Convertibility of IoB, EBC and SABS Methods for Sorghum DP Measurements Using Correlation Coefficients

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ABSTRACT

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The convertibility of the methods for Sorghum malt DP measurement using the correlation coefficient (r) was studied using malts of the white sorghum variety (farafara) from malting outlets, which had been steeped for one day and germinated for four days at 30°C. A correlation of 0.97 ($p < 0.00001$) was obtained between the IoB (Institute of Brewing) and EBC (European Brewing Convention) methods while the correlation dropped to 0.95 ($p < 0.001$) between the SABS (South African Bureau of Standards) and IoB and 0.94 ($p < 0.34$) between SABS and EBC methods. The study was extended to include eleven pure varieties (KSV, ICSV and ICSH entries), which had been steeped for one and a half days and germinated for six days at 25°C. A correlation of 0.968 ($p < 0.000004$) was obtained between the IoB and EBC methods of DP assay. The correlation dropped to 0.90 ($p < 0.0002$) between SABS and IoB and 0.91 ($p < 0.007$) between SABS and EBC assay methods. The IoB and EBC methods are considered suitable for sorghum DP measurements as a way of having the DP in one unit when sorghum and barley malts are used in one mash.

Key words: DP, °L, °WK, SDU, sorghum.

INTRODUCTION

Since 1988, lager beer of European continental style (bottom-fermented, pilsner-like) has been brewed in Nigeria from sorghum (and sometimes maize grits as adjunct) following the ban on malted barley[2]. The ban was necessitated by the need to save the rapidly dwindling foreign reserve of the country. Twelve years later, in the year 2000, the ban was lifted as the government bowed to pressures to liberalize trade. However some breweries still find that brewing with sorghum, using some external enzymes, remains less expensive while others have resorted to the use of barley malt substitution (at a certain percentage of the mash) to eliminate (or minimize) the use of external enzymes.

When sorghum and barley malts are used in one mash, there is a need to standardize their assay units. One such unit is Diastatic Power (DP) expressed as degrees Linter (°L) by the IoB, degrees Windisch-Kohlbach (°WK) by the EBC and Sorghum Diastatic Units (SDU) by the South African Bureau of Standards (SABS). It is widely argued that assay methods for barley malt (especially DP, α- and β-amylases) are not suitable for sorghum[7,14]. Similarly, some assays such as the modified p-hydroxybenzoic acid hydrazide (PAHBAH) method, where starch is reduced with borohydride, are reported to give satisfactory results with sorghum malts but fail to give reliable results with barley malts[4]. Sorghum Diastatic Unit (SDU) is the official unit for DP in South Africa (RSA), where sorghum has long been used in kaffir beer brewing, and there is a strong possibility for its adoption in Nigeria.

Barley malts imported into Nigeria have DP values in their certificate of analysis declared in °L or °WK. With sorghum and barley malts used in one mash, there is a need to express the results of DP in one unit for accurate projection of the total diastatic power of the mixed mash. This study therefore examines the suitability of these three methods for sorghum DP determination and the possibility of inter-converting their units using the correlation coefficient (r).

MATERIALS AND METHODS

Sorghum malts

The malt from the white sorghum variety (*farafara*) was obtained from twelve malting outlets and breweries in Nigeria. The malting procedure in all the maltings started with a first steep in 0.1N $Ca(OH)_2$ for 8 h after which grains were drained and washed with water. An air rest period of 4 h was allowed, followed by a second steeping in 0.5% formaldehyde for 8 h. Grains were drained and washed with water before germination at 30°C for four days. Kilning was carried out at 55°C for 24 h. Ten varieties (KSV 7 and 8, ICSV111, 210, 247, 400 and 401, ICSH 89002, 90003 and 89009) were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Kano, Nigeria, and malted in our laboratory. Malting started with a steep for 12 h in 0.1N $Ca(OH)_2$, followed by an air rest for 4 h and a second steep for 12 h in 0.5% formaldehyde. After a second air rest for 4 h, grains were steeped briefly for 2 h in water, drained and germinated at 25°C for 6 days. Kilning was conducted at 55°C for 24 h. These new varieties are yet to be produced commercially for maltsters to malt.

