FIBRE DETERMINATION BY HYDRAULIC PRESSING—WHICH METHOD IS CORRECT?

By

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KEYWORDS: Quality Components, Routine Analyses, Analytical Accuracy, Error Ratio Tests, Sub-sampling Methodology.

Abstract

ROUTINE ANALYSES of quality components of sugarcane for calibration development of near infra-red spectroscopic applications are enhanced if hydraulic press extraction of juice is used. This results in high-level extraction comparable to that achieved by the first mill in a commercial milling train. A bonus is that fibre also can be estimated with this process. Scepticism has been expressed within the BSES/CSIRO JV breeding team as to the correctness of the method used to determine fibre for the SpectraCane™ calibration. This paper reports on research that establishes the relationship between two methods of determining fibre from hydraulic press data (Berding and Pollock, 1982 (F_B&P), Tanimoto, 1964 (F_T)) and fibre determined by a standard method (F_B). Harvest samples were taken from a replicated final assessment trial containing 2 × 72 entries. Two sub-samples from a sample from each entry were processed to yield data for Brix, expressed juice weight, two fibre determinations (F_B&P and F_T), and polarimeter reading, all from hydraulic pressing, F_B, and moisture content. Total analytical results were accurate with an average mass balance of 1000.9 g/kg. Use of two sub-samples per sample was satisfactory for all traits, with F_B and expressed juice having the lowest error ratios of 6.8 and 6.2, respectively. Mean values for F_B (138.1 g/kg) and F_B&P (137.8 g/kg) were similar while that for F_T was lower (127.2 g/kg). All fibre determinations were highly correlated, particularly F_B&P and F_T but their regressions against F_B produced significantly different regression coefficients ($t_{140} = 3.129^{**}$). Both regressions were skewed, with the latter being more so. The computation of F_B&P adjusts the dry plug weight for residual Brix, taking into account hygroscopic water. The F_T computation does not, which results in underestimation of fibre content. The F_T estimates are consistently lower than F_B&P and F_B values, and so the F_T methodology must be considered flawed.

Introduction

There are two essential requirements in developing applications for near infra-red spectroscopic analyses. Firstly, the variation encompassed in the calibrations should reflect the real-world variation to which the applications will be exposed.
Secondly, the routine laboratory analyses on which the calibrations are based must be accurate. These principles were followed in both near infra-red applications developed at BSES Meringa, the large-cassette module (Berding and Brotherton, 1996) and the high-speed SpectraCane™ analytical system (Berding et al., 2004). In addition to the developments being embedded in a reasonably-sized crop improvement program, and thus incorporating substantial genetic variation in the calibrations, the calibrations also incorporated temporal, geographic, and climatic variation.

Importantly, all juice analyses were based on a high juice-extraction regime, fresh cane being pressed in 70-tonne hydraulic presses. Juice extraction is comparable to that achieved in the first mill in commercial milling trains. An additional advantage of hydraulic pressing is that a fibre determination can be obtained from the pressing process with relatively small additional cost. In both calibration scenarios (Berding and Brotherton, 1996; Berding et al., 2004) within-sample heterogeneity was minimised by imposition of a mixing stage between sample disintegration and scanning. Application of routine analyses for soluble and insoluble carbohydrates to one sub-sample minimises accumulation of possible sub-sampling errors if heterogeneity exists. This is an advantage offered by hydraulic pressing.

Mill technologists at BSES Limited were sceptical of the hydraulic press methodology published by Tanimoto (1964) because the method was theoretically flawed (G.A. Brotherton, pers. comm., 2009). In the late 1970s, development of a rapid screening method for fibre content for crop improvement applications was a priority at BSES Meringa. The location of a 70-tonne hydraulic press at Mulgrave Mill during the 1977 crushing season afforded the opportunity to evaluate hydraulic pressing as a potential method, but one based on theoretically correct principles (Berding and Pollock, 1982).

