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The extended use of fractionation processes

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Winnowing, flour milling and sugar extraction are traditional examples of the mechanical fractionation of agricultural products. Fodder fractionation is more novel. It enables a protein-rich fraction to be separated, for use by people and other non-ruminants, from a more fibrous fraction that can be used as ruminant feed. Because the fibre is partly dewatered in the process, less energy is needed to dry it than to dry the original forage for conservation as winter feed. The 'whey' remaining after separating protein from the expressed juice could simply be returned to the land as a source of nitrogen, phosphorus and potassium – perhaps after being used as a substrate for microorganisms.

Besides being used on crops grown specially for the purpose, fodder fractionation can be applied to various market garden and farm by-products – even to some, such as potato haulm, that are usually considered inedible. The process of extraction destroys detrimental physical structures and the 'whey' removes toxic components.

The production of almost all food involves some fractionation. Cereals are separated from chaff; potatoes are peeled; sugar is extracted from cane or, when cane itself is chewed, the pith is not swallowed; the petioles and outside leaves of many vegetables are rejected. In view of the last example of separation, or selection, it is odd that it has taken so long for the idea of fractionating forages to gain acceptance. We seem content to go on relying on methods of fractionation that are within the capacity of a moderately intelligent monkey or squirrel. The basic motive behind fractionation is simple. The use of a potentially valuable crop component may be restricted because of the presence of a useless or deleterious companion; there may even be several components in a crop, each of which would be useful but for the presence of the others.

FIELD FRACTIONATION

Selection of particular species from a mixed crop is the simplest method of fractionation. Sheep in semi-arid parts of Australia are said to get 80 % of their food from 1 % of the available vegetation: such selectivity would be difficult with mechanical harvesting. It is easy to harvest a crop at different levels. The upper, leafy and protein-rich, part of lucerne (*Medicago sativa*) is sometimes harvested separately (Klimes & Verosta 1962). Sugar cane, where it is harvested unburnt, and sugar beet are always separated in this way though the tops are seldom adequately used. It is also easy, as in combine harvesting, to separate parts of a crop on the basis of their physical characteristics. This technique has been extended to the separation of tree leaves from branches in Russia (Young 1976) and China (Anon. 1974*a*). By an extension of this principle, leaves are partly stripped from forage plants. Thus a suitably adjusted flail does so little damage to lucerne stems that a further crop of leaves grows on them. Ayres (1968) described a harvester which strips off 95 % of the leaf that is more than 20 cm above ground level. The leaf fraction contained 22–28 % crude protein, whereas the whole crop contained 17–21 %. The second harvest of leaf was about as large as the first and regrowth from surviving stems was quicker than from crowns after conventional harvesting.

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FRACTIONS FROM DRIED, OR PARTLY DRIED, FORAGES

Of the fodder conserved in Britain, 76 % is hay in spite of the many well known defects of haymaking as a means of conservation. For example:

The crop cannot be harvested while it is still strongly active photosynthetically, but must be left to mature. This introduces into the harvesting cycle a phase in which the crop is less productive than a crop kept in the juvenile state by frequent cutting;

Unless there is a spell of fine weather after cutting, loss is caused by leaching, moulding and respiration;

The fragile leafy part of the crop is most subject to loss during tedding (Shepherd 1959; Gordon, Holdren & Derbyshire 1969).

Damage after cutting leads to the loss of 30 % of the crop (National Economic Development Office 1974), but the fragility of the protein-rich, leafy parts of a crop can be used, in controlled conditions, as a prelude to fractionation. At the Hannah Dairy Research Institute (1947), broad leaved crops were partly dried so as to make the leaf blades, but not the petioles, brittle. After gentle grinding, the fraction that passed through a sieve contained more crude protein than the fraction retained. The method was not satisfactory on grass (Waite & Sastry 1949). A similar method was used on a factory scale on waste vegetable leaves by Colker, Eskew & Aceto (1948). They do not state the protein content of the starting material, but the best fraction from broccoli (*Brassica oleracea*) leaf contained 35.7 %. They comment on the carotenoid content of the meal and on the consequent brilliant yellow legs (a feature to which importance is attached in the U.S.A.) of poultry fed on it.

Lucerne varieties differ in leaf:stem ratio and therefore in their amenability to dry fractionation. Chrisman, Kohler, Mottola & Nelson (1971) studied several varieties and compared the effectiveness of sieving, and classification in an air current, as methods for separating fractions containing different amounts of protein. They made fractions containing 29 % protein, and argued that the merit of this form of fractionation is that it enables a compounder to sell a product that precisely meets agreed specifications for protein and carotene. This economic point was elaborated further by Vosloh, Kuzmicky, Kohler & Enochian (1972).

FRESH FORAGE FRACTIONATION

Choice between the various processes and extents of fractionation depends on the objective of the operation (Pirie, 1942, 1953, 1966*a*). This is convenient because it enables someone who has unsuccessfully tried to achieve one form of fractionation to become reconciled to a less complete form. Although leaf protein (l.p.) was extracted by Rouelle 200 years ago and has been extensively studied in the laboratory by Osborne, Chibnall and others (for an outline of the history see Pirie (1966*b*, 1971)), proposals that it should be extracted for practical use are recent. Ereky (1927) patented a machine for extracting l.p.; Goodall (1936) patented the use of conventional sugar cane rolls, but he attached more importance to the vitamins in the extract than to the protein. This was also the approach of Schnabel (1938). Slade & Birkinshaw (1939) also patented the extraction of l.p. and the use of various methods of separation that had been used on this and other proteins for a generation. In the light of the earlier academic work it is hard to see what validity these patents had. They have now expired. It is therefore still harder to see what points of any significance are being covered by the steady stream of patents still being allowed.