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Table I. Analysis of variance (ANOVA) from the farafara sorghum malt diastatic power (DP) in °WK, °L and SDU.

Sorghum samples	°WK	°L	SDU
1	33.4	16.9	21.9
2	42.2	21.9	34.5
3	57.6	24.0	37.7
4	41.9	20.8	36.6
5	37.9	16.4	29.8
6	42.2	20.8	31.1
7	41.0	17.8	28.1
8	24.5	12.6	20.2
9	23.4	12.4	20.2
10	25.7	13.1	21.5
11	23.4	11.4	16.3
12	24.5	11.7	17.3

ANOVA [°WK vs °L]

Source of variation	SS	df	MS	F	P-value	F crit
Between group	1870.9	1	1870.9	32.64581	0.00000954	4.300944
Within group	1260.799	22	57.30905			

ANOVA [SDU vs °WK]

Source of variation	SS	df	MS	F	P-value	F crit
Between group	389.6204	1	389.6204	5.083173	0.03445	4.300944
Within group	1686.279	22	76.64905			

ANOVA [SDU vs °L]

Source of variation	SS	df	MS	F	P-value	F crit
Between group	552.96	1	552.96	14.1669	0.001071	4.300944
Within group	858.7	22	39.03182			

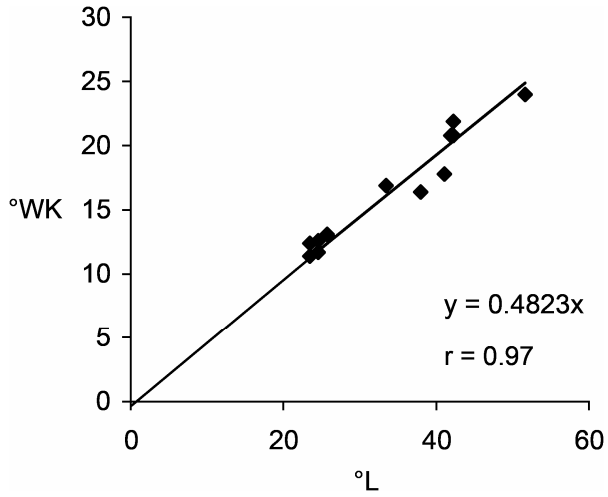

Fig. 1. Correlation between °WK and °L units in sorghum malt (farafara) DP measurement.

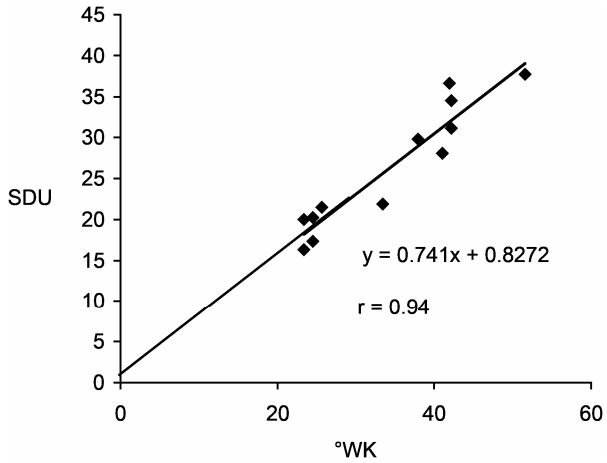

Fig. 2. Correlation between SDU and °WK units in sorghum malt (farafara) DP measurement.

Diastatic power determination

Analysis of diastatic power was carried out using the Institute of Brewing (ferricyanide)[10], European Brewery Convention[9] and South Africa Bureau of Standards[6] methods. Results are the means of three determinations with a standard deviation within ±2.0.

Statistical analysis

Coefficient of correlation analyses were carried out using the Microsoft Excel Analysis Toolpak.

