Journal of Cereal Science **21** (1995) 283–289

The Effect of Malting on the Extractability of Proteins and its Relationship to Diastatic Activity in Sorghum

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Received 10 May 1994

ABSTRACT

Diastatic activity, total protein content, water-extractable protein and water-extractable contents in malted grains of several sorghum cultivars showed wide variation. Protein fractionation studies of malted grain of selected cultivars showed that cultivars with high diastatic activity exhibited high amounts of albumin-globulin fraction in malted sorghum. Diastatic activity was significantly and positively correlated with the water-extractable protein and the water-extractables contents of malted grain. The data indicate that the water-extractable components are good indicators for predicting diastatic activity and indirectly the malting quality. Water-extractable protein and water-extractable determinations are simple and rapid methods that can be used to screen a large number of sorghum cultivars for malting quality.

Keywords: Sorghum malting, diastatic activity, water-extractable protein, protein fractions.

INTRODUCTION

Sorghum grain is used for making various traditional beverages in many countries of Africa. The use of sorghum for lager beer is known in Mexico and Nigeria[1,2]. Aisien and Muts[3] indicated the possibility of using sorghum malt in place of barley for beer production.

The most important characteristics of a good malt are high enzyme levels to degrade starch, and high extract yield. Sorghum malt has low starch-degrading enzyme levels and poor solubility[3,4,5,6]. In lager beer production, simple amino acids and peptides provide a better substrate for yeast than high M_r proteins. Malting of sorghum results in the production of free amino acids and small peptides comprising the free amino nitrogen required for yeast nutrition during fermentation[7]. Although sorghum has been used for lager beer production, the criteria for evaluation of malt quality are not well understood. Variability in grain and malt characteristics can be exploited for better utilisation of sorghum or traditional beverages and lager beer production.

This study describes the variation in grain characteristics that are considered important for malt, and the relationship between malt quality and diastatic activity.

ABBREVIATIONS USED: SDU: sorghum diastatic units; WEP: water-extractable protein; WE: water-extractables.

EXPERIMENTAL

Materials

Sorghum grain

The sorghum cultivars selected for this study included local and improved high yielding cultivars grown in India and Nigeria, and germplasm accessions representing different geographic origins. The grain samples were harvested during the 1988 rainy season from breeding trials conducted at ICRISAT Center, Patencheru, India, and ICRISAT, Bagauda, Nigeria.

0733–5210/95/030283+07 $08.00/0

Malting

Grain samples (5 g) were steeped overnight at 30°C in two replications. The water was drained next morning and excess moisture adhering to the grains was blotted using filter paper. Grains were malted (germinated) on a moist cotton wool layer in petri dishes in an incubator for 96 h at 30°C ± 1°C, with 90% relative humidity. After 96 h, roots and shoots were removed manually from sprouted grains, and the grains were dried at 50°C for 24 h. The 100-grain mass was determined and the loss in weight due to malting was calculated and expressed as malting loss (%). Whole or malted grains were ground in a Udy cyclone mill to pass through a 0·4 mm screen, and the resulting flour was used to determine diastatic activity, total protein content, water-extractable protein and water-extractables (%). All the values are the mean of two determinations.

Diastatic activity

Diastatic activity was determined in malted sorghum grain according to the method of Novellie[8]. The flour (0·25 g) was extracted with 2% (w/v) peptone solution (10 ml) for 2 h 40 min at 30°C. An aliquot of the extract (0·2 ml) was added to a soluble starch solution (10 ml) and incubated at 30°C for 30 min. The reaction was stopped with 0·21 M NaOH (4·8 ml). A blank containing extract (0·2 ml), starch solution (10 ml) and 0·21 M NaOH (4·8 ml) was used. An aliquot (1 ml) of the digest containing reducing sugars was pipetted into 0·025 M alkaline potassium ferricyanide (4 ml) and heated for 20 min in a boiling water bath. The sugar content was determined by adding starch-potassium iodide solution (0·2 ml) and titrating with 0·025 M sodium thiosulphate. The diastatic activity was calculated using the titre value, and the results were expressed as sorghum diastatic units (SDU/g).

Protein content

Total nitrogen was determined using a Technicon auto analyser[9]. After conversion of organic nitrogen in flour into ammonia by digestion with sulphuric acid, the ammonical nitrogen is reacted with sodium phenate in the presence of sodium hypochlorite to form an indo-phenol blue complex. The absorbance of the colour complex was measured at 660 nm using ammonium sulphate standards as calibrants to determine the nitrogen content. Protein content was calculated by multiplying N% by 6·25.

