except with ferulic acid (Table III). *p*-Coumaric acid, phloretic acid, and *p*-hydroxyphenylacetic acid showed up as reddish brown spots after the chromatogram was placed in a humid chamber for 20 minutes, but the dihydric phenols, chlorogenic acid and caffeic acid, reacted immediately, yielding brownish colorations. Phloridzin and its aglycone, phloretin, were oxidized but gave yellow rather than brown colors.

Most of the phenolic acids present in core tissues are potential substrates for apple phenolase (both cresolase and catecholase). Phloridzin gave only a yellow color with apple phenolase and, although a major constituent of core tissues, is not a browning substrate. Its chemical or enzymatic degradation, however, produces phenolic acids (*1*) which brown readily.

This high concentration of phenolic compounds in the core area could account for the browning being localized there. Further research is required, however, to determine whether free phenolics are actually released during the very late stages of 32° F. storage and whether the specific enzymes capable of this release mechanism are present in stored fruit.

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LEAF PROTEINS AS FOODSTUFFS

Amino Acid Composition of Leaf Protein Concentrates

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Leaf protein concentrates, extracted from green leaves by mechanical methods, were analyzed for their amino acid composition. Samples from nine plant species harvested under different conditions of fertilization and maturity did not show large variations in amino acid content. On an amino acid compositional basis, leaf protein concentrate should be a well balanced source of dietary protein, if supplemented with synthetic methionine. Comparisons with other foodstuff proteins are presented and the future role of leaf protein concentrates in increasing the supply of high quality protein is discussed.

IT HAS been estimated that there are 1800 million hungry people in the world whose major nutritional deficiency is an inadequate supply of protein (*27*). Inasmuch as lands suitable for agriculture are already near maximum use, the protein supply could most easily be increased by more efficient utilization of existing protein. Almost all the amino acids consumed by man are initially synthesized in the leaves of green plants. There is some loss when they are concentrated into protein of seeds or tubers and a very great waste when plant protein is converted into animal products for human consumption (*18*). In many operations technological progress has replaced the animal. Perhaps efficiency could be greatly increased if mechanical power and technology were used to separate plant protein from fiber and concentrate it into consumable forms. Mechanical methods of separating and concentrating leaf proteins have been described by Pirie and associates (*3, 22–26, 28, 29*) and Chayen *et al.* (*8*). Leaf protein concentrate (LPC) can be extracted and processed, leaving a residue which might have value as a ruminant feed. This paper presents amino acid analyses of LPC extracted by mechanical processes.

Chibnall, Rees, and Lugg (*9*) reported that proteins extracted by various methods from green plants showed only slight differences in amino acid composition. Wilson (*35*) found that the amino acid composition of leaf proteins from different species was remarkably similar even at various stages of growth and noted that differences in maturity and nitrogen fertilization caused a marked change in the nonprotein nitrogen fraction, depending on the species.

While determination of amino acid provides a guide to the quality of proteins, the final answer to whether LPC could be used as a source of dietary protein lies in feeding experiments. Early feeding experiments suggested a low nutritive value (*4–7, 10–12, 17*), which now seems to have been the result of improper processing of the leaf protein. Recently Duckworth (*15*) found the gross protein value of LPC was 82 compared to 74 for soybean meal. He showed that heating LPC above a critical temperature of about 84° C. drastically reduced the gross protein value. This may explain some of the poor results obtained in earlier feeding trials. Barber (*1*) reported that LPC was nutritionally equal to white fish meal when fed to pigs. Duckworth (*14*), in similar experiments, confirmed Barber's data and concluded that inclusion of relatively small amounts of LPC in the diet improved the efficiency of feed conversion. These data indicate that the feeding value of LPC may be relatively high, if care is taken in processing. Waterlow (*34*) fed human infants recovering from

protein malnutrition LPC plus milk protein or milk protein alone. Average weight gains on the leaf protein plus milk protein were as good as those on milk alone at equal protein levels. Nitrogen balance studies showed that the nitrogen from leaf protein was somewhat less readily absorbed but was retained as well by human infants as nitrogen from milk protein. These data indicate that LPC could be used to supplement human diets and help combat protein malnutrition.

