ON THE ESTIMATION OF THE TOTAL OF VEGETABLE MEMBRANE SUBSTANCES.

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Membrane substances and cell enclosure substances.

The microscopic examination of vegetable materials suggests a partition of plant substances which proves very useful as well for the chemist as for the nutritionist. This is the partition into

1) cell enclosure substances or, plainly, enclosure substances and

2) cell wall substances or membrane substances.

Contrary to the animal tissues the vegetable cell walls are easily distinguishable under the microscope. Also a good deal of the cell contents is detectable without using any dye or other reagent. With the exception of water, some inorganic salts, and pectin compounds which occur both in the cell walls and in the enclosure, the partition is sharp. Substances occurring in the intercellular spaces are regarded as enclosure substances.

So far as the *Phanerogames* are concerned the two main categories of plant substances include the following subdivisions:

Enclosure substances

sugars	organic acids and their esters and salts
dextrins	
starches	alcohols
inulins	hydrocarbons
proteins	chlorophylls
amino acids	glucosides
amides	alkaloids
amines	inorganic salts
lipids	water

Membrane substances .

celluloses and other membrane hexosanes	pectins
	lignins
pentosanes	suberins
hexo-pentosanes	cutins
polyuronides	

This classifying list does not mean to be complete. It also contains some conventional faults; thus e.g., the water and the inorganic salts are ranged only under the Enclosure substances, whereas the pectins are placed under the heading Membrane substances. The different vitamins find their places in definite subdivisions of the enclosure substances.

From the point of view of the nutritionist it is important to notice that, with eventual exception of certain pectin compounds, which possibly are decomposed by the hydrochloric acid in the stomach, the membrane substances can't be disintegrated by the digestive fluids secreted to the alimentary canal from the glands. On the other hand, the substances belonging to the cell enclosure are either absorbable as such or can be disintegrated by the digestive fluids into absorbable components. There are only few and insignificant exceptions from this rule. That a part of the enclosure substances escapes the digestion does not usually depend on the inherent character of the compounds in question but on the protective effect of the cell mambranes.

The usefulness of the above partition is not impaired by the fact that the horse and ruminants can digest the membrane carbohydrates and pectins with the aid of bacteria; for the disintegration of the plant cell walls in the alimentary canal of the named animals is far from being complete, and a high content of membrane substances means altogether a low feeding value of the fodder in question. Concerning the foods of the man and omnivorous animals the pertinence of the partition of vegetable materials into enclosure substances and membrane substances is obvious: the total of the membrane substances approximately represents the indigestible part of the food.

In the teaching of fodder chemistry the above partition has proved very instructive. The one of the authors has also adopted it to his textbooks on animal nutrition (21, 23).

Earlier attempts to determine the total of membrane substances.

It is more than likely that just the microscopic examination of plant tissues led the naturalists of the 18th century to the conception that the cell walls consisted of a single substance, the wood substance, *la matière ligneuse* (2, p. 561). In spite of PAYEN's investigations which led to the discovery of »cellulose» and »les matières incrustantes» from the wood (ref. DUMAS, 3, p. 52, and CZAPEK, 2, p. 522, 561), the erroneous conception of the unity of the wood substance lasted in many laboratories over the middle of the 19th century.

H. DAVY was probably the first to propose a method for estimation of the total of membrane substances. He treated the woody materials to be investigated with boiling water and boiling alcohol, but it is worthy of mention that he did not term the remainder as wood substance but crude fibre (9, p. 303).

It is self-evident that Davy's method was not fitted for investigation of materials containing appreciable amounts of starch or protein. CARL SPRENGEL was presumably the first who described a method for analyzing such materials (26, p. 251). He treated the sample to be analyzed successively with water, alcohol, ether, diluted hydrochloric acid, diluted potassium hydroxide and chlorine water. The residue obtained was called *Holzfaser*. Obviously SPRENGEL had the erroneous concept that this product, as obtained from a definite raw material, was a chemical unity and contained the whole cell wall matter of the raw material in question, although, according to his view, there were different kinds of *Holzfaser* depending on the plant products analyzed, as there are different starches and sugars. SPRENGEL did not know that by the treatment with acid, alkali and chlorine, not only starch, protein and certain coloured substances but also a great part of membrane substances were removed.

