HCHO	62.7^{a}	69.0^{a}	78.1^{a}	9.8^{bc}	60.6^{a}	66.8^{b}	76.2^{ab}	9.1^{ab}	60.7^{b}	67.5^{a}	78.6^{ab}	10.6^{a}
NaOH	62.9^{a}	69.3^{a}	78.3^{a}	10.1^{b}	60.7^{a}	67.5^{a}	76.9^{a}	9.6^{a}	61.2^{a}	67.5^{a}	79.4^{a}	9.7^{b}

[1]To = onset temperature, Tp = peak temperature, Tc = conclusion temperature, ΔH = gelatinization enthalpy.
[2]Means with the same letter in each column are not significantly different ($\alpha = 0.05$) according to Duncan's Multiple Range test.

F-values from analysis of variance of starch thermal properties[1].

Source	Degrees of Freedom	To	Tp	Tc	ΔH
Variety	2	34.24[2]	112.00	77.18	20.66
Treatment	3	36.59	30.33	20.53	3.23†
Variety*Treatment	6	33.87	15.33	4.35	6.46

[1]To = onset temperature, Tc = conclusion temperature, Tp = peak temperature, ΔH = gelatinization enthalpy.
[2]All means significant at $P < 0.05$ except for value marked with †.