PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Legume Proteins in Food Technology

D. J. Wright and M. R. Bumstead

Phil. Trans. R. Soc. Lond. B 1984 **304**, 381-393
doi: 10.1098/rstb.1984.0033

Phil. Trans. R. Soc. Lond. B **304**, 381–393 (1984)
Printed in Great Britain

Legume proteins in food technology

By D. J. Wright and M. R. Bumstead
A.R.C. Food Research Institute, Colney Lane, Norwich, NR4 7UA, U.K.

A number of important aspects concerning the use of legume proteins as ingredients for the processed food manufacturer will be discussed. The conversion of legumes into a material suitable for incorporation into food products involves a series of physical and chemical operations (basically representing various stages of protein purification), each of which can have major implications with regard to the eventual usage of the material. The available processing options will be described as well as their potential ramifications in terms of the nature, composition and properties of the protein fractions obtained. Alternative approaches to the tailoring or modification of the functional behaviour of these fractions will be presented together with a comparative assessment of the basic functional properties of a number of storage globulins from representative members of the Leguminosae.

Introduction

Setting aside nutritional considerations, proteins are used as food ingredients for their functional properties, i.e. to provide a certain specific function in the product. Many examples of such functional properties have been listed (Kinsella 1976), but some of the more important ones relate to a protein's ability to stabilize emulsions and foams or impart textural attributes. The first two examples represent disperse systems in which one phase (air or oil) is dispersed throughout a continuous phase (water). Examples of emulsions are margarine and salad cream, and of foams, whipped desserts and toppings. The two phases are generally immiscible and would revert to two separate phases without the presence of a stabilizing force. The latter can be provided by proteins, which migrate to the air:water or oil:water interface and, on unfolding, form an interface layer with consequent alteration to the surface properties (Halling 1981). The precise mechanism of the action of the protein is unknown but because of the nature of the interface it is not unreasonable to assume that the balance of hydrophobic to hydrophilic regions (and therefore amino acid composition and sequence) in a given protein would have a significant bearing on its ability to stabilize such dispersions. As for the texturing properties of proteins, these originate in the capacity of proteins to heat-set and form a stable matrix or gel. The best example is afforded by meat but other applications include the formation of solid foams as, for example, in cakes. Gelling involves the denaturation of proteins, basically the rupture of intramolecular bonds and unfolding of polypeptide chains, followed by the formation of intermolecular crosslinks between newly exposed residues of the denatured protein. The ability of the protein to gel will depend upon its size, structure and the nature of internal bonding and also upon extrinsic parameters such as concentration and solvent characteristics. In addition, however, the previous history of the protein, in particular whether or not it has been subjected to any denaturing conditions (for example, moist heat, extremes of pH) during isolation and preparation, will be a determining factor.

All this applies equally well to vegetable or legume proteins. If they are to find wide and

continuing use in food processing, then they must be able to perform the necessary functions in the product. This review will be concerned with some factors affecting the expression of the functional properties of legume storage proteins with particular reference to those of soya and pea. Two principal aspects will be considered, first, processing procedures and their potential implications with regard to protein functionality, and secondly the methods available for altering the intrinsic functional properties of either the proteins themselves or of materials prepared from them. It must be stressed, however, that neither of these areas should be considered in total isolation: options chosen in one can often have ramifications with regard to the other.

TABLE 1. COMPOSITION OF SOYBEAN AND PEA (% DRY MATTER)

	soybean	pea
protein	40.4	22.5
oil (lipid)	22.3	2.5
starch	—	47.0
soluble carbohydrates	9.3	7.0
insoluble carbohydrates	22.6	18.8

PROCESSING AND FUNCTIONALITY

Unlike many other raw materials destined for manufacturing into foods, legumes are very rarely available in a form that is immediately useable by the food industry. Varying amounts of processing are essential to transform then into materials or ingredients suitable for the food processor. Currently there are several procedures for achieving this transformation but they all have implications with regard to the properties and therefore subsequent use of the material.

The operations involved in the processing of legumes into raw materials essentially represent stages in the purification of the protein constituents. However a comparison of the respective compositions of two typical members of the legume family suggests that these processing requirements will vary from one legume to another (table 1). The steps involved in the processing of an oilseed such as soybean are shown in figure 1. Damage to protein constituents can occur at several stages in this process. For example, desolventizing after solvent extraction of oil involves moist heat treatment and, as already alluded to briefly, the application of heat to proteins is incompatible with maintenance of a fully native protein and therefore complete retention of functional properties. Similar comments apply to the use of heat for other purposes, such as the reduction of undesirable flavours and inactivation of lipoxygenase in soy. Often a compromise is reached in which the amount of heat applied is dictated by the end-use of the material. To prepare protein concentrates from defatted flour, the protein is immobilized by a choice of treatments to enable removal by washing of the soluble sugars (figure 1). Because of their nature, all these treatments listed leave the functionality of the proteins somewhat impaired, with least damage being inflicted by the dilute acid leach. On the other hand the preparation of protein isolates involves fairly mild conditions (figure 1), but even isoelectric precipitation at pH 4.5 can lead to loss of solubility (Lillford & Wright 1981; Anderson 1974). Neutralization of the protein isolate before drying gives the proteinate that tends to have superior solubility characteristics compared to the isolate, though the increased residual salt levels could pose a problem in subsequent processing and formulation. An even milder procedure for the preparation of isolates was proposed recently by Murray *et al.* (1978). The

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

process involves extraction in salt solution and then precipitation of the protein by reducing the ionic strength through the addition of water. The resultant isolate suffers far less denaturation than a conventional isolate, a fact readily apparent from differential scanning calorimetric studies of the two materials (figure 2) and one that will obviously have a significant bearing on their respective functionalities.