The principle proposed by SPRENGEL was adopted to use in many European laboratories during the next three decades, until the end of 1850's. Only the concentration of the acid and alkali varied in different modifications of the method, and treatment with chlorine was usually omitted. Only the methods of BOUSSINGAULT, WOLFF (ref. HENNEBERG and STOHMANN, 6, p. 511), MOSER, DIETRICH (ref. LEBBIN, 14, p. 214) and HENNEBERG and STOHMANN (6, p. 367) may be mentioned on this paper. Several methods from the same period which were intended for the estimation of cellulose must here be left unconsidered. It is however, important to remember that there was in Germany in those times much confusion about the term *Holzfaser*. Many writers used it as a synonym with the words *Zellstoff* and *Cellulose*, where as some others meant by it the total of membrane substances. It is likely that HENNEBERG and STOHMANN to begin with shared the latter view. Later, as their own investigations proved the erroneusness of this view, they replaced the term *Holzfaser* by the term *Rohfaser*, crude fibre.

It is a very common opinion that the usual, so-called Weende method for crude fibre determination were an invention of HENNEBERG and STOHMANN. As a matter of fact the method, nearly in its final form, was used at the Weende experimental station already before HENNEBERG and STOHMANN. The reason why the method just in the form HENNEBERG and STOHMANN gave to it has made its way through the world, is the fact that particularly in this form it was used at Weende in exeptional numerous analyses, many of which were joined to carefully performed digestion experiments.

Instead of being the inventors of the Weende crude fibre determination method the named workers have by a scientifically accurate way investigated the behaviour of the vegetable cell membranes in the different treatments belonging to this method. Before 1856, however, POGGIALE (ref. KRAUT, 12, p. 19) had already shown that by the acid- and alkali treatments of the usual wood fibre determination methods

a part of the cellulose goes into solution. As the wood fibre (Holzfaser) of the fodder plants was considered to be identical with the wood substance of the proper wood which, in return, was regarded as a prototype of indigestible plant material, it is only natural that even wood fibre of the fodder materials was considered as indigestible. HENNEBERG and STOHMANN (6, pp. 513, 514) were presumably the first who established that the crude fibre (wood fibre) of the hay or straw was not indigestible. They stated that the digestibility of the crude fibre of different fodders varied from 45.5 to 60.4 %. After estimating the elementary composition of the crude fibre of different fodders, of the nitrogen-free extract [1] and of the digestible and indigestible portions as well of the crude fibre as of the nitrogen-free extract of the same fodders, the said investigators were able to draw some important conclusions. They concluded: 1) that in the crude fibre determination the cell wall substances are rather arbitrarily divided into two portions, the one constituting the crude fibre and the other belonging, together with sugar and starch, to the nitrogen-free extract; 2) that the crude fibre is not pure cellulose but contains also encrusts rich in carbon (lignin, suberin and cutin are named), which remain among the feces together with the indigestible part of cellulose, whereas the digestible part of crude fibre is pure cellulose; 3) that in the crude fibre determination the bulk of the lignin and a part of cellulose are dissolved and thus fall into the nitrogen-free extract; 4) that indigestible part of nitrogen-free extract is composed mainly of lignin (6, pp. 367, 511—514; 7, pp. 331—335; see also PALOHEIMO, 16, pp. 9—13).

The criticism directed to the Weende method by HENNEBERG and STOHMANN is so accurate that the later investigators have scarcely been able to add to it anything essential. The Weende method had proved to be no method for estimation of the total of cell wall substances but a very rough and imperfect cellulose determination method. This view was not altered after introduction of the pentosane determinations according to TOLLENS (28, p. 3575—3585), for it was shown that the main part of the pentosanes falls into the nitrogen-free extract (KÖNIG, 11, p. 93). Thus it appeared that not even the lignin-free portion of the nitrogen-free extract was composed merely of cell enclosure substances. Of the later criticism of the Weende method the papers of HOFFMEISTER (8, p. 243), DÜRING (4, p. 87), KÖNIG (1. c.), HONCAMP and RIES (9, pp. 306—317), MAGNUS (15, p. 34), PALOHEIMO (19, p. 281) and NORMAN (ref. ELLIS, MATRONE and MAYNARD, 5, p. 285)[2] may be mentioned. PALOHEIMO (1. c.) points to the fact that cellulose, though resistant as well against diluted acid as against diluted alkali, is partially rendered soluble in alkali when preliminarily treated with acid.

