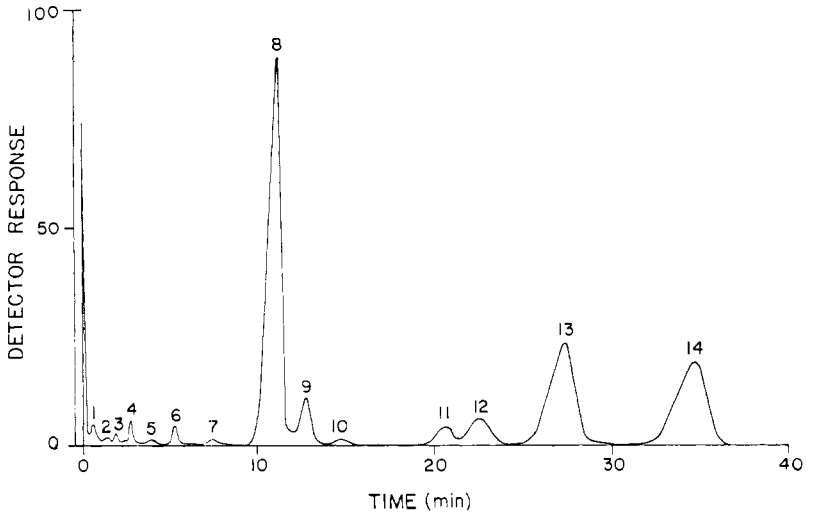

Figure 1. Gas chromatogram of fatty acid methyl esters (DEGS)

1. 8:0	6. 14:0	11. 18:0
2. 10:0	7. 15:0	12. 18:1
3. 11:0 (10:1)	8. 16:0	13. 18:2
4. 12:0	9. 16:1	14. 18:3
5. 13:0	10. 17:0	

linoleic (16.3 to 17.7%), and palmitic (12.9 to 13.4%) acids in green leaves of *Spinacia oleracea* and *Antirrhinum majus*. Burnet and Lohmar (*1*) reported high concentrations of oleic (21.0%), linoleic (12.9%), and linolenic (39.0%) acids in sorghum tissues. Garton (*5*) analyzed a mixture of forage plants and found high levels of unsaturated fatty acids. Sakai (*16*) did not use gas-liquid chromatography but reported that oleic and linoleic acids contributed 87% to the total fatty acids of *Chenopodium album*. Shorland, Weenink, and Johns (*18*) examined the fatty acids of clover-rich pasture and reported values of octadecatrienoic acid as high as 58.9% of total fatty acids.

The high levels of essential linoleic acid and other fatty acids in leaf tissues in general and the leaf protein concentrates in particular indicate that the leaf lipids are valuable human nutrients in terms of both energy contribution and essential nutrients. Attempts should be made to include these nutrients in leaf protein concentrates used for animal and human nutrition and still eliminate undesirable flavors and color. Studies on the peroxidation of these highly unsaturated fatty acids and the effect on protein quality would also be desirable.

Acknowledgment

The authors thank N. W. Pirie and Marjorie Byers, Rothamsted Experiment Station, Harpenden, England, for samples of leaf protein concentrates used in this study.

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Received for review June 4, 1964. Accepted October 19, 1964. Work supported in part by grants from the Herman Frasch Foundation and the Rockefeller Foundation. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

LEAF PROTEINS AS FOODSTUFFS

Nutritive Value of Leaf Protein Concentrate, an In Vitro Digestion Study

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The amino acid composition of leaf protein concentrate (LPC) was reported to be as good as or better than that of many common foodstuffs (*16*). With the exception of methionine, which was low, all the essential amino acids were present in sufficient quantities to constitute a protein of good nutritive value in LPC. This indicated that plant leaves may be an important source of food for human consumption.

Feeding experiments have been conducted. Many early studies suggested that the nutritive value of LPC was low (*5, 7, 8, 10–12, 15*). The poor quality of the LPC used in early studies may have resulted from improper processing. Recent results by Duckworth *et al.* (*13, 14*), Barber (*2*), and Waterlow (*28*) were more encouraging, as they reported that LPC prepared under different conditions may be equal to or better in nutritive value than proteins such as soybean meal, white fish meal, and milk.

