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SYMPOSIUM ON 'FIBRE IN HUMAN NUTRITION'

The chemistry and estimation of fibre

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Crude fibre determination is commonly used to estimate the quality of foods of plant origin on the premise that it constitutes their least digestible fraction. Therefore, a criterion for evaluating fibre methods is the recovery of indigestible plant residues. The so-called Weende method for fibre estimation was not developed at Weende, but at Möglin after 1806 by Heinrich Einhof, who assumed that the fibrous residue left after sequential extraction with solvent, dilute aqueous acid and dilute alkali, represented the indigestible matter. Einhof calculated estimates of nutritive value of vegetables and forages on this basis (von Thaer, 1809).

The deficiencies of Einhof's method became apparent in the nineteenth century (Henneberg, 1859). Over the years many systems of analysis have been proposed for the replacement of crude fibre, but none have been successful in dislodging it as the official method.

The crude fibre method as an estimate of the amount of fibre or plant cell wall in foods has many defects. On average, 80% of the hemicellulose or pentosans and from 50 to 90% of the lignin are removed by the acid and alkaline sequential extraction, while cellulose recovery is 50–80%. Thus, much of the hemicellulose and lignin appears in the nitrogen-free-extract (NFE) to be counted as available carbohydrate (Maynard, 1940). The NFE of straws and grasses may contain as much as 90% of these substances (Nordfeldt, Svanberg & Claesson, 1949; Stallcup, Davis & Ward, 1964; Kim, Gillingham & Loadholt, 1967). Because of the failure of the crude fibre method to recover indigestible substances, NFE appears less digestible than crude fibre in significant numbers of cases (Crampton & Maynard, 1938). In the case of vegetables and cereals the error is less because of the relatively lower content of hemicelluloses and lignin. However, it may be substantial.

There have been various attempts to replace the crude fibre method with a system of analysis which gives a better characterization of the less nutritive fraction of food. Such attempts face several problems, not all of them necessarily analytical.

The problems are (1) conflicting concepts of what constitutes fibre; (2) the definition of lignin, cellulose and hemicellulose; (3) achieving separation of lignin from interfering matter; (4) the isolation of indigestible fibre and its relation to the true fibre of the food; (5) the failure of hemicellulose, cellulose and lignin to be biologically or chemically similar in different plant materials.

It is not possible to review here all attempts to devise new methods for the determination of fibre content, but the various approaches will be considered. Earlier attempts identified fibre with some chemical entity such as cellulose, for which definitive methods were devised (Crampton & Maynard, 1938). This approach did not take into account the importance of lignin, for which other procedures were developed. Later it became apparent that cellulose and lignin are rather variable portions of the cell wall, and that hemicellulose could contribute significantly to the indigestible residue. Various enzymatic and other allied methods have been devised to isolate cell-wall matter (Weinstock & Benham, 1951; Harwood, 1954; Gaillard, 1958; Salo, 1965; Friedemann, Witt, Neighbors & Weber, 1967; Salo & Kotilainen, 1967; Southgate, 1969; Bailey & Ulyatt, 1970; Blake & Richards, 1970). Many of these procedures continue with a partition of the cell wall into subfractions.

Another approach is to isolate the plant cell wall with solutions containing detergents (Van Soest, 1963*a*, *b*; Van Soest & Wine, 1967). This system was based on the fractionation of forages and faeces from balance trials with ruminants, to determine the most meaningful fractionation from a nutritional point of view (Van Soest, 1967). The result of these studies was that the plant cell wall (neutral detergent residue) contained quantitatively the indigestible plant matter. However, a large and variable part of the cell wall was digestible. The indigestible fraction can be estimated by feeding trials or by in vitro fermentation with rumen organisms. The problem remains that there is no chemical procedure which allows the isolation of undiluted indigestible cellulosic material. The existence of this cellulosic fraction has been postulated (Wilkins, 1969; Waldo, 1970) and estimated by means of microbial digestion (Smith, Goering, Waldo & Gordon, 1971; Mertens & Van Soest, 1972).

