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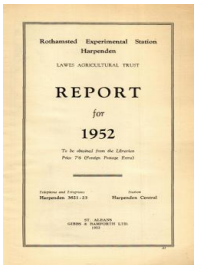
Full Table of Content



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N. W. Pirie

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LARGE-SCALE PRODUCTION of EDIBLE PROTEIN from FRESH LEAVES

By

N. W. PIRIE

The study of plant viruses is the study of leaf protein; for not only are all the known viruses proteins, but to purify them it is necessary to separate large amounts of normal leaf protein from the preparation. Since 1934 we have been engaged in this work and, during the succeeding years, have become increasingly interested in the proteins of the normal leaf. In part this interest was stimulated by war-time food shortages which made it important to see whether leaf proteins could be used as human food and in part it was the result of a recognition of their intrinsic biochemical importance. The study of animal viruses was preceded by a fairly detailed knowledge of the properties of animal proteins and the behaviour of tissue extracts, but during the early phases of work on plant viruses much more was known about the soluble proteins of the infected tobacco leaf than about those of the normal leaf.

The idea that extracted leaf protein could be of nutritional and industrial importance is not new (c.f. Pirie 1942 a and b) and when work, in collaboration with the Food Investigation Board and Imperial Chemical Industries, started in 1940 there was some past experience to build on. Several patents covering leaf-protein preparation had been taken out, some of them dealing with phenomena that have been well known since the pioneer studies of Rouelle* in 1773, but all the experience had been gained on the laboratory scale. No method had been worked out for handling more than a few pounds of leaf and it seemed likely that the conversion of laboratory-scale extractions and fractionations into a large-scale process would prove difficult. It was easy enough to see what we were trying to do; the only problem was how to do it.

During 1940 and 1941 therefore, a series of extraction tests was made with full-sized mills of many types. At first these tests were based on the crudest empiricism but a few principles soon began to emerge. On the one hand significant amounts of protein are not brought out of the leaf by simple pressure, but on the other hand it is not necessary to open each cell. Fine subdivision is indeed, a disadvantage because it is more difficult to separate leaf fragments from the dispersed protein the smaller the fragments are. Some subdivision, coupled with intimate rubbing and bruising of the leaf, releases much of the protein, and the rubbing is done as well by rubbing leaf on leaf as by rubbing the leaves between two elements of the machine. As in most large-scale operations the process should be continuous both in theory and practice. The distinction

* Those who have worked on leaf protein recently have not been deeply interested in the history of science and have not devoted much attention to Rouelle nor even given his initials. I have in the past erroneously attributed this work to G. F. Rouelle but it was, in fact, done by his younger brother Hilaire Marin who succeeded to the demonstratorship at the Jardin du Roi in Paris on the elder Rouelle's death in 1770.

https://doi.org/10.23637/ERADOC-1-74

is important, for some mills seem to be working continuously when they are, in fact, filling up with resistant pieces of fibre, and this may not be recognized on short runs. The mill must cope with occasional stones for these will inevitably accompany agricultural crops. Power consumption must be kept down both for economy and to avoid over-heating the charge. Unless a wasteful cooling system is to be used, it is clear from first principles that the limit comes at about 50 HP for a grinding rate of 1 ton of wet crop per hour. Preliminary drying or adding large amounts of water to the crop are both to be avoided if possible.

This much became clear by 1942 but then work along these lines was stopped. The reasons for this decision were never made clear; it is not, therefore, possible to express an opinion on their validity. At Rothamsted, however, work continued on the laboratory scale and the results were systematized by Crook (1946) who finally managed to extract 95 per cent of the protein in tobacco leaf by very fine grinding and by maintaining mild alkalinity and low salt concentration. Crook and Holden (1948) and others at Rothamsted, using similar techniques, have separated protein from about thirty different species of leaves in varying yield, and we now have enough experience to be able to tell from the appearance and feel of a leaf what its protein content, and the extractability of that protein, is likely to be. This work was done with the idea of large-scale extraction directly in mind, but much of the other work of the Biochemistry Department also gives information about the separation and fractionation of proteins from the leaf. Work on viruses and on pectase, protease, cellulase, normal nucleoprotein and enzymes concerned in the oxidation of manganese has been described in successive Annual Reports; we have also described the action of commercial proteolytic enzymes on leaf fibre and the effect of fertilizers on the protein content of the leaf.