POGGIALE (1. c.) not only criticized the usual crude fibre determination methods but also developed a new method for estimation of the total amount of membrane substances. In his method, which was developed for the analysis of vegetable

[1]) The complex of protein-, fat- and ash-free material dissolved by the treatments belonging to the crude fibre determination method.

[2]) The original paper: NORMANN A. G. 1935. The composition of Crude Fiber. Agr. Res., 25: 529—540.

food-materials, the matter to be analyzed is successively treated with cold water, ether and diastase. From the residue the share of crude protein and ash is to be removed. The remainder was called fibre.

Besides his diastase method POGGIALE proposed a modification in which the treatment with diastase is replaced by boiling in 2 per cent. (about 0.3 normal) hydrochloric acid. In our opinion the latter treatment is far too vigorous and causes dissolving of a great lot of membrane constituents. In our own method a boiling in 0.05 normal acid is used. The ferment method of POGGIALE, although obviously the most accurate of the fibre determination methods of the last century, did not win any success in the time of its inventor and later it was entirely forgotten. The authors who 30, 70 and 80 years later developed analogous methods seem not know the work of POGGIALE.

The first who after POGGIALE proposed a ferment method were STUTZER and ISBERT (27, p. 93). They used not only an amylase-containing solution but also pepsin- and pancreatin solutions. It is obvious that the method is very time-consuming, and it is questionable, nevertheless, whether the protein can be quantitatively disintegrated and extracted by the ferment solutions. REMY (25, p. 2), ANDERSEN (1, p. 52), WILLIAMS and OLMSTEAD (ref. HORWITT, COWGILL and MENDEL, 10, p. 264)[1], HORWITT, COWGILL and MENDEL (1. c.) and WILLIAMS, WICKS, BIERMAN and OLMSTEAD (29, p. 595) have later proposed similar methods with only non-essential modifications.

The principle and general features of the new method.

The principle and the general features of our method for determining the total of vegetable cell membrane substances were described in a preliminary report by L. PALOHEIMO (22, p. 19). A short review may, however, be appropriate.

Our method was developed without knowledge of the methods of POGGIALE (1. c.) or the later modifications of his diastase method, which we came to know only afterwards. It is evident, however, that the new method belongs to the named line of analytical research. The immediate starting point of it was the starch determination method of L. PALOHEIMO (18, p. 150; 20, p. 391; 24, p. 109) and particularly, the observation that a boiling with 0.05 normal acid was sufficient to disintegrate all the starch and to liberate it from the plant cells. Using samples previously extracted with suitable lipid solvents it was obwiously possible after boiling with weak acid to obtain undissolved residues which contained, besides the membrane substances, only proteins and ash constituents. It seemed probable that the treatment would be very cautious as far as the membrane substances are concerned. As the determination of the protein and ash content of the residue is an easy task the approximate membrane content could be estimated by subtracting

[1]) The original paper: R. D. WILLIAMS and W. H. OLMSTEAD 1935. A biochemical method for determining indigestible residue (crude fiber) in feces: lignin, cellulose and non water soluble hemicellulose. J. Biol Chem., vol. 108, pp. 653—666.

from the residue after boiling with 0.05 normal acid its protein and ash. Thus the general features of the method turned out to be as follows.

A sample extracted with an efficient lipid-solvent is boiled with a large volume of 0.05 normal hydrochloric acid solution. The residue separated from the solution by filtering is dried, weighed and incinerated. The nitrogen determinations are made from the lipid-free material and from the filtrate, the nitrogen content of the residue is calculated as difference. The corresponding protein content is calculated using the coefficient 6.25 and the share of the protein is subtracted from the loss of incineration. The difference obtained is the amount of membrane substances.