The detergent system of analysis, described in Table 1, not only allows the estimation of the amount of cell wall but provides relatively rapid procedures for the

Table 1. *Basic scheme for the analysis of forage using detergents*

Fraction	Reagent	Treatment	Yield
Neutral-detergent residue (NDR)	Na lauryl sulphate, EDTA pH 7·0	Boil 1 h	Plant cell wall − pectins
Acid-detergent fibre (ADF)	Cetyl trimethyl-ammonium bromide in 1 N-H_2SO_4	Boil 1 h	Lignocellulose + insoluble mineral
Lignin	640 ml H_2SO_4/l	3 h, 20°	Crude lignin
Cellulose	—	Calculate as ADF − lignin	—
Hemicellulose	—	Calculate as NDR − ADF	—

estimation of lignin, cellulose, and hemicellulose contents, which compare reasonably well with more precise chemical methods (Playne, McLeod & Dekker, 1972). The detergent procedures have been used in balance trials with a number of animal species as shown in Table 2.

Table 2. *Digestibility of cellulose and hemicellulose from lucerne* (Medicago sativum) *and brome grass* (Bromus L.) *in various animal species*

	Lucerne			Brome grass			
Species	Cellulose	Hemi-cellulose	Ratio*	Cellulose	Hemi-cellulose	Ratio*	Reference
Sheep	0·50	0·47	0·9	0·67	0·71	1·1	Keys, Van Soest & Young (1969)
Pigs	0·40	0·43	1·1	0·39	0·47	1·2	
Voles	0·33	0·39	1·2	0·18	0·24	1·3	Keys & Van Soest (1970)
Rats	0·21	0·47	2·2	0·01	0·11	11·0	Keys *et al.* (1969)

*Hemicellulose digestibility: cellulose digestibility.

Non-ruminants digest less cellulose than ruminants, but the digestibility varies with the plant species fed and the animal species consuming it. Non-ruminants are not at as great a disadvantage in digesting hemicellulose as they are in digesting cellulose.

Pectin, hemicellulose and cellulose must be fermented by bacteria to be used at all, since no appropriate enzymes are secreted by the digestive tract. Normal rumen-fermentation products include bacterial lipid and protein, acetic, propionic, butyric and lactic acids, carbon dioxide and methane (Hungate, 1966). Products in the caecum and lower tracts of monogastric animals do not differ greatly from rumen products. It is also apparent from the developing literature on monogastrics that utilization of the fatty acids and bacterial protein is important in their nutritional economy (Maynard, 1957). Certainly the role of fermentation in the human gut should not be overlooked.

The cell walls of edible plants show wide variations in properties. The physical properties of the plant cell walls are influenced by the proportions of lignin, hemicelluloses and cellulose. Lignin has plastic properties and lends wood its rigid character, while hemicelluloses are more or less branched polysaccharides closely associated with the lignin. Fibrousness is contributed by the crystallization of long chains of glucoses linked in a β-1→4 manner. Theoretical chemists have given us this view of cellulose because it is the form found in wood. However, the cellulose of cell walls in parenchymatous tissue, characteristic of the pulp of most vegetables, has little fibrous character and appears as a gel if isolated in a hydrated form. On drying it tends to become hard and cannot be easily reconstituted to its original state. Isolated celluloses have usually been treated with harsh chemicals to remove the contaminating substances. Since strong oxidants and heat are used to remove lignin, and alkali is used to remove hemicelluloses, it is most unlikely that the isolated substance retains its original structure and properties.

The occurrence of relatively pure cellulose, as in cotton, is the biological exception. In plant cell walls, cellulose is intimately combined with hemicelluloses and varying

amounts of lignin. Lignin and hemicellulose are termed encrusting substances and constitute the secondary wall thickening. They are largely, but never completely, removed in the chemical isolation of cellulose. The pentosans that remain in cellulose preparations are impossible to remove without serious degradation. These pentosans are termed micellar and appear less digestible than the extractable fractions (Gaillard, 1962; Lyford, Smart & Matrone, 1963).