Protein is held in many different ways in the leaf and when one particular leaf enzyme is being studied selective methods of extraction are an advantage. When, however, a bulk protein preparation is wanted it is an advantage to get all out in one operation. Protein is held in the leaf in three main ways. It may be dissolved in the fluids liberated when the cell structure is damaged by grinding; it may be present in the chloroplasts, nuclei and other microscopically recognizable cell components; it may be in the cell walls. The intensity of grinding will influence the composition of the mixture by varying the extent to which these components are released. The nutritional and physiological state of the leaf will also effect the composition of the isolated protein, because they affect the ratios in which some different enzymes occur (Holden and Tracey 1948) and it is reasonable to assume that the protein is largely made of enzymes.

For practical purposes a protein may be said to be in solution if it does not settle out under gravity in a few hours. Much of the protein in a leaf extract is soluble initially but coagulates after a few hours at room temperature. Many actions are probably involved in this coagulation; some proteins are so associated with enzymes as to be intrinsically unstable (Pirie 1950) some are probably clotted, as milk is, by leaf proteases (Tracey 1948), while some combine

https://doi.org/10.23637/ERADOC-1-74

with tannins and other leaf components and precipitate slowly. Changes of this type probably also go on quickly and may be responsible for some of the readily sedimentable protein in extracts. Chloroplasts and chloroplast fragments are easily separated from the fibre of many leaves and these may make up the greater part of the readily sedimentable protein in some extracts. In some leaves, however, the chloroplasts do not readily separate from the leaf matrix and in some, precipitation by tannins is so rapid that part of the protein remains in the fibre. Holden and Tracey (1950) have discussed the necessity for assuming that any significant amounts of protein are held in the cell walls. They found that the ratio of nitrogen to chlorophyll is nearly the same in isolated chloroplasts and in washed tobacco leaf fibre. There is no reason to think that protein in the cell wall would be associated with chlorophyll. It is probable, therefore, that most of the introgen remaining in the fibre is present as entangled chloroplasts and chloroplast fragments. Suggestions have been made that part of the lignin of the leaf contains nitrogen, but the total amount that is held in this way is small.

In 1948 a grant from the Agricultural Research Council enabled large-scale work to start again and the survey of existing machinery was continued. Ten different designs of swing-hammer mill were tested under varying conditions and the conclusion was reached that this method of grinding was not suitable because it depends on impact between an unsupported particle and the moving hammer. Wet leaves are not shattered by this type of impact. Designs in which a compacted mass is rubbed or has bars forced through it, as in the domestic meat mincer or the screw expeller, are satisfactory on a small scale but, because the ratio of surface to volume changes when the scale increases, they consume excessive amounts of power when the scale is increased. The idea of continuous rubbing however, was attractive, so having found that none of the existing mills would handle the soggy dough-like mass that results when fresh leaves are ground, a mill was designed that cannot clog and is adjustable to the texture of the material being used.

The basis is a Christy and Norris " coir sifter," designed to separate coconut husk from fibre, and is a drum 4 ft. 6 in. long and 3 ft. in diameter with an axial shaft carrying plain rectangular beater arms. It was fed tangentially at one end and discharged radially at the other. Now it is fed axially at one end and discharges tangentially at the other, many more beater arms have been introduced, so that no space inside the drum more than $\frac{3}{4}$ in. wide is left unswept by an arm, and the arms have been modified so that some have propellor-shaped ends and the others U-shaped ends. By varying the ratio of these two types, the rate of movement of the charge through the machine can be controlled to get the correct amount of grinding. There is no obstruction at the exit; material comes out whatever its state of grinding when it has traversed the mill. This is an important distinction from most types of hammer mill because the charge generally has to stay inside until it has been ground fine enough to get through a screen. Ground leaves soon choke a screen.

The primary merit of this machine is that it works and has run for many hundreds of hours at the Grassland Research Station

https://doi.org/10.23637/ERADOC-1-74

without a breakdown. But it is a makeshift and the next one should be designed from the beginning rather than adapted from an existing machine. It should be smaller, it should be so arranged that it can be opened and cleaned easily, and it should be easy to rearrange the distribution of the two types of beater arm. I am confident that the basic principle is sound and, having made and tested 10-15 types of beater arm, that the beaters are the simplest possible. When fed with succulent crops it handles 4-6 tons an hour and takes 10-20 HP, but the rate of working falls and the power consumption rises with drier crops. Good grass goes through at 1 ton an hour and consumes 20 HP, grinding becomes more extravagant than this only with crops that are so dry and mature that protein extraction is unsatisfactory even in the laboratory.

