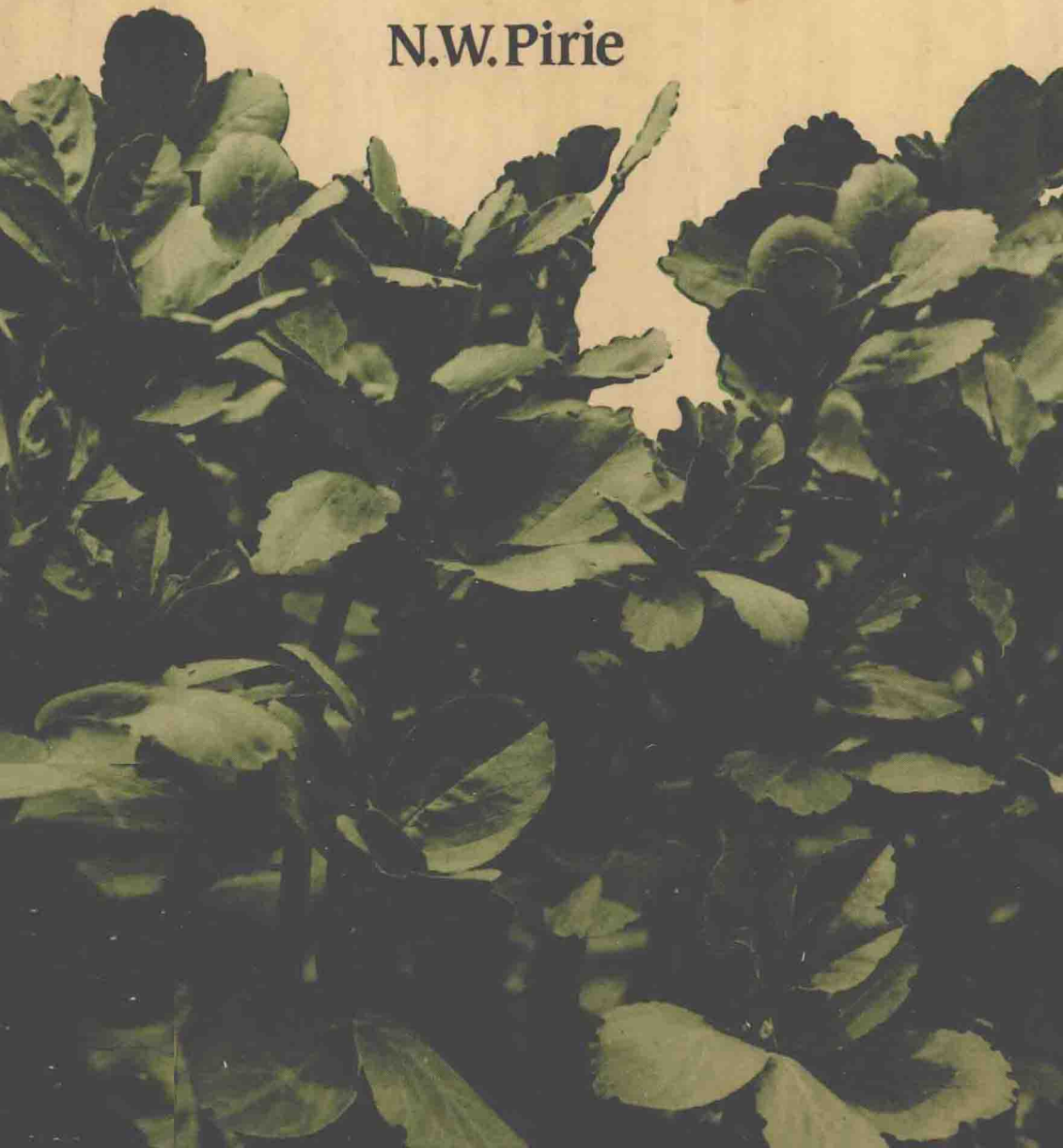
LEAF PROTEIN and its by-products in human and animal nutrition

N.W. Pirie



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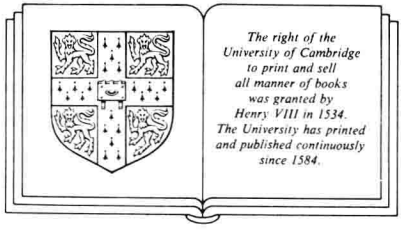
N. W. PIRIE

