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Chemical Changes and Diastatic Activity in Grains of Sorghum (*Sorghum bicolor*) Cultivars during Germination*

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Abstract: Grain samples from nine sorghum (*Sorghum bicolor* (L) Moench) cultivars were germinated for 16, 48, 96 and 144 h, and changes in their diastatic activity, protein, starch, soluble sugars, tannin and total phenols contents were studied. The diastatic activity increased up to 96 h of germination and decreased at 144 h. Diastatic activity showed significant variation among cultivars, which ranged from 10·0 to 88·3 units at 48 h and from 20·0 to 150·4 units at 96 h germination. In general, starch content decreased while soluble sugars increased during germination. Variation in protein content during germination was appreciable among the cultivars.

Key words: Sorghum cultivars, germination, chemical changes, diastatic activity.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L) Moench) grain is used for the production of traditional Pito beer in Nigeria (Olaniyi and Akinrele 1987). After germination the grains are mashed with water and fermented. Grain components such as starch and protein undergo qualitative and quantitative changes during germination. Use of germinated sorghum markedly reduced viscosity in gruel resulting in low bulk with high energy and nutrient density (Mosha and Svanberg 1983). Amylase (diastase) enzyme produced during germination acts on the starch to alter its properties. Diastatic activity is the most important criterion governing malt quality of sorghum (Novellie 1962). The diastatic activity of sorghum malt is rather low. Morrall *et al* (1986) observed a rapid increase in diastatic activity of sorghum grain from 1·5 to 4·0 days of germination. The information available on the changes in diastatic activity during germination of sorghum grain will be useful in considering sorghum for utilisation as malt in brewing and in malt-based foods. Changes in various constituents including diastatic activity during germination in grains of nine sorghum cultivars are reported in this paper.

EXPERIMENTAL

Sorghum grains

Grains of nine sorghum cultivars were grown during the rainy season at the ICRISAT farm. Details of the grains are given in Table 1. The colour of grains was rated using the *Munsell Book of Color* (Munsell Color 1976). One hundred grain mass was determined from three replicates on a dry weight basis, and the mean values are reported.

Germination method

Grain samples (5 g) in three replicates were germinated on moist cotton in petri dishes that were 9 cm in diameter and placed in an incubator. They were germinated at 30°C for 16, 48, 96 and 144 h. A petri dish with water was kept in the incubator during the germination period to

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TABLE 1
Description of grain characters of sorghum cultivars[a]

Cultivar	*Country of origin*	*Grain colour*	*Munsell colour coding*[b]	*100 grain mass (g)*[c]
Lulu dwarf	Tanzania	White	2·5Y 8/2	2·1
M 35-1	India	Pale yellow	2·5Y 8/4	3·9
WS 1297	Ethiopia	White	5·0Y 8/1	3·9
SAR 1	ICRISAT, India	Pale yellow	5·0Y 8/3	2·8
SPV 472	ICRISAT, India	Pale yellow	2·5Y 8/4	3·8
Dobbs	Uganda	Pink	7·5YR 7/4	2·2
IS 7055	Sudan	Reddish brown	5·0YR 3/4	2·8
IS 14384	Zimbabwe	Red	2·5YR 4/8	2·0
Framida	Burkina Faso	Reddish brown	5·0YR 3/4	3·1
	SE			±0·26

[a] Grains were harvested from the crop grown during the rainy season at ICRISAT farm, Patancheru, India.
[b] Munsell colour coding denotes the number for the colour, chroma and hue values respectively.
[c] One hundred grain mass was on dry weight basis.

maintain 90% relative humidity. The incubator was equipped with an exhaust fan for air circulation. The primary roots and shoots from the sprouted grains were removed manually, and the remaining portion of the grain was freeze dried. The 100-grain mass of the germinated samples was determined. The replicates were pooled and used for chemical analysis.