Materials and Methods

Freeze-dried LPC preparations were obtained from two sources. The major part of the material was provided by N. W. Pirie and M. Beyers, Rothamsted Experimental Station, Harpenden, Herts, England, and prepared by the procedure described by Morrison and Pirie (*22*). Nine species were harvested: chenopodium (*Chenopodium amaranticolor*), corn (*Zea mays*), marrow (*Curcubita ovifera*), nasturtium (*Tropaeolum lobianum*), red clover (*Trifolium pratense*), rye grass (*Lolium perenne*), sanfoin (*Onobrychis sativa*), turnip (*Brassica napus*), and wheat (*Triticum vulgare*) (Table I). All samples (except corn 581 and marrow series) were extracted twice in a hammer mill. The extractions were performed on the initial growth in the spring, except for the nasturtium, red clover 528a, rye grass, wheat 549, and wheat 561, which were extracted from the regrowth after the first harvest. In the case of red clover, sample 512 was the first harvest and 528a the regrowth.

Wheat samples 532 and 549 were fertilized with an additional 1000 pounds per acre of nitro chalk (15.5% N) 8 weeks after planting.

Four varieties of corn were sampled at 11.5 weeks (573 series), 14 weeks (577 series), and 16 weeks (581 series) of age. The varieties were caldera 331 (a), caldera 402 (b), orla 226 (c), and pioneer 395 (d), which range in time for maturity, (d) being the latest maturing variety.

Marrow samples were extracted twice with a hand mincer. The proteins were coagulated at 54–55° C., centrifuged, and labeled marrow A. The supernate was further heated to 70° C., and the coagulum was again centrifuged and labeled marrow B. Both precipitates were washed twice with water before freeze-drying.

The IR series (impulse-rendered) (Table I) was obtained from R. H. Smith and I. H. Chayen, British Glues and Chemicals, Ltd., London, England. The method of preparation of these LPC was somewhat different from that of Pirie's samples and is described by Chayen *et al.* (*8*).

Preliminary experiments were carried out to ascertain the most desirable method of hydrolysis. Duplicate samples were hydrolyzed under large volumes of 6*N* HCl (50 and 1000 ml.) as described by Dustin *et al.* (*16*), with and without stannous chloride, under reflux conditions for 24 hours. Since all results were comparable with those of sealed tube hydrolysis, the sealed tube method was used.

Freeze-dried LPC was redried to constant weight over phosphorus pentoxide in a vacuum desiccator and 50 mg. of each sample were suspended in 2 ml. of 6*N* HCl in 18- × 150-mm. borosilicate glass test tubes. The air above the mixture was displaced with nitrogen and the tube was sealed. The suspension was heated in an oven at 110° C. for 22 hours. The hydrolyzate was quantitatively filtered through acid-washed W-40 filter paper and evaporated to dryness three times with vacuum distillation. The hydrolyzate was dissolved in pH 2.2 citrate buffer and diluted to a final volume of 25 ml. A 1-ml. aliquot of this solution was analyzed for its amino acid content. In some cases part of the methionine was oxidized and is expressed as the sum of methionine and methionine sulfoxides.

Acid hydrolysis tends to destroy tryptophan and cystine. Tryptophan was determined after hydrolysis with 5*N* NaOH (*13*). Results for tryptophan are expressed as the average of duplicate analyses corrected for a standard destruction of 6.3%. Cystine was determined as cysteic acid after oxidation with performic acid and acid hydrolysis (*32*). Results are calculated as cystine content.

The amino acids were determined by the ion exchange chromatography method of Moore, Spackman, and Stein (*20, 21, 33*) on a Beckman-Spinco Model 120 amino acid analyzer.