On the basis of amino acid composition, LPC from a wide variety of plant species may have high nutritive value (*16*). However, amino acid composition alone may not give a true picture, since processing may have a deleterious effect on the nutritive value of some proteins (*6, 17*). Carpenter (*6*) showed

The nutritive value of leaf protein concentrate was estimated with an in vitro enzymatic digestion and compared with 12 common food proteins. Digestibility was estimated from the total amount of amino acids released by pepsin followed by pancreatin hydrolysis. The leaf proteins compared favorably with high quality proteins. The biological values of the proteins were estimated from the pepsin pancreatin digest index, which was based on the release of eight essential amino acids. An excellent correlation was observed between this index for the 12 reference proteins and their biological values in the literature. The estimated biological values of leaf proteins were in general lower than values for egg and egg white but higher than beef, casein, soybean, yeast, wheat flour, gluten, zein, and gelatin. The values for leaf proteins were about the same as those of whole milk and lactalbumin.

that digestibility may affect nutritive value. As a further basis for evaluation, the susceptibility of proteins to enzymatic hydrolysis or digestibility was studied. The digestibility was measured in terms of the amounts of amino acid released by a pepsin followed by pancreatin digestion. Akeson and Stahmann (*1*) showed that the biological value of a food protein could be accurately predicted from the amino acids released by a pepsin followed by pancreatin digestion. Using 12 food proteins which covered the entire range of protein quality, they observed an excellent correlation (r = 0.990) between their pepsin pancreatin digest index and the biological values of the proteins obtained from rat feeding trials.

Methods and Materials

Freeze-dried LPC samples were obtained from N. W. Pirie, Rothamsted Experiment Station, Harpenden, England. The LPC was prepared according to the procedure described by Morrison and Pirie (*22*). Information concerning extraction procedure, species, stage of growth, identification of samples, and composition of the LPC samples tested was given by Gerloff, Lima, and Stahmann (*16*). The samples prepared by a somewhat different process, impulse rendered (IR), were obtained from R. H. Smith, British Glues and Chemicals, Ltd., London, England. The method of preparation of these LPC samples was described by Chayen *et al.* (*9*).

The digestion of the protein samples was described by Akeson and Stahmann (*1*). The digests were prepared by incubating 100 mg. of protein with 1.5 mg. of pepsin in 15 ml. of 0.1N hydrochloric acid at 37° C. for 3 hours. Following neutralization with 7.5 ml. of 0.2N sodium hydroxide and addition of 4 mg. of pancreatin in 7.5 ml. of pH 8.0 phosphate buffer, the samples were incubated for an additional 24 hours at 37° C. Enzyme blanks were prepared by incubation under the described conditions with protein samples omitted. Following incubation, 10 ml. of digestion mixture of each sample was added to 50 ml. of 1% picric acid solution and centrifuged for 30 minutes at 1000 × g to remove undigested protein and larger peptides. Fifty milliliters of supernatant was passed through a 12 × 50 mm. column of Dowex 2 × 8 resin in chloride form into a 250-ml. lyophilizing bottle. The column was rinsed three times with 5-ml. portions of 0.02N hydrochloric acid. The samples were dried by lyophilization and then dissolved and diluted to 10 ml. with pH 2.2 citrate buffer. Amino acids were determined by the ion exchange method of Moore, Spackman, and Stein (*20, 21, 27*). The amino acid peaks of the chromatogram were integrated by the method described by Akeson and Stahmann (*1*).

The nutritive values of the leaf protein concentrates were estimated with the pepsin pancreatin digest index described by Akeson and Stahmann (*1*). Total amino acid content of the LPC used in calculating the pepsin pancreatin digest index was that reported by Gerloff *et al.* (*16*). Akeson and Stahmann (*1*) reported that nutritive values were slightly overestimated by the pepsin pancreatin digest index, since whole egg was used as a standard of 100, although it actually had a biological value of 96 (*4*) or 97 (*23*). The authors observed that the corrected values obtained by multiplying the pepsin pancreatin digest index of each protein by the correction factor, average biological value of egg (96.5)/100, were nearly the same as those predicted by the regression equation, $Y = 0.970\ x - 0.54$. Therefore, the biological values of the LPC samples were estimated by multiplying the pepsin pancreatin digest index of each protein by 0.965.

Results and Discussion

Table I shows the levels of individual amino acids released from 12 reference proteins by the pepsin followed by pancreatin digestion. In general, when the level was high, the biological value of the proteins as obtained from the literature was also high, except in the case of yeast and possibly wheat flour, where values were higher than expected, probably because free amino acids were present in the samples at the beginning of the digestion. A positive correlation was observed between the total amino acids released and the biological values for growing rats obtained from the literature. The correlation (r = 0.611) was significant at the 5% level but not at the 1% level.