Second edition of
Leaf protein and other aspects of fodder fractionation



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Leaf protein and its by-products in human and animal nutrition

Introduction

Protein sources dominated discussion on world food problems 30 years ago. That exclusive emphasis caused a natural reaction, and there was a period of preoccupation with energy needs. Those who, early in this century, had been taught of the 'protein-sparing action of the carbohydrates' were puzzled by such exclusive, or either/or, opinions. Unfortunately, the sensible middle-of-the-road outlook was epitomised in the foolish phrase 'protein–energy malnutrition' or PEM. When both protein and energy are scarce, people are simply hungry or half-starved though they may, if the concept PEM is treated logically, be supplied with such dietary components as minerals and vitamins. The simple words *hungry* and *starved* seem preferable to any type of scientific euphemism. Sometimes, all that is needed to remedy matters is an increased supply of the conventional foods of a region, and its equitable distribution both between and within families (Pirie, 1982). Equality of distribution raises political and social issues. Because of the complexity of these issues, it is fortunate that they are not the business of those whose primary concern is the food supply. But it is our business to try to ensure that agriculture produces nutrients in the amounts and ratios needed in each region (Pirie, 1981*a*; Swaminathan, 1981; Pinstrup-Andersen, 1982).

This book is concerned with the merits of one potential source of edible protein. It is therefore not a suitable place to discuss the varied opinions which have been expressed by individuals and international committees about the correct ratio between protein and energy in the diets of apparently well-nourished people. I have discussed that issue in several other publications (e.g. Pirie, 1984*b*, *c*). For a less biassed account, three papers in a recent conference organised by the Rank Prize Funds (Blaxter & Waterlow, 1985)

should be consulted. Some confusion is introduced into the whole subject by misconceptions about the significance of nitrogen (N) balance measurements. Clearly, anyone not in balance, i.e. who is excreting more N than is being absorbed from food, is being depleted and must in time show signs of malnutrition. There is, however, no logical or experimental basis for the assumption that all is well with N metabolism as soon as intake equals output. That assumption confuses a necessary with a sufficient condition. Furthermore, it is not as easy as is often assumed to measure the point of balance. A committee of the United Nations University (1980) concluded that our apparent protein requirement increases as fewer assumptions are made about diet and experimental technique. Suggested values for the amount of protein needed per day have consequently been increasing (e.g. Young & Bier, 1981; Gersovitz *et al.*, 1982). We seem to be slowly returning to the old, and easily remembered, value of 1 g of protein kg^{-1} of body weight. Without a protein concentrate in the diet it would be impossible to approach that value if banana, cassava or sago were the main energy sources, and difficult if the energy sources were potato or rice. Although it has recently been fashionable to dispute statements such as that, disagreement is clearly not taken seriously by those responsible for national policies. Otherwise, less effort would be expended on cultivating seed legumes, fishing, milk production, and poultry husbandry. Work on protein sources other than these is therefore justified.

Leaves are potentially the most abundant source of edible protein (Pirie, 1975*b*, 1981*b*). The best and simplest way to exploit this potentiality is to eat more leafy vegetables in the normal manner (Pirie, 1985*a*). However, the human gut has a limited capacity (which few people approach) to cope with whole leaf. In many parts of the world it would therefore be advantageous to make extracted leaf protein (LP) because:

1. Leaves are the site of protein synthesis and there are losses when protein is translocated to seeds or tubers.
2. Suitable leaf crops maintain a photosynthetically active green cover on the ground throughout the period during which growth is possible. Yields are therefore greater than those from crops which occupy ground while merely ripening. Perennial leaf crops protect ground from erosion.
3. Ruminants make admirable use of nonarable land, but they

convert only 10 to 25% of the protein in their fodder into human food. From cultivated plants, 50 to 65% of the protein can be extracted, and the unextracted protein is still available for ruminants.

