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Determination of Lignin in Feedstuffs from Methoxyl Content in Acid-Lignin Versus, the Calorimetric Method

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THE CALORIFIC value of lignin and separate pure carbohydrates in a crude fibre residue was determined as well as in the original plant residue to check calorimetrically the reliability of the principles applied for lignin determination from methoxyl content (MeO) in acid lignin as described by Moon and Abou-Raya (1966).

Crude fibre residue was prepared from wheat straw and its calorific value was 432 Kcal/100 g of OM when calculated from analysed nutrients (using 6.26, 4.176 and an average of 4.2 Kcal/g as calorific value figures for lignin, cellulose and mixed carbohydrates, respectively). The caloric balance was the same as obtained from direct bomb calorimeter determination which was 432.8. This was also proved with a crude fibre residue from feces of clover hay after being freed from fatty and resinous materials.

The concurrence of the calorimetric method with the chemical method appeared to confirm the lignin determination procedure of Moon and Abou-Raya (1966).

Calorimetric method was suggested for lignin determination in similar residues containing lignin and mixed polysaccharides .Lignin percentage of the residue (Y) could be obtained from a knowledge of the determined calorific value of 100 g OM of the residue (X) by using the following equation :

$$Y = 0.4831\ x\ -\ 202.86$$

Most of the nutritional work on lignin has been handicapped by the lack of reliable method for its determination. The method of Ellis *et al.* (1946) still widely used in nutrition investigations. Critical studies of the quantitative methods of lignin determination using strong acids (mainly 72% w/w H_2SO_4) were undertaken by Abou-Raya (1951), Moon and Abou-Raya (1952a, b and c).

Moon and Abou-Raya (1954) prepared "pure lignin" by ethylacetoacetate to be used as a reference lignin practically free from N. Using an average conversion factor of 5.5 known from the average methoxyl in reference lignin for several feeds (18.2%), it was possible to present a more accurate method for determining lignin based on methoxyl content in acid lignin multiplied by 5.5.

Bergner and Schramm (1968) used samples finely ground and pretreated by refluxing with water, dilute HCl and ETOH — C_6H_6 (1:2). Lignin content was calculated from the heat of combustion of the dried residue.

Material and Methods

The ordinary methods of the A.O.A.C. (1966) were used for the conventional nutritive analysis, lignin determination (PMe lignin × specific conversion factor) was as recommended by Moon and Abou-Raya (1966) and slightly modified by Abou-Raya and Galal (1966).

The procedure of Moon and Abou-Raya (1954) was used to prepare reference lignin (by ethylaceto-acetate from mature plants) in order to calculate the specific conversion factor for common feedstuffs in A.R.E.

"Alkali lignin" was prepared from wheat straw using the procedure of Bondi and Mayer (1948) as slightly modified by Abou-Raya (1951) for calrofic value determinations. When the method of Dorée was used by Ghoneim *et al.* (1966) was used to isolate cellulose (based on delignification by chlorine evaluated from calcium hypochlorite acidified with H_2SO_4 20%), the prepared material contained very high ash as Ca SO_4 up to 37.87%. When replacing dilute HCl acid instead of H_2SO_4 in the chlorinating reagents, the ash content in the cellulose was reduced to 5.93%.

The calorific value determined after such modification was 4.176 Kcal/g. Organic matter was practically the same as recorded figures. Pentosans were determined in crude fibre residue using the method of Launer and Wilson (1939), as indicated by Galal (1969).

The calorific value was determined by an diabetic bomb calorimeter following the British Standard procedure, with crude fibre, cellulose and lignin. The calorific value per g pentosan was calculated from a knowledge of the hexosan formula $(C_6H_{10}O_5)n$, it was possible to prove that each 1 g pentosan could produce 4.28 Kcal. The undetermined fraction of the crude carbohydrate (either

in feed, feces or crude fibre) was considered to belong to true carbohydrates, having a calorific value of 4.28 Kcal/g.

Results and Discussion

1. *The conversion factor of lignin from common feedstuffs in Egypt*

The methoxyl content in reference lignin was determined in order to calculate the specific conversion factor for pure lignin in common feedstuffs in A.R.E. Egypt (Table 1).

TABLE 1. The conversion factor of lignin from common feedstuffs.