Chemical analysis

The ungerminated (control) and germinated grains were ground in a UDY cyclone mill (UDY Corporation, Boulder, Co, USA) to pass through a 0·4-mm screen. The meals were defatted by extracting with *n*-hexane for 8 h in a Soxhlet apparatus. Chemical analyses were made on duplicate subsamples using defatted meal, and mean values were reported. Diastatic activity was determined by peptone (2%) extraction, using 250 mg of undefatted meal from control or germinated grain according to the method of Novellie (1959). The results were expressed as Sorghum Diastatic Units (SDU). Total nitrogen in sorghum meal was determined using a Technicon autoanalyser as described by Singh and Jambunathan (1980). The crude protein content was calculated by multiplying total N% by a factor of 6·25. Starch content was determined using the enzyme glucoamylase (Sigma Chemical Co, St Louis, USA); glucose content was determined in the enzyme digest by the method of Dubois *et al* (1956). Total soluble sugars from sorghum meal were extracted with hot 80% aqueous ethanol. After evaporating the contents *in vacuo*, the residue was dissolved in water and made up to a known volume. Total soluble sugars were estimated by the phenol–sulphuric acid method (Dubois *et al* 1956). The tannin content was determined in 1% acidic methanol extracts of the meal using the modified vanillin–HCl method of Price *et al* (1978). The results were expressed as catechin equivalents. Total phenols were extracted by treating 200 mg meal with 10·0 ml methanol. To 1·0 ml of extract, 1·0 ml of Folin–Ciocalteu's reagent and 2·0 ml of 20% sodium carbonate solution were added. The intensity of the blue colour in the mixture was measured at 560 nm using a spectrophotometer, and reported as absorbance per 100 grains.

RESULTS AND DISCUSSION

Grain mass

The 100 grain mass of the nine cultivars varied from 2·0 to 3·9 g (Table 2). Grain mass decreased from 16 h germination, and the decrease varied among the cultivars indicating a variation in metabolic activity of the cultivars during germination. Germination leads to modification of chemical components and accelerates the metabolic activity of the grain. Changes in grain mass, diastatic activity, starch, total soluble sugars, tannin, total phenols and protein contents during germination were studied in the nine cultivars.

Diastatic activity

During germination, diastase enzyme activity in sorghum grain increases. Diastase is important in brewing as it acts on the starch present in the grain to alter its properties during malting as well as mashing (Novellie 1962, 1968). We observed variation in diastatic activity during germination of the nine sorghum cultivars. Diastatic activity expressed as sorghum diastatic units

TABLE 2
100 grain mass (g) at different stages of germination in sorghum[a]

Cultivar	*Ungerminated grain (control)*	*Germination period (h)*			
		16	*48*	*96*	*144*
Lulu dwarf	2·1	1·9	1·5	1·1	0·8
M 35-1	3·9	3·8	3·2	2·4	1·6
WS 1297	3·9	3·6	3·0	2·3	1·6
SAR 1	2·8	2·6	2·3	1·7	1·0
SPV 472	3·8	3·5	3·1	2·2	1·4
Dobbs	2·2	2·0	1·5	1·0	0·9
IS 7055	2·8	2·5	2·3	1·5	0·8
IS 14384	2·0	1·9	1·6	1·2	0·6
Framida	3·1	3·0	2·9	2·0	1·3
SE	±0·26	±0·25	±0·24	±0·18	±0·12

[a] Values were on dry weight basis.

TABLE 3
Diastatic activity (SDU per 100 grains) at different stages of germination in sorghum

Cultivar	*Control*	*Germination period (h)*			
		16	*48*	*96*	*144*
Lulu dwarf	0·4	4·8	59·3	80·9	20·7
M 35-1	1·2	11·4	32·0	63·1	22·9
WS 1297	1·2	12·2	146·4	219·2	156·6
SAR 1	0·6	7·0	36·3	34·0	3·4
SPV 472	0·4	10·5	101·4	81·8	16·4
Dobbs	0·4	3·8	73·5	89·5	64·5
IS 7055	0·8	7·5	191·4	171·0	65·4
IS 14384	0·4	8·6	137·4	180·5	88·4
Framida	0·3	4·5	165·3	174·0	84·9
SE	±0·08	±0·82	±12·68	±14·76	±10·67

SDU, Sorghum diastatic units.