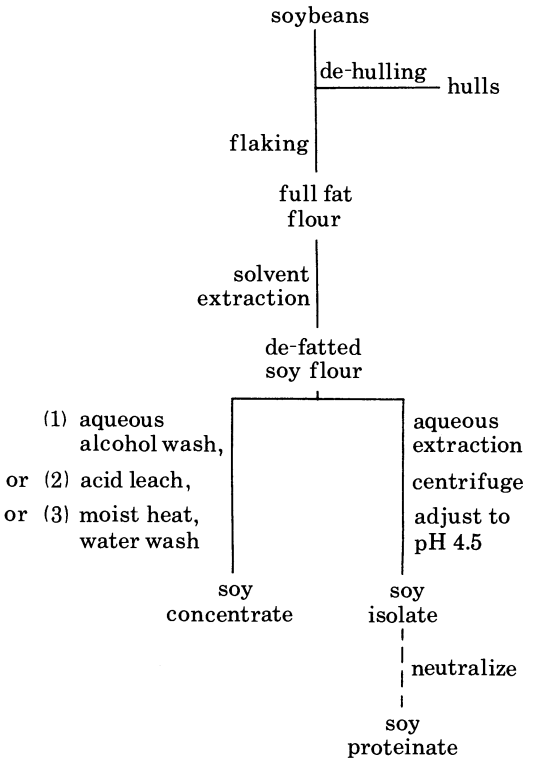

FIGURE 1. Processing of oilseeds (soybean).

For the purposes of comparison, the proximate analyses of the three principal types of ingredient obtained through oilseed processing, namely defatted flour, concentrate and isolate, are presented in table 2. Of course, as highlighted in this last example, this data can only be regarded as a guide to the potential functionality of the material.

Similar materials to soy isolates and concentrates can also be prepared from the non oil-bearing legumes such as pea (Gwiazda *et al.* 1979; Sumner *et al.* 1981), field bean (Flink & Christiansen 1973) and mung bean (Thompson 1977). In these cases, however, there is an alternative and, in many respects, more attractive process available termed air-classification. When applied to a very finely milled flour it yields protein- and starch-enriched fractions (Vose *et al.* 1976). The whole basis of the separation depends on the size differential existing between the protein bodies and the starch granules in the seed. For peas, the protein bodies range in size up to approximately 3 μm (Weber & Neumann 1980) while the average size of the starch granules is roughly 22 μm (Reichert 1981). To improve the yield of protein fraction and thereby increase the overall efficiency of the process, the coarse fraction from the first classification stage is normally re-milled and then subjected to a further classification (Vose *et al.* 1976). The two fine fractions are combined to yield the protein concentrate. The composition of such a

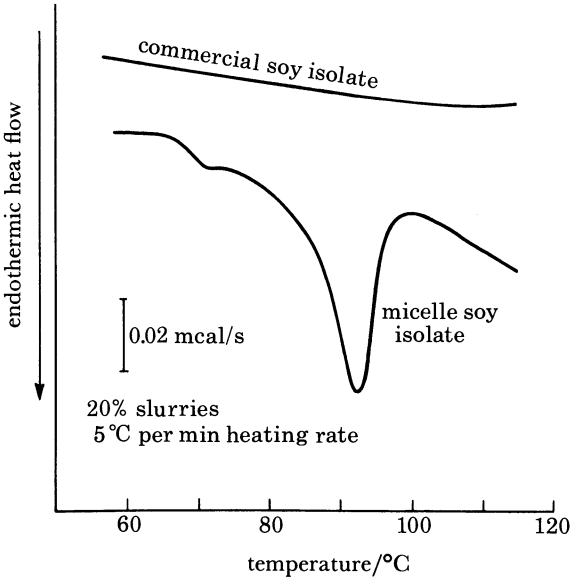

FIGURE 2. Differential scanning calorimetric thermograms of a commercial soybean isoelectric isolate and a soybean micelle isolate. Reproduced from Arntfield & Murray (1981) with permission. Copyright Canadian Institute of Food Science and Technology. 1 mcal = 1 millicalorie. 1 calorie = 4.187 J.

TABLE 2. PERCENTAGE COMPOSITION OF SOY INGREDIENTS†

product	protein	fat	fibre	ash	soluble carbohydrates	insoluble carbohydrates
flour	56.0	1.0	3.5	6.0	14.0	19.5
concentrate	72.0	1.0	4.5	5.0	2.5	15.0
isolate	96.0	0.1	0.1	3.5	0	0.3

† Horan (1974)

TABLE 3. COMPOSITION OF PEA INGREDIENTS (% DRY MATTER)

pea ingredient	protein	starch	lipid	soluble carbohydrate	insoluble carbohydrate
flour	22.5	47.0	2.5	8.0	18.8
protein concentrate	55.1	4.0	7.0	13.0	18.8
protein isolate	84.0	—	12.3	—	—

concentrate prepared from peas is shown in table 3 together with that for the whole pea flour and a wet processed pea protein isolate for comparison. At roughly 55% protein content, these concentrates are more equivalent to a defatted soy flour than a soy protein concentrate, particularly as they still contain appreciable amounts of soluble sugars. Nevertheless, the relatively mild nature of the processing involved in the manufacture of the concentrates ensures that the protein components retain their maximum functionality. One other interesting fact emerges from the data in table 3. It is apparent that the use of either processing method (i.e. aqueous extraction or air classification) results in a fractionation of the lipid component along with the protein. In the case of the isolate, this effect can be significant with as much as 12.3%

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

lipid present compared to approximately 0.1% in a soy protein isolate. Of course, the latter is prepared from a defatted soy flour, but experiments have shown that even the use of a defatted pea flour for the preparation of an isolate produces one containing 7.7% lipid. This residual lipid represents the 60% of the total lipid not extractable with hexane (phospholipids and glycolipids). This high lipid content can have a profound effect on the behaviour of pea protein isolates and concentrates. This is evident from the results shown in table 4 of foaming