Expression of 'water' from leaves

There is less loss when forage is conserved by ensiling than by haymaking because less mature material is harvested and it is carted in immediately so that it does not become fragile. The main source of loss from silage is seepage (Derbyshire, Gordon, Holdren & Menear 1969; Watson & Nash 1960) when lush material is harvested. This loss can be largely prevented by various methods for making the crop wilt, i.e. partly dry out, in the field (Shepperson 1974). When crops are being dried in some form of furnace or oven, the gain from preliminary dewatering is even greater. This is especially true if the advantages of continuous operation necessitate drying material that still has dew, or even rain, on it. From a crop containing 93% water, 13 t of water have to be evaporated to get 1 t of dry matter (d.m.); from a crop containing 80%, 4 t, and from material containing 70%, only 2.3 t. Instead of relying on sun and air for the preliminary dewatering, many institutes and organizations have tried mechanical means. The advantage is at first sight obvious. From suitably pretreated material, juice can be expressed for one thousandth the expenditure of energy that would have been needed to evaporate the same amount of water – assuming that, as in ordinary grass-drying, the latent heat is not being recovered.

The fluid expressed from leaves by pure pressure is mainly vacuolar sap and contains little protein (Phillis & Mason 1937); fluid coming out because of guttation, plasmolysis, or the application of air or water pressure to the petiole is likewise nearly protein-free. In large scale practice it is not feasible to apply pure pressure; there is always some rubbing of leaf against leaf or against parts of the machine and this rubbing liberates protein. When forage is being processed without pretreatment, the juice that is expressed is mainly liberated as a result of this accidental rubbing. It therefore brings with it part of the protein of the leaf. Except when crops containing a large amount of sappy stem are being handled, or when there is surface water on the leaves, the amount of water removed is approximately proportional to the amount of protein removed. The lusher the leaf the greater the protein loss. These points were not appreciated by those who first tried to 'dewater' forage mechanically; judging from the frequent use of such phrases as 'pressing out juice', the points are still not universally appreciated. A great deal of money has been wasted in the past decade because of this and because mistakes made and publicized 30 years ago were made all over again.

Messrs Chivers (Histon) gave up attempts to 'dewater' pea haulm in the late 1930s when they found how much protein was being lost. They had used several extractors, including the now fashionable twin-screw expeller. The need for 'dewatering' is also acute in Florida. Single-screw expellers were used there (Randolph, Rivera-Brenes, Winfree & Green 1958; Casselman, Green, Allen & Thomas 1965), the juice was not collected but trickled out of the mobile unit on to the ground.

The loss of protein that makes it inadvisable to 'dewater' fresh forage can be prevented by heating the leaf so as to coagulate the protein *in situ* and destroy osmotic control so that juice can be easily pressed out. In the late 1930s, North Eastern Grass Driers (West Larmouth) pressed out juice with sugar cane rolls from leaves that had been passed through a tank of water heated to about 80 °C. Processes such as this have several merits (Pirie 1966*a*) if the primary objective is to economize on fuel consumption when drying winter forage for ruminants. They are being developed in Norway (Mathismoen 1974) and France (Gastineau 1976). There is little or no protein in juice pressed carefully from leaves heated to 80 °C. Juice pressed

from some species after heating to only 45–55 °C contains part of what is loosely called the 'cytoplasmic' protein. It is nutritionally more valuable than the 'chloroplast' protein. It could be collected, after coagulation by further heating, and used as food for people and other non-ruminants.

Extraction of protein from leaves

When forage is grown with the amounts of NPK and irrigation water that give maximum d.m. yield, and when it is cut regularly, it cannot be made into hay, it may be too strongly buffered to make silage (Wallace 1975), it would be extravagant to dry it, it contains more protein than a ruminant needs, and the excess protein may cause bloat. When it is fed fresh (zero grazing) the last two defects are sometimes remedied by diluting the forage with hay or some similar low-protein fodder. More often, farmers choose to use less fertilizer and get smaller yields of fodder containing less protein. In Britain 39% of the permanent grass gets no fertilizer (National Economic Development Office 1974); most of this land is too steep or rough to be mown, but much of the grass on land that is, or could be, ploughed would yield more d.m. if more adequately fertilized. Part of the war-time argument for l.p. production (Pirie 1942) depended on that point.

In an ideal fractionation process, each of the fractions is more useful than the original mixture. When l.p. is made, this ideal is nearly achieved. The more complete the liberation of l.p., the less water the pressed fibre will contain. The extracted protein from inedible crops can be eaten by non-ruminants. The 'whey' is a convenient substrate on which to cultivate microorganisms. This, essentially, is what was advocated by Ereky (1927), Slade & Birkinshaw (1939), Pirie (1942), Bickoff, Bevenue & Williams (1947) and Tallarico (1955).

Disintegration and pressing are in principle different processes: logically, therefore, equipment suited to the one would not be suited to the other. Because Rothamsted is primarily an agronomic institute, our early work was concerned more with demonstrating the feasibility of l.p. extraction and with measuring the possible yields from different crops and systems of husbandry, than with efficient methods for extraction in practice. For quantitative work it is essential that the conditions of extraction should be the same in each experiment: that is probably not feasible unless the crop is pulped and then pressed. Pulper and press combinations suitable for work at rates between 1 kg min⁻¹ and 5 t h⁻¹ have been described (Pirie 1971).