Dried isolated celluloses have different digestion characteristics from those of the fresh plant tissue. Dried material has a much slower rate of attack by cellulase (*EC* 3.2.1.4) from *Trichoderma viride* than do undried ball-cotton (*Gossypium barabdense* L.) preparations (Stone, Scallan, Donefer & Ahlgren, 1969). McQueen (unpublished observations) has observed similar effects with cellulase on the cell walls of clover and cauliflower. The isolation and drying of forage cell walls does not reduce the rate of digestion with rumen organisms (Smith *et al.* 1971). The digestibility of isolated cellulose of lucerne is increased by rumen organisms because of lignin removal. Perhaps the large amounts of hemicelluloses and lignin that are closely associated with the cellulose in these instances prevent crystallization of the cellulose.

The hemicelluloses are perhaps the least understood substances of the plant cell wall. They are complex polymers of xylose, arabinose, glucuronic acid, galactose, mannose and sometimes other sugars. Pectins contain mainly galacturonic acid, arabinose and galactose (Aspinall, 1964). Classically the pectins represent the polysaccharides removed by hot ammonium oxalate solution, while hemicelluloses are supposed to be water-insoluble polysaccharides which are, however, soluble in acid and alkali; there is not a clear distinction. Hemicellulose is probably covalently linked to lignin and it becomes more or less water-soluble upon delignification. Since some pectins are isolated with acid, confusion is probable. In theoretical chemistry the term pectin is less often used and the whole complex of structural non-cellulose carbohydrate is termed hemicellulose. This attitude has been adopted in the analysis of vegetables. Salo (1967), for example, has considered all polysaccharides not accounted for by starch or cellulose as hemicellulose. Hemicelluloses and pectin have varying degrees of branching. Arabinofuranosidic groups are hydrolysed in very weak acid (Bailey, 1964) and hydrolysis could occur in the digestive tract without enzymes. Gaillard (1962) has shown that the digestibility of hemicellulosic arabinose is considerably greater than that of xylose in forages fed to sheep. The relative digestibility of hemicelluloses by non-ruminants may be related to the furanosidic linkages. Hemicelluloses are divided in A and B fractions according to their relative solubility in varying concentrations of alkali. This division bears no significance from a nutritive point of view as the fractions do not have intrinsically different digestibilities (Gaillard, 1962).

Lignin is generally regarded as the main non-carbohydrate fraction in plant cell wall and the source of much resistance to microbial degradation. The treatment of vegetable foods and forages with 640 ml H_2SO_4/l tends to isolate much more than the substituted phenylpropane polymer. Cutin, protein-tannin adducts and products of the browning reactions are also isolated and may occasionally be the dominant

components. Provided they are not generated as artifacts in the analytical procedure, they may be regarded as legitimate constituents of crude lignin as they all have the common property of being relatively indigestible.

Lignin is present at higher concentrations in any dried, heated, or cooked food because of the synthesis of Maillard products that result from the degradative condensation, and polymerization of carbohydrates and amino acids (Van Soest, 1965). Lignin values may be corrected by subtracting the value $N \times 6{\cdot}25$, arguing that true lignin contains no N. However, all forage and vegetable lignins, when isolated, contain N (and amino acids) no matter how careful the analysis; the evidence indicates that this N is unavailable (Goering, Gordon, Hemken, Waldo, Van Soest, & Smith, 1972). From the nutrition point of view lignin is a recovery of refractory residues (Pearl, 1967), and therefore the correction for protein content does not seem justified. An exception is the artifact generated by drying of samples in preparation for analysis that some procedures require.

Cutin is the portion of crude lignin that is resistant to strong oxidation (Meara, 1955). This substance is prominent in the peel and on the surface of vegetables and fruits as the polymeric waxy material which protects the surface of plant tissues. Its influence upon the cellulolytic degradation of plant cell walls is very similar to that of lignin.

