Kriek, N. P. J.; Marasas, W. F. O.; Steyn, P. S.; van Rensburg, S. J.; Steyn, M. *Food Cosmet. Toxicol.* **1977**, *15*, 579.
Lindenfelser, L. A.; Ciegler, A.; Hesseltine, C. W. *Appl. Environ. Microbiol.* **1978**, *35*, 105.
Marasas, W. F. O.; Kriek, N. P. J.; van Rensburg, S. J.; Steyn, M.; van Schalkwyk, G. C. *S. Afr. J. Sci.* **1977**, *73*, 346.
Marasas, W. F. O.; Kriek, N. P. J.; Wiggins, V. M.; Steyn, P. S.; Towers, P. K.; Hastie, T. J. *Phytopathology* **1979a**, *69*, 1181.
Marasas, W. F. O.; Leistner, L.; Hofmann, G.; Echardt, C. *Eur. J. Appl. Microbiol. Biotechnol.* **1979b**, *7*, 289.
Marasas, W. F. O.; van Rensburg, S. J.; Mirocha, C. J. *J. Agric. Food. Chem.* **1979c**, *27*, 1108.
Mirocha, C. J., presented at the Workshop on Rapid Analytical Detection Methods at the International Conference on Mycotoxins, Munich, Aug 14–15, 1978.
Mirocha, C. J.; Christensen, C. M. In "Mycotoxins"; Purchase, I. F. H., Ed.; Elsevier: Amsterdam, 1974; pp 129–148.
Mirocha, C. J.; Christensen, C. M.; Nelson, G. H. *Appl. Microbiol.* **1969**, *17*, 482.
Mirocha, C. J.; Pathre, S. V.; Schauerhamer, B.; Christensen, C. M. *Appl. Environ. Microbiol.* **1976**, *32*, 553.
Mirocha, C. J.; Schauerhamer, B.; Pathre, S. V. *J. Assoc. Off. Anal. Chem.* **1974**, *57*, 1104.
Pathre, S. V.; Mirocha, C. J. In "Mycotoxins in Human and Animal Health"; Rodricks, J. V.; Hesseltine, C. W.; Mehlman, M. A., Eds.; Pathotox Publishers: Park Forest South, IL, 1977; pp 229–253.
Pathre, S. V.; Mirocha, C. J. *Appl. Environ. Microbiol.* **1978**, *35*, 992.
Rabie, C. J.; Lübben, A.; Louw, A. I.; Rathbone, E. B.; Steyn, P. S.; Vleggaar, R. *J. Agric. Food Chem.* **1978**, *26*, 375.
Roberts, B. A.; Patterson, D. S. P. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 1178.
Rose, E. F.; McGlashan, N. P. *Br. J. Cancer* **1975**, *31*, 197.
Springer, J. P.; Clardy, J.; Cole, R. J.; Kirksey, J. W.; Hill, R. K.; Carlson, R. M.; Isidor, H. L. *J. Am. Chem. Soc.* **1974**, *96*, 2267.
Thiel, P. G. *Biochem. Pharmacol.* **1978**, *27*, 483.
Thomas, F.; Eppley, R. M.; Trucksess, M. W. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 114.

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Composition of Peas (*Pisum sativum*) Varying Widely in Protein Content

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The composition of one variety of field peas (*Pisum sativum* L. cv. Trapper) containing 14.5–28.5% protein (dry, dehulled basis) was investigated. The variability in protein content did not appear to be related to the degree of maturation. Seventy-two percent of the difference in protein content between the highest and lowest protein content pea was accounted for by starch, 7.9% by lipid, 8.0% by neutral detergent fiber (NDF), 6.8% by sugars, and 3.6% by ash. Starch, lipid, NDF, sucrose, and nonprotein nitrogen content, the nitrogen solubility index, and zinc content were negatively correlated with pea protein content. In addition, the amino acids threonine, cystine, glycine, alanine, methionine, and lysine (expressed as milligrams of amino acid per gram of N) were similarly correlated. Total α-galactosides, verbascose, glutamic acid, arginine, total pigments, and carotenoid pigments were positively correlated with protein content. The amino acid score, an index of nutritional quality, was negatively correlated with protein content. Xanthophylls (monohydroxy type) accounted for most of the difference in carotenoid content between the high and low protein content peas.

