Nutritionally better plant proteins

The most abundant protein in the world is one found in plant leaves. It is an enzyme involved in photosynthesis, bearing the name ribulose diphosphate carboxylase.

Unfortunately, we cannot subsist on most leaves (for example, grass and gum leaves). Although the protein is of good quality, it is enclosed within cell walls that humans cannot digest. We therefore rely mainly on seeds, tubers, or animal products to provide protein.

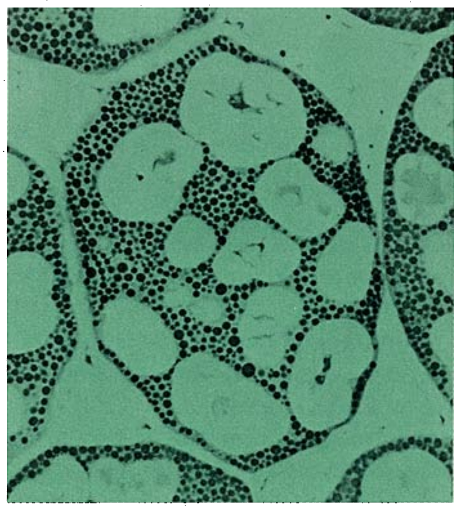
Research at two CSIRO Divisions — Plant Industry and Protein Chemistry — aims at improving the nutritional quality of plant proteins, particularly legumes. If this can be achieved, the effect on the world's food supply could be substantial.

Proteins are composed of amino acids. Our bodies contain 20 different amino acids, linked together by the hundred, or sometimes the thousand, to form a myriad of different proteins. An ordinary liver cell, for example, contains more than 1000 distinct proteins, each characterized by a particular arrangement of amino acids.

On a global scale, most of the world's edible protein comes from seeds.

Eight of the 20 amino acids are called 'essential' because our bodies cannot synthesize them: we must obtain them directly from food. On this basis, we can assign a 'protein score' to individual foodstuffs. A score of 100 (human milk and hen's eggs are so rated) means that the food contains the essential amino acids in the exact proportions needed for the body's protein-building. Lesser scores indicate that the body utilizes the protein in the food less efficiently. (For example, only about half the total amino acids in corn, with a score of 49, can be built up into proteins.)

Corn is low in the amino acids tryptophan and lysine. Navy beans, which are low in methionine, have a protein score of 44. This doesn't mean that corn and beans are necessarily poor food value. Corn's deficiencies can be made up by



The dots are protein bodies in the cell of a pea.

the beans, and *vice versa*. A meal of corn tortillas and fried beans has a much higher protein score than either component.

The Food and Agriculture Organization of the United Nations has concluded that most adults can easily get by on 40 grams of high-quality (high protein score) protein daily. Even 20 grams will do if the body obtains sufficient food energy (protein will be burned up for its energy if there is insufficient food).

On a global scale, most of the world's edible protein (about 70%) comes from seeds. More than half (55%) comes from the cereal grains wheat, rice, and corn; another 13% is provided by legume seeds — soybeans, peanuts, lentils, and the like. Roots and tubers provide only 7% of the total, and leaves 5%.

Animal products supply 20% of the world's dietary protein, most of it consumed in the affluent western world. As much as 90% of the protein fed to animals is used to maintain them, leaving only some 10% to be recovered for human consumption. The world production of corn is nearly as large as that of wheat or rice, but corn supplies only about 5% of our food because most of it is used as animal feed, along with wheat, skim milk, fish-protein concentrate, and other fortified feed supplements. Human beings and domesticated animals are, in a sense, competing for the same food.

It seems inevitable that in the long term, as the world's population increases, there will be a trend in all countries towards direct consumption of plant proteins for human nutrition. We will be less able to afford the luxury of feeding grain to livestock to produce meat containing only a fraction of the amount of protein we began with. Instead, technology may create from seed proteins products analogous to meat in taste and texture; indeed, the marketplace already contains the early products, more or less convincing, of this technological effort.

However good the protein quality of a legume, it stands for little if the seed is indigestible or toxic.

High-scoring plant protein

In this light, the dietary quality of plant proteins becomes a matter of concern. Cereals such as wheat, maize, and barley are low in lysine and tryptophan, and rice is also low in threonine.

Legume seeds, on the other hand, characteristically lack adequate cystine and methionine. The amino acid imbalance is not a serious problem in developed countries, where many sources contribute protein to the diet. However, in poor countries, protein intake is low and often restricted to a narrow range of staple foods.