The estimated biological values calculated from the pepsin pancreatin digest index (*1*) showed an excellent correlation (r = 0.990) with the literature values. We conclude that the percentage of all the amino acids released by the pepsin followed by pancreatin digestion may be useful in giving a rough measure of the biological value. However, the biological values were predicted more accurately with the pepsin pancreatin digest index, which is calculated from the amount of each essential amino acid released by the enzymes.

Table II shows the levels of individual amino acids released from several LPC samples by the pepsin followed by pancreatin digestion. The last column shows the biological value estimated from the pepsin pancreatin digest index. There was little variation between samples in the amounts of each amino acid released. The levels of all essential amino acids released from the LPC samples were as great or nearly as great as those released from whole egg and egg white, with the exception of methionine and possibly phenylalanine in the case of egg white (Table I). Similarly, LPC and whole milk released nearly equivalent amounts of each amino acid, except lysine, which was high in milk. In general, the LPC samples compared favorably with the high quality reference proteins in the levels of each essential amino acid released. When total amino acid released is used as a measure of digestibility, these data suggest that most LPC samples were nearly equal to lactalbumin and beef in digestibility; slightly lower in total amino acid release than whole egg, egg white, and whole milk; and higher than casein, soybean, zein, and gelatin. The authors concluded that the nutritive value of these LPC samples was not significantly affected by digestibility of the protein. Thus, the enzymatic release of amino

Table I. Release of Amino Acids by an in Vitro Pepsin Followed by Pancreatin Hydrolysis of Representative Food Protein Samples[a]

Food Protein	Essential[b,c]							Nonessential[b]								Total	Estimated Biological Value	Literature Biological Value for Growing Rat
	Lys	Phe	Met	Thr	Leu	Ileu	Val	His	Tyr	Arg	Asp	Ser	Glu	Gly	Ala			
Whole egg	2.4	3.1	1.7	0.5	4.9	1.0	1.0	0.7	2.4	2.8	0.2	1.3	0.9	0.2	1.1	24.2	97	96[d], 97[e]
Egg white	1.6	3.7	2.0	0.4	5.1	0.9	0.9	0.9	2.7	2.0	0.2	1.2	0.7	0.1	1.0	23.4	87	83[d], 82[f], 97[g]
Whole milk	3.7	2.4	1.0	0.7	5.1	1.0	1.1	0.4	2.3	2.2	0.2	1.5	0.9	0.4	0.8	23.7	83	90[d,f], 84[d], 84[e]
Lactalbumin	4.5	1.8	1.4	0.5	5.1	0.8	1.0	0.4	1.9	1.5	0.6	1.0	0.4	0.2	0.6	21.7	84	85[d], 84[f]
Beef	3.1	2.5	1.3	0.5	4.6	0.8	0.8	0.6	1.9	3.3	0.2	0.9	0.8	0.3	1.0	22.6	75	76[d,f]
Casein	2.9	2.0	0.8	0.3	3.7	0.5	0.5	0.2	1.8	2.1	0.1	0.8	0.2	0.2	0.4	16.5	76	73[f], 69[d], 78[h]
Soybean	1.7	2.3	0.5	0.3	3.5	0.8	0.6	0.3	1.7	3.5	0.1	0.8	0.4	0.2	0.4	17.1	65	Raw-57[d], 59[h] Heated-75[d], 74[h]
Yeast	4.1	2.8	0.8	0.9	4.3	1.4	1.5	0.6	2.3	3.7	0.4	1.5	5.1	0.6	3.4	33.4	71	63[d], 69[f]
Wheat flour	1.2	2.4	0.9	0.5	4.4	1.3	1.3	0.6	1.9	2.3	0.3	3.0	0.8	0.3	1.1	22.4	50	52[d,f]
Gluten	0.7	1.7	0.6	0.2	3.0	0.9	1.0	0.4	1.3	1.6	0.2	2.2	0.3	0.1	0.6	14.8	45	40[g], 61[h]
Zein	0.1	1.7	0.2	0.3	3.0	0.5	0.6	0.2	1.4	0.5	0.2	1.2	0.3	0.1	1.1	11.4	26	...
Gelatin	1.5	0.7	0.4	0.1	0.9	0.3	0.2	0.3	0.4	4.1	0.1	0.3	0.2	0.4	0.3	10.2	17	25[d], 0[i]

[a] Grams of amino acid released per 100 grams of amino acid in LPC.
[b] Required by adult human (*24*, *25*).
[c] Tryptophan destroyed by picric acid precipitation step.
[d] Block and Mitchell (*4*).
[e] Sommer (*26*).
[f] Mitchell and Block (*19*).
[g] Mitchell and Beadles (*18*).
[h] Rippon (*23*).
[i] Bender, Miller, and Tunnah (*3*).