All BSES juice laboratories possessed SpectraCane™ high-speed analytical systems (Berding et al., 2004) for quality component analyses by the start of the 2009 season. Subsequently, scepticism was expressed within the BSES/CSIRO JV breeding team about the correctness of the Berding and Pollock (1982) methodology used in the development of the calibrations supplied with the SpectraCane™. Use of the Tanimoto (1964) method at one centre produced results at variance with those from the Berding and Pollock (1982) method, as would be expected. This paper addresses this scepticism by examining the theory behind each method and applying the resulting equations to data from a replicated trial, harvested during the 2009 season, to evaluate the validity of each method relative to a modified, standard bag technique for fibre determination (Skinner, 1969).

Materials and methods

Sampling and analytical methods

Six-stalk samples were drawn from plots of the plant crop of Final Assessment Trial SJO 08-34 located on the farm of Graham Whitaker, Spanos Road, Silkwood. The trial contained 72 plots in two replicates. Samples were disintegrated
through a Dedini laboratory disintegrator (Dedini S/A Indústrias de Base, Piracicaba, Brazil) and analysed spectrally through the SpectraCane™. Samples were contained in 40-L, lidded pails. Sample exposure was minimised through the sub-sampling process and all sub-samples were taken from beneath the surface of the material in the pail. Three additional analyses were done:

1. Two sub-samples of disintegrated cane, each of known weight, \( M_c (g) \), of about 500 g, were taken from each plot sample (average \( \approx 11 \) kg) and hydraulically pressed in a Model OB700 70-tonne press (Pinette Emicedau Industries, Chalon sur Saone, France) for 60 s. Weights of the resultant plug, \( M_{wp} (g) \), and expressed juice, \( M_{ej} (g) \), from each sub-sample were recorded. The latter were unified to a common scale of g/kg by applying the computation \( 1000 * M_{ej} / M_c \) on a sub-sample basis. The plugs were placed in #7223 aluminium food trays (Confoil Pty Ltd, Bayswater, Victoria) for drying. After drying at 70°C for 7 d, the weights of the dry plugs, \( M_{dp} (g) \), were recorded. Each expressed juice sample was subjected to routine analyses for refractometer-determined soluble solids (Brix - \( B_{ej} \)) and polarimeter-determined optical rotation (°Z).

2. Two sub-samples of disintegrated cane, each of known weight of about 150 g, were placed in #7119 aluminium food trays (Confoil Pty Ltd, Bayswater, Victoria) for processing. Sub-samples were dried at 70°C for 7 d and the weight of the dried cane recorded for independent determination of moisture content (\( M_m \)).

Two sub-samples of disintegrated cane, each of known weight of about 100 g were taken and placed in standard fibre bags. The fibre sub-samples were spin washed with cold water for 5 min and spin dried for 3 min in a laundry centrifuge, and then washed in hot water using the heavy duty cycle of an automatic, top-loading washing machine.

The fibre sub-samples, submerged under grid mesh, were boiled vigorously in clean water for 15 min, and again spin washed and dried, for 5 and 3 min, respectively. The fibre sub-samples were dried at 70°C for seven days and the dry weights recorded for determination of fibre content, \( F_B \).

For all three determinations, sub-samples were held at -18°C between the time they were taken and until drying could proceed.

**Data calculations**

The following data were available for each hydraulically pressed sub-sample:

\[
M_c = \text{mass of disintegrated fresh cane sampled (g)}.
\]
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$M_{dp} = \text{mass of the dried plug (g)}.$
$M_{wp} = \text{mass of the plug (wet) removed from the hydraulic press (g)}.$

Berding and Pollock (1982) showed algebraic manipulation of the mass balance equations

$$B_p = B_{ej} \times (W_p - W_f)/(100 - B_{ej}),$$
$$F_p = 100 - B_p - W_p,$$ and
$$F_c = M_{wp} \times F_p / M_c,$$ where

$B_{ej} = \text{Brix (°) of the expressed juice.}$
$B_p = \text{Brix of the plug.}$
$F_c = \text{fibre content (%) of the cane.}$
$F_p = \text{fibre content (%) of the plug.}$
$W_f = \text{hygroscopic moisture content (%) of plug,}$
$W_p = \text{moisture content (%) of the plug}$

and making the assumption that $W_f = F_p / 4$, yielded the formula:

$$F_c = 80 \times (100 \times M_{dp} - B_{ej} \times M_{wp})/[M_c \times (80 - B_{ej})].$$

This estimate of fibre content (F_c) will be referred to as F_{B&P}.