(SDU) increased progressively from 16 to 96 h of germination (Table 3). Appreciable variation among the cultivars was found only from 48 h of germination onwards. The SDU values ranged from 36·3 to 191·4 at 48 h and from 34·0 to 219·2 at 96 h germination. The cultivars SAR 1 and M 35-1 had low values even at 96 h. The cultivars WS 1297, IS 14384, Framida and IS 7055 had higher values than other cultivars at 48 and 96 h. The SDU values decreased at 144 h over 96 h values for all the cultivars. This indicates that prolonged germination beyond 96 h may reduce the diastatic enzyme activity. These data suggest that cultivar variation exists for diastatic activity development during germination. This also confirms the observation of the existence of cultivar variation in SDU values as reported by Olaniyi [illegible] that cultivars WS 1297, IS 14384 and IS 7055, due to their ability to produce high SDU during germination, have good potential for use in a breeding programme to incorporate the above trait into high-yielding cultivars, if germinated or malted flour is to be used for specific end uses such as porridges, opaque beer or lager beer.

Starch

The major grain constituents of interest that undergo changes during germination are starch, total soluble sugars, and protein. Starch content varied from 1·38 to 2·77 g per 100 grains between the nine cultivars (Table 4). Starch is the major constituent in sorghum grain and undergoes modification during malting (Novellie 1977).

TABLE 4
Starch content (g per 100 grains) at different stages of germination in sorghum

Cultivar	*Control*	*Germination period (h)*			
		16	*48*	*96*	*144*
Lulu dwarf	1·52	1·33	1·08	0·72	0·50
M 35-1	2·77	2·74	2·25	1·62	1·01
WS 1297	2·66	2·49	2·11	1·54	0·95
SAR 1	2·02	1·92	1·62	1·27	0·67
SPV 472	2·66	2·38	2·07	1·51	0·82
Dobbs	1·44	1·35	1·00	0·60	0·46
IS 7055	1·85	1·66	1·45	0·95	0·44
IS 14384	1·38	1·24	1·02	0·82	0·31
Framida	1·85	1·88	1·76	1·24	0·63
SE	±0·184	±0·183	±0·163	±0·127	±0·087

TABLE 5
Total soluble sugars content (mg per 100 grains) at different stages of germination in sorghum

Cultivar	*Control*	*Germination period (h)*			
		16	*48*	*96*	*144*
Lulu dwarf	29·6	19·8	68·9	72·3	47·5
M 35-1	54·6	50·2	116·5	186·5	172·6
WS 1297	50·7	35·3	143·4	179·4	92·5
SAR 1	33·0	24·7	130·0	113·6	55·8
SPV 472	44·1	36·8	132·7	198·4	151·2
Dobbs	39·4	28·2	92·6	114·4	89·6
IS 7055	47·0	34·0	117·5	117·2	79·2
IS 14384	30·4	25·1	89·1	127·9	82·2
Framida	78·1	46·2	120·1	209·6	135·2
SE	±5·07	±3·37	±8·04	±15·89	±14·31

tern of decrease in 100 grain mass and starch content is similar for all the cultivars studied (Tables 2 and 4). Though a decrease in starch was observed at 16 h when compared with that of ungerminated grain, appreciable reduction in starch content was observed only after 48 h germination. This coincides with the development of diastatic activity (Table 3). The reduction in starch content varied from 33·0 to 58·4% in 96-h germinated grain as compared with the values for ungerminated grain. In general, the reduction was higher in cultivars with high diastatic activity. This indicates that starch modification also varied among the cultivars.

Total soluble sugars

The total soluble sugars content in ungerminated grain varied from 29·6 to 78·1 mg per 100 grains among the cultivars (Table 5). In contrast to starch, the total soluble sugars showed appreciable changes in their quantity during 16 to 144 h germination. The total soluble sugars content decreased in all the cultivars at 16 h germination, but later on it increased up to 96 h germination in all the cultivars. The presence of fermentable sugars in the malt is important for its utilisation in wort for brewing. Increase in total sugars was higher in Framida, WS 1297, SPV 472, and M 35-1 from 48 to 96 h than in other cultivars. The quantity of sugars decreased from 96 h to 144 h in all cultivars (Table 5). We did not observe any relationship between SDU and total sugars contents at all stages of germination.