4. The processes of extracting and separating LP disintegrate the leaf and remove toxic or ill-flavoured components. Species normally rejected as human or animal food can therefore be used.
5. Forage from which LP will be made is harvested when young. The fibre is therefore less lignified than when a crop is taken for hay. Furthermore, the crop is not at risk from pests and diseases for so long.
6. The process of extraction removes much of the water from the fibrous residue. When ensiled, there is therefore less drip; when conserved by drying, less fuel is needed. Field wilting is a common technique for achieving these results. But 30% of a wilted crop may not be gathered up. It is easy to collect 95% of a crop which is cut and harvested in one operation.
7. If made with reasonable care, LP has better nutritive value than the usual seed proteins; it is as good as fish or meat, but not egg or milk. Like other foods containing unsaturated fats, it is damaged by inept handling.
8. Although people with European or North American prejudices find the appearance of LP unusual on first contact, it is readily accepted by adults and children when intelligently presented.
9. The technique of extraction is simple; equipment has been designed, and is being constantly improved, for production on the domestic, village and commercial scale.
10. Several countries which are so wealthy as to have little need for LP as a human food, depend on imported groundnuts and soya beans for pigs and poultry. LP could largely replace these imports.

The validity of these points is now gaining acceptance, especially among those who are concerned with feeding animals. This is partly because increases in the cost of oil necessitate less waste of fuel, and partly because people who heard of fodder fractionation when young are now reaching influential positions. The present degree of interest is shown by the need for a second edition of this book and by the appearance of two other books (Costes, 1981; Telek & Graham, 1983). Increasing international interest is shown by the

conferences on LP, in India in 1982 (N. Singh, 1984) and Japan in 1985 (Tasaki, 1986), which have, after a long interval, followed the conference in India in 1970 (Pirie, 1971*a*). A conference in Italy is planned for 1989.

Abstracts of about 500 papers dealing with LP and its use as human food, as feed for nonruminant animals such as pigs and poultry, and with the use, as ruminant fodder, of the residue from which LP has been extracted, which have appeared since the first edition of this book, have been collected and circulated in nine numbers of a *Leaf Protein Newsletter* (Matai, 1984). We hope to continue this service.

National and international organisations concerned with research on food have shown little interest in the use of LP as a human food. Charitable organisations, notably 'Find Your Feet', have been mainly responsible for the progress which has been made. Elsewhere (e.g. Pirie, 1976*a*), I have discussed some reasons for official reluctance to admit that, with the present rate of population increase, reliance on a steady increase in agricultural productivity may be mistaken, and new methods of food production may be needed. I have also censured the tendency, in some quarters, to try to solve the food problem by changing the assumptions made about our food requirements. Both points have recently been forcefully emphasised by Miller (1983).

Though welcome, this widespread acceptance of the idea of fodder fractionation encourages the assumption that we now have adequate knowledge about what should be grown for fractionation, how it should be fractionated, and how the products should be used. This is far from the case. The amount of research that has been done is trivial compared to the amount that has been, and is being, done on projects of comparable complexity and smaller potential yield, such as the cultivation of microorganisms or the processing of fish. Some of the more obvious topics on which much more research is needed are outlined in this book. It is well to remember that haymaking and ploughing are ancient arts on which useful research is still being done.

An anomalous feature of agricultural research is that, while magnificent work is done on increasing the efficiency of primary photosynthetic production, on ensuring that a plant's roots are adequately supplied with mineral nutrients, and on selecting varieties with increased Harvest Index (the ratio of total dry matter to

conventionally useful DM), little attention is paid to making optimal use of crops which already have Harvest Indexes approaching 1, i.e. leafy crops.