Lignin reduces the digestibility of the carbohydrates with which it is associated. An often-quoted mechanism for this effect is the theory of incrustation, which states that a physical layer of lignin covers the cellulose, protecting it from attack. Alternative hypotheses include enzyme inhibition and covalent linkage of carbohydrate to lignin. Actually, hemicellulose is associated with the lignin and forms part of the encrusting substance. An extension of the incrustation idea is the enclosure of intracellular materials by indigestible cell walls so that they are protected from digestion. This idea has been occasionally used to explain the increased loss of protein in faeces on increasing the fibre content of diets.

The hypothesis is not a reasonable one because if it was correct, the several analytical methods for isolating plant cell wall with enzymes or neutral reagents could not work. More probable explanations for the effect of fibre on other dietary constituents include the increase in their rate of passage in the presence of fibre and of cellulosic fermentation.

The composition of plant cell wall material has a great effect not only on its physical properties but also on its biological degradation. There is a great deal of variation in the composition and digestibility of vegetable fibres as shown in Table 3. Crude fibre is most closely related to cellulose, which forms a variable part of the plant cell wall as estimated by neutral-detergent residue. In wheat bran, crude fibre constitutes only one-quarter of the estimated cell wall material. This situation exists in the graminaceous materials which have generally a higher hemicellulose content (Van Soest, 1969). The digestibility of the cell wall is more closely related to the lignin:cellulose ratio than to any other measurement. Digestibility values obtained with rumen organisms or with cellulase from *Trichoderma viride* are essentially similar (McQueen & Van Soest, 1973).

Table 3. *Composition of dry matter (g/kg) and digestibility of fibre of various vegetable species*

Vegetable	Crude fibre	NDR*	Cellulose	Lignin	Lignin: cellulose	NDR digestibility† 15–20 h Rumen	TV
Cauliflower	107	151	102	5·5	0·5	0·93	0·86
Rutabaga	74	102	78	6·2	0·8	0·89	0·83
Potato	18	47	18	1·8	1·0	0·86	—
Carrot	55	92	77	2·7	0·4	0·86	0·81
Apple	37	76	44	3·8	0·9	0·80	—
Lettuce	135	173	140	20·3	1·5	0·73	0·73
Onion	59	99	49	4·5	0·9	0·57	—
Orange	27	35	31	6·2	2·0	0·50	—
Wheat bran	120	470	90	39·0	4·3	0·33	0·26

*Neutral-detergent residue.
†Digestibility of NDR by rumen organisms or *Trichoderma viride* enzymes (TV).

It is important to notice that the digestibilities of cell walls of onion and wheat bran are much lower than those of other vegetables. Hoppert & Clark (1945) reported that the digestibility of cellulose from wheat bran is lower and values for fruits and vegetables are much higher in man. As estimates of transit times in humans are generally greater than 15 h (Burkitt, Walker & Painter, 1972) it can be suggested that digestion of vegetable fibre should be quantatively important and that significant variation will occur as a result of the source and type of vegetable fibre.

The rate of digestion of cell wall material from many of the common vegetables is extraordinarily rapid, more than 90% of the maximum digestion occurring before 15 h (Table 4). Forage fibre ferments more slowly than that of vegetables and that from cotton and prepared wood cellulose (Whatman filter paper) ferments still more slowly. Although cotton and wood cellulose are very digestible in the long term, the rates of digestion are very slow. As these materials contain no lignin, the intrinsic properties of cellulose are emphasised.