The protein content of pea seeds appears to be highly variable and is influenced by both genetic and environmental factors. Slinkard (1972) analyzed 1452 pea varieties from the U.S. Department of Agriculture world collection grown in Saskatchewan. Protein contents ranged from 15.5 to 39.7%. Gottschalk et al. (1975) found that genetically identical pea plants grown in the same year on the same field produced seeds containing protein contents ranging from 19.3 to 25.2%. The 1970 field pea crop in Saskatchewan was seeded mostly to the Century variety and showed nitrogen contents of 2.43–4.06% (McLean, 1972). Since then a new variety (Trapper) has been introduced which presently accounts for over 75% of the acreage seeded to peas. A survey by the authors of farmers peas (n = 198) obtained in 1979 showed that these ranged from 13.3 to 27.1% protein on a dry, whole seed basis.

The environmental factors responsible for the very wide range in pea protein content are not fully understood. McLean et al. (1974) reported that pea protein contents were increased from 20 to 30% by application of nitrogen fertilizer. Holl and Vose (1980) found that seed protein was deposited early in the development of the seed but decreased (as a component of dry weight) with maturation. The largest decrease (27.5% protein at 15 days after anthesis to 20.7% at 80 days) was observed in the absence of added nitrogen fertilizer. Eppendorfer and Bille (1974) reported that increasing soil phosphorus and decreasing soil potassium increased pea nitrogen content from 3.5 to 4.9%. Robertson et al. (1962) analyzed pea seed protein contents up to the 30th day after flowering and found that protein synthesis was markedly delayed and reduced at low growth temperatures. Application of s-triazines to the leaves of young plants increased the protein content of bean and pea seeds (Singh et al., 1972).

The objective of this study was to determine the chemical composition of pea samples as a function of a very wide range in protein content (14.5–28.5% on a dry, dehulled seed basis). The components that make up the large difference in protein content and the correlations between protein content and chemical components were determined. Compositional data of this nature is important from the nutritional standpoint, since field peas are des-

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tined largely for human consumption in both domestic and export markets. From an industrial standpoint, this compositional data is valuable since small variations in the initial pea composition appear to have an amplified effect on the concentration of constituents in protein concentrates or isolates prepared by air classification (Reichert, 1982) or wet milling, respectively. Both of these processes have recently been commercialized in Canada.

EXPERIMENTAL SECTION

Sample Preparation. Smooth, yellow, field peas (*Pisum sativum* L. cv. Trapper) were obtained from Saskatchewan farmers in 1979 by sampling 198 bins with a probe. Following analysis of seed protein content, four samples ranging from 14.5 to 28.5% protein (dry, dehulled seed basis) were chosen for detailed chemical analysis. The seeds were dehulled in a Currier-type plate mill followed by air aspiration. To obtain a fine flour the peas were milled in an Alpine pin mill, Model 250 CW.

Analytical Methods. Protein ($N \times 6.25$) was determined by the automated Kjel-Foss method [AACC method 46-08 (American Association of Cereal Chemists, 1969)], while ash and moisture were determined by standard Association of Official Agricultural Chemists (1975) procedures.

Starch was assayed by using a modification of the dual enzyme semimicromethod of Banks et al. (1970) using α-amylase and amyloglucosidase (Tenase and Diazyme L-100, respectively; Miles Laboratories Inc., Elkhart, IN). The chromogen used in conjunction with the glucose oxidase–peroxidase system was 2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid) from the Sigma Chemical Co., St. Louis, MO.

Neutral detergent fiber was determined by using AACC method 32-20 (American Association of Cereal Chemists, 1969).