Table II. Release of Amino Acids by an in Vitro Pepsin Followed by Pancreatin Hydrolysis of Leaf Protein Concentrate Samples[a]

Species	Essential[b,c]							Nonessential[b]								Total	Estimated Biological Value
	Lys	Phe	Met	Thr	Leu	Ileu	Val	His	Tyr	Arg	Asp	Ser	Glu	Gly	Ala		
Chenopodium	2.2	2.7	0.5	0 4	3.9	0.7	0.7	0.4	2.3	3.1	0.2	0.7	0.4	0.2	0.7	19.1	84
Turnip 437	2.3	2.7	0.6	0.4	4.0	1.0	0.8	0.4	2.2	3.5	0.2	0.8	0.5	0.3	0.8	20.5	82
Sanfoin	1.7	2.7	0.4	0.5	3.9	0.9	0.9	0.3	2.2	2.0	0.3	0.9	0.5	0.4	1.0	18.6	79
Nasturtium	2.7	3.1	0.7	0.7	4.6	1.2	1.3	0.5	2.6	3.3	0.3	1.3	0.5	0.4	1.3	24.5	83
Clover 528a	2.2	3.0	0.5	0.6	4.5	1.0	1.0	0.4	2.4	2.9	0.2	1.0	0.6	0.4	1.0	21.7	84
Rye grass 525	2.0	3.1	0.7	0.5	4.6	1.0	1.0	0.5	2.3	2.8	0.3	0.9	0.6	0.3	1.2	21.8	86
Wheat																	
539	2.5	2.6	0.8	0.4	3.9	0.9	0.9	0.5	2.1	3.7	0.2	0.8	0.5	0.3	0.9	21.0	89
549	2.6	3.1	0.7	0.6	4.7	0.9	1.0	0.4	2.4	3.7	0.2	0.9	0.6	0.3	1.1	23.0	87
552	2.5	2.8	0.6	0.5	4.2	0.9	0.8	0.4	2.3	3.5	0.2	1.0	0.5	0.2	1.0	21.4	87
561	2.3	3.0	0.6	0.5	4.3	1.1	0.9	0.3	2.3	3.3	0.1	0.9	0.5	0.2	1.0	21.3	79
Corn																	
573a	2.5	2.8	0.9	0.7	4.7	1.1	1.2	0.5	2.3	3.6	0.2	1.1	0.7	0.3	1.2	23.8	89
577a	2.3	2.8	0.6	0.6	4.5	0.9	1.2	0.4	2.2	3.1	0.2	1.0	0.6	0.3	1.2	21.9	83
581a	2.5	2.7	0.8	0.5	4.4	1.0	1.0	0.4	1.9	3.5	0.2	1.0	0.6	0.3	1.0	21.8	78
581b	2.4	2.7	0.7	0.5	4.5	1.0	1.1	0.4	2.1	3.3	0.2	0.9	0.6	0.3	1.1	21.8	84
																Av.[d]	83
IR1 corn	1.7	2.7	0.5	0.4	3.9	1.0	1.0	0.4	2.2	3.2	0.2	0.7	0.3	0.2	0.7	19.1	78
IR2 alfalfa	2.3	2.8	0.6	0.4	4.0	0.9	0.9	0.4	2.1	3.2	0.2	0.8	0.5	0.2	0.8	20.1	85
IR3 mixed grass	2.1	3.4	0.6	0.5	4.6	1.0	0.9	0.4	2.7	3.0	0.3	0.7	0.5	0.2	0.8	21.7	81
IR4 alfalfa	1.9	2.6	0.7	0.5	4.0	1.1	1.0	0.4	2.2	3.0	0.4	1.0	0.5	0.3	0.9	20.5	73
																Av.[e]	79

[a] Grams of amino acid released per 100 grams of amino acid in LPC.
[b] Required by adult human (*24*, *25*).
[c] Tryptophan destroyed by picric acid precipitation step.
[d] Average of samples from N. W. Pirie.
[e] Average of samples from R. H. Smith.

acids reported in this study and the favorable amino acid composition found by Gerloff *et al.* (*16*) suggest that LPC samples have high nutritive value.