Protein fractionation

Fractionation of protein into fractions I-V was carried out using the extraction method of Landry and Moreaux[10] with minor modification. 0·5 M sodium chloride solution, 70% (v/v) propan-2-ol and 70% (v/v) propan-2-ol with 0·6% (v/v) 2-mercaptoethanol were used to extract albumin-globulin (fraction I), prolamin (fraction II) and cross-linked prolamin (fraction III), respectively, from flour. Glutelin-like proteins (fraction IV) and glutelins (fraction V) were extracted using borate buffer, pH 10·0, containing 0·6% (v/v) 2-mercaptoethanol, and borate buffer, pH 10·0, containing 0·6% (v/v) 2-mercaptoethanol and 0·5% (w/v) sodium dodecyl sulphate, respectively. The residue from fraction V was extracted with 0·1 M sodium hydroxide and referred to as alkali-extractable protein (fraction VI). The nitrogen content in each fraction was determined and expressed as a proportion of total nitrogen (%) in the flour.

Isolation of water-extractable protein and water-extractables

Either malted or whole grain flour (1 g) was extracted with water (25 ml) for 1 h at ambient temperature (25 ± 2°C). The mixtures were centrifuged for 5 min at 3000 ***g*** and the supernatant transferred to a 50 ml volumetric flask. The residue was further extracted twice with 15 ml and 10 ml each time, respectively, for 30 min and centrifuged for 5 min. The supernatants were pooled and made up to 50 ml. After mixing well, the solution was filtered using a Whatmann No. 541 filter paper. The filtrate was taken for the determination of water-extractable protein (WEP) and water-extractables (WE).

The malted or whole grain flour sample was also extracted with water at 60 ± 1°C for 1 h in a water bath, with minor modifications of the method of Morrall *et al.*[4]. The extracts were used to determine WEP and WE and expressed as hot water-extractable protein (HWEP) and hot water-extractables (HWE).

Determination of water-extractable protein

The water extract (10 ml), either WEP or HWEP, was pipetted into a Technicon digestion tube and

evaporated almost to dryness. The residue was digested with sulphuric acid and the protein content was determined by the Technicon auto analyser method[9]. Water-extractable protein content was expressed as g/100 g total protein.

Determination of water-extractables

The water extract (10 ml), either WE or HWE, was pipetted into a pre-weighed aluminum dish and evaporated overnight at 110°C. The aluminum dish was cooled in a dessicator and weighed. The proportion (%) of WE was calculated and expressed as g/100 g flour.

RESULTS AND DISCUSSION

Protein extractability in unmalted and malted grain sorghum

Grains of seven sorghum cultivars, ISCV 145, CSV 13, CSH 1, M 35-1, IS 18519, TAM 2566, and IS 14384, were chosen for this study. The malting characteristics, such as diastatic activity and extractability of protein, were determined using uniform and standard conditions. Although the optimum malting conditions may vary for different cultivars, our aim was to compare the above characteristics under identical conditions. The cultivars showed a wide range of diastatic activities for the malted grains, ranging from 11·3 to 141·4 SDU/g (Table I). The variation for SDU among the cultivars was significant ($P<0·01$). Wide variation in the diastatic activity of sorghum malts has been reported previously[11]. The diastatic activity of sorghum malts includes both *alpha*- and *beta*-amylase activities, although the *beta*-amylase content is markedly lower than for barley malts[8]. Malted sorghum contains higher level of *alpha*-amylase than *beta*-amylase[12]. The cultivars, IS 14384, TAM 2566, and IS 18519 had high diastatic activities, while ICSV 145 and CSV 13 had low diastatic activities. The protein contents of the seven cultivars ranged from 8·6 to 11·4% in the unmalted grain and from 5·3 to 9·1% in the malted grain.

Malting increased the proportion of salt-extractable protein (fraction I). This fraction consists of albumin-globulin and non-protein nitrogen. The cultivars IS 14384, TAM 2566, and IS 18519 had high SDUs, and malting of these cultivars resulted in 2·3 to 3·8-fold increases in the amount of fraction I protein. The cultivars CSV 13, CSH 1, and M 35-1 had low SDUs, and malting of these cultivars resulted in 1·5 to 1·9-fold increases in the amount of fraction I protein (Table I). The cultivar IS 14384 had high amounts of fraction I protein.