TABLE 4. FOAMING PROPERTIES OF PEA INGREDIENTS

	protein content, %†	foam expansion (per g ingredient)‡
flour: full fat	18.8	81±3
defatted	20.3	169±7
isolate: full fat	79.1	28±2
defatted	84.6	226±8
concentrate: full fat	50.0	154±2
defatted	44.8	275±4
whey protein: full fat	26.8	407±9

† N × 6.25.
‡ 0.6% dispersion, pH 7.0. Method from Leatherhead Food R.A. Research Report No. 358, 1981. The functional properties of milk proteins.

experiments conducted on these materials. The removal of the neutral lipid by hexane extraction is seen to enhance greatly the foaming properties, particularly for the protein isolate. In addition, however, it is clear that the storage proteins are not the sole dictators of foaming properties since, on a protein basis, both the flour and the protein concentrate are more effective than the isolate. The answer appears to be connected with the whey protein fraction (a by-product of isolate production) which exhibits very good foaming behaviour (table 4). The high foaming capacity of the whey protein is probably attributable to the presence of such compounds as peptides and glycosides. These will also be present in the flour and the concentrate but not, because of extraction procedures, in the isolate. Apart from its effect on functional properties the high lipid levels of the pea concentrates and isolates may also present a problem with regard to storage of these materials because of the various problems associated with lipid oxidation (Wolf 1975; St Angelo & Ory 1975; Ory & St Angelo 1982).

These few examples illustrate the point that processing can be all important in determining the functional properties and, ultimately, the uses of vegetable protein materials and also that simple compositional analyses are frequently not sufficient in themselves to characterize fully the behaviour of such materials. Once control of the processing factors has been achieved then it is worth considering other options for the improvement or alteration of the intrinsic functional behaviour of these proteins.

MANIPULATION OF FUNCTIONAL PROPERTIES

It is now generally accepted that the storage protein of legumes is made up of two types of protein designated 7 *S* and 11 *S* or vicilin and legumin (Derbyshire *et al.* 1976). Recent evidence pointing to the existence of extensive heterogeneity in these proteins suggests that each would be more correctly referred to as a family of closely related proteins rather than to single

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

molecular species (Iibuchi & Imahori 1978; Kitamura *et al.* 1980; Utsumi & Mori 1980; Gatehouse *et al.* 1981; Casey 1979; Gillespie & Blagrove 1975; Utsumi *et al.* 1981). However, for the purpose of the following discussion it will be assumed that functionally each of the protein families acts as a single entity. As it is, currently there is no published evidence that either supports or refutes this assumption. On the other hand, if the functional properties were to differ

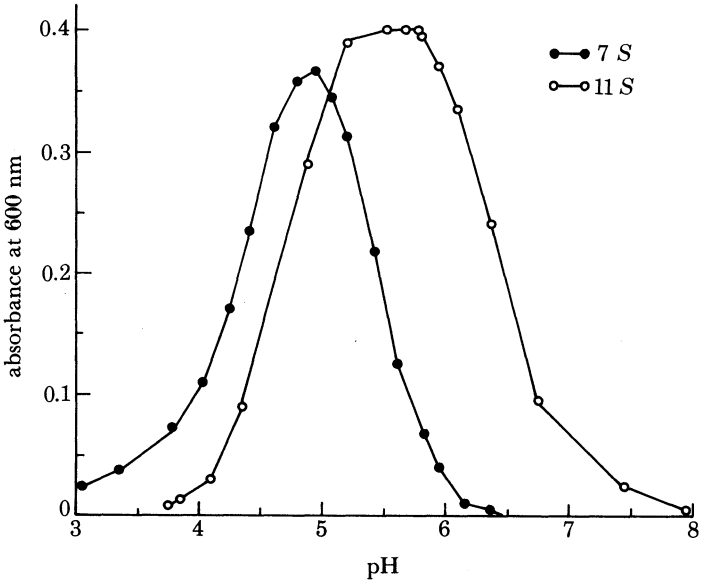

FIGURE 3. Effect of pH on precipitation of 7 *S* and 11 *S* soy globulins in 0.06 M Tris-HCl buffer. Reproduced from Thanh & Shibasaki (1976) with permission. Copyright American Chemical Society.

significantly *between* the two protein (7 *S* and 11 *S*) classes, then the manipulation of the relative amounts of these two proteins in the processed material could afford a means of modifying the functional behaviour of the latter. There are a number of ways in which the ratio of 7 *S* to 11 *S* globulin could be altered. Firstly it could be achieved via processing operations through the use of inherent differences in the properties of the two protein types. For example, differences in the pH–solubility profiles of the 7 *S* and 11 *S* globulins of soy have been used in preparative procedures (Thanh & Shibasaki 1976) (figure 3) and also more recently, in a patent covering the preparation of 7 *S* and 11 *S*-enriched fractions from legumes in general (Lehnhardt *et al.* 1983). In another instance, the differential effect of divalent cations such as Mg^{2+} and Ca^{2+} on the precipitability of 7 *S* and 11 *S* soy globulins has been used to effect their separation (Koshiyama 1965; Saio *et al.* 1973; Rao & Rao 1977) (figure 4).

A second option for altering the balance of 7 *S* and 11 *S* globulins is to employ any naturally occurring variation and then to enhance this through the selection of either high 7 *S* or high 11 *S* lines in a breeding programme. Current research into both the synthesis and the genetics of inheritance of these storage proteins should help to indicate what constraints are likely to govern such a breeding exercise (Croy *et al.* 1980*a*; Higgins & Spencer 1981; Beachy *et al.* 1980; Thomson & Schroeder 1978; Casey 1979). Published values for the range in 7 *S*:11 *S* globulin ratios for three legumes are listed in table 5. It is immediately apparent that none of these legumes individually covers the entire range of 7 *S*:11 *S* globulin ratios encountered.