The nitrogen-free organic substances dissolved by the acid treatment are called by the authors easily soluble carbohydrates. The percentage of this fraction actually composed as well of easily soluble as of easily hydrolyzable nitrogen-free non-lipid substances can be calculated by subtracting from 100 the percentages of water, ash, lipids, crude protein and membrane substances. It is obvious that for securing a correct value for the group of easily soluble carbohydrates the lipid-solvent used must not dissolve any non-lipid substances. In many cases it is advisable to fit an after-extraction with a lipid-solvent in between the filtration and incineration.

It is important that the volume of the acid solution is large enough to prevent any significant change of the H-ion concentration.

A detailed description of the method.

In the following the method is described chiefly in that form the authors have employed it. Some suggestions however are made to other possible manners of procedure.

Preliminary treatment and preservation of the samples. — Materials containig water more than about 15 per cent are dried at 60° C with efficient motor-driven ventilation. The air-dry material obtained is ground by a Wiley mill with 1 mm screen. In some cases the grinding is easier if the material is quite dry. Then the air-dry material is dried in a vacuum oven at 60° C before grinding. After grinding the material is dried in a vacuum at 60° C for about 10 hours and placed in a preservation pot with tight stopper; if it need not be stored for a longer time it can be placed air dry in the pot.

Determination of water. — If not only the percentage composition of the dry matter, but also that of the original watercontaining material is to be determined, the above manipulations must be completed by some weighings fitted in appropriate points. When in the later course of the analysis dry-matter determinations are to be made it is preferable to perform the drying in a vacuum oven at 70° C for 10 hours. This is recommendable especially for the sake of fructose.

Extraction of lipids. — The most efficient lipid-solvent the authors have tested is the ethanol-benzene (32 parts by weight ethanol and 68 parts by weight benzene). It can however be used in our method only when the material is preliminarily

extracted with cold water. The authors have, in fact planned and applied a more complete scheme of analysis to which belongs the extraction with cold water, and by means of which the group of easily soluble carbohydrates can be divided into two subsections: 1) water soluble carbohydrates and 2) easily hydrolysable carbohydrates.[1] In that system the extraction with ethanol-benzene is appropriate.

Usually the authors have made the first extraction with ether and the after-extraction (see above p. 6) with ethanol or with ethanol-benzene. As well the ether as the sample to be extracted (8—10 g) must be quite dry. Either Soxhlet- or Twisselmann-apparatus has been used; The after-extraction has always been made with Soxhlet-apparatus, the middle part with the siphon being isolated with paper. By this arrangement it is possible to retain the temperature of the solvent around the sample quite near the boiling point. In the after-extraction the residue after the acid-treatment need not be dry. If one prefers the drying before the extraction, it may be done even in a common drying oven at 100—105° C. The drying of the extracts is performed in a vacuum at 70° C. In some instances, especially when the after-extract contains water, it is advisable to put in the flask some slips of filter paper which have been dried and weighed together with the flask before the extraction. When only the determination of membrane substances is to be made, without paying attention to the other groups of substances, materials poor in lipids may be analyzed omitting the preliminary extraction with ether.

Boiling with acid, filtration. — The material extracted with ether is spread on a watch glass or a Petri disk and let stay over-night for attaining an air-dry state. Then three samples are weighed: 1) for determination of dry matter, 2) for nitrogen determination, and 3) for boiling with acid.

For the last purpose 1—2 g are weighed; of materials rich in mambrane substances 1 g is adequate. The sample is placed in a 600 ml beaker and mixed with some cold distilled water. If the material contains hard particles with compact formations of solid gels of starch, pectin or protein, the weighed sample must be crushed in a mortar before transferring into the beaker.[2] The beaker has a mark at 400 ml. Boiling water is added to the beaker not quite up to the mark and the mixture is brought up to boil. 20 ml of 1-normal hydrochloric acid is added and made up to 400 ml with boiling water. The mixture is boiled for 30 minutes by compensating the loss of evaporation with boiling water.

When examining succulent materials poor in lipids, such as potatoes, beets or different fruits, analysis can be made of the fresh material directly. A sample of 5—10 g of grated pulp is weighed for the acid treatment. Generally it is advisable to crush the weighed sample in a mortar before boiling with acid.