RESULTS AND DISCUSSION

Diastatic power in brewing is the total activity resulting from the simultaneous action of α- and β-amylases, R-enzyme and limit dextrinase on malt starch during mashing, which results in the generation of fermentable extract. In barley malt, about 80% of this activity comes from β-amylase, while in sorghum the reverse is the case, with diastatic activity produced principally (50–80%) by the α-amylase[7,13].

Table II. Analysis of variance (ANOVA) of the diastatic power (DP) of some sorghum varieties in °WK, °L and SDU.

Sorghum samples	°WK	°L	SDU
KSV 7	132	67	87
KSV 8	96	50	78
ICSV 111	91	43	60
ICSV 210	78	39	68
ICSV 247	80	35	63
ICSV 400	94	46	69
ICSV 401	76	35	52
ICSH 89002	75	33	51
ICSH 90003	62	32	50
ICSH 89009	56	29	47
Farafara	85	37	65

ANOVA [°WK vs °L]

Source of variation	SS	df	MS	F	P-value	F crit
Between group	10429.14	1	10429.14	39.90001	0.00000364	4.35125
Within group	5227.636	20	261.3818			

ANOVA [SDU vs °WK]

Source of variation	SS	df	MS	F	P-value	F crit
Between group	2510.227	1	2510.227	8.921936	0.007287	4.35125
Within group	5627.091	20	281.3545			

ANOVA [SDU vs °L]

Source of variation	SS	df	MS	F	P-value	F crit
Between group	2706.182	1	2706.182	19.83343	0.000244	4.35125
Within group	2728.909	20	136.4455			

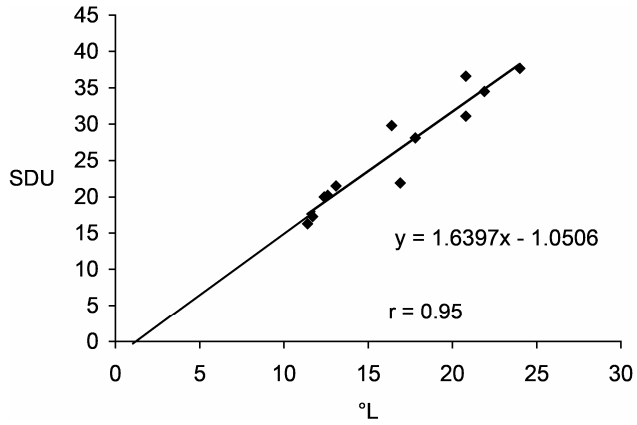

Fig. 3. Correlation between SDU and °L units in sorghum malt (farafara) DP measurement.

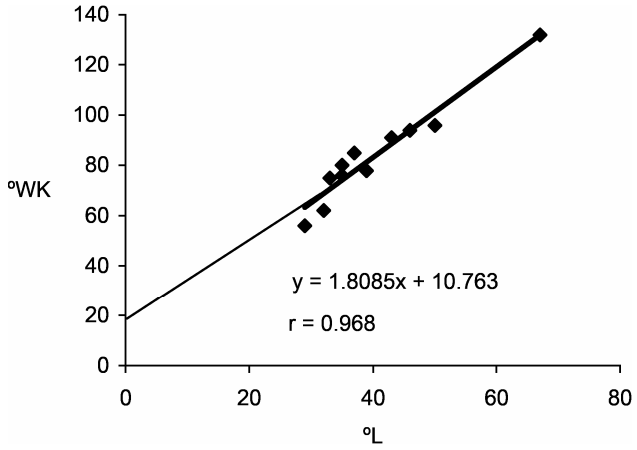

Fig. 4. Correlation between °WK and °L units in the malt DP measurement of 11 sorghum varieties.