Tanimoto (1964) approached the determination of fibre content in cane, using hydraulic press data, via a different set of equations.

$$F_c = M_f \times 100 / M_c,$$
$$M_f = M_c - M_j,$$
$$M_j = M_{ej} + M_{rj},$$
$$M_{rj} = W_{wp} \times 100 / W_j,$$ and
$$M_w = M_{wp} - M_{dp},$$ where

$M_{ej} = \text{mass of expressed juice from } M_c \times (g).$
$M_f = \text{mass of fibre in } M_c \times (g).$
$M_j = \text{mass of total juice in } M_c \times (g).$
$M_{rj} = \text{mass of residual juice in } M_{wp} \times (g).$
$M_w = \text{mass of water in } M_{wp} \times (g).$
$W_j = \text{moisture in juice (%)}. $
Substitution and algebraic manipulation of the above five equations from Tanimoto (1964) yields the following formula expressed in terms of the data collected:

\[ F_T = 100 \times \left[ (100 \times M_{dp} - B_{ej} \times M_{wp}) / \left[ M_c \times (100 - B_{ej}) \right] \right]. \]

This estimate of fibre content \((F_T)\) will be referred to as \(F_T\).

The overall accuracy of the laboratory analyses of whole cane was assessed by mass balance summation for each sub-sample. These data were subjected to a sub-sampling analysis of variance. Mass balance \((MB)\) was determined as follows:

\[ MB = B_c + F_{B&P} + M_m, \]

where

\[ B_c = \text{Brix in cane, and} \]

\[ B_c = B_{ej} \times (970 - F_{B&P}) / 1000, \]

The components \(B_c\), \(B_{ej}\), \(F_{B&P}\), and \(M_m\) were all expressed in g/kg. As the latter three were determined independently, the expectation was that \(MB = 1,000\) g/kg if the individual analyses were accurate.

**Statistical analyses**

Where necessary, data were subjected to routine sub-sampling analyses of variance. An error test \((s\sigma_e^2 / \sigma_s^2)\) was calculated. Phenotypic \((r_P)\), genotypic \((r_G)\), and environmental \((r_E)\) correlations were computed using unadjusted cross sums of products from analyses of covariance. Homogeneity of regression coefficients for \(F_{B&P}\) vs \(F_B\) and \(F_T\) vs \(F_B\), was tested using the procedure outlined in Steel and Torrie (1960, p. 173). Hypotheses as to whether the regression coefficients differed from 1.0, and whether the regression lines passed through zero were tested using procedures from Steel and Torrie (1960, pp. 169–170 and 179–180, respectively).

**Results and discussion**

One would expect that the sum of weights of the two components exiting the hydraulic press, the wet plug \((M_{wp})\) and the expressed juice \((M_{ej})\), should approximate the weight of the fresh cane \((M_c)\). Values for the check sum \(M_c - (M_{wp} + M_{ej})\) ranged from -1.14 to 19.2 g for a mean \(M_c\) value of 506.1 g.

There are two sources of error in this method that are very difficult to manage. The complete retrieval of all fibre in the wet plug from the pressure cage is problematic, but any error from this can be sensed more readily than the non-retrieval of juice from the pressure cage, piston, and collecting stage of the press. The latter was considered the major source of error, despite drainage time being allowed, and so the best estimate that can be used for expressed juice weight was taken as:

\[ M_{ej} = M_c - M_{wp}. \]
Analyses of variance revealed there were highly significant clonal effects for all seven harvest traits and mass balance (Table 1). In all analyses except that for mass balance, the test of plot error against that for sub-sampling error also was highly significant. For all of these, the error ratio test exceeded the rule-of-thumb value of 3.0, indicating that the sub-sampling strategy of taking two sub-samples per six-stalk plot sample for analysis was perfectly acceptable. However, there was considerable variation for this ratio, ranging from 6.2 for juice content, to 138.9 for Brix in juice (Table 1). Even for the traits with the lowest error ratio, $F_B$ (6.76) and juice content (6.20), the ratios are comfortably above the threshold of 3.0.