Extraction in one operation is obviously preferable when measurements are not being made, and all that is required is a supply of leaf extract or of partly dewatered fibre. Three-roll sugar cane mills were for a time reintroduced but no longer have any advocates. Several institutes use various types of screw expeller. The principle of the screw expeller is admirable for work on well lubricated materials in which the fluid that is to be expressed has already been released from cells or similar structures. It is for this reason that oilseeds are usually cooked before pressing. A conventional screw expeller will not extract protein-rich leaf juice if it is truly efficient as a press: inefficiency in the application of pressure leads to some incidental rubbing which releases juice. Furthermore, most expellers exert excessive pressure. After pressure at as little as 2 kgf cm⁻² (200 kPa) for a few seconds on a grid, the d.m. of a layer of leaf pulp 10 mm thick increases to over 30%.

In the late 1940s a large cordite mixer (Pfleiderer) was used at Rothamsted to produce batches of pulp with which presses could be tested. This demonstrated that slow rubbing was an effective method for liberating protein-rich juice from leaves. It should be possible to design

a unit in which little energy is wasted in friction between the smooth scroll of an expeller and the mass of leaf in it. By breaking the scroll into a set of angled paddles mounted on a cone, the mass would be continually rearranged, and the application of pressure would be the result of useful rubbing instead of being the primary objective. Because crops, unlike the materials usually fed into screw expellers, vary greatly from day to day in texture and in the ratio of juice volume to fibre volume, the unit must allow two adjustments to be made easily. The output of the feeding auger should be controllable to suit the amount of rubbing needed, and the extent to which the cone is inserted into the cage should be controllable so that paddles at either end of the cone sweep volumes that are in the correct ratio. With these principles in mind, the first steps have been taken towards designing a simple and effective unit (Pirie 1977). The economics of leaf fractionation will be falsely assessed until more effort is put into designing equipment for this precise purpose.

Feeding trials on whole juice

A joint paper from the Rowett Research Institute and the National Institute for Research in Dairying (Houseman & Connell 1976), and recent Annual Reports from the Agricultural Institute at Castleknock (Dublin) have commented briefly on the satisfactory performance of calves and pigs on diets supplemented with whole juice from grass or lucerne. Lucerne juice has also been used in Russia (Naumenko, Tarasenko & Kinsburgskii 1975). This procedure is obviously convenient, especially if forage is being fractionated on the farm, but it has defects. Depending on whether the crop is harvested young, semi-mature, with dew or rain on it, or at the end of a sunny afternoon, the protein content of the juice can vary by a factor of 5 and its d.m. content by a factor of 3. This complicates the calculation of diets. Soon after extraction, coagulation and proteolysis start in all the juices that have been tested, and these processes proceed at different rates in different species. The products of proteolysis probably have the same nutritive value as the original protein, but the presence of curd may make juice distribution troublesome. Some components of juice from all species are of doubtful nutritional value in non-ruminants, and soluble components from some species, e.g. kale (*Brassica oleracea*), are harmful. Furthermore, although adult pigs do well on whole lucerne juice (Braude 1976), young pigs do not like it (Connell 1975). For all these reasons, some separation of curd from 'whey' seems advantageous.

Separation of protein from leaf extracts

The protein in leaf extracts can be coagulated by heating or acidification. Heating is preferable because it inactivates enzymes and produces a dense, coherent, coagulum that is easy to separate from the 'whey' (Pirie 1971). Enzyme inactivation is important with crops, such as lucerne, rich in chlorophyllase. If juice is heated slowly so that the enzyme is allowed to hydrolyse phytol from chlorophyll, the resultant chlorophyllide may then lose magnesium. The resultant pheophorbide can make animals photosensitive (Lohrey, Tapper & Hove 1974). This is a very slight hazard with quick heating as in the original (Morrison & Pirie 1961) separation procedure. It can be prevented completely by injecting steam into a stream of leaf extract so as to heat it quickly to 100 °C (Arkcoll & Holden 1973). When coagulation, rather than enzyme inactivation, is all that is required, it is sufficient to heat to 70 °C, but quick heating remains advantageous. It is also advantageous to separate the curd from the 'whey' quickly to minimize combination of the protein with phenolic substances in the extract.

The effects of different processing conditions on the quality of l.p. are discussed more fully elsewhere (Pirie 1975*a*).

At first, part of the coagulum usually floats because of air trapped in it. Almost all of it can be made to float by bubbling air (or ideally N_2) into a tank of coagulated extract. If deaerated, the curd sinks. The volume of the sediment depends on the protein content of the extract, but the composition is fairly constant: 10–15% of the wet weight is protein. Because of its greater uniformity, and of the partial removal of the soluble components of the leaf, the sediment would seem to be the most suitable material for adding to liquid feeds for calves or pigs. Furthermore, because of the diminution in volume and removal of much buffering material, less formic, propionic or phosphoric acids, or sulphite, will be needed if an uneven supply of forage necessitates short term conservation.

Coagulated l.p. can be separated from 'whey' in standard equipment used in processing such materials as yeast, dyestuffs and sewage sludge. Material intended as human food should be pressed so as to separate as much 'whey' as possible from the curd, the curd should then be suspended in water at about pH 4 and filtered off again. This removes most of the leaf flavour and makes the second filtration easier. Obviously, as much 'whey' as possible should be pressed out each time so as to increase the effectiveness of washing. The pressed curd contains 60–65% water and, being acid and having been pasteurized, has the keeping qualities of cheese. Obviously, it should be used in the moist state whenever possible. It can be pickled, salted or dried by the methods commonly used with lipid-containing proteins.

As Rouelle observed, carefully controlled heating coagulates that fraction of the protein which is associated with chlorophyll; if this fraction is removed, nearly colourless protein can be separated by heating the fluid to a higher temperature. It is also possible to sediment all the chlorophyll-containing protein by centrifuging unheated juice at above 20 000 relative centrifugal force (e.g. Pirie 1950), but such a procedure is not likely to be feasible in large scale practice.

The quality of leaf protein

Protein made from many species, without separating the green and white fractions, contains 9–11% N. The green fraction contains 1 or 2% less, and the white fraction 1 or 2% more, N than the unfractionated material. Some species consistently yield products containing less N than that. If juice is properly strained before coagulation, l.p. will contain less than 1% of fibre. From some species, notably pea haulm (*Pisum sativum*) taken as a by-product from canning or freezing, it contains as much as 10% of starch.

Whole l.p., and the green fraction from it, contain 20–30% of highly unsaturated lipid (Lima, Richardson & Stahmann 1965; Buchanan 1969; Hudson & Karis 1973). Chlorophyll, its breakdown products, and part of the lipid are easily removed by non-polar solvents. This has caused some authors to underestimate the amount of lipid present. Complete lipid extraction necessitates the use of polar solvents. Partial lipid extraction is sometimes advocated by those who think a green product will be unacceptable. This fear is baseless: 1–2 weeks experience is sufficient to remove any initial unfavourable reaction. Furthermore, l.p. contains 0.1–0.2% of β -carotene which would be removed by solvent extraction. Vitamin A deficiency is often associated with protein deficiency; a few grams of l.p. daily would ameliorate or prevent both.

Surface dust or mud on leaves will appear in the final l.p. When human food is being made the crop should therefore be washed before being extracted. Properly pressed and washed l.p. from many species, should contain < 1% of material soluble in water, < 3% of ash and

< 1 % of acid insoluble ash. In spite of careful washing, leaves from species that contain much hydrated silica may yield l.p. containing more ash than that. When allowance is made for the presence of fibre, starch, lipid and ash, preparations usually contain less N than would be expected. The results of Allison, Laird & Synge (1973) suggest that this is in part the result of fixation of phenolic compounds by amino groups in the protein. When the ϵ -amino group of lysine is involved, this combination is nutritionally detrimental. Varieties of the same plant species differ in the amounts of phenolic material present; when herbage is to be fractionated to produce l.p. it will probably be worthwhile searching for varieties relatively free from phenolic compounds.

Byers (1971) found small, but probably real, differences in the amino acid compositions of bulk l.p. made from different species, and also differences between protein fractions made from the same extract. The relative amounts of 'chloroplastic' and 'cytoplasmic' protein in an extract depends on the age of the leaf. Until l.p., and protein fractions from it, from leaves of various ages and differing nutritional status have been compared, it would be premature to conclude that differences between species are larger than those between fractions, or between products from leaves of differing maturity. Similarly, the formation of complexes between unsaturated fats, phenolic compounds, etc. has so much effect on digestibility that it is reasonable to assume that the observed differences in nutritional value are the consequence of uncontrolled differences in the precise technique used in making the preparations.

Amino acid analysis suggested that l.p. would be surpassed in nutritive value by casein and egg protein but would be better than the seed proteins that are available in bulk. This suggestion was confirmed *in vivo*. The early measurements on animals were surveyed by Woodham (1971); and those on children by Singh (1971). Trials are now being made on many species both in laboratory conditions and in conditions which approximate to those of normal life. Examples are: Cowey, Pope, Adron & Blair (1971), Olatunbosun, Adadevok & Oke (1972), Anon. (1974*b*), Kawatra, Garcha & Wagle (1974), Toosy & Shah (1974), Kamalanathan, Karupiah & Devadas (1975) and Kanev, Boncheva, Georgieva & Iovchev (1976). L.p. was a consistently useful supplement, though, in spite of the apparent presence of adequate methionine in it, its value is enhanced by supplementation with methionine.

The use and conservation of the fibre residue

The precise manner in which the initial d.m. of the crop is distributed between extracted l.p., fibre residue, and 'whey', depends greatly on the species, physiological state of the crop, and the conditions of extraction used. In all practical circumstances, the fibre residue is the dominant fraction; plans for its effective use have therefore been assigned an essential part of the case for making l.p. (e.g. Pirie 1942). Neglect of this obvious point was responsible for some gloomy economic forecasts.

During the extraction of l.p., salts, carbohydrates and other substances are also extracted. Consequently, if half the protein is extracted from a crop, the N content of the residue is not halved. Byers & Sturrock (1965) analysed the residues from 17 species harvested at different stages of maturity. From grass containing on average 16.5 % crude protein, Maguire & Brookes (1973) made a residue containing 12.7 %; Connell (1975) from lucerne containing initially 2.5–4.8 % N, made residues containing 2.4–4.3 %. Although the N content of the residue is diminished, there are two reasons to expect its nutritive value to be greater than that of forage containing initially the same amount of N: the residue comes from younger and less lignified

material, and more of the N in it is true protein N. Experiment bears out this expectation, though few animals have so far been used and the experiments have been published in incomplete form only (Ulyatt 1971; Greenhalgh & Reid 1975). There are also comments in recent Annual Reports from the Rowett Research Institute and the National Institute for Research in Dairying, and in recent Proceedings of Technical Alfalfa Conferences (U.S. Department of Agriculture). All reports agree that the nutritive value of the residue is similar to that of the original crop. In some trials it was even better, perhaps because the fibres had been so extensively broken up during the extraction.

Silage was made from the residue at Rothamsted as soon as large scale work started. To ensure good fermentation, and to help in excluding air from the silage, some of the 'whey' was returned to it. Ulyatt (1971) pointed out that 'whey' would also return minerals otherwise deficient in silage made from pressed residue. No problems were encountered in making silage at most of the centres where l.p. is being studied (e.g. Oelshlegel, Schroeder & Stahmann 1969; Vartha, Fletcher & Allison 1973; Mungikar & Joshi 1976).

The advantages of fractionation are still greater if the residue is to be conserved by drying rather than ensiling because of the 'dewatering' that has already been commented on. Fibre that has been so thoroughly pressed that it is 35% or more d.m. is indeed so dry that it is not easy to control in some conventional grass driers. Driers can, however, be modified, and such a pressed residue can be dried in a current of air.

The significance of this diminution in the drying load depends on the ratio of energy expended in pulping and pressing, to energy expended in evaporation. Figures for the work done in extracting juice to different extents from a range of crops have not yet been published. General experience is that about 10 kW h (36 MJ) are needed for 1 t of an average crop, and about half the mass of the crop will appear as juice. The juice will contain about 450 kg water. Evaporating that amount of water would consume 1100 MJ. Sceptics and advocates comment in antithetical ways on this apparent immense saving of energy. On the one hand, latent heat of vaporization could, in principle, be recovered, and energy liberated by burning fossil fuel is applied directly to the crop during evaporation whereas there are losses when it is converted to mechanical energy. On the other hand, so few arbitrarily chosen conditions of pulping have been tested that it is reasonable to assume that more economical conditions will be found. The energy 'wasted' in a diesel engine appears at a site where it could be used to warm air to dry the fibre residue, and not much more than half the energy of combustion is actually used in any existing forage drier. Practical experience may not ultimately show that there is a 30-fold economy of energy when water is extracted, but the conclusion seems inescapable that the saving will be considerable.

These uncertainties have not discouraged economic forecasting at various times during the past 30 years. The conclusions need not be summarized because they depended on the initial prejudices of the assessors more than on the facts of the case, and the facts were changing: during this period, precariously financed work at Rothamsted increased the yield of l.p. per ha fivefold and decreased the energy expended on pulping threefold.

The use of the 'whey'

The composition of the 'whey' pressed from curd after heat coagulation depends on the weather at the time of harvest, the species that is processed, its stage of growth, and the amount of water that remains on the herbage after washing dust from it. The ranges in analyses

(Festenstein 1972) on 61 samples were: d.m. 11–47 g l⁻¹, carbohydrate 2.2–22 g l⁻¹, and N 0.25–1.2 g l⁻¹. Carbohydrate soluble in 80 % ethanol (i.e. sugars rather than polysaccharides) was 49–90 % of the total carbohydrate. The 'whey' contains about 15 % of the d.m. of the crop; a larger fraction of the d.m. is in the 'whey' from young than from mature crops, and a larger fraction of the N is in 'whey' from legumes than from other species. Most of the K and much of the P of the leaf is also in this fraction but there have been no systematic analyses. Various (unpublished) analyses of 'whey' made at other centres fall within these ranges.

The simplest way to dispose of the 'whey' is to sprinkle it back on an area of ground similar to that from which the crop came. The NPK in it is useful, and on some soils the carbohydrate in it stimulates bacteria that improve soil structure (Pirie 1973). When concentrated, it can depress regrowth of some crops and should not therefore be returned to a small area. In Hungary (Hollo & Koch 1971) it is evaporated *in vacuo* and the concentrate is mixed with the coagulum. The advantage of complicating the process in this way, rather than spray-drying the whole extract (Hartman, Akesson & Stahmann 1967), is that a fluid that does not coagulate on heating can be processed in an economical multiple-effect or vapour-compression unit. In spite of the thermal economies thereby attainable, it seems likely that only a 'whey' with unusually large initial d.m. content would contain enough nutritionally valuable material to be worth concentrating.

Ideally, 'whey' would be used as a substrate on which to grow microorganisms (Pirie 1951): their readiness to grow on it is embarrassing in summer. Jönsson (1962) grew 7 organisms on it, Shah (in Pirie 1971) extended the list, which has been still further extended by Paredes-Lopez & Camagro (1973) who found that 4 yeasts grew well on lucerne 'whey' and 3 would not grow. Commercial exploitation cannot be expected until a regular supply of 'whey' is assured.

Sources of leaf

Species that are already being grown for conservation by conventional means are obvious candidates for fractionation. The grasses and lucerne are most often used commercially, but rape (*Brassica napus*) and kale are useful (Hollo & Koch 1971) for extending the working season. The merits of many other species, as sources of l.p., were commented on in the Rothamsted Annual Reports (1952–72) and by Lexander *et al.* (1970). Trials on many species growing in India were surveyed recently (Pirie 1976*a*).

The two methods of fresh forage fractionation discussed here, because they alter the texture of the forage and remove much soluble material in the 'whey', can, in principle, make useful fibre and l.p. from unpalatable or toxic material (Pirie 1962). So much effort has been devoted to the selection of productive varieties of conventional forage plants that toxic plants are not likely to be used soon. Fractionation would however be useful with crops such as kale that are only slightly toxic. This is an aspect of fodder fractionation that has had too little attention. Potato haulm (Carruthers & Pirie 1975) has potentialities and so have several species of water weed that are at present wastefully destroyed (Boyd 1971; Pirie 1976*b*).

Commercial production of leaf protein

There is work on l.p. in research institutes in Australia, Belgium, Denmark, Eire, Hungary, India, Mexico, Netherlands, New Zealand, Nigeria, Norway, Pakistan, Philippines, Spain, Sri Lanka, Sweden, Taiwan, U.S.A., Vietnam, West Germany, and possibly other countries. In some of these countries there is also commercial research, and interest may by now have

resulted in the establishment of production units. It is difficult to get reliable information. Some of this work is supported by grants from the E.E.C.; a National Science Foundation committee recommends that the scale of work in the U.S.A. should be increased to 108 'scientific man years' (N.S.F.); a J.C.O. Report (1976) recommends increased work in the U.K.

In Britain, France and Hungary there is commercial production as well as academic research. Production started earlier in Hungary than elsewhere; the amount that is now being made is not known. France Luzerne (Chalons sur Marne) produced 3 t a day during 1975 and plans greater productivity in 1976. The product contains 50 % protein, but it is mainly valued for its xanthophyll content. More refined material, suitable for use as human food, will be made later. With cooperation from companies interested in manufacturing equipment, Dengie Crop Driers Ltd (Southminster, Essex) has just started production. Arrangements for the production and/or use of l.p. are being made by various feeding stuffs compounders, e.g. B.O.C.M. Silcock in the U.K., and there is interest, but apparently no commercial production, in Japan and the U.S.A.

FUTURE DEVELOPMENT OF FODDER FRACTIONATION

Fodder fractionation will probably enable us to exploit more fully the potential productivity of leaf crops; it will also cheapen the production of winter feed for ruminant and non-ruminant animals. These probabilities should not make us lose sight of the fact that the object of agriculture is the production of human food. Animal conversion is a pleasant but extravagant means towards that end. Therefore, when we strive to produce very large yields of fodder so good that it has to be fractionated before being conserved or even fed fresh, we should wonder whether, with comparable effort, edible vegetables could have been grown. General awareness of the productivity of leafy vegetables is hindered by the almost universal failure of those concerned with research on vegetable crops to publish figures from which the yield of edible material can be calculated (Pirie 1975*b*). Perhaps market gardeners do not realize that their product has nutritional as well as aesthetic value!

In countries that regularly get prolonged spells of fine weather, it may be reasonable to fractionate dry, or partly dry, material. Elsewhere, one of the methods in which juice is expressed before drying would be preferable. Choice between pressing heated leaf, so that most of the protein remains in the leaf, and extracting most of the protein along with the juice, depends on the scale of work envisaged and on the use that can be made of the extracted protein. Pressing heated leaf seems to have few advantages as a farm operation, whereas protein extraction on the farm has great potentialities as an adjunct to pig and chicken feeding.

The primary reason for suggesting that fodder should be fractionated by either method is that less energy would be needed to evaporate water when dry winter fodder is made. As with many other energy conserving projects, this theoretical advantage would be nullified if the necessary equipment must be very elaborate and expensive. Equipment designed long ago, or for another purpose, is adequate for use in experimental work to discover the potentialities of new crops, and to work out methods for using the extracted protein and the fibre residue; the economics of the processes cannot be assessed until simple, robust equipment has been designed. This equipment should be designed for both farm and factory scale. For economy, and also to avoid local pollution, proper use of the 'whey' will ultimately be essential. This problem does not arise if whole juice is fed to animals on a farm. On a factory scale, a practical

but crude solution is to pump it back on to the land. This is a facet of the general problem of the economical disposal of effluents and it would be a pity if anxiety about it delayed studies on problems that are more specifically associated with fodder fractionation.

REFERENCES (Pirie)

- Allison, R. M., Laird, W. M. & Synge, R. L. M. 1973 Notes on a deamination method proposed for determining 'chemically available lysine' of proteins. *Brit. J. Nutr.* **29**, 51.
- Anon. 1974a *J. Flour & Animal Feed Milling* **156** (7), 45.
- Anon. 1974b Alfalfa protein for human use. *Agric. Sci. Rev.* **11** (2), 55.
- Arkcoll, D. B. & Holden, M. 1973 Changes in chloroplast pigments during the preparation of leaf protein. *J. Sci. Fd Agric.* **24**, 1217.
- Ayres, G. E. 1968 Field harvesting alfalfa leaves. *Proc. 10th Tech. Alfalfa Conf. U.S.D.A.* 1968, p. 97.
- Bickoff, E. M., Bevenue, A. & Williams, K. T. 1947 Alfalfa has a promising chemurgic future. *Chemurgic Digest* **6**, 215.
- Boyd, C. E. 1971 Leaf protein from aquatic plants. In *Leaf protein: its agronomy, preparation, quality and use*, I.B.P. Handbook **20** (ed. N. W. Pirie), p. 44. Oxford: Blackwell.
- Braude, R. 1976 New sources of protein for pigs. *Proc. Nutr. Soc.* **35**, 93.
- Buchanan, R. A. 1969 *In vivo* and *in vitro* methods of measuring nutritive value of leaf protein preparations. *Brit. J. Nutr.* **23**, 533.
- Byers, M. 1971 The amino acid composition and *in vitro* digestibility of some protein fractions from three species of leaves of various ages. *J. Sci. Fd Agric.* **22**, 242.
- Byers, M. & Sturrock, J. W. 1965 The yields of leaf protein extracted by large-scale processing of various crops. *J. Sci. Fd Agric.* **16**, 341.
- Carruthers, I. B. & Pirie, N. W. 1975 The yields of extracted protein, and of residual fibre, from potato haulm taken as a by-product. *Biotechnol. & Bioeng.* **17**, 1775.
- Casselmann, T. W., Green, V. E., Allen, R. J. & Thomas, F. H. 1965 Mechanical dewatering of forage crops. *Univ. Florida Agric. Exp. Stn., Tech. Bull.* **694**.
- Chrisman, J., Kohler, G. O., Mottola, A. C. & Nelson, J. W. 1971 High and low protein fractions by separation milling of alfalfa. *U.S. Dept Agric. Agric. Res. Serv.* 74-57.
- Colker, D. A., Eskew, R. K. & Aceto, N. C. 1948 Preparation of vegetable leaf meals. *U.S.D.A. Tech. Bull.* **958**, p. 53.
- Connell, J. 1975 The prospects for green crop fractionation. *Span* **18**, 103.
- Cowey, C. B., Pope, J. A., Adron, J. W. & Blair, A. 1971 Studies on the nutrition of marine flatfish. Growth of the plaice *Pleuronectes platessa* on diets containing proteins derived from plants and other sources. *Marine Biology* **10**, 145.
- Derbyshire, J. C., Gordon, C. H., Holdren, R. D. & Menear, J. R. 1969 Evaluation of dewatering and wilting as moisture reduction methods for hay-crop silage. *Agron. J.* **61**, 928.
- Ereky, K. 1927 Vegetable foods and medicines for men and animals. *Brit. Pat.* 270629.
- Festenstein, G. N. 1972 Water-soluble carbohydrates in extracts from large-scale preparation of leaf protein. *J. Sci. Fd Agric.* **23**, 1409.
- Gastineau, C. 1976 Advertising material from France Luzerne.
- Goodall, C. 1936 Improvements relating to the treatment of grass and other vegetable substances. *Brit. Pat.* 457789.
- Gordon, C. H., Holdren, R. D. & Derbyshire, J. C. 1969 Field losses in harvesting wilted forage. *Agron. J.* **61**, 924.
- Greenhalgh, J. F. D. & Reid, G. W. 1975 Mechanical processing of wet roughage. *Proc. Nutr. Soc.* **34**, 74A.
- Hannah Dairy Research Institute 1947 *15th to 18th Annual Report*, p. 14.
- Hartman, G. H., Akeson, W. R. & Stahmann, M. A. 1967 Leaf protein concentrate prepared by spray-drying. *J. agric. Fd Chem.* **15**, 74.
- Hollo, J. & Koch, L. 1971 Commercial production in Hungary. In *Leaf protein: its agronomy, preparation, quality and use*, I.B.P. Handbook **20** (ed. N. W. Pirie.), p. 63. Oxford: Blackwell.
- Houseman, R. A. & Connell, J. 1976 The utilization of the products of green-crop fractionation by pigs and ruminants. *Proc. Nutr. Soc.* **35**, 213.
- Hudson, B. J. F. & Karis, I. G. 1973 Aspects of vegetable structural lipids: I. The lipids of leaf protein concentrate. *J. Sci. Fd Agric.* **24**, 1541.
- Joint Consultative Organization Report. 1976 *Protein feeds for farm livestock in the U.K.* London: A.R.C.
- Jönsson, A. G. 1962 Studies in the utilization of some agricultural wastes and by-products by various microbial processes. *Kungl. Lanibrukshögskolans Ann.* **28**, 235.
- Kamalanathan, G., Karupiah, P. & Devadas, P. R. 1975 Supplementary value of leaf protein and groundnut meal in the diets of preschool children. *Ind. J. Nutr. Dietet.* **12**, 203.