Advocates of different methods of analysis for sorghum malt DP have based their arguments on the bound form of sorghum β-amylase[13] in the grain and the indirect assay methods employed. While in barley, β-amylase is produced by *de novo* gibberellic acid (GA) induced synthesis in the aleurone layer[11,15], sorghum β-amylase develops by transformation from a bound form to a free or active form[3,13] during germination. This explains the use of peptone solutions to liberate the bound β-amylase, which consequently has resulted in a higher DP of the sorghum malts in coloured and bird-proof varieties[13,14]. The indirect assay method for estimating sorghum β-amylase from the difference between total amylase (DP) and α-amylase, after β-amylase had been inhibited (by heat[13,14] or mercuric chloride[8]) has been criticized for use with sorghum, where β-amylase is particularly low. Direct assay of sorghum β-amylase using the specific betamyl substrate has equally resulted in low values for this enzyme in sorghum. While Taylor and Robbins[16], using the betamyl substrate, reported values of 16.6–57.2 μ/g for some South African low tannin varieties, Agu and Palmer[1] obtained values as high as 109.3 μ/g for KSV13 from Nigeria. These values however are low compared to barley[1] (chariot) with β-amylase activity of 479 μ/g using same substrate. The use

Table III. Comparison of procedures for the measurement of diastatic power (DP) in malts.

Procedure	EBC [°WK]	IoB [°L]	SDU
Wt. of ground malt/mL	(20 g/520 g)	10 g/200 mL	25 g/500 mL
	38.5 mg/mL	50 mg/mL	50 mg/mL
Medium of extraction	Dist. water	Dist. water + 0.1N NH_3	Dist. water or/peptone
Temp of extraction (°C)	40	20	30
Vol of filtrate/supernatant for diastasis (mL)	5	3	2, 5, 10 (depending on activity)
Wt. of malt in filtrate for diastasis (g)	0.2	0.15	0.1, 0.25, 0.5
Starch	Merck 1252	ASBC	Merck 1252
Vol of starch (mL)	100	100	200
Amt of malt in mg filtrate/mL starch	0.2	0.15	0.125
Temp of diastasis (°C)	20	20	30
Duration of diastasis (min)	30	60	30
Determination of sugars	Ferricyanide	Ferricyanide	Ferricyanide
Expression of results	Maltose/100 g ext	mL of extract	KDU/g
Calculation formula	34.2 (B – A)	23 [(B – A)/V](200/250)	40 (B – A)(F/V)

V = mL of malt extract used for diastasis
F = Factor of 0.05 sodium thiosulphate used
100/(100 – M) = To correct for moisture in all three methods

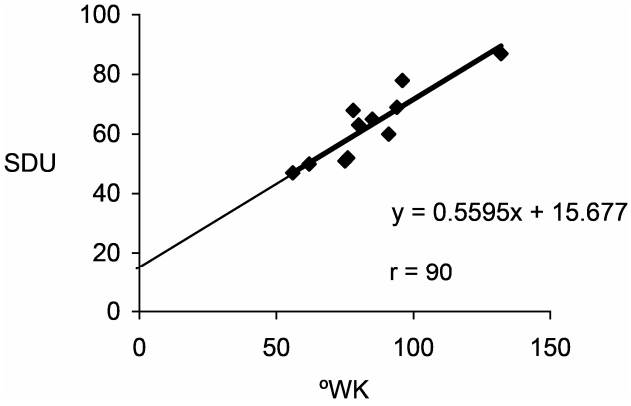

Fig. 5. Correlation between SDU and °WK units in the malt DP measurement of 11 sorghum varieties.

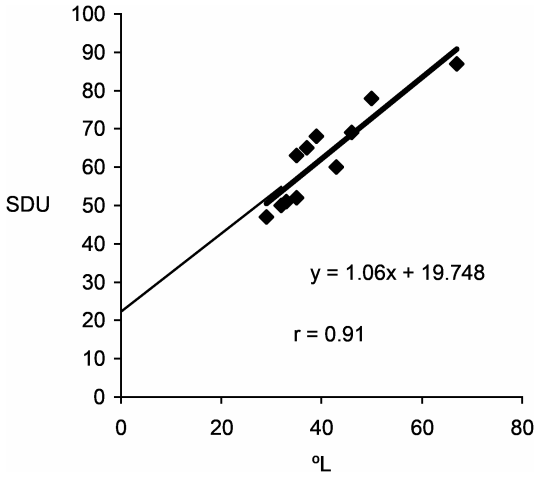

Fig. 6. Correlation between SDU and °L units in the malt DP measurement of 11 sorghum varieties.

of the betamyl substrate only serves to confirm earlier findings, that this enzyme is low in the sorghum malts[8,12,14]. As β-amylase is low in the sorghum malts, and its measurements attracts a lot of controversy, the diastatic power measurement remains critical and of utmost importance. There is a compelling reason for adopting units of measurement that are universally accepted or convertible.