Table 1—Summary analyses of variance and key genetic statistics for seven key harvest traits, including three fibre determination methods and mass balance, a statement of analytical accuracy.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Trait</th>
<th>Brix in cane (g/kg)</th>
<th>Brix in juice (g/kg)</th>
<th>Fibre – bag (g/kg)</th>
<th>Fibre – B &amp; P (g/kg)</th>
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<tr>
<td>Reps</td>
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<td>142.48</td>
<td>183.68</td>
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<td>204.98**</td>
<td>321.77**</td>
<td>591.46**</td>
<td>565.16**</td>
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<td>54.28**</td>
<td>81.85**</td>
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<td>137.8</td>
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<td>112.8</td>
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<td>$g^2$</td>
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<td>0.594</td>
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<th>d.f.</th>
<th>Trait</th>
<th>Fibre – Tanimoto (g/kg)</th>
<th>Juice content (g/kg)</th>
<th>Moisture (g/kg)</th>
<th>Mass balance (g/kg)</th>
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<td>4.9</td>
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<td></td>
<td>8.1</td>
<td>24.4</td>
<td>13.3</td>
<td>2.34</td>
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<tr>
<td>$g^2$</td>
<td></td>
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<td>0.865</td>
<td>0.771</td>
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<td>31.30</td>
<td>6.20</td>
<td>73.01</td>
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</table>

1 Calculated from hydraulic press data using Berding and Pollock (1982)
2 $Lsd_{(0.05)} = $ least significance difference, $P \leq 0.05$; $g^2 = $ degree of genetic determination; GCV% = Genetic coefficient of variation; and error ratio test $= \frac{2\sigma_e^2}{\sigma_s^2}$.
3 Calculated from hydraulic press data using Tanimoto (1964).
One can speculate why this variation in error ratio occurred. The disintegrated sample, averaging around 11 kg, was passed through the SpectraCane™ twice and this was assumed to provide sufficient mixing. This was in contrast to the earlier development of near infra-red calibrations for the large cassette module (Berding and Brotherton, 1996) and the HSSA (Berding et al., 2004) where each sample was prepared in a rotary drum mixer for 90 s, which minimised the heterogeneity in distribution of tracer pellets through the sample. The lower error ratio for the determination of FB, based on a sub-sample of about 100 g, suggested that there was still residual heterogeneity in the sample. The moisture determination, based on a sub-sample of about 150 g, produced an error ratio of 73. All other determinations came from hydraulically pressed sub-samples of about 500 g, so a range in error ratio from 6.2 for juice content to 138.9 for Brix in juice is difficult to reconcile with a hypothesis that residual heterogeneity in the sample, because of inadequate mixing, was being countered with a larger sub-sample size. There is no reasonable explanation why data for expressed juice weight, even when derived indirectly as the difference between the weight of fresh cane pressed and the weight of the wet plug, shows a markedly reduced error ratio.

There was ample significant variation for all traits, e.g., indicated by Range/lsd0.05. The mean Brix was high (234.7 g/kg). Means for FB, 138.1, and FB&P, 137.8 g/kg, were very similar, but both were higher than the mean for FT, 127.2 g/kg. The range of fibre values evident for FB&P, 115 – 162 g/kg, is typical for the BSES Meringa core program. Values ≤10.0 and ≥18.0 are rarely seen. Broad sense heritabilities (g²) varied considerably, with those for the fibre determinations being highest (0.771 – 0.865), and that for Brix in cane the lowest of the traits (0.581; Table 1). Genetic coefficients of variation ranged from 1.78% for moisture to 8.3% for FB&P, although the values for the three fibre measures were comparable (Table 1).

The mass balance mean of 1000.9 g/kg (Table 1) showed there was, on average, an error of only 0.09%, indicating that in total the analyses summed over the independent determinations of Brix in cane, FB&P, and moisture were accurate. Precision was high, with a sampling standard deviation (σs), extracted from Table 1, of 1.63 g/kg. The standard deviation for the raw data (n = 288) was 1.86 g/kg. Interestingly, there was a highly significant genetic effect for this trait indicating that a consistent error was occurring in some of the component analyses for some of the clones involved. Estimates of the broad sense heritability and genetic coefficient of variation were low and small, respectively. Data for the cellulose-water isotherm reveals that at 70°C and 10% R.H., hygroscopic water should approximate 2% of the fibre mass (da Motta Lima et al., 2004). The drying method used in this research would have removed the bulk of the hygroscopic water from the fibre, and so application of mass balance summation is appropriate.