Tannin/polyphenols

The presence of tannin or polyphenols in sorghum grain interferes with the bioavailability of nutrients (Jambunathan and Mertz 1973). Grains of WS 1297, Dobbs, IS 7055 and Framida contained tannin (Table 6), whereas grains of other cultivars had no tannin. The tannin (catechin equivalents) content decreased until 48 h germination in all the cultivars. However, a small

TABLE 6
Tannin content (mg CE[a] per 100 grains) at different stages of germination in sorghum

Cultivar	*Control*	*Germination period (h)*			
		16	*48*	*96*	*144*
WS 1297	34·3	13·0	8·1	13·8	8·3
Dobbs	59·8	39·6	38·3	44·8	51·2
IS 7055	57·7	34·8	29·4	30·6	19·2
Framida	58·6	41·4	35·7	35·8	26·0

[a] CE, Catechin equivalents.
Tannin was not detected in the grains of the other five cultivars used in this study.

TABLE 7
Total phenols content (A_{560} g^{-1}) at different stages of germination in sorghum

Cultivar	*Control*	*Germination period (h)*			
		16	*48*	*96*	*144*
Lulu dwarf	2·1	1·9	3·0	4·4	4·0
M 35-1	3·9	3·8	3·2	2·8	6·4
WS 1297	3·9	3·6	6·0	4·6	8·0
SAR 1	2·8	2·6	2·3	3·4	4·0
SPV 472	3·8	3·5	6·2	4·4	4·4
Dobbs	24·2	18·0	18·0	21·0	23·4
IS 7055	25·2	17·5	18·4	22·5	12·8
IS 14384	6·0	3·8	4·8	2·4	3·6
Framida	34·1	21·0	26·1	26·0	26·0
SE	±4·13	±2·63	±2·90	±3·29	±2·90

increase was observed in WS 1297 and Dobbs at 96 h. Tannin content decreased due to germination of sorghum (Novellie 1977; Osuntogun *et al* 1989). It appears that there exists genotypic variation in changes of tannin content during germination. Thus, cultivars such as IS 7055 with high SDU may still have the potential for use in malt foods or in brewing although they may have tannin in ungerminated grain which reduces on germination. However, the nature of the tannin and its effects on products such as beer need further investigation. Total polyphenols in ungerminated grains of three cultivars (Dobbs, IS 7055 and Framida) were high (Table 7). The grains of these cultivars have brown testa. Other cultivars had comparatively low quantities of phenols (Table 7). Polyphenols concentration varied during the course of germination. The significance of variation among the different cultivars requires further study.

Protein

In general, protein content decreased during germination. The protein content of the nine cultivars varied from 198 to 443 mg per 100 grains. In barley it has been reported that protein content has to be low, although nitrogenous substances in the malt play an important role in brewing (Thomas and Pyler 1986). During 96 to 144 h of germination the protein content in germinated grain decreased in all the cultivars (Table 8). Although the protein content decreased appreciably, the diastatic activity levels increased and the extent of the increase was variable among the cultivars. The results in this study indicate that reduction in protein content is independent of SDU development during germination in sorghum.

CONCLUSIONS

Our study suggests that diastatic activity increased in some cultivars irrespective of the changes in the quantities of protein or starch. It appears that changes in chemical composition are independent of development

TABLE 8
Protein content (mg per 100 grains) at different stages of germination in sorghum

Cultivar	*Control*	*Germination period (h)*			
		16	*48*	*96*	*144*
Lulu dwarf	201·6	176·7	112·5	79·2	59·2
M 35-1	386·1	361·0	265·6	208·8	147·2
WS 1297	393·9	360·0	270·0	190·9	144·0
SAR 1	201·6	171·6	136·4	107·1	74·0
SPV 472	361·0	322·0	297·6	167·2	134·4
Dobbs	198·0	188·0	97·5	53·0	45·0
IS 7055	310·8	272·5	250·7	109·5	65·6
IS 14384	214·0	212·8	179·2	102·0	66·6
Framida	443·3	429·0	423·4	224·0	193·7
SE	±32·86	±31·69	±34·93	±20·35	±17·36

of diastatic activity during sorghum grain germination. The reasons for high diastatic activity in a few cultivars need detailed investigation. Further, the influence of temperature and relative humidity during germination on diastatic activity in addition to genotypic variation is important. Such information will be useful for ascertaining the possible potential utilisation of germinated flour for traditional and industrial food uses.

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