Results

Amino Acid Composition. Table I shows the per cent amino acid composition of the protein in LPC and other foodstuffs. The protein content of the LPC preparations varied from 83.8 to 31.5%, a range of 52.3%, with an average of 58.0%, but the amino acid content of the protein did not show this large variation. The largest range was 3.2 and 3.0% for proline and tyrosine, respectively. The narrow range of variation in the amino acid content of the leaf protein suggested that protein of a uniform composition could be extracted from a wide variety of green leaves. Comparing average essential amino acid values of LPC protein to those of milk, cheese, meat, poultry, fish, and egg protein, only isoleucine, lysine, and methionine in leaf preparations were consistently lower than those in the animal products. Threonine was consistently higher, while the remainder of the amino acids were in favorable balance. The nonessential amino acids were not compared to other proteins, but the over-all balance indicated that none would be detrimentally low and that there would be an adequate amount of each to help provide well balanced dietary protein. The IR samples obtained from R. H. Smith, which were produced under different conditions during extraction and processing, had about the same relative amino acid composition as leaf protein extracted by N. W. Pirie.

Conditions of Crop. Table I shows the effect due to condition of the crop on the amino acid composition and protein in the LPC. Protein in the nasturtium (dry, weedy) and the sanfoin (dry, weedy, mature) samples had essentially the same amino acid composition as the protein extracted from the green lush samples. These data indicated that proteins extracted from plants under vastly different conditions were remarkably similar. However, LPC samples extracted from plants in the green lush condition contained roughly 1.3 to 2 times as much protein as the dry, weedy and dry, weedy, mature samples. This indicated that the optimum time of harvest was the green lush condition.

Precision of Extraction and Processing. The amino acid composition of the corn 577 series demonstrated that the percentage of protein in the LPC varied somewhat because of the extraction procedure. Sample d contained 10% more protein in the LPC than the rest of the series, although all were sampled at approximately the same time under the same conditions. When the extraction and subsequent processing were carefully carried out, a uniform LPC was produced, as demonstrated by the corn 573 and 581 series.

The importance of further processing was illustrated by the marrow samples. Marrow A was heated to 54–55° C., while marrow B was heated to 70° C. The resulting LPC contained 37.6 and 83.8% amino acids, respectively. The remarkably similar amino acid composition of both protein fractions demonstrated that the protein content of the LPC depends on the amount of heating used in the coagulation procedure.

Species, Variety, Maturity, and Fertilization. Nine species, including four corn varieties sampled at three stages of growth, were analyzed for amino acid content of protein and LPC. Because of the influence of the condition of crop and the precision of extraction, no significant direct effect could be identified with species, variety, or maturity of the samples studied. Fertilized wheat samples 532 and 549 also showed no significant difference in amino acid composition or amount of the protein in LPC for both initial growth or regrowth.

Discussion

A primary concern was whether LPC production would aid in relieving protein malnutrition. Figure 1 compares the median per cent of the essential amino acids of protein in all LPC samples with that in meat, poultry, and fish protein. Methionine, lysine, and isoleucine were lower in LPC protein than in the animal proteins. The remainder of the essential amino acids were higher. Thus, from a dietary standpoint, LPC protein with methionine supplementation may

Table I. Amino Acid Composition of Protein in Leaf Protein Concentrates and in Other Foodstuffs