Immediately after boiling, the mixture is filtered through a coarse sinter of glass or quartz covered with a layer of quartz sand. A water-jet vacuum pump is used. The filtration must go rapidly; if a pellicle rendering the filtration difficult is formed on the surface of the sand layer, it must be teared up with a rough-edged glass rod. The residue on the filter is washed with about 70 ml of boiling water;

[1]) This system of analysis will probably be published in the near future.

[2]) Many prepared foods, e.g. bread, belong to this category.

the filtrate with the washing waters (30 ml for the washing of the filter-flask) is transferred in to a 500 ml measuring flask and reserved for nitrogen determination, eventually also for starch determination according to the iodine colorimetric principle (24).

After-extraction, incineration. — After-extraction, the ethanol or ethanol-benzene extraction of the residue from the boiling with acid, is indispensable also, and especially, in those cases where the preliminary extraction with ether is omitted. The residue with quartz sand is transferred into a thimble made of filter paper. Also may the whole sinter crucible be put into the Soxhlet apparatus if only the dimensions of the apparatus permit it. Further description of the extraction procedure is given already on page 7.

After extraction the residue with the sand is transferred into an ignited crucible. The crucible with contents is dried, weighd and placed in an incineration oven. After incineration at red heat the loss of incineration is estimated. If a sinter crucible of quartz has been used and the after-extraction made without removing the residue from the crucible, also the incineration can be made in the same crucible.

Protein correction, calculation of results. — The loss of incineration is compozed of membrane substances and of the protein not dissolved by the acid treatment. The latter component can be estimated by subtracting from the amount of crude protein before the boiling the amount dissolved by boiling with acid. — As mentioned above, the filtrate was reserved for nitrogen determination in a 500 ml measuring flask. After making the flask up to the volume an aliquot, at least 200 ml, is taken for the nitrogen determination.

The calculation of the percentage of membrane substances needs no further description. For attaining a more comprehensive picture of the nature of a food or feed the percentages of water, ash, lipids (crude fat), crude protein and easily soluble carbohydrates must be known. For computing the percentage of lipids the amount resulting from the after-extraction is to be added to the yield from the preliminary extraction. The amount of the easily soluble carbohydrates is obtained by subtracting from 100 the percentages of the other constituents. If a part of filtrate after boiling with acid is used for starch determination the fraction of easily soluble carbohydrates can be divided into two subdivisions: starch and other easily soluble carbohydrates (see p. 7).

Criticism and applications.

The authors have not as yet performed any detailed studies on the behaviour of different plant substances in the proposed method, and especially in the treatment with weak acid. However, some plain observations and simple experiments are rather encouraging as to the usefulness of the method.

By testing with iodine the residues resulting from the acid treatment of different vegetable products it was proved that only insignificant traces of starch were still present among the membrane substances. It may therefore be concluded that the

Table 1. Content of membrane substances, lipid-, protein-, and ash-free residue after boiling with water, and crude fibre of different vegetable materials poor in starch. (The figures are percentages on dry matter basis.)

	Membrane substances	Lipid- protein-, and ash-free residue after boiling whit water	Crude fibre
Filter paper	99.6	99.9	89.8
Spruce wood	90.9	96.3	73.8
Birch wood	90.8	94.3	59.5
Hulls of sunflower seeds	87.5	90.5	64.6
Cork	85.1	86.1	22.3
Peat litter	85.1	88.3	42.9
Peanut hulls	83.8	85.1	71.9
Rye straw	82.1	89.1	51.1
Oat straw	73.8	83.3	47.0
Corn cobs	72.0	90.1	36.1
Rye chaff	66.8	77.5	34.3
Barley chaff	58.6	72.4	27.9
Red clover stalks	55.3	64.1	40.6
Oat chaff	51.3	62.7	25.7
Alfalfa hay	42.5	50.1	32.0

complex of membrane substances obtained by the method in question is free from cell enclosure carbohydrates.

It is obvious that for the competence of the method *the inviolability of the cell walls* is not less important than the complete dissolving of the cell enclosure carbohydrates. Considering the great number of cell wall constituents and their very variable susceptibility to acid hydrolysis it is to be expected that even so weak an acid as 0.05 normal may have a dissolving effect upon some cell wall substances. In the authors' opinion it is even impossible to find an acid treatment by which it were feasible to dissolve all the enclosure carbohydrates without affecting the integrity of the complex of membrane substances. It is, however, essential that a method planned for the determination of the complex of membrane substances, as unbroken as possible, does not give yields that include also a part of cell enclosure carbohydrates. Thus, although boiling with water may be considered a treatment by which the membrane substances are dissolved hardly in any degree, this treatment is inadequate to liberate all the starch from the tissue.