The protein fractions II (prolamin) and III (cross-linked prolamin) decreased on malting in all cultivars except M 35–1. Taylor[7] reported that prolamin was degraded during the malting of sorghum grain. The total prolamin (fractions II + III) was lowest in the three cultivars with highest diastatic activity. Variations in the levels of fractions IV and VI between unmalted and malted grains were small and not consistent among different cultivars. The amount of fraction V protein was diminished on malting in all the cultivars. The differences in the amounts of protein in fractions I, II, III, V, VI and in total extractable protein between cultivars and interaction with malting were significant ($P<0·01$). The differences in the proportion of fraction IV protein was not significant between unmalted and malted samples, however. Albumin and globulin proteins increased in quantity when sorghum was malted[7]. The quantity of fraction I protein was correlated significantly ($r=0·82$, $P<0·05$) with the diastatic activity of malted grain, indicating that cultivars with high quantities of fraction I have high diastatic activity. This indicates that malting results in the production of enzymes that hydrolyse a portion of the prolamin and glutelin into simpler forms. A similar observation was reported for sorghum by Taylor[7]. Since fraction I showed appreciable variation in the seven cultivars, another experiment was conducted involving 10 germplasm accessions to study the variability of WEP, WE, HWEP, and HWE, and their relationship with diastatic activity of the malted grain.

Diastatic activity and malt extractability

The malting quality parameters for 10 germplasm accessions are given in Table II. The protein content of malted grain was lower than that of unmalted grain. The diastatic activity of malted grain ranged from 32·3 to 153·0 SDU/g. The water-extractable protein (WEP) content of malted sorghum expressed as a proportion (%) of total protein ranged from 11·0 to 34·5%, and HWEP ranged from 19·3 to 44·1%. The water extractables (WE) expressed as proportion (%) of flour weight ranged from 10·7 to 27·7%, and HWE from 18·3 to 38·9%, The water-extractable protein content

Table I Protein distribution in whole and malted sorghum grains

Cultivar	SDU/g	Protein[a] (%)	Protein fractions[b]						
			F I	F II	F III	F IV	F V	F VI	Total
Unmalted grain									
ICSV 145	—	9·8	15·5	8·4	17·6	5·6	29·6	3·2	79·9
CSV 13	—	9·1	19·1	13·0	20·5	6·1	28·2	5·1	92·0
CSH 1	—	9·3	16·7	11·2	21·1	4·1	35·4	1·4	89·9
M 35–1	—	9·7	15·7	10·1	22·2	5·2	34·3	2·4	89·9
IS 18519	—	8·6	5·9	4·3	10·9	13·3	37·9	1·0	73·3
TAM 2566	—	11·4	16·7	10·3	17·6	7·9	24·0	8·9	85·4
IS 14383	—	8·7	19·9	10·8	15·8	10·1	28·2	5·4	90·2
Malted grain									
ICSV 145	11·3	9·1	34·2	7·5	16·3	5·9	18·6	2·9	85·4
CSV 13	21·3	9·0	27·7	10·2	13·1	9·1	26·4	4·1	90·6
CSH 1	28·2	8·5	30·0	6·7	20·3	4·5	30·9	1·3	93·7
M 35–1	34·6	9·1	29·1	11·2	24·1	3·4	20·8	2·3	90·0
IS 18519	74·3	5·3	22·5	2·4	1·2	12·9	30·6	1·0	70·6
TAM 2566	95·9	8·7	40·6	7·1	12·1	7·5	19·2	2·7	89·2
IS 14384	141·4	6·0	46·3	6·0	5·9	8·6	18·6	3·2	88·6
$LSD_{1\%}$ (Cultivars × malting)	7·07[c]	0·23	2·38	0·99	3·95	1·16	1·81	0·72	5·09

[a] Moisture-free basis.
[b] Proportion (%) of total nitrogen.
[c] LSD for cultivars.