Unfortunately, simple supplements do not seem to be the answer. Trials in Tunisia, where wheat products were supplemented with lysine, and in Thailand, where rice was supplemented with lysine and threonine, have not been judged successful.

A far more useful approach has been the development by scientists and plant breeders of cereals with improved protein quality. In particular, high-lysine lines of maize, barley, and sorghum have been developed.

However, the same approach has not been applied to legume seeds, perhaps because they are not as important

Experimental plot of rape.

economically on a world scale. Yet peas, lentils, kidney beans, lima beans, chickpeas, mungbeans, cowpeas, soybeans, peanuts, and broad beans are the most important high-protein foods in many poor countries.

Legumes contain 15–40% protein, which means that most of them contain more protein than cereals, and even than meat (although of a lower quality). They contain much more iron than the same amount of cereals, and about five times as much riboflavin and ten times as much thiamine. Legumes also have the advantage of being able to fix nitrogen through bacteria growing on their roots — they therefore can grow on poor land with a lesser amount of fertilizer than cereals require.

Although the total protein fraction of legume seed may be unbalanced, in the dietary sense, some of the individual proteins making up this fraction may have a very favourable amino acid composition. With this consideration in mind, the CSIRO scientists have been investigating the possibility of developing nutritionally improved legumes. In coming to grips with the problem, the scientists were encouraged by some degree of success with cereals and with the knowledge that the number of storage proteins is less in legumes than in cereals, making the task theoretically easier — another reason for aiming at improving legumes.

Storage proteins, which make up the bulk of a seed's protein, comprise the food store the plant lays down in its seeds in order that the seeds will have the wherewithal to germinate and grow to a stage where photosynthesis can take over as the energy source.

The immediate aim of the research program is to study the processes involved in their formation.

The scientists want to learn how the processes of laying down protein are governed by the plant at the genetic, physiological, and biochemical levels. The longer-term aim is to use this knowledge to manipulate the plant's protein production by breeding chosen lines and by providing the optimum in nutrients and environment.

Plant breeders may have a less tortuous path to success if they have a detailed knowledge of the basic plant processes involved in protein accumulation. Breeding strategies based only on selecting plants with high protein yields overlook many of the interacting factors that are at work.

At the Division of Plant Industry in Canberra, Dr Don Spencer and his colleagues are concentrating their efforts on understanding a single legume, the garden pea (*Pisum sativum*). This is a convenient experimental plant for many reasons. Its seed protein is typical of legumes in being low in the sulphur-containing amino acids, including methionine, and it can be grown to seed-set stage fairly rapidly under standardized glasshouse conditions. It is excellent for genetic studies because of extensive knowledge already available (dating back to Mendel), and its storage proteins are closely related to those of other legumes. A number of interesting findings have already come to light.

More or less

A decade ago it was generally accepted that there are two sorts of legume storage protein — legumin, which contains useful amounts of the wanted sulphur-containing amino acids, and vicilin. This is a great over-simplification, as work by Dr Spencer, Dr T. J. Higgins, and Professor John Thomson (formerly of the Division and now at Sydney University) has shown. They have found that legumin and vicilin are each highly complex mixtures of closely related proteins. Detailed studies of the biosynthesis of these proteins has shown that they undergo an extensive series of modifications during seed development.

Parallel microscopic studies by Mr Stuart Craig and Dr David Goodchild have given an insight into the changes that occur in the internal structure of the cells during accumulation of the storage protein. The structure has to be completely reorganized during the process so that the stored protein can be packaged into minute 'protein bodies'.

Present work suggests that improvement of seed protein quality by genetic means is most likely to be achieved by altering the relative proportions of proteins already present (that is, more legumin, less vicilin). The alternative of breeding mutations with seed proteins containing different amino acids runs the risk of reducing, or destroying, the viability of the seed.

Professor Thomson has been studying the many individual legumin and vicilin components to ascertain how many different genes control their chemical structures and amounts and how the genes are linked in pea chromosomes. This will provide an invaluable foundation when plant breeders try to manipulate the individual storage protein components.

Of course, the question still remains as to how much variation in the proportions of the proteins the seed can tolerate while maintaining viability. After all, the biological functioning of the seed has different requirements from those for satisfying human nutrition. However, experiments suggest that, in legumes anyway, such variations would not be a serious constraint.

Dr Peter Randall of the Division grew peas in soils containing levels of sulphur ranging from none to very high. When Professor (then Dr) Thomson analysed the peas, he found that, if no sulphur was available to it, the plant manufactured a seed that contained no legumin at all. Significantly, the seed was still viable. Unfortunately, no amount of added sulphur could persuade the plants to increase the proportion of legumin — the sulphur-containing fraction — beyond normal levels.

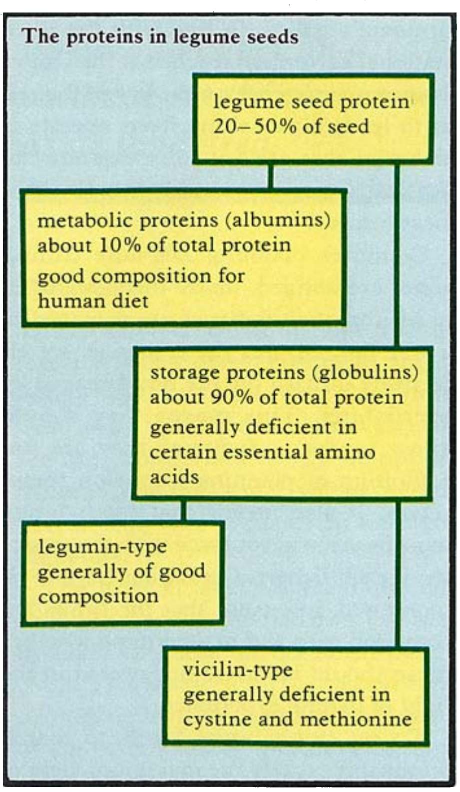
Under some circumstances, the proportion of legumin could be increased, but usually at the expense of stressing the plant. Growing a plant with gross deficiencies of potassium or phosphorus is not recommended.

Anybody for lupins?

Dr Morton Gillespie, Dr Robert Blagrove, and other scientists at the Division of Protein Chemistry in Melbourne work in collaboration with the Canberra team, with the narrow-leafed lupin (*Lupinus angustifolius*) as the focus of their attentions.

Lupin seed was chosen because of its potential as a substitute for soybean. Although lupin seeds have been used for food since ancient times, only recently has the cultivation of low-toxicity, low-alkaloid varieties become common in Australia. Modern lupin breeding has changed a wild plant into one suitable for large-scale seed production by introducing characters such as low alkaloid level, earlier flowering, permeable seed coat, disease resistance, and non-shattering pods. However, little attention has been paid to the improvement of protein quality, especially an increase in the level of sulphur-bearing amino acids.

Dr Gillespie and Dr Blagrove developed a simple and rapid procedure to screen lupin proteins (and other legume proteins too) by electrophoresis. In 10 minutes the method resolves the storage proteins into their components, allowing the relative proportions to be estimated.



To improve the nutritional value of legume seed protein, we should aim for increased levels of legumin.

Lupin seeds contain three storage proteins — mostly conglutin *alpha* and conglutin *beta*, but also a minor component, not previously recognized, named conglutin *gamma*. As a consequence of their differences in amino acid composition, increasing the proportion of conglutin *gamma* relative to the others should result in a seed with a higher content of many of the essential amino acids.

The scientists have screened many lupin cultivars and species for favourable composition of storage proteins. They found considerable variation in the proportions of storage protein types, encouraging Dr Rex Oram of the Division of Plant Industry to undertake breeding trials with some of the varieties.

A single gene was found, which markedly reduces the proportion of one poor-quality protein, and tends to increase a good-quality component. If more such genes can be uncovered, combinations of them could significantly improve amino acid balance.

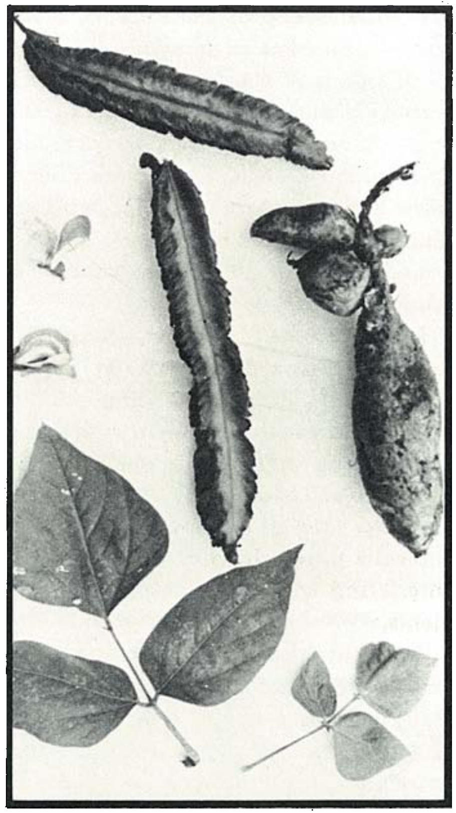
A completely edible plant

More recently, the scientists at the Division of Protein Chemistry have been looking at another legume, the winged bean (*Psophocarpus tetragonolobus*). It is suited to growth in the humid tropics and has nutritional advantages over the soybean (containing even more lysine) and lacks the somewhat bitter, beany flavour of the latter. Nearly all the parts of this perennial climbing plant can be eaten: the beans, pods, tuberous roots, leaves, and flowers. The ripe seeds contain 30–40% protein.

When the scientists applied electrophoresis to winged beans, they found that the storage proteins separated into three fractions, named psophocarpins A, B, and C. Of particular interest is the C component, normally a minor constituent, which contains a high level of sulphur-rich amino acids, just like the conglutin *gamma* of the lupin. Unfortunately, the seed storage proteins in the 80 examples collected from the highlands of Papua New Guinea show little diversity, leaving little scope for improvement by selective breeding among these lines.

Anti-nutritional factors

However good the protein quality of a legume, it stands for little if the seed is indigestible or toxic. For this reason, the Division of Protein Chemistry has been investigating the anti-nutritional factors in legumes.



They're all edible — the flowers, pods, leaves, and tubers of the winged bean.

Soybeans are known to contain anti-vitamin, anti-enzyme, goitrigen, oestrogen, and other toxins. Feeding raw soybeans to rats leads to poor growth, enlarged pancreas, excessive enzyme secretion, and general metabolic disturbance. Fortunately, many of the toxins are removed by soaking the beans in water or by cooking. The extent to which they are detrimental in the diet is therefore difficult to assess.

Dr Alex Kortt of the Division of Protein Chemistry is seeking to isolate anti-enzymes and to characterize their structure and function. These materials inhibit digestion by binding with digestive enzymes. They occur in lupin seeds in only small amounts, but in winged bean they constitute up to 5% of the protein.

Some scientists have speculated that the anti-enzymes may be part of the plant's defence mechanism against attack by insects. Insects dislike indigestion too! Furthermore, a single plant leaf wounded by an insect also induces anti-enzyme to accumulate throughout the plant. And if birds or other animals eat the seeds, the anti-enzyme will improve the chances that the seed will be excreted in a viable condition.

Another group of plant toxins, under study by Dr Michael Jermyn, is the family of lectins — plant proteins that interfere with digestion by binding to cell walls. When incorporated into the diet, they inhibit growth. Lectins were identified almost 90 years ago through their ability to clump red blood cells. They account for about 20% of the protein in winged bean.

Little is known of them, except that certain lectins preferentially act on tumour cells, introducing the possibility of a use in cancer therapy. Lectins may act as plant antibodies, produced in response to soil bacteria. Transferred to the digestive tract of an animal, they attack the cells lining the intestinal wall, thus interfering with the absorption of nutrients.

Anti-nutritional factors cause problems in some non-legume seeds as well. Rape seed is a useful oilseed crop in Australia, and the protein meal left after extraction of the oil has a well-balanced amino acid composition. However, in older cultivars, toxic constituents make the meal unsuitable for human consumption and of only limited use for livestock. Dr John Kirk and Dr Oram, at the Division of Plant Industry, are having considerable success in breeding lines of rape seed free of these constituents.

The future

Despite many recent advances in our knowledge of seed protein structure and function, there is still a long way to go before we can direct a seed to produce the proteins we want.

Nevertheless, progress is being made and, besides the CSIRO groups involved, considerable overseas research interest is devoted to this area. But, progress or not, it seems inevitable that the direct consumption of seed protein will become more widespread. Acquiring a taste for beans may be useful preparation for the future.

Andrew Bell

More about the topic

The biology of plant storage proteins and oils. D. Spencer. *CSIRO Division of Plant Industry, Annual Report*, 1978, 27–31.

Review — plant protein research. *Review and Abstracts, CSIRO Division of Protein Chemistry*, 1974, 3–25.

Plant protein research in the CSIRO Division of Protein Chemistry. R. J. Blagrove. *Food Technology in Australia*, 1979, **31**, 492–7.