However, so far as materials poor in starch are concerned, it is obvious that the lipid-, protein-, and ash-free portion of the residue after boiling with water must not be much greater than the corresponding result of the membrane substance determination. In tables 1 and 2 the comparison in question is presented comprising a series of vegetable materials poor in starch. The tables show also the corresponding percentages of crude fibre.

Tables 1 and 2 suggest that the plant cell walls do not remain intact in the treatments of our method. It is also obvious that the margin between the percen-

Table 2. Content of membrane substances, lipid-, protein-, and ash-free residue after boiling with water, and crude fibre of some grasses at different stages of growth.
(The figures are percentages on dry matter basis.)

	Membrane substances	Lipid-, protein, and ash-free residue after boiling with water	Crude fibre
Phleum pratense			
leaf stage I	50.3	53.1	22.6
leaf stage II	54.2	58.0	26.9
at beginning of bloom	62.1	65.6	32.8
Alopecurus pratensis			
leaf stage	47.7	53.1	23.3
with ears, before blooming	55.9	59.0	26.7
at full bloom	58.4	64.9	28.9
Poa pratensis			
leaf stage	51.9	55.4	21.3
with panicles, before blooming	56.6	61.5	28.0
at full bloom	60.0	67.1	29.2
Festuca pratensis			
leaf stage	42.0	50.6	22.4
with panicles, before blooming	42.8	54.4	23.1
at beginning of bloom	53.7	60.7	26.8
Avena elatior			
leaf stage	47.1	49.0	22.3
with panicles before blooming	52.8	58.4	28.6
at beginning of bloom	65.1	68.8	32.6
Trifolium repens			
leaf stage	26.8	31.6	15.6
at beginning of bloom	27.7	35.8	21.5
after full bloom	43.1	49.7	32.9

Table 3. Behaviour of pentosanes in boiling with 0.05 normal acid.
(The figures are percentages on dry matter basis.)

Material	Pentosanes total	Pentosanes dissolved	
		in ½ hour's boiling with acid	in 1 hour's boiling with acid
White clover grass	11.7	4.5	5.1
Wheat bran I	24.0	17.1	18.3
Wheat bran II	25.4	18.0	19.1

tages of the water-insoluble fraction and the »membrane substances» in many cases is so broad that it cannot be covered with the whole pectin content of the material in question, not to mention the fact that a part of pectins is dissolved already by the hot water. It is conspicuous that the said margin is especially broad in some very coarse feed materials as in different kinds of straw and chaff, whereas

it is rather narrow in different sorts of hay and even in samples of hay cured at a very early stage of growth. On the other hand, the margin is narrow also in some coarse materials not suitable for feeds.

At the present state of our researches it is difficult to decide what are the substances which, in addition to a part of pectins, are soluble in boiling 0.05 normal acid although insoluble in boiling water. Pentosane determinations according to Tollens cannot, owing to the non-specificity of the method, afford much light on this question. Table 3 shows that at least in some cases a rather great portion of furfurol-forming substances is soluble in the acid treatment of our method. Concerning the wheat bran II we know that the percentage of »pentosanes» dissolved in boiling water is 7.3.

Tables 1 and 2 not only disclose the incompleteness of our method but, on the other hand, reveal its superiority as *compared with the crude fibre determination.* The difference between the percentage of membrane substances determined by our method and the percentage of crude fibre is, as a rule, very great, in many cases about a half of the amount of membrane substances. As we may conclude that our method gives residues free from lipids and cell enclosure carbohydrates, and as the protein correction cannot involve any appreciable errors, the membrane substance values in Tables 1 and 2 may not prove to be too high. On the contrary, they may be regarded as minimum values, the actual values being as a rule somewhat higher. Thus they, compared with the corresponding crude fibre figures, reveal the very violent manner in which the treatments of the crude fibre determination attack the vegetable cell wall.