In theory the amount of work that has to be done in grinding a mass of leaves is extremely small, so that in the earlier phases of this work there was always the hope that a much more efficient arrangement might be found. So far nothing has made it seem likely that this will prove possible and all the other arrangements consume more power. Tracey has carried out some (unpublished) experiments in which a weight was dropped on to 20 g. lots of grass and the percentage of protein liberated was measured after different amounts of work had been done. Satisfactory liberation required 6×10^9 ergs. If this could be replicated on a large scale, it would mean that a grinding rate of 1 ton per hour would take a little over 10 HP, which suggests that stamping mills would merit more thorough investigation than they have yet received.

In this connection it is interesting to consider what success animals have had in solving this problem. The bullock grinds grass with its teeth and tongue. Figures for its performance are somewhat approximate, but the ones given have been chosen so as to favour the efficiency of the bullock rather than the reverse. The jaw muscles of an 11 cwt. animal weigh 5 lb. and its tongue also weighs 5 lb. This weight is not all muscle used for chewing, but we will assume that it is and also that its rate of working is 0·01 HP per pound. This is the rate that Gray (1936) found for the strenuous conditions of dogs running on a treadmill and men rowing; it is, therefore, certainly a greater rate than would be compatible with the placid expression of a chewing bullock. We may be sure, therefore, that not more than 0·1 HP is being expended during 8 hours in which it collects grass and chews it roughly and the further 8 in which it chews the cud. After this the mass has about the consistency at which we aim for satisfactory protein extraction. The bullock eats 30 lb. dry matter or 150 lb. of fresh grass during the 16 hours, so that its 0·1 HP machinery is handling material at 9·4 lb. an hour. To get a rate of 1 ton per hour by replicating the same machinery we would need $2{,}240 \times 0{\cdot}1/9{\cdot}4 = 24$ HP. The actual rate of working may be only half this but it would seem that the course of evolution has not produced a mechanism much more efficient than our hasty adaptation.

The problem of pressing the juice from the ground leaf mass does not seem to be so nearly solved. On the laboratory scale it is easy, and on a large scale it is also easy if small molecules are the only valuable components of the extract. But much of the leaf protein

https://doi.org/10.23637/ERADOC-1-74

is present as particles up to 5μ in diameter and such particles are easily held back by tightly compacted masses of fibre. Arrangements that expose large bulks of material to high pressures are not therefore well adapted to our purpose. Moderate success has been achieved with a machine made at the National Institute for Agricultural Engineering. This has a perforated steel drum which is supported on three rollers inside it. Opposite each roller a larger wooden roller presses the drum on the outside, these outside rollers are driven and carry the drum around by friction. Ground leaves are fed on to the outside of the drum and are carried by it under each of the three wooden rollers in turn so that at each nip juice is pressed through the drum and into a tray inside. With this simple arrangement many tons of juice have been made but it is difficult to keep the layer of material on the drum even and the time during which pressure is applied in passage through the nips is too short for the juice to run away effectively.

Juice is so easily pressed by hand from minced leaves enclosed in a cloth and it is so easy, by continual hand pressing, to get a product containing only 65 per cent of water, that it is tempting to underestimate the problem of large-scale juice extraction. The pressure applied by hand is only about 30 lb. per sq. in., but it is maintained for many seconds, the charge is continually being rearranged, so that new parts are brought near the filtration surface by finger action. This is not an action that it would be easy to simulate with a machine, but it should not be needed if the thickness of the layer being pressed is kept small. With this in mind, new designs for a press are being discussed and in them three principles stand out clearly ; the layer, after pressing, should not be more than $\frac{1}{4}$ in. thick; pressure should be maintained for a few seconds ; there should be no movement between the charge under pressure and the filtration surfaces. There are so many ways of achieving these desiderata that we can be confident of success as soon as sustained work on the problem starts.