Table 4. *Extent of digestion of plant cell wall (mg/g) after 15 h and 127 h incubation with cellulase (EC3.2.1.4) and with rumen organisms*

	Substrate solubilized by enzymes of *Trichoderma viride*			Substrate solubilized by rumen organisms		
		Maximum digestion			Maximum digestion	
Substrate	15 h	127 h	15 h:127 h	15 h	72 h	15 h:72 h
Tomato NDR*	0·87	0·97	0·90	0·69	0·83	0·83
Cauliflower NDR	0·86	0·93	0·92	0·82	0·97	0·85
Carrot NDR	0·77	0·95	0·81	0·80	0·92	0·87
Lettuce NDR	0·72	0·83	0·87	0·70	0·79	0·89
Rutabaga NDR	0·81	0·92	0·88	0·87	0·95	0·92
Lucerne NDR	0·39	0·49	0·80	0·32	0·59	0·54
Wheat-straw NDR	0·25	0·40	0·63	0·12	0·47	0·25
Cotton linters	0·16	0·67	0·24	0·18	0·99	0·18
Whatman cellulose	—	0·54	—	0·26	0·97	0·27

*Neutral-detergent residue.

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A common mistake made by nutritionists in formulating experimental diets containing fibre, is to choose a source of wood cellulose on the assumption that it represents natural dietary fibre or pure cellulose. Neither is true. Solka floc (cellulose) and other preparations from wood are not satisfactory as standard celluloses because they vary in composition and digestibility (Table 5). Paper varies in quality, reflecting different sources and manufacturing techniques (Mertens & Van Soest, 1971). Sometimes prepared celluloses are fed to non-ruminants to supply inert ballast. Although some wood products may be indigestible, it can by no means be guaranteed.

Table 5. *Composition (g/kg dry matter) and digestibility of various sources of paper*

Paper	Ash	NDR	Cellulose	Lignin	Digestibility*
Manchester Guardian	300	680	590	30	0·99
Solka floc (cellulose)	3	990	840	50	0·97
Whatman No. 41	2	980	890	50	0·91
Brown cardboard	17	940	720	120	0·77
Playboy	240	650	510	90	0·65
Christian Science Monitor	4	970	600	210	0·31
Washington Post	4	940	550	260	0·32

*Digestibility of organic neutral-detergent residue (NDR) by incubation for 48 h with rumen organisms.

Furthermore, since celluloses and hemicelluloses are degraded during isolation, it is doubtful that the feeding of preparations will produce the same responses as the intact natural material.

In conclusion, the properties of vegetable cell walls which may have important influences in the human diet are (1) the properties of vegetable cell walls vary and are different from prepared celluloses, which usually have been dried or treated with caustic chemicals; (2) the biological properties of plant cell walls vary with plant species and reflect their chemical and physical composition, cell walls from fleshy vegetables may be very digestible; (3) the fibrous quality of plant cell walls depends on their morphology and composition, and is not necessarily identical with the nutritionally unavailable residue; (4) the fermentation generated by the plant cell wall must influence the environment of the lower digestive tract, and, therefore, must be considered a factor in the quality of dietary fibre and of the whole diet.

REFERENCES

Aspinall, G. O. (1964). In *Symposium Internationale sur la Chimie et la Biochimie de la Lignine, de la Cellulose et des Hemicelluloses*. p. 421. Grenoble, France: Les Imprimeries Réunies de Chambery.
Bailey, R. W. (1964). *Oligosaccharides*. London: Pergamon Press.
Bailey, R. W. & Ulyatt, M. J. (1970). *N.Z. Jl agric. Res.* **13**, 591.
Blake, J. D. & Richards, G. N. (1970). *Aust. J. Chem.* **23**, 2354.
Burkitt, D. P., Walker, A. R. P. & Painter, N. S. (1972). *Lancet* ii, 1408.
Crampton, E. W. & Maynard, L. A. (1938). *J. Nutr.* **15**, 383.
Friedemann, T. E., Witt, N. F., Neighbors, B. W. & Weber, C. W. (1967). *J. Nutr.* **92**, Suppl. 2, 1.
Gaillard, B. D. E. (1958). *J. Sci. Fd Agric.* **9**, 170.
Gaillard, B. D. E. (1962). *J. agric. Sci., Camb.* **59**, 369.
Goering, H. K., Gordon, C. H., Hemken, R. W., Waldo, D. R., Van Soest, P. J. & Smith, L. W. (1972). *J. Dairy Sci.* **55**, 1290.
Harwood, V. D. (1954). *J. Sci. Fd Agric.* **5**, 270.
Henneberg, W. (1859). *J. Landwirt.* **3**, 299.