Protein Source[a]	Age of Crop, Weeks	Condition of Crop[b]	% Protein[c] in LPC	Amino Acids[c]																
				Essential[d]										Nonessential						
				Lys	Phe	Met[e]	Thr	Leu	Ileu	Val	Try	Arg	His	Tyr	Cys	Asp	Ser	Glu	Pro	Gly	Ala
Leaf Protein Concentrates																				
Chenopodium	...	D	63.9	7.2	5.8	2.0	5.2	9.2	5.2	6.4	1.6	6.6	2.8	4.4	0.9	9.7	4.9	11.0	5.3	5.6	6.3
Turnip	...	GL	62.0	6.6	6.5	1.6	5.3	9.7	5.3	6.5	1.3	6.6	2.9	4.4	0.6	10.0	4.5	11.1	5.3	6.0	5.8
Sanfoin	...	DW	47.6	6.3	5.9	1.9	5.5	9.7	5.3	6.3	1.3	6.3	2.5	4.4	0.6	10.1	4.8	11.8	5.5	5.7	6.3
Nasturtium	5	DWM	31.5	6.7	5.7	1.9	5.1	9.2	5.4	6.0	1.6	6.3	2.5	4.1	0.9	10.1	4.8	11.7	5.4	6.0	6.3
Clover																					
512	...	W	56.6	6.5	6.2	1.6	5.5	9.5	5.5	6.5	1.6	6.5	1.8	4.4	0.9	10.2	4.6	11.5	5.3	5.6	6.2
528a	...	GL	59.0	6.8	6.1	1.7	5.4	9.5	5.3	6.8	1.7	6.4	2.5	4.4	0.7	10.0	4.6	11.5	5.1	5.4	6.1
Rye grass	2–3	GL	66.7	6.6	6.1	2.1	5.1	9.1	5.2	6.4	1.8	6.9	2.8	4.5	0.9	9.4	4.2	11.2	5.2	5.5	6.6
Wheat																					
532	16	GL	63.8	6.1	6.1	2.0	5.3	9.4	5.0	6.4	2.2	6.7	2.2	3.8	0.6	9.9	4.7	11.6	5.0	6.0	6.9
549	4	M	61.9	7.3	5.8	2.4	5.3	9.7	5.0	6.3	1.6	7.3	2.4	3.7	0.8	9.7	4.2	11.5	4.8	5.7	6.5
539	18	GL	65.9	6.5	6.1	2.6	5.2	9.6	4.9	6.2	1.5	6.7	2.3	4.1	0.8	9.9	4.4	11.4	5.3	5.8	7.0
552	21	DM	60.7	6.6	6.3	2.5	5.1	9.6	4.9	6.1	1.6	6.6	2.3	4.0	0.8	9.4	4.6	11.5	5.3	5.9	6.9
561	4–6	...	62.4	4.5	6.6	2.6	5.8	9.5	5.0	6.3	1.6	6.6	1.3	4.6	1.0	9.0	4.8	12.3	5.6	6.1	7.1
Corn																					
573a	11.5	GL	59.9	5.8	6.2	2.2	5.7	10.2	5.0	6.3	1.5	5.8	1.8	4.0	0.3	9.0	5.2	12.5	5.8	5.7	6.8
573b	11.5	GL	61.6	6.3	4.9	2.8	4.7	10.1	5.2	6.5	1.3	6.6	2.1	3.1	0.3	10.4	5.2	12.2	5.2	5.7	7.0
573d	11.5	GL	60.2	7.1	5.5	2.7	5.1	10.1	5.0	6.1	1.8	7.0	2.3	3.3	0.7	9.8	4.7	12.0	5.0	5.3	6.5
577a	14	G	53.3	6.2	6.0	2.3	4.3	10.7	5.2	6.9	...	7.1	2.1	3.9	0.8	9.8	5.1	11.2	5.4	5.8	7.1
577b	14	G	53.5	6.7	5.6	2.1	5.0	9.9	5.0	6.0	1.7	6.7	2.1	3.7	0.6	10.3	5.2	11.6	5.4	5.6	6.7
577c	14	G	53.7	6.5	6.0	2.0	4.7	10.0	5.0	6.1	1.7	6.5	2.0	4.1	0.7	10.4	5.0	11.4	5.6	5.4	6.7
577d	14	G	66.4	5.6	6.3	2.4	5.3	10.7	5.4	6.6	1.2	5.9	1.8	4.4	0.5	10.8	5.4	11.1	3.5	5.9	7.2
581a	16	GM	62.6	6.4	5.8	2.1	4.8	9.9	6.4	6.7	1.6	5.8	1.8	4.0	0.6	9.7	5.0	11.8	5.0	5.8	7.0
581b	16	GM	63.6	6.0	5.5	2.4	5.0	9.7	5.2	6.4	1.3	6.3	1.9	4.1	0.6	10.0	5.3	12.3	5.5	5.5	6.9
581c	16	GM	66.0	5.9	6.5	2.3	5.3	10.3	5.3	6.5	1.4	6.1	2.0	4.4	0.8	10.8	5.3	11.2	3.5	5.8	6.8
581d	16	GM	61.2	6.4	5.6	1.5	5.4	10.0	5.2	6.4	1.3	6.9	2.1	4.4	0.6	9.3	4.9	11.6	6.7	5.6	6.2
Marrow																					
A	12	...	37.6	6.4	6.1	1.6	4.8	10.1	5.6	6.4	...	6.4	1.9	3.7	0.8	10.4	5.3	12.8	5.6	5.8	6.4
B	12	...	83.8	6.3	6.4	2.4	5.4	9.9	5.3	6.1	...	7.3	2.6	6.1	1.1	9.9	3.8	11.7	3.5	6.0	6.3
IR corn			43.1	4.6	6.0	2.1	5.1	10.0	4.9	6.5	1.9	5.8	1.9	3.9	0.7	10.0	5.1	13.2	5.1	5.8	7.4
IR alfalfa			66.8	6.3	6.4	1.9	5.1	9.6	6.6	6.3	1.9	5.8	2.1	4.5	0.6	10.2	4.3	11.4	4.8	5.7	6.4
IR mixed grass			55.9	7.2	6.1	1.6	5.2	9.5	5.0	6.1	2.1	6.1	2.1	4.1	0.9	10.0	5.4	11.6	5.0	5.7	6.3
IR alfalfa			32.2	6.2	6.8	0.9	5.3	9.9	5.3	5.9	1.6	6.5	2.5	4.0	0.9	10.2	5.3	11.2	5.3	5.6	6.5
Average			58.0	6.3	6.0	2.1	5.2	9.8	5.3	6.3	1.6	6.5	2.2	4.2	0.7	9.9	4.8	11.7	5.1	5.7	6.6
Range			52.3	2.8	1.9	1.9	1.5	1.6	1.7	1.0	1.0	1.5	1.6	3.0	0.8	1.8	1.5	1.8	3.2	0.8	1.6
Other Foodstuffs[f]																					
Milk				8.2	5.7	3.4	4.5	11.3	8.5	8.5	1.6	4.3	2.6	5.3							
Cheese				8.5	6.5	3.6	3.6	8.9	7.3	7.7	1.5	4.2	3.0	6.9							
Meat, poultry, and fish				8.1	4.9	3.3	4.6	7.7	6.3	5.8	1.3	7.7	2.9	3.4							
Wheat gluten				2.0	5.5	1.5	2.7	7.5	3.7	4.2	1.0	3.9	2.2	3.8							
Corn grits				0.8	6.4	2.5	4.1	15.0	6.4	5.3	0.7	3.1	1.6	6.7							
Egg				7.2	6.3	4.1	4.3	4.2	8.0	7.3	1.5	6.4	2.1	4.5							
Beans and nuts				4.0	5.1	1.4	2.7	6.4	4.1	4.0	0.8	9.4	1.7	4.4							
Potatoes, vegetables, and fruits				5.7	4.5	2.3	4.1	6.6	3.6	4.4	1.9	7.0	2.1	5.4							

[a] Number or letters of species refer to information regarding production, explained in text.
[b] Dry (D), weedy (W), mature (M), green (G), and lush (L).
[c] Amino acids expressed as per cent calculated from total amino acids recovered. When calculations were based on crude protein (N × 6.25), differences were within experimental error. Since amino acid content and not total nitrogen limits nutritive value of crude proteins, and little nonprotein nitrogen was present, protein content was expressed as total amino acid residues.
[d] Required by adult man (*30*, *31*).
[e] Sum of methionine and methionine sulfoxides.
[f] Data for other foodstuffs from Block and Weiss (*2*).

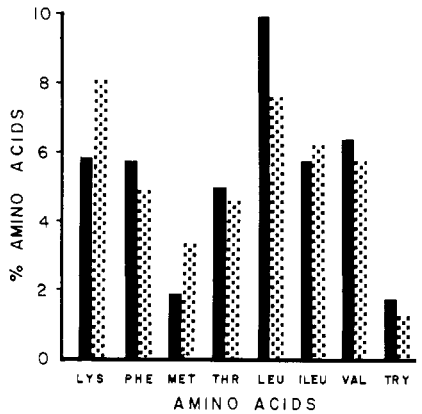

Figure 1. Essential amino acid content of leaf protein concentrates and of meat, poultry, and fish

Dark bars. Median value of leaf protein concentrates calculated from Table I

Checked bars. Values of meat, poultry, and fish protein taken from Block and Weiss (2)

compare favorably with the proteins from commonly accepted and widely used protein sources.

Table II compares the grams of protein per day required for an adult man to maintain nitrogen balance. The minimum amount was determined by the limiting amino acid in the LPC and foodstuff protein. Methionine was admittedly low and not considered in either the LPC or foodstuff protein. Even in the extreme case in which all the protein came from LPC and was supplemented with synthetic methionine at the rate of 0.5 gram per day, 1 kg. ($3.15) of methionine would last one man about 5.5 years (*19*). This would be a relatively inexpensive way to improve the protein quality of LPC. The limiting amino acids which determined the level of protein intake for LPC were phenylalanine and tryptophan. This meant that the low level of lysine noted in Figure 1 was not detrimental to the protein from a dietary standpoint. The amount of foodstuff protein was determined by the level of phenylalanine, lysine, tryptophan, and leucine. To illustrate the comparison further, if whole milk were used, an adult man would have to consume 39 grams of milk protein per day, contained in approximately 1100 grams of milk, while in the case of clover, he would get 38 grams of LPC protein from 58 grams of dry LPC.

Table II. Amount of Protein in Leaf Protein Concentrates or in Other Foodstuffs Providing Recommended Level of Each Essential Amino Acid to Maintain Nitrogen Balance in Adult Man

Species or Foodstuff	Amino Acids[a] Lys	Phe	Met	Thr	Leu	Ileu	Val	Try	Minimum Amount Needed[b]
	Recommended Grams Per Day[a] 1.6	2.2	2.2	1.0	2.2	1.4	1.6	0.5	
LPC Protein									
Chenopodium	22	37	105	19	23	26	24	31	37
Turnip	25	36	147	20	24	28	26	42	42
Sanfoin	28	42	138	20	25	29	28	42	42
Nasturtium	26	42	123	21	26	28	29	33	42
Clover[c]	26	38	138	20	25	28	26	31	38
Rye grass	25	37	110	20	25	28	25	28	37
Wheat[c]	27	37	129	19	24	29	26	29	37
Corn[c]	27	40	92	21	23	28	26	36	40
Marrow[c]	26	37	110	20	23	27	27	..	...
Foodstuff Protein									
Milk	20	39	65	22	20	17	19	31	39
Cheese	19	34	61	28	25	19	21	33	34
Meat, poultry, and fish	20	45	67	22	29	22	28	38	45
Wheat gluten	80	40	147	37	29	38	38	50	80
Corn grits	200	34	88	24	15	22	30	72	200
Eggs	22	35	54	23	53	18	22	33	54
Beans and nuts	40	43	157	37	34	34	40	63	63
Potatoes, vegetables, fruits	28	49	96	24	33	39	36	26	49

[a] Grams of protein needed to provide amino acid to maintain nitrogen balance in adult man. Dietary levels according to Rose (*30*).

[b] Total grams protein needed to provide sufficient essential amino acids (except methionine) to maintain nitrogen balance in adult man. Methionine supplementation assumed.

[c] Average values of all samples analyzed.

Conclusions

The amino acid composition of protein in LPC was not greatly altered by any of the factors studied. Protein content was subject to marked differences, depending on condition of the crop, the extraction procedure, and subsequent processing. The effect due to species, variety, maturity, or fertilization was relatively insignificant from a per cent dry weight standpoint. It was, however, reasonable to assume that plants which contained high nitrogen and produced more dry matter per unit area yielded the largest amount of LPC.

The range, median, and arithmetic average of the amino acid composition of the protein in LPC compared favorably with those of other foodstuffs. Only methionine and lysine were consistently lower than in animal products. From a dietary standpoint, the LPC protein had a favorable balance of essential and nonessential amino acids, the only exception being methionine, which was low in all cases.

LPC could be prepared from many species of land and water plants that are at present economically unimportant. The choice of plants is of considerable importance. At present only from 7 to 18% of the plant protein is concentrated into edible protein by animals (*18*); the LPC samples used in this study represented 48.5% of the total plant protein. On a per unit area basis the production of LPC would more than double the amount of edible protein now being obtained. This represents a large increase in efficiency, which could still be improved by techniques to give more complete extraction and by utilization of the remaining residue.

Protein from the green plant obtained in the form of LPC is of sufficiently high quality to be used as a dietary supplement to help eliminate the world's protein malnutrition problem and this justifies increased research to solve problems of LPC production and utilization.

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LEAF PROTEINS AS FOODSTUFFS

Fatty Acids in Some Leaf Protein Concentrates

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The total fatty acid composition of leaf protein concentrates from seven plant species was studied by gas-liquid chromatography of their methyl esters. In general, the concentrates contained from about 3 to 8% fatty acids, more than 75 to 80% of which were linolenic, palmitic, and linoleic acids. Lesser amounts of oleic, palmitoleic, and stearic acids contributed 12 to 17% of the total fatty acids. Small amounts of other saturated and unsaturated fatty acids were detected. There appeared to be no gross qualitative differences in the composition of fatty acids from the leaf protein concentrates from different species.

Leaf protein concentrates may furnish adequate amounts of many essential amino acids, although they are low in methionine and cystine (*6*). The same concentrates contain significant levels of lipid, which could make a substantial contribution to the nutritional quality of the concentrates.

Since the early work of Smith and Chibnall (*19, 20*) on the lipids of forage grasses, a number of papers have appeared on the fatty acids of leaf tissue. The advent of gas-liquid chromatography has greatly facilitated studies of this nature (*1–3, 5, 8, 17, 18, 22, 23*).

The present paper deals with the total fatty acid composition of leaf protein concentrates used in the previous amino acid studies primarily in terms of the polyunsaturated fatty acids (PUFA) which affect the nutritional and keeping qualities of the concentrates. To the authors' knowledge, no studies have been published on the fatty acids of these leaf protein concentrates.

Experimental

Samples. Leaf protein concentrates of *Brassica napus* (turnip), *Chenopodium amaranticolor*, *Curcubita ovifera* (marrow A), *Lolium perenne* (rye grass), *Trifolium pratense* (red clover), *Triticum vulgare* (wheat preparation 539), and *Zea mays* (corn preparations 577–d and 581–d) were obtained from N. W. Pirie, Rothamsted Experimental Station, Harpenden, Herts., England. Their preparation has been described (*12*).

Extraction of Lipids. One-gram portions of each sample, dried and finely powdered (60-mesh), were extracted with shaking at room temperature (23° C.) four times each for 2 hours with 20 ml. of chloroform-methanol (2 to 1, v./v.). After each extraction the samples were filtered through Whatman No. 1 paper on a Büchner funnel under suction. The final volumes of each extract were measured and 3-ml. aliquots were taken to determine total lipid extracted after complete removal of the solvent in vacuo. Four extractions removed all but negligible amounts of total lipid-solubles. The combined extracts were concentrated in vacuo at about 20 mm. of Hg and 30° to 40° C. The crude lipids were saponified by refluxing for 2 hours in 0.5*N* ethanolic potassium hydroxide (20 ml. per gram) as described by Weenink (*23*). After cooling, the unsaponifiable components were extracted from the soap solution by three extractions with 20 ml. of Skellysolve B. The remaining solution was acidified with hydrochloric acid and the total fatty acids were extracted with four 25-ml. portions of Skellysolve B. The solutions of fatty acids were transferred to tared tubes and yields were determined after complete removal of the solvent in a stream of dry nitrogen at 23° C.

Preparation of Methyl Esters. The fatty acids were converted to methyl esters by the procedure of Radin, Hajra, and Akahori (*14*) using 2,2-dimethoxypropane (DMP) as a water scavenger. To 30 mg. of fatty acid in a test tube, 2 ml. of DMP and 2 ml. of methanolic hydrochloric acid were added. The tube was stoppered and left for 1 hour at room temperature. The esters were isolated by addition of water and extraction with Skellysolve B. The extracts were washed with 0.2*N* sodium bicarbonate solution, the solvent was removed under nitrogen, and the methyl esters were examined by gas-liquid chromatography.

Gas-Liquid Chromatographic Analysis. Samples of methyl esters were chromatographed on an Aerograph Hi-Fy Model 600-B equipped with a hydrogen flame ionization detector. Stationary phases and solid supports were 15% diethylene glycol succinate (DEGS) on 60/80-mesh firebrick GC-22 and 15% Apiezon M on 60/80-mesh base-washed Chromosorb W. The columns were packed in a 5 foot × 1/8 inch stainless steel tube. The DEGS column was