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Pirie, N. W. 1967. Leaf protein research in the International Biological Program. *Agricultural Science Review*. 5 (4), pp. 17-21.

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LEAF PROTEIN RESEARCH

In the International Biological Program

N. W. PIRIE

DURING our lifetime the higher plants will probably become the main source of the extra food that the world needs. Algae, in normal circumstances, offer no advantages. Micro-organisms will be valuable for converting various wastes and by-products into food, and they will be especially useful if the suggestion that they can be grown in edible form on coal and oil proves well founded. Chemical synthesis is not likely to be cheaper than agriculture as a method of getting protein or carbohydrate.

The food plants that are now grown extensively were all known to primitive man. They were chosen primarily because they gave some product—a seed or a tuber—that could be stored so as to tide over periods of drought or cold weather. With modern techniques of storage, this limitation no longer operates so strongly and choice can be wider. This means that leaves and immature flowers, which at present serve mainly to make meals more interesting rather than more nourishing, could play a larger part. Nothing but habit keeps this from being done in Europe and the United States. But in many parts of the tropics, the familiar vegetables do not grow well. Immigrants to these parts tend, therefore, to depend on imports, and the original inhabitants eat very little fresh vegetation.

Three courses could be followed to overcome this situation: (1) More vigorous efforts could be made to introduce the vegetables already used in South China and parts of Indonesia; (2) a wider range of temperate varieties, especially some varieties not found suitable in temperate regions, could be tested in the new environment to see whether they would flourish in it; and (3) selection and breeding could be started on the local plants. The last is the most interesting approach. It involves doing quickly with modern techniques the job that took centuries by the hit-or-miss methods of the mediaeval gardener. These things will indubitably be done but, even under ideal circumstances, vegetables are likely to supply only 2 to 5 percent of a person's daily protein intake. They do, of course, supply other nutrients besides protein. But protein deficiency is the world's most widespread dietary fault, so that attention may usefully be concentrated on it.

Leaves are the primary source of almost all our food but, because of the amount of fiber in them, they can be eaten only sparingly by people. Hence most plants are allowed to go on growing till protein has moved out of the leaves into seeds and tubers. Or they are fed to animals. We are accustomed to these altered forms and relish them, but

movement or conversion wastes time, or food, or both. As soon as this is realized, the advantages of making food from leaves directly by mechanical processes are obvious. This is a logical extension of well-established processes such as the extraction of sugar from cane or beet, and oil from olives or soya.

Work on the extraction of edible protein from leaves started 30 years ago; machinery suitable for large scale extraction was designed 20 years ago; and 10 years ago reasonably satisfactory units were working at Rothamsted. There are units—either made at Rothamsted or based on our designs—in India, New Guinea, Nigeria, Uganda and the United States. Machinery working on a more elaborate variant of the process was in use in Israel. Nevertheless, considering the potentialities of this method of making protein concentrates, especially in the wet tropics, the scale of research is still extremely small. The total effort devoted to research on leaf proteins is only a tiny fraction of that devoted to the preparation of palatable products from oil-seed residues, fish, yeast, algae and other microbial materials. This disparity in the scale of research should be born in mind when the quality of the different end products is being compared.

The extraction process is extremely simple. It is summarized in Table 1 and its historical development has been given in some detail elsewhere (13).¹ For large-scale (0.7 to 1.5 tons wet weight per hour) work we use a 25 h.p. pulper (2) that delivers the pulped leaf to a 1 h.p. press (4) which extracts the

protein-containing juice. Seventy percent of the protein in young lush leaves can be extracted, and these soft leaves are obviously the easiest to pulp. We also use a smaller machine (3) that pulps 100 to 200 kg. batches of leaf and presses out the juice at the same time. Protein is coagulated by heating the juice quickly to 70° C. or higher (12); it is then filtered off in conventional equipment and washed. At this stage, it is a dark green cake with the consistency and perishability of cheese. We generally use protein in this form for cooking, but it can be preserved by drying, salting, pickling, or canning, and it can be extracted with solvents so as to make a gray-brown powder.

Feeding trials on pigs (6), rats and chicks (5, 16) infants (15), and rats (7)—all show that leaf protein undamaged through inappropriate drying methods has a food value as great as that of fish meal or the best seed proteins but less than that of casein or egg protein. Food value does vary, however, depending on the species and age of the leaf (7), for which no fully satisfactory explanation has as yet been offered.

Need for International Cooperation

WORK on leaf protein had reached this stage by the end of 1963, when, after a somewhat hesitant start which I have discussed elsewhere (14), the decision was formally taken to establish the International Biological Program (IBP). It seemed, for three reasons, that international cooperative research on leaf protein would be a suitable facet of work within the IBP.

¹ *Italic numbers in parentheses refer to Literature Cited, p. 21.*

TABLE 1.—*Pulped green leaves are separated into:*

Juice which gives after coagulation	{ coagulum containing fluid containing	{ proteins fats starch amino acids amides sugars salts, etc.	{ Food for man and other non-ruminants. Medium for the growth of micro-organisms.
Fibrous residue containing	{ most of the some of the	{ cellulose hemicelluloses lignins pectin proteins fats starch	{ Still a fodder for rumi- nants and a substrate for microbial fermenta- tion.

1. The need for new sources of protein is most acute in countries with little capacity for doing the research and development needed before protein can be produced from novel sources.

2. Interesting research on plant physiology and agronomy is involved in the husbandry of the crops from which leaf protein would probably be made.

3. The presentation and acceptability of any novel food should be studied in as many environments as possible.

The International Sectional Committee for "Use and Management of Biological Resources" (UM) agreed in 1965 that the project was relevant to the IBP (8, 11) and it was one of the themes discussed at an IBP/UM Working Group meeting on "Novel Protein Sources" in 1966 (9).

Early in 1966 the Royal Society, acting on the recommendation of the UK National Committee for the IBP, made a grant for the design and manufacture of equipment with which the extractability of leaf protein could be measured on a laboratory scale in a consistent and standardized manner. This procedure is a necessary step in Phase I of the IBP, which is concerned with design and feasibility, for there is no reason to think that all the apparent differences in the extractability of protein from different plant species are real and not the consequence of the use of widely different extraction methods.

Protein Extraction

THE extraction of leaf protein consists of two processes: the disintegration of the leaf, and the expression of the protein-containing juice. A domestic meat mincer, either hand or power driven, has been used for so many years for making leaf pulps at Rothamsted and in other laboratories that we decided to use it in the first instance and study the expression of juice. It is already well known that the conventional screw press is not suitable; the mass of pulp in it is so thick that the fiber acts as an ultrafilter and holds back protein as soon as any significant amount of pressure is applied. Most of the measurements made hitherto have depended on hand squeezing in a cloth and they have established the broad outlines of our knowledge of suitable plants from which to make protein. There is, however, little prospect of getting consistent results with hand squeezing. There are uncontrolled variations in the amount of pressure applied, in the rate at which it is applied, and in the extent to which



the pulp is rearranged within the cloth during extraction.

When protein is being made on the large scale, the pulp is pressed between a smooth belt and a perforated roller. There is no differential movement between these two and the layer of pulp after pressing at 1.5 to 2.0 kg./cm.² is about 5 mm. thick. Our press simulates these conditions and so should give results comparable to bulk production. We are well satisfied with the repeatability of the measurements made with this press. However, having got consistent pressing results, we are becoming increasingly dissatisfied with the domestic mincer as a means of making the initial pulp and we have begun to design a pulper that will, on 2 to 3 kg. samples of leaf, simulate the action of the large machine. If our grant is renewed in Phase II of the IBP, this pulper will be built.

Yields and Sources

EXPERIENCE with large scale extraction, accumulated up to 1964 (1), made it clear that 1 ton of extracted protein could be obtained from 1 hectare in 1 year at Rothamsted. Since then we have raised the yield to 1.3 tons; in India it is 3 tons because of the longer growing season. This, although a considerably greater yield of edible protein than can be got in any other way, is only the beginning. We are using those crops with which farmers are already familiar such as wheat, barley, rye, rape, tares, maize, kale, clovers, etc. These crops have been selected because of many qualities other than the ability to give a good yield of extractable protein. In Phase II of the IBP we hope that several institutes, in different climatic zones, will get laboratory-scale equipment similar to ours and make systematic measurements on different crops, harvested at different stages of maturity and after different fertilizer treatments, so as to work out a system of agronomy adapted to leaf-protein production.

Ideally, the leaves used would be the byproduct of some other form of production—cotton, jute or sugar—or would be unused weeds floating or growing round the edges of bodies of water where they could be mechanically harvested. Such plants would provide something for nothing but there would be little control over the quality of the starting material. In that way, an existing waste or the growth on extensive unexploited areas of water and swamp would

be used. Less extensive areas of little used land lie along the margins of seasonal lakes and rivers. These often remain moist too briefly for them to carry a conventional crop through to normal harvest. They would be useful as a source of such leaves as can be harvested a month after sowing. Green manures and cover crops are already grown extensively; they are incompletely or inadequately used because, in hot climates, most of the protein in them is destroyed and denitrified by soil micro-organisms.

Without wishing to belittle the importance of these crops as a means for preserving soil texture, it may be suggested that the leaf residue after protein extraction would probably serve as well as the intact crop. This is a matter for experiment. In all these examples, custom or the physical condition of the area used for growth controls the species of plant that would be used. More freedom of choice is given when crops are grown primarily as sources of leaf protein, though, with the subsidiary prospect that the fiber residue would form the basis of a cattle fodder. The range of species worthy of study is immense. It is not limited to existing crop plants, so that the possibility exists of developing a new style of agriculture—especially in the wet tropics. Any leafy plant that grows, or is expected to grow, exuberantly in a region is a potential protein source provided it is amenable to propagation, fertilization, and harvesting. This point is important: Ultimately, what is harvested will be what has been cultivated. This is not a method for using the sparse unmanured growth from unused land.

Future Prospects

INTEREST in leaf protein production and use was already being shown in other countries before the inception of the IBP—as the scattered distribution of extraction machinery shows. Under the IBP, research should become more systematic. At the suggestion of Professor Burström of Sweden, work on leaf protein is included in the Swedish IBP program and one of his colleagues worked with us on a grant from the UK/IBP committee. The IBP has not been long established in India, but two scientists have already come to Rothamsted on IBP grants to learn our methods, and we are making plans to start cooperative IBP projects at two centers there. Preliminary arrangements have been made for a similar project in Nigeria. We will be very willing to cooperate with any other country that wishes to start work.

Agricultural Science Review

Allocation of responsibility for different research projects within the IBP is, to some extent, arbitrary. The agronomic aspects of work on leaf protein could logically come within the compass of the section devoted to "Production Processes". A suggestion has been made (10) that a joint PP/UM subcommittee should be set up to consider the various ways in which plant proteins could be more adequately used. This committee would be a suitable parent-body to control leaf protein research. Similarly, when laboratory scale work has advanced so far as to make it reasonable to study bulk production within the framework of the IBP, intensive research on handling, presentation and acceptability will be needed. This aspect of the work has already been discussed informally with the section on "Human Adaptability" and a joint HA/UM subcommittee will probably be set up later. There is no reason why work on acceptability in any region should be delayed until production starts there. Proteins from

many different leaf species have similar properties, and supplies for the initial trials can be sent from Rothamsted.

So little work has been done on leaf protein that confident predictions about future developments cannot be made. It is possible that the enzymic methods that we use in the laboratory will be preferable to mechanical methods of extraction. It is possible that, as with fish protein concentrate, it will be necessary to subject the product to more intensive and expensive refining before it is acceptable. Furthermore, it is quite likely that some of the other novel proteins will prove so amenable to local production that they will meet all local needs. Obviously, I do not think these things probable or I would not be working along present lines. One very important advantage of gaining the interest of the international biological program in the project is that these points should soon be settled.

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