Feed	Source	MeO% in ref. lignin	Conversion factor
Wheat, Triticum spp.	Straw	17.80	5.62
Bean, Vicia faba equina	Straw	17.66	5.66
Maize, Zea mays	Stalks	17.89	5.59
Cotton, Gossypium spp.	Stalks	17.60	5.68
Rice, Oryza sativa	Straw	18.38	5.44
Clover, Trifolium alexandrinum	Straw	18.14	5.51
Sugar cane, Saccharum officinarum	Bagase	18.20	5.49
Average of conversion factors			5.57

Owing to the fact that N content in prepared "reference lignin" could be practically neglected, therefore, MeO% was recorded with out correction for N content.

Results in Table 1 show that MeO% in legume "reference lignin" ranged between 17.66 and 18.14, while in cereals the range was 17.80 to 18.38. The corresponding range of the conversion factor was 5.51 to 5.66 and 5.44 to 5.62. The average for the seven cases was 5.57. Moon and Abou-Raya (1954) obtained for legumes and cereals (but correcting for N content) a range from 5.27 to 5.64 with an average of 5.5. The range and average here were

practically the same as those of Moon and Abou-Raya (1954). Therefore, the suggested conversion factor average (5.5) by Moon and Abou-Raya (1954) appeared to be a suitable average for common feed-stuffs in A.R.E. Egypt.

2. *Caloric balance in plant and fecal materials in relation to lignin determination*

Results with wheat straw crude fibre (Table 2) indicated that the calorific value per 100 g calculated from the summation of calorific values of lignin, cellulose, pentosan and the undetermined fraction was 429.50 Kcal in OM and 432.0 Kcal in OM. The corresponding figures obtained from direct bomb calorimetry were 430.40 and 432.80, the difference being negligible not exceeding 0.18%. These results were in favour of the analytical methods used for cellulose, lignin and pentosan determination as well as the physico-chemical method for estimating calorific value of such ingredients.

Particularly with lignin, it appears that the method of Moon and Abou-Raya (1966) (based on acid lignin OMe $\times$ conversion factor) produced a reliable estimate of lignin content.

Owing to the close calorific value of cellulose and pentosans, it might be more practical and simpler to consider that the whole carbohydrate fraction (mainly cellulose and pentosans) contains 4.20 Kcal per g. Testing such consideration in this case, the OM of the crude fibre containing 6.14% lignin and 93.86% carbohydrate would contain by calculation ($93.86 \times 4.20 + 6.14 \times 6.27$) an amount of 432.7 Kcal. This was the same as obtained from direct calorimetry or from the summation of the calorific values of separate ingredients. The suggested scheme would save cellulose and pentosan determination. The carbohydrates would be obtained by difference, *i.e.* organic matter minus lignin.

Alternatively, from a knowledge of the calorific value determined calorimetrically per 100 g CF (on OM basis); it is possible to determine indirectly lignin content in the CF using a very simple equation as follows :

Assuming Y = % lignin in organic matter, the amount of carbohydrate would be (100-Y)%. Taking 100 g OM of the crude fibre :

Table 2. . The calorific value of crude fibre and the distribution of the calorific value among its nutrients.

a. Results with wheat straw CF.

Nutrient	Kilocal/1g nutrient	Analysis of 100 g DM	Calorific value in nutrients of DM	Analysis of 100 g OM	Calorific value in nutrients of OM
Ash	-	0.57	-	0.00	-
Lignin	6.27	6.11	38.30	6.14	38.50
Cellulose	4.18	82.49	344.80	82.96	346.80
Pentosan	4.28	10.51	45.00	10.57	45.20
Undetermined fraction	4.28	0.32	1.40	0.33	1.50
Total	-	100 g	429.50	100 g	432.00
Determined calorific value/100 g CF	-	-	430.40	-	432.80

b. Results with faecal CF from hay :

Ash	-	4.80	-	0.00	-
Lignin	6.27	23.44	146.97	24.62	154.37
Cellulose	4.18	50.60	211.51	53.15	222.17
Pentosan	4.28	10.80	46.22	11.34	48.54
Ethanol-benzen extraction	9.50	2.47	23.50	2.59	24.60
Undetermined fraction	4.28	7.89	33.80	8.20	35.10
Total	-	100 g	462.00	100 g	484.78
Determined calorific value/100 g CF	-	-	461.70	-	484.48

$$\text{Therefore } 432.8 = 4.20\ (100\text{-}Y) + 6.27\ X$$
$$= 420 - 4.20\ Y + 6.27\ Y$$
$$= 420 + 2.07\ Y$$
$$\therefore \quad Y = \frac{432.8 - 420}{2.07} = 6.18$$
$$= \frac{\text{Calorific value in 100 g OM} - 420}{2.07}$$