- Kanev, S., Boncheva, I., Georgieva, L. & Iovchev, N. 1976 Tests of a protein concentrate from lucerne for fattening pigs and poultry. *Nutr. Abs. Rev.* **46**, 282.
- Kawatra, B. L., Garcha, J. S. & Wagle, D. S. 1974 Effect of supplementation of leaf protein extracted from berseem (*Trifolium alexandrinum*) to wheat flour diet. *J. Fd Sci. Tech.* **11**, 241.
- Klimes, I. & Verosta, B. 1962 Neue Methode der Erzeugung von Eiweiss-Vitamin Konzentrat aus Futterpflanzen. *Arch. Tierernahrung* **11**, 393.
- Lexander, K., Carlsson, R., Schalen, V., Simonsson, A. & Lundborg, T. 1970 Quantities and qualities of leaf protein concentrates from wild species and crop species grown under controlled conditions. *Ann. appl. Biol.* **66**, 193.
- Lima, I. H., Richardson, T. & Stahmann, M. A. 1965 Fatty acids in some leaf protein concentrates. *J. agric. Fd Chem.* **13**, 143.
- Lohrey, E., Tapper, B. & Hove, E. L. 1974 Photosensitization of albino rats fed on lucerne-protein concentrate. *Brit. J. Nutr.* **31**, 159.
- Maguire, M. F. & Brookes, I. M. 1973 The effects of juice extraction on the composition and yield of grass crops for dehydration. *First Internat. Green Crop Drying Cong.*, p. 346.
- Mathismoen, P. 1974 Stord twin screw presses: design and application on alfalfa. *Proc. 12th Tech. Alfalfa Conf.* 1974, p. 135.
- Morrison, J. E. & Pirie, N. W. 1961 The large scale production of protein from leaf extracts. *J. Sci. Fd Agric.* **12**, 1.
- Mungikar, A. M. & Joshi, R. N. 1976 Studies on the ensilage of the residues left after the extraction of leaf protein from lucerne and hybrid napier grass. *Ind. J. Nutr. Dietet.* **13**, 39.
- National Economic Development Office 1974 U.K. farming and the Common Market: grass and grass products. London: N.E.D.O.
- National Science Foundation (to be published). A comprehensive analysis of protein resources: present status, future requirements and research needs.
- Naumenko, V., Tarasenko, A. & Kinsburgskii, Z. 1975 Juice from lucerne in feeds for young pigs. From *Nutr. Abs. Rev.* **45**, 580.
- Oelshlegel, F. J., Schroeder, J. R. & Stahmann, M. A. 1969 Protein concentrates: use of residues as silage. *J. agric. Fd Chem.* **17**, 796.
- Olatunbosum, D. A., Adadevoh, B. K. & Oke, O. L. 1972 Leaf protein: a new protein source for the management of protein calorie malnutrition in Nigeria. *Nigerian med. J.* **2**, 195.
- Paredes-Lopez, O. & Camagro, E. 1973 The use of alfalfa residual juice for production of single-cell protein. *Experientia* **29**, 1233.
- Phillis, E. & Mason, T. G. 1937 Concentration of solutes in vacuolar and cytoplasmic saps. *Nature, Lond.* **140**, 370.
- Pirie, N. W. 1942 Direct use of leaf protein in human nutrition. *Chem. & Ind.* **61**, 45.
- Pirie, N. W. 1950 The isolation from normal tobacco leaves of nucleo-protein with some similarity to plant viruses. *Biochem. J.* **47**, 614.
- Pirie, N. W. 1951 The circumvention of waste. In *Four thousand million mouths* (eds F. LeGros Clark & N. W. Pirie), p. 180. Oxford University Press.
- Pirie, N. W. 1953 Large-scale production of edible protein from fresh leaves. *Rep. Rothamsted exp. Stn for 1952*, p. 173.
- Pirie, N. W. 1962 Progress in biochemical engineering broadens our choice of crop plants. *Economic Botany* **15**, 302.
- Pirie, N. W. 1966a Fodder fractionation: an aspect of conservation. *Fertil. feed. Stuffs J.* **63**, 119.
- Pirie, N. W. 1966b Leaf protein as a human food. *Science* **152**, 1701.
- Pirie, N. W. (ed.) 1971 *Leaf protein: its agronomy, preparation, quality and use*. I.B.P. Handbook 20. Oxford: Blackwell.
- Pirie, N. W. 1973 Effects of leaf protein 'whey' on soil. *Rep. Rothamsted exp. Stn for 1972*, p. 117.
- Pirie, N. W. 1975a The effect of processing conditions on the quality of leaf protein. In *Protein nutritional quality of foods and feeds*. (ed. M. Friedman), p. 341. New York: Marcel Dekker.
- Pirie, N. W. 1975b The potentialities of leafy vegetables and forages as food protein sources. *Baroda J. Nutr.* **2**, 43.
- Pirie, N. W. 1976a Food protein sources. *Phil. Trans. R. Soc. Lond. B* **274**, 489.
- Pirie, N. W. 1976b Leaf protein. In *Food from waste* (eds G. G. Birch, K. J. Parker & J. T. Worgan), p. 180. London: Applied Science.
- Pirie, N. W. 1977 A simple unit for extracting leaf protein in bulk. *Exp. Agric.* **13**, 113.
- Randolph, J. W., Rivera-Brenes, L., Winfree, J. P. & Green, V. E. 1958 Mechanical dewatering as a potential means for improving the supply of quality animal feeds in the tropics and sub-tropics. *Proc. Soil Crop Sci. Soc. Florida* **18**, 97.
- Schnabel, C. F. 1938 Vitaminic product from grass juices. *U.S. Pat.* 2,133,362.
- Shepherd, W. 1959 The susceptibility of hay species to mechanical damage. I. Effects of growing and curing conditions. *Aust. J. agric. Res.* **10**, 788.
- Shepperson, G. 1974 Dry conservation of grass. *Agric. Engineer* **29**, 40.
- Singh, N. 1971 Feeding trials with children. In *Leaf protein: its agronomy, preparation, quality and use*, I.B.P. Handbook 20 (ed. N. W. Pirie), p. 131. Oxford: Blackwell.

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- Slade, R. E. & Birkinshaw, J. H. 1939 Improvements in or relating to the utilization of grass and other green crops. *Brit. Pat.* 511525.
- Tallarico, G. 1955 Information from advertising material produced by Prodotti Alimentari Sistema Tallarico, Rome.
- Toosy, R. Z. & Shah, F. H. 1974 Leaf protein concentrate in human diet. *Pak. J. Sci. ind. Res.* **17**, 40.
- Ulyatt, M. J. 1971 Nutritive value of the residue from protein-extracted herbage. *J. Sci. Fd Agric.* **22**, 1791.
- Vartha, E. W., Fletcher, L. R. & Allison, R. M. 1973 Protein-extracted herbage for sheep feeding. *N.Z. J. exp. Agric.* **1**, 171.
- Vosloh, C. J., Kuzmicky, D. D., Kohler, G. O. & Enochian, R. V. 1972 Alfalfa products from separation milling – an economic study. *Proc. 11th Tech. Alfalfa Conf.*, p. 136.
- Waite, R. & Sastry, K. N. S. 1949 The composition of timothy (*Phleum pratense*) and some other grasses during seasonal growth. *Emp. J. exp. Agric.* **17**, 179.
- Wallace, G. M. (ed.) 1975 *Leaf protein concentrates (New Zealand scene)*. Ruakura: Agric. Res. Cent.
- Watson, S. J. & Nash, M. J. 1960 *The conservation of grass and forage crops*. Oliver & Boyd.
- Woodham, A. A. 1971 In *Leaf protein: its agronomy, preparation, quality and use*, I.B.P. Handbook 20 (ed. N. W. Pirie), p. 115. Oxford: Blackwell.
- Young, H. E. 1976 Muka: a good Russian idea. *J. For.* **74**, 160.

Immediately after the Royal Society meeting, the British Grassland Society and British Society for Animal Production covered the subject comprehensively in a symposium that has been published as *Green crop fractionation*, ed. R. J. Wilkins, Occasional Symposium 9 of the British Grassland Society. At the beginning of 1978, *Leaf protein and other aspects of fodder fractionation* (N. W. Pirie) will be published by Cambridge University Press.