Any robust press designed to work quickly will allow some leaf fragments to pass through into the juice, so that a further stage of straining is needed before the protein can be separated. This presents no difficulties. The protein is then coagulated by heat or by adding acid. With many batches of juice, acid gives the better yield ; it also gives a purer product but one that is more difficult to handle because it is finely divided. If heat is applied rapidly with live steam, the curd is coarse and easily filtered off. From this stage on the protein is handled by normal chemical engineering methods. So far the substances remaining soluble in water after heating or acid coagulation have been discarded but, as is well known, the leaf at various stages in its growth contains valuable amounts of carbohydrate, and non-protein nitrogen part of which appear in this juice. It is therefore, essential that methods of using it should be explored.

By this sequence of grinding and pressing it is easy to get out in the form of protein a quarter of the nitrogen in leaves containing more than 2·6 per cent of nitrogen and possible, by rewetting the pressed mass and pressing again, to get out a third. The yield is lower than that reached in the laboratory but this is to be expected.

https://doi.org/10.23637/ERADOC-1-74

The remainder of the nitrogen is either soluble or else it remains as unextracted protein in the pressed residue. There would be obvious advantages in getting this out also; the question of how much it is economic to extract depends simply on the costs of extraction compared with those of growing more leaves. Further grinding is an obvious step but as already mentioned it has defects. We have made a fairly through study of the enzymic degradation of leaves (Holden, Pirie and Tracey 1950), mainly because of an interest in the liberation of viruses, but also with the application to protein extraction in mind. The enzymes used were juices or extracts from snails and various fungi ; these would hardly be practical for large-scale use but the work showed that leaf residues were easily digested. In practice it would be easiest to seed the mass of leaf residue with a culture of a cellulase producing micro-organism and to let growth and fibre digestion proceed together. Hitherto cellulase has been an unreasonably neglected enzyme, but during the past few years it has begun to get the attention that its academic and practical interest warrants.

Protein which it is not economic to extract from the fibre will not, however be wasted. Cattle eat the residue readily, both when it is fresh and after drying, and it is very easy to ensile. The idea of drying it as winter feed is particularly attractive because although the nitrogen content is generally only 1 ·5 to 2 per cent, it is economical to dry and is satisfactorily handled by a rotary drier. Normal grass drying is not the unqualified success it was expected to be and one reason is that the protein content of a leaf is approximately proportional to its water content. The more worthwhile it is to make the dried product, therefore, the more water has to be dried off to get it. Thus really good leaves with 4 ·8 per cent of N on the dry matter may contain 93 per cent of water when cut, whereas those with only 2 ·4 per cent may contain only 75 per cent of water; to get a ton of dry matter from the former necessitates drying off 13 tons of water and from the latter 3 tons. The former is an extreme case ; much of the dried " grass " at present being made in Britain is of the low quality of the latter. But if there were that extension in the use of fertilizers and irrigation water that is widely, and rightly, advocated, much more of the material coming to the driers would have such a high nitrogen and water content as to make drying doubtfully economic. Many proposals have been made for resolving the dilemma that the better the technique used in growing a forage crop the more expensive it becomes to dry it. To them we may add the proposal that the crop should first be processed to get out much of the protein and most of the water, so that only the residue containing about 65 per cent of water, would be dried. The proposal is that protein preparation should be a supplement to grass drying. It is easy to make a rough estimate of the protein and water content of a batch of leaves visually, and each load that arrives at a processing station should be sent straight to the drier if it is of low quality but should be used first for protein production if it is of high quality.

One of the difficulties encountered in the introduction of modern agricultural methods into undeveloped areas is the lack of power to run tractors and pumps. The residue of leaves from which protein has been extracted might be a valuable fuel. It could be used either

https://doi.org/10.23637/ERADOC-1-74

directly or after fermentation to give alcohol or methane ; the first course is simpler and more economical and the granular texture of the residue as it comes out of a press make it much more suitable for mechanical handling than the other agricultural wastes with which it is sometimes proposed that furnaces should be stoked. Research is already going on on the design of engines to run on low grade fuel ; it would seem that this is one of the fuels that should be tried.