Table II Diastatic activities (SDU/g), protein, water-extractable protein (WEP), hot water-extractable protein (HWEP), water-extractables (WE) and hot water-extractables (HEW) contents of unmalted grain flour (UF) and malted grain flour (MF) of sorghum

Cultivars	SDU/g	Protein [a] (%)		WEP (%)		HWEP (%)		WE (%)		HWE (%)	
		UF	MF	UF	MF	UF	MF	UF	MF	UF	MF
IS 155	48·8	9·7	9·4	6·8	29·0	15·1	31·1	6·5	19·2	12·1	25·1
IS 5758	153·0	12·2	10·2	7·7	23·8	14·2	44·1	6·7	21·8	11·0	38·9
IS 6414	76·2	7·8	6·8	6·6	34·5	20·7	38·1	6·4	23·3	16·7	32·9
IS 14384	124·6	8·3	5·5	6·1	34·2	15·7	40·2	5·7	27·7	11·0	36·4
IS 18643	32·3	7·9	7·7	6·4	17·2	17·9	22·8	5·8	14·4	15·3	22·0
IS 18519	74·3	8·5	5·6	5·9	16·8	11·1	22·6	5·1	17·0	10·0	24·0
IS 19902	111·0	11·6	11·4	9·1	26·1	15·2	33·5	7·5	19·0	12·8	30·0
IS 25474	33·8	10·7	10·1	6·5	11·0	11·2	19·3	5·7	10·7	10·8	18·3
ET 125	57·0	11·8	11·6	7·9	17·3	14·1	25·9	7·1	16·3	12·7	24·5
ET 155	41·3	11·0	10·8	6·7	25·6	13·3	27·4	5·3	19·2	9·8	23·1
$LSD_{1\%}$ (Cultivars × malting)	7·36[b]	0·63		1·04		2·00		1·11		2·14	

[a] Moisture-free basis.
[b] LSD for cultivars.

increased with malting in IS6414, IS 14384, IS 555, ET 155, and IS 5758 (Table II). Taylor[7] observed a more than 9-fold increase in free amino nitrogen in sorghum during 7 days of malting.

Malted grain of IS 5758 showed high SDU, HWEP, and HWE, whereas malted grain of 18643 showed low SDU, HWEP, and HWE. The proportions (%) of HWEP and HWE was higher than WEP and WE for all the cultivars, indicating that the extractability of protein and water extractable solids of malted grain were higher at 60°C. The extractability of nitrogen at 60°C increased re-

Table III Correlation coefficients (*r*) between diastatic activity (SDU/g) and extractibility characteristics of sorghum malt

Characteristics	*r*
SDU *vs.* Water-extractable protein (%)	0·92*
SDU *vs.* Hot water-extractable protein (%)	0·84*
SDU *vs.* Water-extractables (%)	0·69*
SDU *vs.* Hot water-extractables (%)	0·46

* Significant at 1% level.
$n = 10$

markably for IS 5758, IS 6414, and IS 14384 on malting. Jayatissa *et al.*[6] reported that the hot water extract values varied considerably among sorghum cultivars. The contents of WEP, HWEP, WE, and HWE increased 3·4-, 2·0-, 3·0-, and 2·3-fold, respectively, on malting. The variation among cultivars and the interaction between malting and cultivars for the above characteristics were significant ($P<0·01$).

The cultivars with high SDU values showed high WEP and WE values at room temperature and at 60°C. There were significant correlations between the diastatic activities of malted grains and the WEP and WE values (Table III). The correlation coefficients between SDU and water-extractable protein were 0·92 ($P<0·01$) for room temperature extraction and 0·84 ($P<0·01$) for extraction at 60°C.

To confirm the above observation, we analysed another set of 26 cultivars for the different malt characteristics. It has been reported that cold water extract values for malted sorghum indicated good modification of the grain constituents during malting[6]. Therefore, a simple and rapid method was chosen to extract the water-extractable protein of

Table IV Malting losses, diastatic activities, and water-extractable protein (WEP) and water-extractables (WE) contents of sorghums