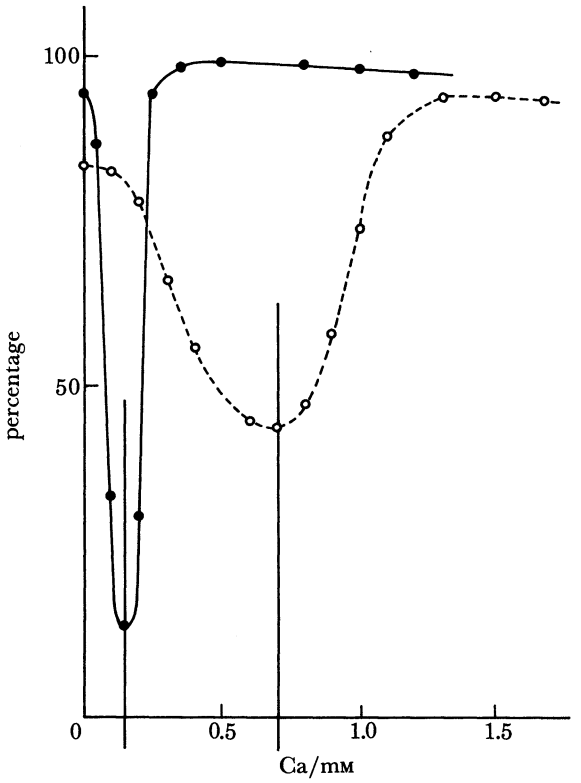

FIGURE 4. Effect of calcium on precipitation of 7 *S* and 11 *S* soy globulins. Vertical axis represents transmission of supernatant at 500 nm after centrifugation. Reproduced from Saio *et al.* (1973) with permission. Copyright Institute of Food Technologists.

TABLE 5. VARIATION IN 7 *S*:11 *S* GLOBULIN RATIO IN VARIOUS LEGUMES

legume	7 *S*:11 *S* ratio (range)	reference
Vicia faba	0.3–0.5	Gatehouse *et al.* 1980
	0.4–0.6	Martensson 1980
Pisum spp.	0.7–5.0	Casey *et al.* 1982
Glycine max	0.6–1.25	Wolf *et al.* 1961
	1.1–2.0	Saio *et al.* 1969

Hence a more versatile approach to obtaining 7 *S*- or 11 *S*-enriched materials might be to use a legume that is particularly rich in the desired protein component, for example, *Vicia* spp. for 11 *S* globulin and *Pisum* spp. for 7 *S* globulin. However this does contain the added assumption that across the Leguminosae functional characteristics of 7 *S* and 11 *S* globulins are more or less conserved. To test this hypothesis one can compare the known physicochemical and structural properties of either 7 *S* or 11 *S* globulins from different legumes and infer that any similarity in these will be automatically reflected in a corresponding similarity in their functional properties. This is not unreasonable because these properties must be a reflection of the protein's intrinsic amino acid sequence and structure. Alternatively one could measure the functional properties of the proteins directly, a more preferable but unfortunately much more difficult option.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

To date, the accumulating weight of evidence supports the existence of a gross homologous relation between globulins from different legumes. This homology is especially apparent in the case of the 11 *S* or legumin-like globulins where studies on the physicochemical characteristics (Derbyshire *et al.* 1976; Wright 1983), thermodynamic properties (Wright & Boulter 1980; Wright 1983) and primary structure (Gilroy *et al.* 1979; Moreira *et al.* 1979; Casey *et al.* 1981 *a*, *b*) have revealed extensive similarities. Selected properties of three 11 *S* globulins (from soya,

Table 6. Selected properties of 11 *S* globulins†

	molecular mass / kDa	subunit structure	dissociation behaviour	average hydrophobicity / kcal per residue‡	denaturation temperature T_{max} / °C	denaturation enthalpy / cal g^{-1}‡
glycinin (*G. max*)	320000	6	11 *S* → 7 *S* → 3 *S* → 2 *S*	0.94	94	5.6
legumin (*V. faba*)	330000	6	11 *S* → (?) → 3 *S* → 2 *S*	0.96	97	5.4
legumin (*P. sativum*)	350000	6	11 *S* → (?) → 3 *S* → 2 *S*	0.93	94	5.4

† Wright 1983; Derbyshire *et al.* 1976.
‡ 1 kcal = 1 kilocalorie. 1 calorie = 4.187 J.

pea and field bean) are presented in table 6. The one aspect currently in dispute concerns the quaternary structure of the 11 *S* globulin. A hexagonal and a trigonal arrangement of subunits have been proposed for this protein from soybean and pea respectively (Badley *et al.* 1975, Casey *et al.* 1980) with the latter also the preferred configuration for the apparently legumin-like 11 *S* globulins from sunflower and rapeseed (Plietz *et al.* 1983). Since the necessary assumptions inherent in some of these studies together with the limitations of the methods used could invalidate both interpretations, conclusive proof for either of the suggested models is still lacking. As for the 7 *S* globulins, there is less certainty over the existence of one universal protein and, in fact, current evidence would suggest the presence of at least two types in legumes (Croy *et al.* 1980*b*; Derbyshire *et al.* 1976; Wright & Boulter 1980; Koshiyama & Fukushima 1976*a*, *b*). However, it is not inconceivable that these may merely represent extreme members of a very heterogeneous class of proteins (Gatehouse *et al.* 1981; Thanh & Shibasaki 1978; Yamauchi *et al.* 1981; Sykes & Gaylèr 1981). On the basis of the evidence to date, it is not unreasonable to conclude that globulins (more notably the 11 *S* globulins) extracted from different legumes would exhibit fairly significant similarities in their functional behaviour.

As for the actual measurement of the functional properties of individual globulins, few results are available. However, what has been published indicates little difference in one property, emulsifying capacity, of 11 *S* globulins derived from three legumes, soybean, pea and field bean (Wright 1983). The overriding problem associated with such studies is that most of the functionality tests were devised with gram quantities of material in mind and such amounts of purified legume proteins are not readily or easily obtainable. To overcome this problem has involved both the scaling-up of protein preparative procedures and the development of micromethods for the evaluation of functional properties (Utsumi *et al.* 1982).