Terminology is important in every subject. Agreement about it is convenient, but it is more important to ensure that it is neither ambiguous nor misleading. There is no ambiguity about the equivalent words 'juice' and 'extract'. The coagulum separated from leaf juice, but not further fractionated, is called leaf protein (LP) in this book. It is often called leaf protein concentrate (LPC) by others, and some recent publications have called it leaf nutrient. The last name is suggested because of the valuable presence of β carotene (provitamin A) and traces of other vitamins. But it will cause confusion in indexes because leaf nutrient already means something which is sprayed on to the leaves of a growing plant. As normally made, LP is a mixture of many proteins; the intrusion of the word *concentrate* suggests that a product is being referred to which has a greater content of true protein than some parent substance that would be called leaf protein. It can be argued that it should be called leaf lipoprotein because it contains 30 to 40% of non-protein material – most of it lipid. That would imply that most of the lipid was attached to, rather than merely mixed with, most of the protein. This is probably not the case. When LP is fractionated, the usual products are a green mixture of chloroplasts, their fragments, and fragments of fibre, etc., not removed by straining the juice. Here this is loosely called 'chloroplast' protein. The other, paler, product is equally loosely called 'cytoplasm' protein although much of it was originally in the chloroplasts. It is important not to give the residue from which protein has been extracted a misleading name. Here it is called fibre – which is brief. It could be called extracted residue. The most misleading name for it is pressed crop: that perpetuates the widespread illusion that pressing is the important feature of fractionation. Here, juice from which protein has been removed is called 'whey'; that is brief and the metaphor is obvious. It is sometimes longwindedly, but not misleadingly, called brown juice or deproteinised juice.

In some publications it is not clear whether the yields given are for moist press-cake of LP, dry LP, or the true protein component of the LP. Here, wherever the contrary is not stated, the yields given are for 100% protein, and they are usually calculated by multiplying the N content by six.

Abbreviations

ARC	Agricultural Research Council; now Agricultural and Food Research Council, AFRC
cm	centimetre
DGLV	dark green leafy vegetable
DM	dry matter
FAO	Food and Agriculture Organisation of the United Nations
g	gram
g	normal gravitational acceleration, 9.8 m s^{-2}
ha	hectare; ha^{-1} = per hectare (the same convention is used for other quantities)
HP	horse-power = 746 watts (W)
IBP	International Biological Program; this has now ended
J	joule = 1 watt second = 0.239 small calories
kg	kilogram (kgf = kilograms force)
km	kilometre
kPa	kilo Pascal = 1/98 kgf cm^{-2}
kWh	kilowatt hour, often loosely called a unit of electricity
l	litre
LP	leaf protein (unfractionated)
m	metre
mg	milligram
ml	millilitre
mm	millimetre
MJ	megajoule = 1 million joules = 239 kilocalories
M£	1 million pounds sterling
μg	millionth of a gram
μm	millionth of a metre

N	nitrogen (except when gaseous N_2 is referred to)
RNA	ribonucleic acid
RuBP	ribulose-1,5-bisphosphate carboxylase-oxidase = rubisco = fraction 1 protein
s	second
t	ton or tonne (tf = tons force)
TCA	trichloroacetic acid
UNICEF	United Nations Children's Emergency Fund
UNU	United Nations University
USDA	United States Department of Agriculture
WHO	World Health Organisation

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1

Historical and anatomical background

It is seldom possible to date precisely the beginning of a new line of research. Preliminary vague hints, coming from folk medicine, primitive technology or some such sources, are as a rule slowly integrated into a new fabric. Leaf protein is different. The date of the first publication on it is 1773. The chemical category now known as 'protein' was not recognised at that time: the word itself was not coined until 1838 when Berzelius used it in a letter to Mulder. Presumably because of some similarity in texture and sheen, wheat gluten was called *mien chin*, literally muscle of wheat, centuries ago in China. When Beccari studied gluten in 1728 (published in 1745) he emphasised a further relationship between gluten and animal products – they stank similarly when putrid or heated. The same criterion led Rouelle (1773) to call the material he isolated from leaves *matière glutineuse ou végéto-animale*. The 'smell of burnt feathers' retained a place in textbooks, as a means of protein recognition, for many years.

About 1785, Berthollet found N in this group of substances, the colour test known as the xanthoproteic reaction was described by Fourcroy and Vauquelin in 1800, and Fourcroy stressed the importance of N in the nutrition of plants and animals in about 1806. These observations were reasonably factual. Then, as was his way, Liebig confused the issue by using his imagination rather than his undoubted experimental skill. He decided that there were only four proteins and asserted that the curds separating from boiled vegetable juices were indistinguishable from those separating from such animal fluids as blood and egg white. As Berzelius remarked in 1842: 'This easy kind of physiological chemistry is created at the writing desk, and is the more dangerous, the more genius goes into

its execution.' The individuality of proteins was not established till many years later.