Cultivators	Malting loss (%)	Diastatic activity (SDU/g)	WEP (%)		WE(%)	
			UF	MF	UF	MF
ICSV 1	19·9	11·3	12·4	17·1	7·1	15·1
ICSV 145	24·1	12·8	12·0	16·6	8·3	16·3
ICSV 272	20·8	34·0	14·3	23·1	88·3	18·7
ICSV 209	23·0	27·8	16·5	24·7	7·9	16·8
CSV 13	24·0	21·3	13·9	20·9	7·6	17·4
ISCH 11	23·4	28·7	14·7	23·9	7·6	16·0
ICSH 110	22·3	19·6	16·0	18·5	8·4	14·9
CSH 1	20·3	28·2	12·6	19·7	7·1	12·7
CSH 9	23·7	20·5	14·8	18·7	9·2	15·8
M 35–1	18·6	34·6	14·3	20·7	6·2	15·9
TAM 2566	26·7	95·9	13·2	26·7	7·9	29·5
Naga white	21·4	53·0	12·5	21·5	5·8	19·7
Farafara	28·2	75·4	14·2	24·4	8·7	18·2
KSV 8	26·5	73·0	11·9	29·0	7·8	22·1
SK 5912	27·2	66·8	13·7	22·6	8·7	20·1
CO 4	20·1	14·7	15·3	17·4	9·1	13·6
Dobbs	20·6	46·2	9·2	24·8	5·4	14·0
ET 3491	22·2	40·2	11·7	25·7	7·7	19·0
Framida	20·1	86·7	9·7	24·8	5·4	24·1
IS 14384	24·7	141·4	8·7	36·0	6·8	31·2
IS 15255	21·2	11·7	16·0	17·1	6·2	11·0
IS 20940	23·7	37·9	11·6	22·9	10·3	17·1
IS 22472	21·8	72·0	12·3	30·4	7·6	16·9
IS 24885	20·6	71·2	14·8	27·7	7·5	12·5
IS 25359	23·0	39·4	10·1	24·0	5·2	16·9
RSA	26·8	26·3	8·3	21·5	5·2	14·3
$LSD_{1\%}$ (Cultivars × malting)	2·70[a]	8·01[a]	2·58		1·75	

UF: Unmalted grain flour; MF: Malted grain flour.
[a] LSD for cultivars.

flour at room temperature. The malting losses of sorghum grain germinated for 96 h varied from 18·6 to 28·2% among the 26 cultivars (Table IV). The malting loss was higher for the cultivars TAM 2566, RSA, KSV 8, SK 5912 and Fara Fara than for others. Earlier workers reported about 20% malting loss of sorghum[13]. The present study confirms our earlier observation[11] of wide variation in the diastatic activity in malted sorghum (Table IV). The diastatic activity varied from 11·3 to 141·4 SDU/g for the 26 cultivars. All 26 cultivars could be grouped into 3 categories based on SDU values: low (<30 units), medium (31–60 units), and high (>60 units). The cultivars IS 14384, Framida, TAM 2566, IS 22472 and IS 24885, and local cultivars from Nigeria, such as SK 5912, KSV 8, Fara Fara, and Naga white, had high diastatic activities in the malts.

The water-extractable protein contents of the sorghum malts varied from 17·1 to 36·0% and water-extractables from 12·5 to 31·2% among the 26 cultivars studied (Table IV). The water-extractable protein and water-extractables content increased by 79·2 and 139·2% on malting. Thus, the extractability of protein in malted sorghum varied among the cultivars selected. This is of significant importance as sorghum is known to release less protein during malting[7]. In all the cultivars tested, the extractable-protein levels increased on malting at least two-fold compared with unmalted grain. It is noteworthy that in cultivars such as TAM 2566, Framida and IS 14384, the water-extractables were four-fold higher in malted grains than in unmalted grains. These cultivars also had high diastatic levels and water-extractable protein content in the malts. Sorghum malt has been shown to have a significant amount of alpha-amino nitrogen due to the malting of grain[14].

Analysis of variance (ANOVA) showed that the water-extractable protein and water-extractables content of malted grains were higher ($P<0·01$) than those of unmalted grains. Diastatic activity was correlated positively and significantly with water-extractable protein ($n=26$, $r=0·87$, $P<0·01$) and water-extractables ($r=0·87$, $P<0·01$) content of malted sorghum. These results confirmed our earlier observations using 10 sorghum cultivars. The results indicate that the determination of water-extractable protein and water-extractables content in malted sorghum may provide a rapid and easy method of indicating the diastatic activity.

The WEP content can be dertermined using conventional and simple microKjeldahl procedure or a automated procedure, such as the Technicon auto analyser used in this study. The methodology for WEP and WE involve only limited laboratory facilities and are simple and rapid to determine. They can be used to indicate the diastatic activity in sorghum malt, and may be useful for screening a large number of lines and germplasm accessions in a breeding program.

Acknowledgement

We thank K. A. R. Zafar for technical assistance and K. Vidyasagar Rao for suggestions on statistical analysis.

Submitted as J.A. 1578 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India.

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