Results of some of our recent studies on the emulsifying and foaming capacities of 7 *S* and 11 *S* globulins from both soybean and pea are presented in table 7. The data on emulsifying

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

capacities confirm the previous findings of little variation between 11 *S* globulins from different legumes (Wright 1983). There is, however, a substantial difference in the emulsifying capacities of the 7 *S* globulins from soy and pea, an indication perhaps of their differing natures as discussed above, but this is not reflected in their foaming properties. The foaming capacities of the 7 *S* globulins are seen to be superior to those of the 11 *S* globulins but, considering the experimental errors involved, the overall variation may not be that significant.

TABLE 7. FOAMING AND EMULSIFYING PROPERTIES OF GLOBULINS FROM PEA AND SOY

protein	emulsifying capacity† (ml oil g^{-1})	foaming capacity‡ (% of solution foamed)
soy 7 *S*	11.4±0.3	41±3
pea 7 *S*	9.0±0.3	44±3
soy 11 *S*	9.8±0.3	39±4
pea 11 *S*	9.6±0.0	32±3

Results are means and standard errors of a minimum of two determinations.
† pH 7.5. Method adapted from Inklaar & Fortuin (1969).
‡ 0.2% solution, pH 7.0, using small scale sparging procedure.

Saio *et al.* (1969, 1971) have studied the gel formation of 7 *S*-rich and 11 *S*-rich fractions from soybean. Their preparations contained roughly 68% and 62% respectively of the relevant globulin component and so the results must be viewed with some reservation. Nevertheless, they observed significant differences in the properties of gels prepared from the two materials, in particular, gels made from 11 *S* globulin were harder and exhibited higher water holding capacities than their 7 *S* analogues. Differences in gel characteristics were ascribed to the contribution of disulphide bonds in the 11 *S* globulin to the gel matrix. In contrast to these results, Shimada & Mutsushita (1980) found that the 7 *S* globulin of soy formed firmer gels than the 11 *S* globulin. Although their protein preparations were of somewhat higher purity (95% 11 *S* and 80% 7 *S*) the differences in observed behaviour is more probably attributable to the differing fractionation procedures adopted by the two groups. Specifically, Saio *et al.* (1969, 1971) used calcium precipitation to effect separation of 7 *S* and 11 *S* globulin and therefore there exists the possibility of residual protein-bound calcium affecting the behaviour of the proteins.

Mori *et al.* (1982) took the investigation of the gelling properties of soy 11 *S* globulins (glycinin) a step further in their study of 'pseudoglycinins', i.e. artificial glycinin reconstituted from the isolated acidic and basic subunits, with each pseudoglycinin made up of only one type of acidic subunit. Their observation that the measured gel strength varied according to which acidic subunit was present combined with the known variation in subunit composition of glycinin isolated from different soybean cultivars (Utsumi *et al.* 1981; Staswick & Nielsen 1983) would suggest the possibility of naturally occurring glycinins with differing functional properties. To what extent the experimentally observed differences would be manifested in material processed from soybean varieties would depend largely on the degree of heterogeneity present in the protein and also, at a more fundamental level, on whether the 11 *S* globulin existed as a family of assorted homopolymers or a mixture of heteropolymers.

Overall, the differences apparent in certain aspects of their behaviour could make selection (via breeding or processing) for 7 *S* or 11 *S* globulins a viable proposition. As for the other suggestion of selecting specific legumes for either 7 *S* or 11 *S* globulins, too little information

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

is available on comparative functional properties of these proteins from different legumes; what is known so far, however, does appear to support the existence of a functional homology within the 11 *S* globulin class.

Finally, a more direct approach to the modification of functional properties is through the application of enzymic or chemical processes to alter the structure or composition of the protein. The most obvious example of this involves the preparation of protein hydrolysates by enzyme (for example, pepsin), acid or, less commonly, alkali treatment (Smith & Circle 1972; Lewis & Chen 1979). The result is a material with increased solubility and with enhanced foaming and emulsifying properties. Careful control of the degree of hydrolysis is necessary to maximize functional performance and also important is the choice of hydrolysis procedure to ensure that the production of bitter-tasting peptides is minimized (Adler-Nissen & Olsen 1979).

An alternative means of altering protein behaviour is through the chemical derivatization of specific residues. The complex nature of proteins has meant that a seemingly ever-increasing arsenal of reagents and procedures is now available for their modification (Means & Feeney 1971). This has proved particularly useful for the chemist who wishes to either provide protection for active groups and centres, elucidate structure and mechanisms, or merely facilitate analysis of proteins. However, applications in the food area are not so widespread. The reason is that the range of materials and processes that can be used is restricted because of necessary legal, toxicological and economic considerations. So far the most common type of modification procedure adopted has been acylation, specifically acetylation or succinylation involving reaction with either acetic or succinic anhydride (Kinsella & Shetty 1979). Under the conditions normally employed for the reaction, both reagents acylate amino (principally lysine) and tyrosyl groups, but whereas acetylation replaces a cationic amino group with an electrically neutral acetyl group, succinylation converts them to anionic residues. This complete reversal of charge frequently results in the dissociation of aggregated or multi-subunit proteins with a concomitant increase in solubility. As with most functionality studies on legume proteins, these modification procedures have been applied to mixtures of proteins, as prepared, for example in the form of protein isolates, rather than to separate globulin components. Hence there is no information as yet on the individual response of the two major globulins to such reactions. Nevertheless, some general conclusions can be drawn from the range of studies conducted on mixed protein fractions derived from soybean, peanut and field bean (Franzen & Kinsella 1976; Barman *et al.* 1977; Beuchat 1977). Both acetylation and succinylation have been reported as causing extensive dissociation of legume proteins (Barman *et al.* 1977; Shetty & Rao 1978), a result, presumably, of either a decrease in attractive forces or an increase in repulsive forces between polypeptide chains arising from the altered charge characteristics of the latter. However, whereas acetylation reduced water binding and emulsifying properties and left foaming capacity largely unaffected (Barman *et al.* 1977; Schmandke *et al.* 1981; Franzen & Kinsella 1976), succinylation brought about a significant improvement in both emulsifying and foaming properties when compared to the unmodified proteins (Franzen & Kinsella 1976; Beuchat 1977; Rauschal *et al.* 1981). The effect of both modifications was to eliminate the gelling ability of the proteins (Melnychyn & Stapely 1973; Barman *et al.* 1977). Much of the improvement in functional properties can be ascribed to a loosening of the protein structure with a resultant enhancement of solubility properties, particularly in the region of the isoelectric point, and also to an apparent increase in thermal stability, or more correctly, a much reduced tendency to coagulate or precipitate upon heating. As many functional properties (such as