Especially striking is the low crude fibre value of cork, a result of the disintegrating effect of the boiling with alkali. A considerable part of the margin between membrane substances and crude fibre may, as a rule, be covered with the alkali-soluble fraction of lignin. The fact that the said margin in spruce wood is relatively narrow depends on the resistance of coniferous lignin against diluted alkali (see PALOHEIMO, 17).

It is obvious that *our method is to a certain extent conventional.* As the 0.05 normal acid is capable of dissolving a portion of the cell wall substances during a half an hour's boiling, it is to be expected that a doubling of the acid concentration or of the duration of the boiling will result in a lower yield compared with the result obtained by the conventional boiling procedure. Table 4 shows, however, that lengthening the duration of the boiling with half an hour does not appreciably reduce the yield; neither has the doubling of the acid concentration any great effect on the result of analysis, as seen from table 5. It is especially remarkable that a prolonged boiling appears to result in a rather slight increase in the amount of dissolved pentosanes of clover grass and wheat bran (see table 3). As the cell wall substances of the wheat bran as a whole evidently are exceptionally sensitive to the boiling with acid, it remains an interesting problem to solve what are those sensitive components.

Obviously the size of the sample to be boiled in 400 ml of acid solution is not without importance. Therefore we have arranged comparative experiment's with

Table 4. Effect of the duration of boiling with 0.05 normal acid.
(The figures are percentages on dry matter basis.)

Material	The protein-, ash-, and fat-free residue after boiling with 0.05 normal acid	
	½ hour's	1 hour's
Oat straw	73.8	69.9
Alopecurus pratensis	62.9	59.5
Palm kernel cake	51.7	51.7
Alfalfa	48.8	48.5
Trifolium repens	43.1	40.3
Coconut cake	32.2	31.5
Red clover leaves	30.7	29.0
Wheat bran	23.0	19.0
Linseed cake	22.9	21.8
Wheat bran I	21.5	16.5
Cottonseed cake	17.4	16.5
Sunflower-seed cake	19.9	15.5
Carrots	12 8	12.2
Rutabagas	12.7	12.0
Red beets	10.7	9.2
Fodder beets	9.9	9.1
Potatoes	3.9	3.6

Table 5. Effect of the raising of acid concentration from 0.05 normal to 0.10 normal.
(The figures are percentages on dry matter basis).

Material	The protein-, ash-, and fat-free residue after ½ hour's boiling in acid solution	
	0.05 normal	0.10 normal
Oat straw	73.8	68.7
Wheat bran III [1]	31.5	25.7
Red clover leaves	30.7	29.3

air-dried samples of 1, 2, and 3 g. The results which are seen in table 6 show that the method is not very sensitive to such variations.

From the results of the last-mentioned experiments also the conclusion can be drawn that the pH of the acid solution may not be essentially influenced by the small sample of vegetable material. This circumstance the authors have directly studied only with a sample of young red clover grass. While the blind test gave the pH value 1.27, the solution with 1 g of air-dry grass sample gave the value 1.33. A sample boiled with 400 ml destilled water only gave the value 6.62.

[1] A part of the starch removed by washing with cold water.

Table 6. Effect of the size of the sample to be boiled with 400 ml 0.05 normal acid. (The figures are percentages on dry matter basis.)

Material	Air-dried sample of		
	1 g	2 g	3 g
Hay, mixed	49.8	50.5	—
Wheat bran	22.1	22.1	—
Barley corns, with hulls	18.7	18.6	—
Spinach	18.0	—	18.4
Cabbage, head without stem	14.0	—	14.6
Apples, fruit flesh	6.3	—	6.5
Potatoes, peeled	2.8	—	3.1
Wheat flour, Graham	4.8	—	4.7
Wheat flour, white	0.5	—	0.8

Table 7. Solubility of crude protein by the boiling with 0.05 normal acid.

Material	Crude protein, dissolved %
Trifolium repens, leaf stage	33.7
Alfalfa hay	49.3
Rye straw	40.4
Cabbage	75.9
Carrots	78.8
Potatoes	92.3
Apples	93.8
Wheat flour, Graham	82,5
Soya meal	70.1
Cottonsead meal	89.7
Aspergillus niger	20.9
Yeast, baker's	100.0
Pseudomonas fluorescens	98 2
Bacillus subtilis	100.0

The importance of quick filtration has already in a previous chapter been pointed out. The fulfilment of this requirement seems not cause any trouble, for all materials analyzed by the authors have given by boiling with 0.05 normal acid readily filtrable mixtures. Nor has the boiling with acid met with any noteworthy difficulties.