Hoppert, C. A. & Clark, A. J. (1945). *J. Am. diet. Ass.* **21**, 157.
Hungate, R. E. (1966). *The Rumen and Its Microbes.* New York: Academic Press.
Keys, J. E. & Van Soest, P. J. (1970). *J. Dairy Sci.* **53**, 1502.
Keys, J. E., Van Soest, P. J. & Young, E. P. (1969). *J. Anim. Sci.* **29**, 11.
Kim, J. T., Gillingham, J. T. & Loadholt, C. B. (1967). *J. Ass. off. Analyt. Chem.* **50**, 340.
Lyford, S. J. Jr, Smart, W. W. G. Jr & Matrone, G. (1963). *J. Nutr.* **79**, 105.
McQueen, R. E. & Van Soest, P. J. (1973). *J. Dairy Sci.* **56**, 680.
Maynard, L. A. (1940). *J. Ass. off. agric. Chem.* **33**, 156.
Maynard, L. A. (1957). *Annls Nutr. Aliment.* **11**, A179.
Meara, M. L. (1955). In *Moderne Methode der Pflanzenanalyse* vol. 2, p. 317 [K. Paech and M. V. Tracey, editors]. Berlin: Springer Verlag.
Mertens, D. R. & Van Soest, P. J. (1971). *J. Anim. Sci.* **33**, 293.
Mertens, D. R. & Van Soest, P. J. (1972). *J. Anim. Sci.* **35**, 286.
Nordfeldt, S., Svanberg, O. & Claesson, O. (1949). *Acta agric. suec.* **3**, 177.
Pearl, I. A. (1967). *The Chemistry of Lignin.* New York: M. Dekker.
Playne, M. J., McLeod, M. N. & Dekker, R. F. H. (1972). *J. Sci. Fd Agric.* **23**, 925.
Salo, M. (1965). *Maataloust. Aikakausk.* **37**, 187.
Salo, M. (1967). *Suom. maatal. Seur. Julk.* **107**, 101.
Salo, M. & Kotilainen, K. (1967). *Maataloust. Aikakausk.* **39**, 38.
Smith, L. W., Goering, H. K., Waldo, D. R. & Gordon, C. H. (1971). *J. Dairy Sci.* **54**, 71.
Southgate, D. A. T. (1969). *J. Sci. Fd Agric.* **20**, 331.
Stallcup, O. T., Davis, G. V. & Ward, D. A. (1964). *Arkans. agric. expl Stn Bull.* p. 684.
Stone, J. E., Scallan, A. M., Donefer, E. & Ahlgren, E. (1969). *Adv. Chem. Ser.* **95**, 219.
Van Soest, P. J. (1963*a*). *J. Ass. off. agric. Chem.* **46**, 825.
Van Soest, P. J. (1963*b*). *J. Ass. off. agric. Chem.* **46**, 829.
Van Soest, P. J. (1965). *J. Ass. off. Analyt. Chem.* **48**, 785.
Van Soest, P. J. (1967). *J. Anim. Sci.* **26**, 119.
Van Soest, P. J. (1969). *Proc. Cornell Nutr. Conf.* p. 17.
Van Soest, P. J. & Wine, R. H. (1967). *J. Ass. off. Analyt. Chem.* **50**, 50.
von Thaer, A. (1809). *Gründsätze der rationellen Landwirtschaft* Vol. 1, p. 275. Berlin: Die Verlag der Realschulbuchhandlung.
Waldo, D. R. (1970). In *Proceedings of National Conference on Forage Quality and Utilization* p. E-1 [R. F. Barnes, editor]. Lincoln, Nebraska: University of Nebraska.
Weinstock, A. & Benham, G. H. (1951). *Cereal Chem.* **28**, 490.
Wilkins, R. J. (1969). *J. agric. Sci., Camb.* **73**, 57.

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