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

foaming) depend upon the presence of a soluble protein fraction, any alteration in the latter will be reflected in a corresponding change in performance (Hidalgo 1978).

Apart from modifying functional properties, chemical derivatization of proteins can be used to provide protection against deteriorative reactions occurring as a result of processing operations. For example, acetylation, though relatively unimportant in terms of improving functional properties as such, affords a useful means of minimizing the occurrence of undesirable Maillard, condensation or cross-linking reactions involving lysine in particular (Friedman 1977). At the same time, there is some evidence indicating that the modified residue is still hydrolysable by renal enzymes (LeClerc & Benoiton 1968).

This last example represents an application of the chemical modification of a protein as a preventive measure. There also exists the possibility of using similar procedures for curative purposes, in other words, to improve the functional properties of a material that has been affected detrimentally by processing or storage treatments.

To conclude, in this paper I have attempted to highlight those factors that through their potential impact on the functional properties of legume proteins, could ultimately determine the extent to which they are useful in the food processing industry. It is clear that though extra processing may be involved in their preparation, these proteins could be rare amongst food proteins in affording such diverse opportunities for the manipulation of functional properties. Most importantly, perhaps, this diversity arises naturally out of the heterogeneous nature of the storage proteins and need not be implanted artificially, though as discussed, this option does remain. Further detailed research is required to establish the structural and physical basis of these functional differences between storage globulins and this, in itself, may aid in an improved understanding of the general relation between protein structure and functionality.

References

Adler-Nissen, J. & Olsen, H. S. 1979 The influence of peptide chain length on taste and functional properties of enzymatically modified soy protein. In *Functionality and protein structure* (ed. A. Pour-El), pp. 125–146. Washington: American Chemical Society.

Anderson, R. L. 1974 Ultracentrifugation and binding studies of acid-sensitive soybean proteins. *Cereal Chem.* **51**, 707–717.

Arntfield, S. D. & Murray, E. D. 1981 The influence of processing parameters on food protein functionality. I. Differential scanning calorimetry as an indicator of protein denaturation. *Can. Inst. Fd Sci. Tech. J.* **14**, 289–294.

Badley, R. A., Atkinson, D., Hauser, H., Oldani, D., Green, J. P. & Stubbs, J. M. 1975 The structure, physical and chemical properties of the soybean protein glycinin. *Biochim. biophys. Acta* **412**, 214–228.

Barman, B. G., Hansen, J. R. & Mossey, A. R. 1977 Modification of the physical properties of soy protein isolate by acetylation. *J. Agric. Fd Chem.* **25**, 638–641.

Beachy, R. N., Barton, K. A., Thompson, J. F. & Madison, J. T. 1980 In vitro synthesis of the α and α′ subunits of the 7 *S* storage proteins (conglycinin) of soybean seeds. *Pl. Physiol.* **65**, 990–994.

Beuchat, L. R. 1977 Functional and electrophoretic characteristics of succinylated peanut flour protein. *J. Agric. Fd Chem.* **25**, 258–261.

Casey, R. 1979 Genetic variability in the structure of the α-subunits of legumin from *Pisum* – a two-dimensional gel electrophoresis study. *Heredity* **43**, 265–272.

Casey, R., Hills, G. J. & Shaw, P. 1980 The 3-dimensional structure of legumin from *Pisum sativum*. *John Innes A. Rep.* 20–21.

Casey, R., March, J. F. & Sanger, E. 1981*a* N-terminal amino acid sequence of β-subunits of legumin from *Pisum sativum*. *Phytochemistry* **20**, 161–163.

Casey, R., March, J. F., Sharman, J. E. & Short, M. N. 1981*b*. The purification, N-terminal amino acid sequence and some other properties of an α^{M}-subunit of legumin from the pea (*Pisum sativum* L.) *Biochim. biophys. Acta* **670**, 428–432.

Casey, R., Sharman, J. E., Wright, D. J., Bacon, J. R. & Guldager, P. 1982 Quantitative variability in *Pisum* seed globulins: its assessment and significance. *Qual. Pl. Pl. Fds Hum. Nutr.* **31**, 333–346.

Croy, R. R. D., Gatehouse, J. A., Evans, I. M. & Boulter, D. 1980*a* Characterisation of the storage protein subunits synthesised in vitro by polyribosomes and RNA from developing pea (*Pisum sativum* L.) I. Legumin. *Planta* **148**, 49–56.

Croy, R. R. D., Gatehouse, J. A., Tyler, M. & Boulter, D. 1980*b* The purification and characterisation of a third storage protein (Convicilin) from the seeds of pea (*Pisum sativum* L.) *Biochem. J.* **191**, 509–516.

Derbyshire, E., Wright, D. J. & Boulter, D. 1976 Legumin and vicilin, storage proteins of legume seeds. *Phytochemistry* **15**, 3–24.