Phenotypic, genetic, and environmental correlations between FB&P and FT were extremely high (Table 2). Those between FB and FB&P and between FB and FT also were extremely high, with the rG values being very close to that between the two
press fibre determinations while the $r_P$ and $r_E$ values were marginally lower. All fibre measures were strongly negatively correlated with moisture content, with the $r_G$ values (-0.874, -0.852, and -0.828 for FB, F_B&P, and FT, respectively) being higher than the $r_P$ values (-0.840, -0.822, and -0.795) which, in turn, were higher than the $r_E$ values (-0.610, -0.615, and -0.547; Table 2). All correlations between the three fibre measures and juice content were moderate, ranging from -0.460 to -0.564 for all three correlations. Juice content does not offer a viable correlated screening method for fibre content.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Correlation</th>
<th>F_B&amp;P</th>
<th>FT</th>
<th>Juice weight</th>
<th>Moisture</th>
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<td>0.983</td>
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<td>-0.840</td>
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<td>0.995</td>
<td>-0.564</td>
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<tr>
<td></td>
<td>$r_E$</td>
<td>0.900</td>
<td>0.891</td>
<td>-0.460</td>
<td>-0.610</td>
</tr>
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<td>$r_P$</td>
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<td>0.999</td>
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<td>$r_E$</td>
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</table>

1 Calculated from hydraulic press data using Berding and Pollock (1982).
2 Calculated from hydraulic press data using Tanimoto (1964).

The regression of data, resulting from use of each of the hydraulic press methods against FB, yielded different results (Figure 1). The regression equation for F_B&P vs FB,

$$\hat{Y} = 4.799 + 0.963 \times X,$$

differs considerably from that calculated for FT vs FB, which was:

$$\hat{Y} = 6.278 + 0.876 \times X.$$

The two methods generated populations with different means (137.8 vs 127.2 g/kg; Table 1). Regression analyses showed that both populations had a skewed relationship with FB. The regression coefficients for F_B&P vs FB, $b_1 = 0.963$, and FT vs FB, $b_1 = 0.876$, differed highly significantly ($t_{140} = 3.129, P \leq 0.01$). The first regression coefficient did not differ significantly from 1.0 ($t_{70} = -1.881; P = 0.064$) whereas the second regression coefficient did ($t_{70} = -6.254; P = 0.000$). A test of the hypothesis as to whether the regressions passed through zero revealed that the first regression did ($F_{70} = 3.10; P = 0.083$) while the second did not ($F_{70} = 5.22; P = 0.025$).
The equations resulting from the Berding and Pollock (1982) and Tanimoto (1964) derivations are obviously different, but attempts to algebraically resolve the difference in terms of parameters used have failed. Berding and Pollock (1982) showed bag fibre and press-determined fibre, using a population of 47 clones in two replicates, were highly correlated \( r = 1.0 \), means were close (12.94 and 13.12, respectively), and ranges near identical (9.7 – 16.3 and 9.7 – 16.4).

Tanimoto (1964) presented little comparative data for his press-determined fibres vs the standard-method fibres, determined by pol-ratio analysis (Payne and Mahon, 1956). The only data presented were samples from a nitrogen fertilisation trial \( n = 12 \) and a repeatability sub-sampling exercise of 10 sub-samples from two cultivars \( n = 20 \). No statistical evaluation accompanied these data. Regression analysis of these data showed the relationship was strongly skewed \( b_1 = 0.636 \), and while the relationship between the two measures of fibre was still strong \( r = 0.898 \), this was weaker than those obtained for the comparisons presented here (Table 2). Neither Payne and Mahon (1956) nor Tanimoto (1964) presented data that allows judgment of the accuracy of the pol-ratio fibre determination. Payne and Mahon (1956) presented duplicate fibre data for 900 g sub-samples taken from 30 x 182 kg plot samples. The mean, range, and sampling standard deviation for these data are contrasted with the three determinations reported in this paper (Table 3). The mean and range are lower, and although the sample and sub-sample sizes and methodology
differ radically from those used here, the sub-sampling standard deviation for the pol-ratio fibre was bettered by all except that for F_B (Table 3). This suggests that the poor relationship between hydraulic press and pol-ratio determined fibre could, in part, be due to poor precision of the latter. This is supported by Foster’s (1963) observation that fibre data, obtained from development of an analytical procedure using wet disintegration, a method similar to the pol-ratio method, lacked precision.