The determined lignin content was 6.14, the difference between direct and indirect calorimetric method per lignin determination was only 0.65%. These results might be in favour of the method of Moon and Abou-Raya for lignin determination and encouraging to use indirect calorimetric method for lignin determination confirming the results of Bergner and Schramm (1968). The calorimetric method appeared easier and fairly rapid so long there was a fair agreement with direct methods.

Therefore, a new CF residue was prepared in a second trial from hay faecal material (Table 2b) a satisfactory result was also obtained which confirmed that obtained with wheat straw CF.

3. *Proposed scheme for indirect determination of lignin calorimetrically*

From the previous study, it appeared possible to predict the amount of lignin in a plant material after being pretreated to remove rude fat, resinous material and nitrogenous material. In other words, if the pretreated residue contained lignin and polysaccharides (assuming 6.27 kcal per g lignin and 4.20 kcl per g carbohydrate), a knowledge of the calorific value per 100 g OM of the pretreated residue x (determined directly in the bomb calorimeter) would enable prediction of the lignin content in the residue. Then, it could be easily related to the original plant material by a knowledge of the percentage residue in the material.

Using the equation deduced before, the lignin percent in the OM of the residue would be :

$$Y = \frac{X - 420}{2.07} - \frac{1}{2.07} X - \frac{420}{2.07}$$

$$^{*}_{**} \; Y = 0.483 \times - 202.86$$

Such equation could be plotted in a straight line curve. When lignin (Y) is zero, X would be 420 kcal/100 g OM of the residue, and when Y is 100, X would be 627 Kcal.

This field appeared promising, but further studies are needed to determine the suitability of the pretreated residues used for acid lignin determination for applying such an equation. Checking the results should be undertaken by direct acid lignin determination based on methoxyl content in the residue.

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تقدير اللجنين فى مواد العلف على طريق المحتوى الميثوكسيلى للجنين المحضر بطريقة الحامض وما يقابلها بطريقة حرارية

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تم تقدير القيمة الحرارية لكل من اللجنين والكربوايدرات النقية للالياف الخام المحضرة وكذلك للمادة النباتية المحضرة وذلك لمعرفة نسبة اللجنين بطريقة حرارية ومدى تعزيزها لطريقة تقـــدير اللجنين المحضر بطريقة الحامض عن طريق محتواه الميثوكسيلى كما اوضحه مون وابو رية ١٩٦٦ .

وقد تم تحضير الياف خام من تبن القمح وكانت الطاقة المحسوبة هى ٤٣٢ كيلو كالورى/١٠٠ جم مادة عضوية (باستخدام القيم الحرارية ٦ر٢٦ ، ١٧٦ر٤ ، ٢ر٤ كيلو كالورى/ جم لكل من اللجنين ، السليلوز ، مخلوط الكربوايدرات على التوالى) وقد وجد تطابق بين القيمة الحرارية المحسوبة (كل مركب × قيمته الحرارية) والقيمة الحرارية المقدرة مباشرة بالحرق فى بمبة المسعر حيث كانت ٨ر٤٣٢ كيلو كالورى/١٠٠ جم مادة عضوية وقد ثبت هذا التطابق ايضا فى عينة الياف خام اخرى محضرة من روث دريس البرسيم بعد استخلاص المواد الدهنية واللدائنية منها .

وهذا التطابق السابق بين التقديرين الكيماوى والمباشر اظهر تأكيدا لطريقة مون وابو رية ١٩٦٦ لتقدير اللجنين المحضر بطريقة الحامض عن طريق محتواه الميثوكسيلى لهذا اقترحت طريقة حرارية لتقدير اللجنين فى المتبقى من مواد العلف والتى تتكون اساسا من اللجنين ومخلوط السكريات العديدة عن طريق المعادلة الاتية :ـ

س = ٤٨٣١ر٠ ص ـ ٨٦ر٢٠٢

وذلك بفرض ان النسبة المئوية للجنين هى (س) والقيمة الحرارية لكل ١٠٠ جم مادة عضوية فى مادة العلف السابقة التجهيز هى (ص) .