The advantage of developing these techniques for separating protein from leaves depends on three propositions : That the leaf is the best place to look for further supplies of protein : That the protein and other components of the leaf are of more value to us after they have been separated from each other than they were when they were mixed : That there is no better method of making the separation. There will be little argument about the first, all the terrestrial protein sources now used on a large scale, e.g., beans, meat and milk depend on the leaf. Yeasts and some other micro-organisms can make protein directly from ammonium salts or even from atmospheric nitrogen and fish depend mainly on algæ and unicellular plants, but these sources should be looked on as complements rather than as alternatives to leaf protein. The only unusual feature of leaf protein separation is the intimacy with which the useful and the less useful parts are mixed in the starting material. In principle it is comparable to such well established separations as grain from chaff, oil from oil seeds and sugar beet tops and crowns from sugar beet. These separations are well known to be advantageous because by them the value of at least one of the products is enhanced. If leaves are to be used as a source of protein in the human diet, the only alternative would be to grow leaves with an exceptionally high protein content. Research on the conditions needed for a plant to produce high protein leaves regularly and a search for the species that can be easily made to do this would be both interesting and valuable, but for some time it is likely to be easier to grow a lower quality leaf and then to separate the digestible protein from the indigestible fibre. These are all issues that have been argued at greater length elsewhere (Pirie 1951, 1952, 1953).

Under existing conditions there are two main ways in which the separation is brought about. In the plant, the growth of seeds and tubers entails the translocation of protein, so that the fibre remains in the sere leaf and the protein appears in a digestible form along with fat and carbohydrate. Ruminant animals also separate the protein for us when they feed on leaves. Each process involves waste and the waste is especially great with animals because their value as sources of concentrated protein is a consequence of the fact that they are even more wasteful of carbohydrate than they are of protein. It is this fact that enables a bullock to lay down meat containing 60-70 per cent protein, in terms of dry matter, when fed on a diet containing only 10 per cent. Few will dispute that the products of animal conversion have more gustatory appeal than the products made from leaves are likely to have in the near future, but culinary enterprise can often bring about surprising changes. Furthermore a policy of leaf-protein production would probably not diminish the amount of food available for animals. First, there would be low-grade batches of protein suitable for pig and chicken

https://doi.org/10.23637/ERADOC-1-74

food ; second, there would be the leaf residue suitable for cattle food, and third, leaf protein production depends on the growing of high quality leaf. With leafy crops the improvements of quality by manuring, irrigation and frequent cutting would be accompanied by a total increase in yield both of protein and dry matter. This increase, it is true, could be achieved without the further step of making leaf protein, but the idea has never proved particularly attractive because such intensely cultivated crops tend to have too high a protein content for any but the most productive milking cows.

These advantages have naturally not escaped general notice and several commercial projects for making protein or protein concentrates have been started. Information about these projects is not always easily obtained but they seem to have the common defect that an attempt is made to get out the protein in one operation and to use one machine for all types of leaf. In practice the attempt does not succeed and the crop is passed several times through the same machine. Rollers and oil expellers or modifications of them are most commonly used. There seems to be no advantage in passing the charge through the same machine twice rather than passing it successively through two machines, or even three, each designed for the particular job to be done. *A priori* it is unlikely that a machine which is efficient at grinding leaves would also be efficient at separating the juice from the ground mass. All the evidence from our own work and the work of others suggests that it is better to start with an adjustable mill that can produce an approximately standard product from a wide range of raw materials, and then to feed this product into a press. Research is still needed until a workable unit has been built. This work can with advantage be done by anyone who knows the starting material, knows the aim, and has the enthusiasm to do it. The results will bear the same relationship to the final design that Trevethick's steam engines bear to those used now and the metamorphosis will call for the most expert available engineering skill, but some sort of working unit is a necessary first step.

Besides the work that is needed on the machinery there is also much scope for botanical and agricultural work. First there is the choice of crop. Hitherto a forage crop has had to have a texture and flavour acceptable to stock. A mill is less exacting and opens up wide botanical possibilities. Most of the necessary research to find which plants give the biggest return of dry matter and extractable protein per acre can be done on small scale plots. But once some conclusions have been reached they need confirmation by large-scale extraction, partly to confirm the laboratory results and partly to get enough protein to be sure that it has the expected feeding value. It may be that no crops better than those already in use, either in Britain or overseas, will be found at an early stage in the work but the standard crops offer much scope for variation. The effect of fertilizers and irrigation is already being actively studied at Rothamsted; this work could usefully be supplemented by a study of the extractability and quality of the protein. All the leafy agricultural wastes also need examination as do plants such as bracken and sedges that grow on uncultivated areas.

Work on the large-scale extraction of leaf protein has now been

https://doi.org/10.23637/ERADOC-1-74

going on in an uncertain manner with support from various Government departments for thirteen years. It seems to have got to a stage at which, with little more effort, a conclusion could be reached and machinery designed which would be suitable for use both in Britain and overseas.

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