The estimated biological values calculated from the pepsin pancreatin digest index gave a much better measure of the relative nutritive value of the protein samples than the amino acid composition alone. This value is based on the amount of each essential amino acid released by the digestive enzymes as compared to that released from the proteins of whole egg. Akeson and Stahmann (*1*) showed that the pepsin pancreatin digest index gave better correlation with biological values from the literature for 12 reference proteins than did indexes calculated from the amino acid composition. Although some variation was observed in the estimated biological values of the LPC samples, all samples were sufficiently high in nutritive value to indicate that they would be high quality protein sources for animal or human consumption. The estimated biological values of most of the LPC

samples were nearly equal to the values for milk and lactalbumin, slightly lower than egg white (except for wheat samples 539, 549, and 552, and corn 573a, which were about equal to egg white), and much lower than whole egg. On the other hand, all LPC samples except IR4 had estimated biological values higher than those of soybean, yeast, wheat flour, gluten, zein, and gelatin.

There was relatively little difference among the leaf proteins from nine species of the plants in their amino acid composition, hydrolysis by digestive enzymes, and estimated biological value. This suggests that leaf proteins from a large number of plant species growing in different localities would have uniformly high biological value. The plant species appear to differ mainly in the total yield of LPC which can be obtained from the leaves, not in the digestibility or amino acid composition of leaf proteins.

The authors have concluded that leaf proteins may be an excellent source of protein for animal or human consumption. Furthermore, leaf protein concentrates could be prepared in nearly every part of the world where green plants grow.

Acknowledgment

The authors thank N. W. Pirie and R. H. Smith for supplying samples of leaf protein concentrates.

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Received for review June 4, 1964. Accepted October 19, 1964. Work supported in part by grants from the Herman Frasch Foundation. Published with the approval of the Director of the Wisconsin Agriculture Experiment Station.

LOW-COST PROTEIN SOURCES

Protein-Rich Mixture Based on Vegetable Foods Available in Middle Eastern Countries

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A protein-rich mixture of vegetable foods available in Middle Eastern countries consists of 47% autoclaved chick peas, 35% defatted sesame flour, and 18% heat-processed low-fat soybean flour, and contains 37.8% protein. Its biological value is 74 and its protein efficiency ratio (assessed on young rats in 28 days' assays) is 2.90. Comparison with the FAO egg protein pattern suggests that the order of limiting amino acids is methionine, followed by tryptophan. The mixture is a good source of B vitamins, calcium, and iron.

In various regions of the world where protein deficiency in infants constitutes a major problem of public health and nutrition, efforts are being made to introduce into the diet protein from locally available sources, particularly of vegetable origin. The preparation must be cheap and the population concerned be well acquainted with its constituents. Such protein-rich foods, which may be made of various components, have been used in India (*11*, *19*) and Central America (*6*, *18*).

Protein deficiency in infancy is frequently encountered in Middle Eastern countries (*9*, *14*, *17*, *22*). Therefore, a protein-rich mixture of locally available cheap foods was needed, particularly for infant feeding. Since high nutritive value of the protein was thought to be of great importance, the concentrations of essential amino acids per gram of nitrogen should not be much less than those suggested by the Food and Agriculture Organization (*8*). Various mixtures containing different proportions of wheat flour, parboiled wheat (local name, burgul), soybean flour (*Glycine max.* L.), sesame flour (*Sesamum indicum* L.), chick peas (*Cicer arietinum* L.), and sunflower seed meal (*Helianthus annus* L.) were studied, and the amounts of the amino acids limiting the nutritive value of most proteins—i.e., methionine, lysine, and tryptophan—were determined. After several trials a mixture of chick peas, defatted sesame flour, and toasted, defatted soybean flour seemed to deserve further study. Since the mixture is intended to be used by small children, the chick peas were autoclaved in order to avoid possible intestinal disturbances.

Materials and Methods

Protein Sources. Chick peas obtained on the open market were autoclaved for 30 minutes at 15 pounds' pressure, dried in the sun, and finely