Flink, J. & Christiansen, I. 1973 Production of a protein isolate from *Vicia faba*. *Lebensm.-Wiss. Technol.* **6**, 102–106.

Franzen, K. L. & Kinsella, J. E. 1976 Functional properties of succinylated and acetylated soy protein. *J. Agric. Fd Chem.* **24**, 788–795.

Friedman, M. 1977 Effects of lysine modification on chemical, physical, nutritive, and functional properties of proteins. In *Food proteins* (ed. J. R. Whitaker & S. R. Tannenbaum), pp. 446–483. Westport, Connecticut: AVI.

Gatehouse, J. A., Croy, R. R. D., McIntosh, R., Paul, C. & Boulter, D. 1980 Quantitative and qualitative variation in the storage proteins of material from the EEC joint field bean test. In *Vicia faba. Feeding value, processing and viruses* (ed. D. A. Bond), pp. 173–190. The Hague: Martinus Nijhoff.

Gatehouse, J. A., Croy, R. R. D., Morton, H., Tyler, M. & Boulter, D. 1981 Characterisation and subunit structures of the vicilin storage proteins of pea (*Pisum sativum* L.) *Eur. J. Biochem.* **118**, 627–633.

Gillespie, J. M. & Blagrove, R. J. 1975 Variability in the proportion and type of subunits in Lupin storage globulins. *Aust. J. Pl. Physiol.* **2**, 29–39.

Gilroy, J., Wright, D. J. & Boulter, D. 1979 Homology of basic subunits of legumin from *Glycine max* and *Vicia faba*. *Phytochemistry* **18**, 315–316.

Gwiazda, S., Rutkowski, A. & Kocon, J. 1979 Some functional properties of pea and soybean protein preparations. *Nahrung* **23**, 681–686.

Halling, P. J. 1981 Protein-stabilized foams and emulsions. *Crit. Rev. Fd Sci. Nutr.* **15**, 155–203.

Hidalgo, J. 1978 Functional properties of food proteins from a biophysical point of view. In *Biochemical aspects of new protein food* (ed. J. Adler-Nissen, B. O. Eggum, L. Munck & H. S. Olsen), pp. 89–98. Oxford: Pergamon Press.

Higgins, T. J. V. & Spencer, D. 1981 Precursor forms of pea vicilin subunits. Modification by microsomal membranes during cell-free translation. *Pl. Physiol.* **67**, 205–211.

Horan, F. E. 1974 Soy protein products and their production. *J. Am. Oil Chem. Soc.* **51**, 67A–73A.

Iibuchi, C. & Imahori, K. 1978 Heterogeneity and its relation to the subunit structure of the soybean 7 *S* globulin. *Agric. Biol. Chem.* **42**, 31–36.

Inklaar, P. A. & Fortuin, J. 1969 Determining the emulsifying and emulsion stabilizing capacity of protein meat additives. *Fd Technol.* **23**, 103–107.

Kinsella, J. E. 1976 Functional properties of proteins in foods: a survey. *Crit. Rev. Fd Sci. Nutr.* **7**, 219–280.

Kinsella, J. E. & Shetty, K. J. 1979 Chemical modification for improving functional properties of plant and yeast proteins. In *Functionality and protein structure* (ed. A. Pour-El), pp. 37–63. Washington: American Chemical Society.

Kitamura, K., Toyokawa, Y. & Harada, K. 1980 Polymorphism of glycinin in soybean seeds. *Phytochemistry* **19**, 1841–1843.

Koshiyama, I. 1965 Purification of the 7 *S* component of soybean proteins. *Agric. Biol. Chem.* **29**, 885–887.

Koshiyama, I. & Fukushima, D. 1976*a* Identification of the 7 *S* globulin with β-conglycinin in soybean seeds. *Phytochemistry* **15**, 157–159.

Koshiyama, I. & Fukushima, D. 1976*b* Purification and some properties of γ-conglycinin in soybean seeds. *Phytochemistry* **15**, 161–164.

LeClerc, J. & Benoiton, L. 1968 Further studies on ε-lysine acylase. The N-acyl-diamino acid hydrolase activity of avian kidney. *Can. J. Biochem.* **46**, 471–475.

Lehnhardt, W. F., Gibson, P. W. & Orthoefer, F. T. 1983 U.S. patent no. 4370267.

Lewis, B. A. & Chen, J. H. 1979 Effect of conformation and structure changes induced by solvent and limited enzyme modification on the functionality of soy proteins. In *Functionality and protein structure* (ed. A. Pour-El), pp. 27–35. Washington: American Chemical Society.

Lillford, P. J. & Wright, D. J. 1981 Influence of isoelectric precipitation on the solubility of soya bean proteins. *J. Sci. Fd Agric.* **32**, 3125–3327.

Martensson, P. 1980 Variation in legumin: vicilin ratio between and within cultivars of *Vicia faba* L. var. *minor*. In *Vicia faba. Feeding value, processing and viruses* (ed. D. A. Bond), pp. 159–172. The Hague: Martinus Nijhoff.

Means, G. E. & Feeney, R. E. 1971 *Chemical modification of proteins*. San Francisco: Holden-Day, Inc.

Melnychyn, P. & Stapley, R. 1973 U.S. patent no. 3764711.

Moreira, M. A., Hermodson, M. A., Larkins, B. A. & Nielsen, N. C. 1979 Partial characterization of the acid and basic polypeptides of glycinin. *J. Biol. Chem.* **254**, 9921–9926.

Mori, T., Nakamura, T. & Utsumi, S. 1982 Formation of pseudoglycinins and their gel hardness. *J. Agric. Fd Chem.* **30**, 828–831.

Murray, E. D., Myers, C. D. & Barker, L. D. 1978 Canadian patent no. 1028552.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Ory, R. L. & St Angelo, A. J. 1982 Effects of lipid oxidation on proteins of oilseeds. *Am. chem. Soc., Symp. Ser.* **206**, 55–65.