Table 3—Contrast of mean fibre values (g/kg), ranges, and sampling standard deviation for fibre determination by pol-ratio method (Payne and Mahon, 1956) and three determinations reported here.

<table>
<thead>
<tr>
<th>Determination</th>
<th>Mean</th>
<th>Range</th>
<th>Sampling standard deviation ($\sigma_s$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre – pol-ratio</td>
<td>115.17</td>
<td>100.1 – 127.2</td>
<td>2.384</td>
</tr>
<tr>
<td>F_B$^1$</td>
<td>138.06</td>
<td>112.8 – 163.6</td>
<td>2.998</td>
</tr>
<tr>
<td>F_B&amp;P$^2$</td>
<td>137.77</td>
<td>114.6 – 162.4</td>
<td>1.105</td>
</tr>
<tr>
<td>F_T$^3$</td>
<td>127.19</td>
<td>106.3 – 150.4</td>
<td>1.025</td>
</tr>
</tbody>
</table>

1  Modified standard bag fibre.
2  Calculated from hydraulic press data using Berding and Pollock (1982).
3  Calculated from hydraulic press data using Tanimoto (1964).

Berding and Pollock’s (1982) equation used to calculate F_B&T from hydraulic press data corrects the plug weight for residual soluble solids. Brix of the residual and expressed juices was assumed to be identical. The correction, however, recognises that soluble solids are present only in non-hygroscopic moisture. Foster (1956) determined that ‘By extrapolation it appears that cane fibre at 20°C and 100% R.H. would contain about 25% hygroscopic water based on the dry weight of fibre’. Earlier work determined that the amount of water adsorbed by cane fibre (from solutions of sucrose, electrolytes, or non-electrolytes) is driven by the solutions’ water content (Kelly and Rutherford, 1957).

Interestingly, they noted genetic variation for hygroscopic water content among cultivars. In further work, Foster (1962) obtained estimates of between 10 and 18% for hygroscopic water content of fibre, but discounted these because of the extreme pressures used and sucrose adsorption on fibre was ignored. He regarded these as comparable with his earlier estimate of 25% (Foster, 1956), the value currently used in cane analysis and milling calculations. Foster (1963) found hygroscopic water varied from 25 to 40% of fibre weight. More sophisticated analytical techniques were used in subsequent work. For a data set of >250 observations, Mangion and Player (1991) reported 1) a mean of 20.6 ± 2.2% for estimated hygroscopic water; 2) cultivars differed for hygroscopic water content; and 3) a cultivar’s hygroscopic water was largely independent of the environment in which it was grown. Qin and White (1991) reconfirmed that hygroscopic water content 1) decreased with increasing juice concentration; 2), was relatively unaffected by temperature; and 3) varied among tissues tested, rind, fibre, and pith. Their data
suggested that at low Brix values, ≈ 1°, hygroscopic water varied from 19 – 29%. Use of 25% of fibre content as a value for hygroscopic water is reasonable given the variation evident in available estimates and the genetic variation revealed for this trait.

The question of hygroscopic water in the Tanimoto (1964) method, and its implication in the correction of residual Brix in the pressed plug, is as relevant as for the Berding and Pollock (1982) method. However, such a correction is not evident in the computations. Tanimoto’s method assumes all water in the plug contains solubles.

The mass of juice extracted and the mass of juice retained in the plug are added. This over-estimates the Brix retained in the plug which results in under-estimation of fibre. The results presented here are consistent with this.

The method of determining fibre content from hydraulic-press data using the Berding and Pollock (1982) method is theoretically rigorous, produces accurate results, and is precise. Its use for providing data for near infra-red spectroscopic applications is justified.

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REFERENCES