The results in Table I and Fig. 1 show a good correlation ($r = 0.97$, $p < 0.00001$) of sorghum DP measurement using the IoB and EBC methods of assay. A good, but lower, correlation ($r = 0.94$, $p < 0.034$) was obtained in Table I and Fig. 2 when the sorghum diastatic units (SDU) were compared with the Windisch-Kohlbach (°WK) units and the degrees Linter ($r = 0.95$, $p < 0.001$) in Fig. 3. As sorghum malt characteristics are usually variety dependent, ten new sorghum varieties were incorporated into this study. Results in Table II and Fig. 4 show a similar correlation ($r = 0.968$, $p < 0.000004$) between the IoB (°L) and EBC (°WK) methods of assay. Lower correlation was still the case when, as shown in Table II and Fig. 5, sorghum diastatic units (SDU) were compared with the EBC ($r = 91$, $p < 0.007$) and IoB units ($r = 0.90$, $p < 0.0002$) in Fig. 6.

The lower correlation with the degrees Windisch-Kohlbach (°WK) by the EBC and sorghum diastatic units (SDU) by the SABS method prompted a comparison of the analysis procedures of the three methods. This is shown in Table III. The distinguishing features of the SABS method are (i) addition of peptone to extraction medium (not necessary in white low tannin varieties), (ii) extraction at 30°C (40°C for EBC, 20°C for IoB), (iii) double volume of starch for diastasis (200 mL) and (iv) a temperature of 30°C for diastasis (20°C for EBC and IoB). While the features of the SABS method are designed to ensure maximum quantification of the low diastatic enzymes of sorghum, the lower correlation may likely come from the constant in the SABS method formula for DP. These differences not withstanding, the convertibility of SDU to °WK and °L, is highly desirable and can be done using the correlation equations in Figs. 2 or 5 ($y = 0.741x + 0.8272$ or $y = 0.559x + 15.677$) and Figs. 3 or 6 ($y = 1.6397x - 1.0506$ or $y = 1.06x + 19.748$) respectively.

As stated earlier, this study arose from the need to express the diastatic measurements of sorghum and barley malts analyses in one unit, for a good projection of the

total activity in the mash when both are used together. Though the EBC diastatic power method has been criticized for underestimation, and the P-hydroxybenzoic acid hydrazine (PAHBAH) assay reported[4,5] as a better alternative, more evidence is still needed. Meanwhile the IoB and EBC units still remain the best alternatives with greater international acceptability.

The °WK unit would be more preferable for studies with sorghum malt since the values here are higher. In sorghum, the ratio between the two amylases (α and β) is a constant and fundamental feature and is unaffected by moisture and temperature of germination[7,14]. Taylor and Robbins[16] reported a correlation of 0.9855 between sorghum β-amylase and DP when the Delcour and Verchaeve PAHBAH method was used. This correlation dropped to 0.67 with the β-amylase specific betamyl substrate. With this correlation, Taylor and Robbins[16] concluded that in a breeding programme, initial selection of sorghums for high β-amylase could simply be conducted on the basis of DP. The high correlation with the PAHBAH method was achieved after heat/Ca^{2+} inactivation of the β-component – a method strongly criticized for inactivating some of the α-component.

CONCLUSIONS

The conclusion therefore is that sorghum DP should be expressed in °WK and °L. These units enjoy greater international acceptability and allow for good projection of total DP when sorghum and barley malt are used in one mash. These units (preferably °WK) are also best for research in sorghum breeding programmes and should be taken to be principally α-amylase activity. Sorghum β-amylase, where the need arises, should be specifically measured with the betamyl substrate and compared for now with a good barley variety. Samples with DP given in SDU can also be converted to °WK and °L as a good correlation exists.

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