Plietz, P., Damaschun, G., Mueller, J. J. & Schwenke, K. D. 1983 The structure of 11 *S* globulins from sunflower and rapeseed. A small-angle X-ray scattering study. *Eur. J. Biochem.* **130**, 315–320.

Rao, A. G. A. & Rao, M. S. N. 1977 A method for isolation of 2 *S*, 7 *S* and 11 *S* proteins of soybean. *Prep. Biochem.* **7**, 89–101.

Rauschal, E. J., Linow, K.-J., Pahtz, W. & Schwenke, K. D. 1981 Chemische modifizierung von Proteinen. 8. Mitt Beeinflussung physikochemischer und funktoneller Eigenschaften von Proteinen aus Ackerbohnen durch Succinylierung. *Nahrung* **25**, 241–248.

Reichert, R. D. 1981 Quantitative isolation and estimation of cell wall material from dehulled pea (*Pisum sativum*) flours and concentrates. *Cereal Chem.* **58**, 266–270.

Saio, K., Kajikawa, M. & Watanabe, T. 1971 Food processing characteristics of soybean 11 *S* and 7 *S* proteins. 2. Effects of sulphydryl groups on physical properties of Tofu-gel. *Agric. Biol. Chem.* **35**, 890–898.

Saio, K., Kamiya, M. & Watanabe, T. 1969 Food processing characteristics of soybean 11 *S* and 7 *S* proteins 1. Effect of difference of protein components among soybean varieties on formation of Tofu-gel. *Agric. Biol. Chem.* **33**, 1301–1308.

Saio, K., Watanabe, T. & Kaji, M. 1973 Food use of soybean 7 *S* and 11 *S* proteins. Extraction and functional properties of their fractions. *J. Fd Sci.* **38**, 1139–1144.

Schmandke, H., Maune, R., Schuhmann, S. & Schultz, M. 1981 Contribution to the characterization of acetylated protein fractions of *Vicia faba* in view of their functional properties. *Nahrung* **25**, 99–109.

Shetty, K. J. & Rao, M. S. N. 1978 Effect of succinylation on the oligomeric structure of arachin. *Int. J. Peptide Protein Res.* **11**, 305–313.

Shimada, K. & Mutsushita, S. 1980 Gel formation of soybean 7 *S* and 11 *S* proteins. *Agric. Biol. Chem.* **44**, 637–641.

Smith, A. K. & Circle, S. J. 1972 *Soybeans: chemistry and technology*. Westport, Connecticut: AVI.

St Angelo, A. J. & Ory, R. L. 1975 Effects of lipoperoxides on proteins in raw and processed peanuts. *J. Agric. Fd Chem.* **23**, 141–146.

Staswick, P. E. & Nielsen, N. C. 1983 Characterization of a soybean cultivar lacking certain glycinin subunits. *Arch. Biochem. Biophys.* **223**, 1–8.

Sumner, A. K., Nielsen, M. A. & Youngs, C. G. 1981 Production and evaluation of pea protein isolate. *J. Fd Sci.* **46**, 364–366, 372.

Sykes, G. E. & Gayler, K. R. 1981 Detection and characterization of a new β-conglycinin from soybean seeds. *Arch. Biochem. Biophys.* **210**, 525–530.

Thanh, V. H. & Shibasaki, K. 1976 Major proteins of soybean seeds. A straightforward fractionation and their characterization. *J. Agric. Fd Chem.* **25**, 1117–1121.

Thanh, V. H. & Shibasaki, K. 1978 Major proteins of soybean seeds. Subunit structures of β-conglycinin. *J. Agric. Fd Chem.* **26**, 692–695.

Thompson, L. U. 1977 Preparation and evaluation of mung bean protein isolates. *J. Fd Sci.* **42**, 202–206.

Thomson, J. A. & Schroeder, H. E. 1978 Cotyledonary storage proteins in *Pisum sativum*. II. Hereditary variation in components of the legumin and vicilin fractions. *Aust. J. Pl. Physiol.* **5**, 281–295.

Utsumi, S., Inaba, H. & Mori, T. 1981 Heterogeneity of soybean glycinin. *Phytochemistry* **20**, 585–589.

Utsumi, S. & Mori, T. 1980 Heterogeneity of broad bean legumin. *Biochim. biophys. Acta.* **621**, 179–189.

Utsumi, S., Nakamura, T. & Mori, T. 1982 Micro-method for the measurement of gel properties of soybean 11 *S* globulin. *Agric. Biol. Chem.* **46**, 1923–1924.

Vose, J. R., Basterrechea, M. J., Gorin, P. A. J., Finlayson, A. J. & Youngs, C. G. 1976 Air-classification of field peas and horsebean flours: chemical studies of starch and protein fractions. *Cereal Chem.* **53**, 928–936.

Weber, E. & Neumann, D. 1980 Protein bodies, storage organelles in plant seeds. *Biochem. Physiol. Pfl.* **175**, 279–306.

Wolf, W. J. 1975 Lipoxygenase and flavor of soybean protein products. *J. Agric. Fd Chem.* **23**, 136–141.

Wolf, W. J., Babcock, G. E. & Smith, A. K. 1961 Ultracentrifugal differences in soybean protein composition. *Nature, Lond.* **191**, 1395–1396.

Wright, D. J. 1983 Comparative physical and chemical aspects of vegetable protein functionality. *Qual. Pl. Pl. Fds Hum. Nutr.* **32**, 389–400.

Wright, D. J. & Boulter, D. 1980 Differential scanning calorimetric study of meals and constituents of some food grain legumes. *J. Sci. Fd Agric.* **31**, 1231–1241.

Yamauchi, F., Sato, M., Sato, W., Kamata, Y. & Shibasaki, K. 1981 Isolation and identification of a new type of β-conglycinin in soybean globulins. *Agric. Biol. Chem.* **45**, 2863–2868.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES