Pirie, N. W. 1972. Reserach on leaf protein and its applications. *Archivos Latinoamericanos de Nutricion.* 22 (4), pp. 507-517.

Research on leaf protein and its application

N. W. Pirie
Rothamsted Experimental Station, Harpenden, Herts. U. K.

SUMMARY

Leaves are, potentially, the most abundant source of protein.

This source can be most simply and immediately exploited by popularising green vegetables.

When vegetable consumption is accepted, protein extracted from leaves should be used as a supplementary protein concentrate - especially in wet tropical regions.

Leaf protein can be made from vegetable discards. When made from forage crops, the protein yield per hectare and year is greater than that of any other form of agriculture. The methods are well-adapted for use on a small as well as a large scale.

Leaf protein is better nutritionally than any seed protein, as good as fish though not as good as milk, and it is a useful source of β-carotene.

The fibre residue is an easily conserved fodder, and yeasts and other microorganism can be grown on the soluble non-protein leaf components.

There is so much underexploited savannah in Latin America that there seems to be no immediate need for research there on new sources of food protein. McMeekan (1) argues there are 80 million hectares of potential grazing land in Colombia and 400 million hectares unused in other regions. With better manuring, and possibly by introducing more productive strains of herbage, meat production in countries that are already large producers could unquestionably be increased. Nevertheless, populations are increasing, meat is expensive everywhere, and there are extensive regions unsuited to animal husbandry. Tropical rain forest is the largest of these and it is at present the climatic zone that is least adequately used. Discussion about new sources of protein may therefore, for brevity, be restricted to a source with potentialities in a

Recibido: 11-4-1972.

rain forest region, without implying that in all other circumstances this source would be the best.

Leaf protein was separated 199 years ago, 50 years ago its use as human food was suggested, and 30 years ago equipment suitable for largescale separation was designed and its potentialities were stressed (2). Since then much has been published, sone, from several institutions, and a comprehensive book on leaf protein (3), resulted from a conference held in India in 1970 under the auspices of the International Biological Program. This article can therefore be limited to a general account of the processes used in making and handling leaf protein, and to its quality and use, without giving references. Some material published after the conference will also be discussed.

The crop. It is not realistic to think of any starting material that could not be harvested mechanically from arable land. Hand harvesting is obviously useful in small-scale or experimental work, but leaves that could probably never be harvested mechanically e. g. from large trees or rough ground, need not be considered. Unless a leaf is soft, lush, and rich in protein, extraction is poor. Is is also poor from acid leaves, and separation is difficult from leaves that are glutinous or slimy. In spite of these limitations, the range of possibilities is enormous. Extraction is satisfactory from more than 100 species and the search for suitable species has hardly begun. Leaves that accumulate as a byproduct e. g. from sweet potato or the utside leaves of vegetables, would be used wherever they are available. The idea of using sugar cane is attractive and worth study, but the leaves are tough and contain little protein. A crop grown specially for protein production would be the usual source. Two points need emphasis. There is little advantage in extracting protein from something that is already eaten as a leafy vegetable: few communities eat vegetables to the extent that is nutritionally advisable. The first step in winning acceptance for leaf protein is to increase the prestige of leafy vegetables - I will return to that point later. The mixed, untended growth on waste land is not a reasonable source. It is inadequately fertilised, and if land is of such character that the growth on it can be harvested mechanically, it should be ploughed and reseeded. That condemnation of weeds does not extend to water weeds. They are often well-nourished, a single species tends to dominate an area of water, and mechanical harvesting from a barge would be easy.

The advantage of using arable land to produce leaf protein rather than a conventional crop is that the yield of protein is thereby greatly enhanced. At Rothamsted we can get 2 tons (dry weight) of extracted protein from a hectare in a year, in India the yield is 3 tons. This will not be the limit when skill in harvesting a sequence of crops has been aquired: the graph shows how our skill has increased. We expect to get 3 tons; in a tropical region with abundant water the yield should be 5 tons. The yield from conventional crops that produce a protein concentrate is less than a ton, and the yield from fodder converted to animal protein is much less than that.

About a third of the protein is not extracted and remains in the leaf fibre. This can be used as ruminant fodder. The soluble components of the leaf include carbohydrates and nitrogen compounds, these can be recovered in a useful form by growing yeast or other microorganisms on the effluent. The overall efficiency of the process as a means of using land to produce human food is therefore unprecedentedly great.

The process. Extraction is extremely simple. The freshly harvested crop is pulped, juice is pressed out from the pulp, protein is coagulated from the juice and washed if need be. Satisfactory pulpers and presses have been made that take from 120 to 3000 kg (fresh weight) of crop per hour. The smaller machine is intended for laboratory studies on agronomy, and for pilot-plant production. In my opinion, the large unit is about the maximum efficient size. If a greater throughput than that is called for it would be better to use several units in parallel, as is done in the vegetable oil industry, rather than make one very large unit.

Protein is most conveniently coagulated by heating the leaf extract to 70°. Quick heating is essential to form a dense curd that can be filtered off easily. There is also coagulation when the extract is allowed to stand for a day but there is then some loss of protein because of the presence of proteases in many leaves. Acid coagulates the protein but my guess is'that heating will be the most satisfactory method of coagulation; this is a matter for experiment. From most species of leaf the curd is then washed, using methods similar to those used in preparing cassava or sago, to remove the flavour of the leaf, and poisonous components if the leaf should happen to contain them. The addition of a little acid is essential at this stage to promote easy filtration and ensure complete removal of alkaloids.

At first, the protein would be used in the fresh state. Its keeping qualities are similar to those of cheese because the curd is slightly acid and contains only 60% of water. Various methods of pickling the moist product have been devised so as to give it a long shelf-life. Canning raises no problems. If the protein is simply dried in air, or in an oven at less than 90°, there is no loss of nutritional value but the product is horny, dark and unattractive. A better product can be made by drying in two stages (at this state it can be further crumbled between the fingers without smearing them green), it is then finely ground while drying goes to completion. The product is pale green and attractive.

Character of the protein. When made from suitable leaves by the method outlined, the dry material contains 60 to 70% of protein and 20 to 30% of fats. The latter are highly unsaturated. This causes trouble during preservation and storage because unsaturated fats combine with protein in a manner that makes it less digesteble and keeps some of the constituent amino acids from being effectively metabolised. These fats also undergo "oxidative rancidity". This enhances the value of products such as tea and smoked fish but it is usually regarded as detrimental in other foods. Dry leaf protein that has been stored in air slowly develops a characteristic flavour. As with tea and smoked fish, one learns to like it but, in the early phases of popularisation, the less flavour leaf protein has the better. The protein does not extract well from leaves rich in tannin or phenolic compounts. Even small amounts of these substances, though they may not prevent extraction, combine with the protein as unsaturated fats do. Differences in the extent to wich these complexes were formed during extraction or storage, probably explain the differences in nutritional value that have sometimes been observed between samples of protein with similar amino acid compositions.

It will probably not be possible, in the course of bulk production, to prevent combination between protein and phenolic compounds. That form of damage could be circumvented by selecting species and stages of growth that ensure that the leaves are relatively free from phenols. Unsaturated fats, and also the chlorophyll that makes leaf protein preparations dark green, are easily removed by solvent extraction. I hope that this will not be necessary. Leaf protein production has not only the merit of yielding an unprecedentedly large amount of protein, it is also such a simple process in essence that it could become a small-scale industry run by any community able to manage a tractor. It would lose that merit if solvent extraction became necessary.

Quality of the protein. Interest in leaf protein as a human food was engendered by early measurements of the amino acid composition. Several hundred preparations have now been analysed from many species. The analyses are similar - as would be expected from the fact that what we loosely call leaf protein is a mixture of hundreds, or even thousands, of different proteins. A leaf is able to perform many more syntheses and other enzymic processes than any animal tissue, each probably involves several enzymes, and it is excessively improbable that the same amino acid excess or deficit would appear in each of the enzymes. Individual differences will be smoothed out by the complexity of the mixture. The composition is not quite so favourable for human nutrition as that of the protein in milk or eggs, but it is as good as that of meat and fish and better than that of any of the unfractionated seed proteins. The widely quoted dictum that plant proteins are less valuable than animal proteins is based on analysis of seeds, it is not true of leaf protein nor, for that matter, of the protein of potato tubers.

Analysis leads us to expect that properly made leaf protein will have good nutritional value. This expectation was borne out by experiments on the growth rate of chickens, pigs and rats and by brief experiments on the amount of nitrogen retained by infants fed on milk alone and on mixtures of milk and leaf protein. These results have been confirmed and extended by a six month experiment in which school children, on a diet consisting predominantly of ragi (*Eleusine coracana*), were given supplements containing 10 g of protein per day in the form of lucerne (*Medicago sativa*) leaf protein or sesame seed (*Sesamum indicum*). Leaf protein gave better results, assessed from height, weight and haemoglobin content, than sesame although the protein in the latter is regarded as one of the best seed proteins (4).

Acceptability. Leaf protein should not be regarded as a medicine and eaten in the form of pills. It should rather become one of the many components from which a mixed diet is made up. But people do not, as a rule, think of their diets in terms of so much energy, so much protein and so on per dey. They eat foods with familiar appearance for traditional reasons. Methods must, therefore, be found for presenting any novel foodstuff in a familiar and acceptable way. The experience we have of the acceptability of leaf protein in Britain is of little relevance in many other countries. Experience that may be more relevant to Latin America has been gained in India. The colour blends easily with local dishes and the slight flavour is not obtrusive in the presence of more highly flavoured materials: now that the novelty has worn off, we find the flavour attractive. The next stages in popularisation cannot, and should not, be taken until a continuing supply of leaf protein is assured. This is not the place to consider the ethics and strategy of innovation. I have discussed these issues at some length in a "Penguin" book, *Food Resources: Conventional and Novel* (5).

Problems of presentation arise with every novelty - yeast, fish flour, groundnut protein, soy protein, etc. It may be a little easier during the first few days of a trial to win acceptance for a white or pale coloured product, but our experience is that the doubts arising because of the unfamiliar green colour are quickly allayed. New members of staff, and visitors to Rothamsted, become accustomed to the colour in 1 or 2 weeks. We have the advantage that it is already customary in Britain to eat green vegetables. In countries where it is not customary, the first step should be their popularisation. That would be beneficial in any event because vegetable production is one of the more efficient ways of using land to produce human food, and the nutritional merits of green vegetables are well known. Acceptance will also be easy to win in countries where it is traditional to use curries and various other dark coloured powders in preparing food. In many parts of the world, dried whole leaves are traditionally used as a relish.

One of the unfortunate features of the present-day scene is that traditional styles of food preparation, into which leaf protein would fit easily, are being given up because of the example set by Europeans and Americans, before there is any possibility of producing enough of the now-favoured types of food. In the past, and in various parts of the world, people have had the same tendency to copy diets that only the rich could afford; foods that make economical use of what is available locally then get disparaged. Thus Homer (Odyssey chapters 18 and 20) praised a goat's paunch stuffed with fat and blood and then tossed to-and-fro in front of a fire till coagulated; and Gervase Markham wrote (6) "Pudding which is called the Haggas of Haggus, of whose goodness it is vain to boast". Few would now relish Homer's mixture, and haggis, made from oatmeal and various parts of an animal that are usually considered inedible, is no longer esteemed in England though it still is in Scotland. The trend is inevitable and in many ways commendable, but it makes the problem confronting those wishing to introduce a new dietary component more difficult. The novel foods are usually powders or pastes that could easily be incorporated into gruels or foods such as haggis, and they are becoming available just when, as a result of example and advertising, the old foods are losing prestige.

Beta carotene. The yield of protein per hectare and year is so much greater from leaves than from any other method of using land in a wet tropical region, that it would be worth while making leaf protein simply as a protein source. The freshly-made, dark green cake is also a useful source of carotene. This is a good reason for studying methods of preservation other than solvent extraction - for that would remove the carotene. We do not yet have an extensive series of carotene measurements on protein from different species, harvested at different ages, but some contain 4 mg beta carotene per g of protein and many contain 1 mg per g. During extraction, some carotene is lost; this loss is minimised by working quickly, especially in the presence of alkali. There is also loss on storage in air; this can be minimised by excluding air or adding antoxidants (7). Much of the variability in the preparations we have studied is probably a consequence of uncontrolled losses. If, as is likely, we can develop methods for ensuring the invariable presence of 1 to 2 mg beta carotene per g of protein, it could become an important source of vitamin A.

The World Health Organization suggests that children under 7 need 1.8 mg of beta carotene per day and adults 4.5 mg. These requirements would be met by 10 to 30 g (dry weight) of leaf protein per week. The methods of presentation that have been used in Britain and India are designed to put 5 to 10 g of protein into each helping. Therefore, if leaf protein appeared on the table three of four times a week there should be no vitamin A deficiency even if the diet contained no other vitamin A or carotene source. At present vitamin A deficiency causes at least ten thousand children to lose their sight each year in India. The number in Latin America is not so definitely known - but it is probably large.

Conclusion. The feasibility of extracting and using leaf protein has now been amply demonstrated. As with every other novel foodstuff, the costs of production cannot be stated until some experience of large scale production has been gained, and until the exact source of the protein has been decided. It is however certain that, when proper attention is paid to the value of the by-products, this will be as cheap as any other protein concentrate that is not itself a by-product. My estimate is 18 to 25 pence per kg when a crop is grown solely as a source of leaf protein, and less when by-product leaves are used (8).

In any country where it seems likely that leaf protein production would be useful, the first step in a systematic scientific investigation would be to set up some detailed agronomic experiments. A few species that give good results elsewhere would be sown at different densities, with different manurial treatments and harvested at different ages. Only 10 to 20 m^2 would be needed for each. In most species, the extractability of the protein in the leaves begins to decline at a stage of maturity at which the total protein in the above-ground parts of the plant is still increasing. There is therefore an optimum time of harvest which will be different for different species and will be influenced by such factors as temperature, humidity, and length of day. Figure 2 shows this for 4 crops grown at Rothamsted. Similar studies are being made in the Indian Statistical Institute in Calcutta, the Department of Botany at Aurangabad and in Nigeria. In Brazil, California and New Zealand large-scale production has started without this preliminary survey. A familiar crop, usually lucerne, is chosen and harvested at an arbitrarily selected stage of growth. There are merits in both methods of tackling the problem. If the second is successful, a great deal of time will be saved; if it is unsuccessful, nothing will have been demonstrated except that the crop was unsuitable, or the time for harvest was badly chosen.

In some parts of the world need for protein could be most economically satisfied by growing yeast or some other microorganism on molasses or oil. In others the best source may be a seed such as groundnut or soya. But in tropical regions with regular, or even excessive rainfall, leaves are the most productive protein source, and these regions are at present the worst fed parts of the world.

IMPROVEMENTS IN THE ANNUAL
YIELDS OF EXTRACTED LEAF PROTEIN

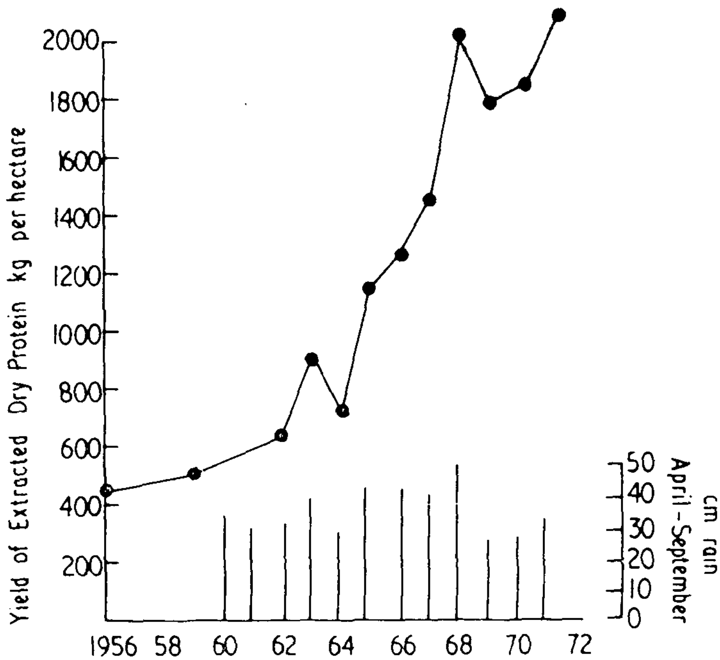

Figure 1.—The cropping year in these experiments ran from October to October and a succession of crops was grown on the same plot. The sequence varied, but always started with winter wheat (Triticum aestivum). This was followed by mustard (Sinapis alba), fodder radish (Raphanus sativus or vetch (Vicia sativa). The plot was not irrigated; breaks in the improvement curve were probably caused by dry summers.

THE EFFECT OF MATURITY ON THE EXTRACTABILITY OF PROTEIN FROM DIFFERENT LEAF SPECIES

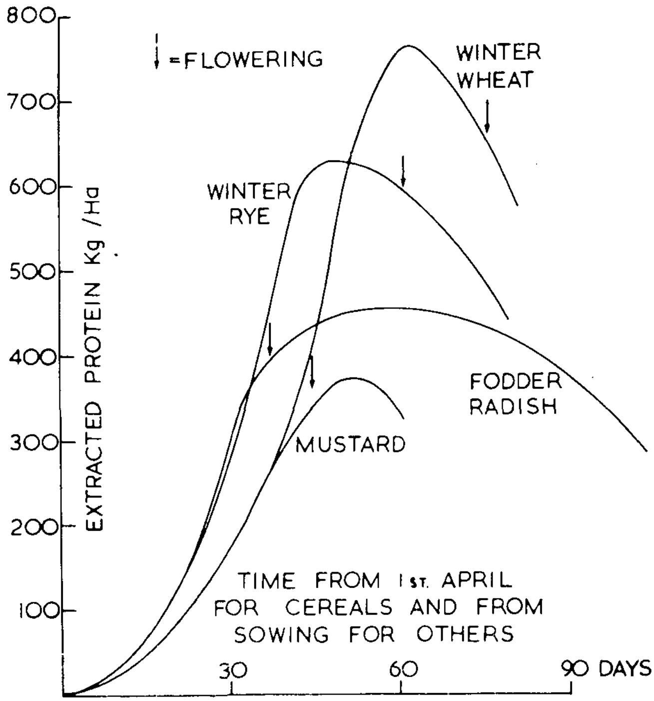

Figure 2.—The ordinate is the yield of extracted dry protein from a single harvest of leaves taken at different ages. With rye (Secale cereale) and wheat (Triticum aestivum) there is little growth during winter, the abscissa therefore starts at 1st April. The optimal time of harvest with fodder radish (Raphanus sativus) is less sharp than with mustard (Sinapis alba), partly because it matures later in the year when it is cooler, and probably also because of an intrinsic difference between the species. The effect of maturity is still less marked with some Chenopodiaceae such as Chenopodium quinoa.

RESUMEN

Investigaciones sobre proteínas de hojas y sus aplicaciones

Potencialmente, las hojas son la fuente proteica más abundante. La utilización más sencilla e inmediata sería a través de una popularización del consumo de vegetales. Si el consumo de vegetales es aceptado por una población, proteínas extraídas de hojas podrían usarse como suplemento, especialmente en regiones tropicales húmedas.

Para la producción de proteínas de hojas se pueden usar desechos de vegetales. Si se usan forrajes como materia prima, el rendimiento en proteínas/ha es mayor que con cualquier otra forma de producción agrícola. Los métodos de producción se adaptan bien para escalas pequeñas y grandes.

Proteínas de hojas son superior desde el punto de vista nutricional a todas las proteínas de semillas y son tan buenas como las de pescado aunque inferior a las de leche; además son una buena fuente de β-caroteno. El residuo de fibra puede servir de alimento animal. La parte soluble noproteica de los componentes de hojas sirven para el cultivo de levaduras y otros microorganismos.

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