The protein correction, which is an essential point in our method, contributes in many instances to the inaccuracy of the results. The authors have conventionally, and certainly erraneously, assumed that the nitrogen content of the crude protein of all materials analyzed, as well in the fraction dissolved by the 0.05 normal acid as in the fraction of the residue, is the same, viz. 16 %. In fact these circumstances are rather unsufficiently known and we have no idea especially of the possible differences in the nitrogen content of the nitrogenous compounds of

the two fractions named above. The fraction of crude protein soluble in the 0.05 normal acid during a half an hour's boiling is greatly variable depending on the botanical nature of the sample and also on its degree of fineness. Table 7 shows the percent solubility of the crude protein of some vegetable materials of different type. (It is worth to remember that the filter used by the authors was rather coarse.) Usually the error rising from the protein correction is relatively the smaller the greater the ratio membrane substances/undissolved protein. In cases where the matter to be analyzed contains chitin substances it is perhaps advisable to omit the protein correction.

It is obvious that all errors attached to the different determinations belonging to our system of analysis have their effect upon the percentage of *»easily soluble carbohydrates»*. To these errors belongs also a conventional error connected with the determination of lipids: the sum of the preliminary extract obtained with ether and of the after-extract obtained with ethanolbenzene is always somewhat smaller than the total of lipids because a part of lipids falls into the filtrate after boiling with acid. It is also evident that omitting the nitrogen of the lipids causes an error in the percentage of the total crude protein. This circumstance as well as the conventional errors attached to the determination of ash are universally known.

Conclusions.

In the preceding chapter the authors have criticized rather strictly their own method. Its defects should, however, not be exaggerated. Compared with the Weende crude fibre estimation method and with its modifications the new method has the great advantage that the plant substances are fractionated by it, if not exactly, at least rather accurately, into histologically natural groups. It helps to give a correct picture of vegetable materials and offers a rather useful basis for a deeper going and more detailed analysis.

Summary.

The authors refer to the chapter »The principle and general features of the new method» and to the »Conclusions».

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SELOSTUS.

KETTOAINEIDEN KOKONAISPALJOUDEN MÄÄRITTÄMINEN KASVIKUNNAN TUOTTEISTA.

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Kotieläintieteellinen laitos, Helsingin yliopisto..

Käytännössä oleva weendeläinen raakakuidunmääritysmenetelmä on kovin sovinnainen ja luonnontieteellisesti epäpätevä. Karkein puutteellisuus tässä menetelmässä lienee, että pääosa ligniniaineista, jotka muodostavat ruoka- ja rehuaineiden ravinnollisesti negatiivisimman osan, liukenee kuitumäärityksen käsittelyissä ja joutuu siten typettömien uuteaineiden joukkoon yhdessä sokerin ja tärkkelyksen kanssa. Tekijät ovat suunnitelleet menetelmän, jolla kasvikunnan tuotteisiin sisältyvien kettoaineiden kokonaispaljous saadaan melko tarkoin määritetyksi. Tähän menetelmään kuuluu: 1) rasva-aineiden poistaminen tutkittavasta näytteestä, 2) näytteen keittäminen runsaassa määrässä 0,05 normaalista suolahappoliuosta, 3) happokeitossa liukenematta jääneen osan eroittaminen siivilöimällä, 4) liukenemattomaan osaan sisältyvän raakavalkuaisen määrittäminen rasvattoman näytteen raakavalkuaismäärän ja happokeitossa liuenneen raakavalkuaisen eroituksena ja 5) liukenemattoman jäännöksen hehkutuskevennyksen määrittäminen. Kun hehkutuskevennyksestä vähennetään siihen sisältyvä, edellä esitetyllä tavalla laskettu raakavalkuaisen määrä, saadaan selville kettoaineiden paljous. Saadut kettoaineprosentit ovat useimmiten 150—200 % korkeammat kuin vastaavat raakakuituprosentit.