The brothers Rouelle, though very unlike in temperament and appearance, are often confused. Their portraits have even been misassigned. Guillaume François (1703–70) was an enthusiastic and excitable teacher. His manner of teaching was described as '*...souvent incorrecte et familière, mais toujours animée et pittoresque,...*' and he enlivened proceedings on some occasions by partly undressing, and by explosions. Even when young he was megalomaniac, accusing many contemporaries of plagiarism, using such phrases as '*Ecoutez-moi! car je suis le seul qui puisse vous démontrer ses verités*'; in 1756 he claimed to have a secret weapon with which he could destroy London and the British navy. Patriotic feeling was so strong that he refused a tempting offer from a London publisher; his lectures remained unpublished. Lavoisier and Rousseau were among his distinguished pupils, and it is after him that the rue Rouelle in Paris is named. Because of increasing eccentricity he resigned his professorship at the Jardin du Roi (now Jardin des Plantes) in 1768. At his request, his brother, Hilaire Marin (1718–79), succeeded him – but only as demonstrator.

By contrast, H. M. Rouelle was neat and tactful; an experimenter rather than a forceful teacher. He did not share his brother's chauvinism, but became a corresponding member of the Royal Society of Arts (London) on 24 October 1770. There is no record of him playing an active role in the Society. J. d'Arcet wrote an *éloge* in *Observations sur la Physique* (**16**, 165, 1780). He is less often mentioned than his brother, and gets less space when mentioned in biographical dictionaries in spite of a distinguished research record. He found potassium in 'cream of tartar' and went on to isolate tartaric acid from unfermented grape juice and to make tartarates of several metals. He found formic acid in ants, and made urea, or possibly the hydrate of urea and sodium chloride, from urine. This was not a novelty: Boerhaave probably made it in 1729. He confirmed the presence of iron in blood. This hardly needed confirmation, for the removal of rust stains from blood-soiled clothing must have been a familiar problem. He extended his brother's studies on various oils and resins. In this work he pioneered the use of solvents in sequence. At that time, starch and fat were considered the nutritionally important components of foodstuffs. Rouelle, having begun to recognise proteins as a chemical

category, stressed their nutritional importance. Clearly, he helped to establish the science of biochemistry: he has been called its father. However, he had not quite shaken off the older outlook. For example, he did not completely reject the possibility of alchemical transformations, and argued that, although we could not make an animal or a plant, it did not follow that a metal, without structure or parts and therefore presumably lifeless, could not be made. The argument that the characteristic feature of an organism was its heterogeneity had already been used by Jean Rey in 1630 (cf. Pirie, 1964*a*).

Rouelle (1773) published two papers on leaf protein. The second contains so much information that it can be taken as the origin of our subject. Its title, '*Sur les Fécules ou parties vertes des Plantes, & sur la matière glutineuse ou végéto-animale*', shows his recognition that plant and animal products are similar. He pounded leaves of several species in a marble mortar, pressed out the juice and heated it. A green coagulum, which he could decolourise by washing with alcohol, separated at about the temperature at which he could no longer keep his finger in the hot juice.* He filtered off that coagulum and got a pale coagulum on further heating. Although this paper is now often referred to, and since about 1952 the correct initials are usually given to its author, it seems to have been seldom read. Patent Office inspectors are particularly remiss and allow patents that cover points clearly established in 1773! I therefore give a free translation of the paper in an appendix to this book. Rouelle's work was extended by Vauquelin and Fourcroy (1789) a few years later.

In spite of the defects and tediousness of contemporary methods for measuring N, Boussingault concluded in 1839 that atmospheric N_2 (dinitrogen) and food N were not interchanged during animal metabolism. The generalisation that N_2 is little, if at all, used by animals, or made by them from their food, is still accepted by most scientists though there are a few puzzling apparent exceptions. Mulder attached particular importance to plant proteins as the source from which animals derive their protein and other nitro-

* Farenheit made thermometers and developed the idea of a temperature scale in 1742: Martel described the Centigrade scale in the same year. This scale is sometimes, absurdly, called the Celsius scale although Celsius had it upside down with water boiling at 0°. Meteorologists adopted thermometers quickly – chemists did not, for example: thermometers are not mentioned in Macquer's (1766) *Dictionnaire de chymie*.

genous substances. An animal, as Johnson phrased it in 1867, 'moulds over these vegetable principles into the fibrine, albumin and casein of its muscle and other tissues, of its blood, milk and other secretions'. As a step towards assessing their value as fodder, forages and other animal feeding stuffs were analysed in many laboratories – notably at Rothamsted by Lawes and Gilbert.

Beddoes suggested in 1792 that leafy material should be made into human food (Levere, 1984). Like most of Beddoes' suggestions, this was ridiculed : he figures in scientific literature mainly because he started Davy on his career. The suggestion was probably made again during the 19th century because Lawes (1885) remarked 'It might be possible by some chemical process to produce from grass a nutritious substance which a man could use as food, but food so extracted would be far more costly than as it existed in the grass, and no one would think of preparing such a food for oxen or sheep, as their machinery is quite competent to separate the nutritious from the indigestible portion of the food.' Lawes' comment on ruminants is justified: it is fortunate that his comment on the extraction of human food was not widely known when work on LP extraction started at Rothamsted!

Winterstein (1901) extracted protein from dried, ground leaves with dilute alkali. Sustained work started 20 years later when Osborne temporarily forsook his studies on the seed proteins. Osborne & Wakeman (1920) and Osborne *et al.* (1921) made preparations from spinach (*Spinacia oleracea*) and lucerne (*Medicago sativa*) by pulping the fresh leaf, removing the coarser particles by centrifuging or filtering, and coagulating with alcohol.

Osborne (1924) was well aware of the importance of this work, but he was distressed by the properties of the material. As he put it: 'Our present meagre knowledge of the protein constituents of living plants is chiefly due to the difficulties encountered in separating the contents of the cells from the enveloping walls. Attempts to grind the fresh leaf and extract the contents of the cells with water result in mixtures that cannot be filtered clear, and consequently appear to present no opportunity to obtain the protein in a state fit for chemical examination.' As Vickery (1956) put it: '...when Chibnall came to the laboratory in 1922 for a two-year period, Osborne was happy to turn this difficult problem over to him...' Chibnall had already worked with extracts from cabbage (*Brassica oleracea*) and runner bean (*Phaseolus vulgaris*). One chapter

in the book he wrote in 1939, *Protein metabolism in the plant*, describes his work.

The work of Chibnall and Osborne established LP as a reasonable material for biochemical investigation. A steady flow of papers on techniques of extraction in the laboratory soon started e.g. Davies (1926); Lugg (1932, 1939); Kiesel *et al.* (1934); Yemm (1937); Foreman (1938); Crook (1946); Crook & Holden (1948); Holden & Tracey (1948); Bryant & Fowden (1959); and Festenstein (1961). Because leaves from different species, and of differing age and nutritional status, were studied the conclusions reached in these papers are not identical. However, the general conclusion was that the younger the leaf and the greater its protein and water content, the greater the percentage extraction of protein. Also, thorough subdivision or rubbing, and the maintenance of alkaline conditions during the extraction, are advantageous.

During the past 30 to 35 years the total amount of biochemical research has increased enormously. The amount of research on plants has not increased correspondingly, and that research tends to be concerned with alkaloids, pigments and the components of seeds and tubers. Nevertheless, much more work has been done on proteins in leaves than it would be useful to describe in detail in a book primarily concerned with practical problems arising when an edible protein is being extracted in bulk. Early academic work on leaf proteins is described in several reviews, e.g. Vickery (1945), Pirie (1955, 1959*a*), and various specialised aspects of the subject are covered regularly in review journals. It may, nevertheless, be useful to give a brief survey of the subject so as to explain the reasons for some methods and precautions adopted during the production of LP.

Even casual study of pieces of leaf under the microscope shows that the green colour is concentrated in chloroplasts: the colour of leaf extracts shows that chloroplasts, or their fragments, are being extracted. Methods for separating them from the other types of protein will be discussed later (p. 66). Here, all that need be said is that there is much research on their isolation in physiologically active forms. Those from some species are extremely fragile, while from others they are robust enough to withstand the disintegration of the leaf during digestion by *Clostridium roseum* (White *et al.*, (1948). Morris & Hall (1982) comment on the unusual stability of chloroplasts from quinoa (*Chenopodium quinoa*). The durability of