Neutral lipids were extracted by refluxing 5 g of pea flour in 50 mL of hexane. The hexane was separated from the flour, and the flour was thoroughly washed with clean hexane. The extract was taken to dryness, and the lipid material was weighed. The hexane-extracted flour was further extracted with chloroform–methanol (2:1) by the same procedure to determine the polar lipid content.

Oligosaccharides soluble in 80% aqueous methanol (sucrose, raffinose, stachyose, and verbascose) were quantitated by GLC essentially using the procedure of Vose et al. (1976). Total sugar present in the extract was determined by the phenol–sulfuric acid method of Dubois et al. (1956) using raffinose as the standard.

Amino acids were determined by hydrolyzing vacuum-oven-dried samples in a sealed tube under nitrogen atmosphere with distilled 6 N HCl for 22 h at 110 °C. Hydrolysates were evaporated to dryness in vacuo, dissolved in buffer, and analyzed on a Beckman amino acid analyzer, Model 119BL, by using the single-column procedure. Amino acid values were calculated by using hydrated molecular weights. Cystine was calculated from the sum of cysteine and cysteic acid. Methionine was determined by gas chromatography of the reaction product (methyl thiocyanate) between cyanogen bromide and methionyl residues of the protein (Finlayson and MacKenzie, 1976; MacKenzie, 1977). The amino acid score was determined by using the limiting sulfur-containing amino acids, methionine and cystine (Food and Agriculture Organization, 1973).

The nitrogen solubility index was determined by AOCS method Ba 1165 (Americal Oil Chemists Society, 1976). Pea flour (5 g) was stirred in 200 mL of distilled water for 2 h at room temperature (pH was not adjusted). Following centrifugation (10400*g*; 15 min) an aliquot of the supernatant was analyzed for nitrogen to determine the soluble N. Nonprotein nitrogen was estimated (Bhatty et al., 1973) by determining the percent of sample nitrogen extracted by 70% ethanol (22 °C at a flour to solvent ratio of 1:20).

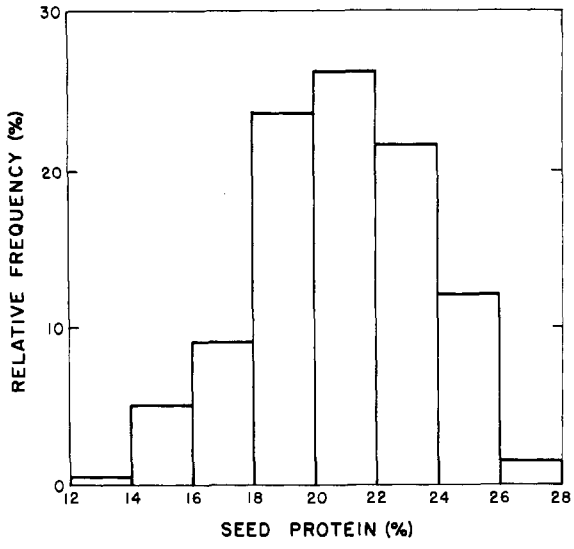

Figure 1. Distribution of seed protein content (dry, whole seed basis) of 198 pea samples grown in Saskatchewan.

Atomic absorption was used to quantitate Mg, Zn, Cu, Mn, and Fe following digestion according to AOAC method 7.084 (Association of Official Analytical Chemists, 1975). For K the same digestion was used followed by measurement by flame photometry. For Ca and P, samples were dry-ashed according to AOAC method 7.080 (Association of Official Analytical Chemists, 1975). Calcium was determined by titration with EDTA while P was determined by AOAC method 7.103-7.106 (Association of Official Analytical Chemists, 1975). Molybdenum was determined by the method used by Stewart et al. (1979), whereas boron was determined according to the procedure of Gupta and Stewart (1975).

Carotenoids were determined by using the hot saponification procedure [AOAC method 43.020a (Association of Official Analytical Chemists, 1975)] followed by column chromatographic procedures 43.021a (total carotenes) and 43.021c (total xanthophylls). Because of the low concentration of carotenoids in the pea flour, 40 g was extracted instead of 4 g, and the quantities of other reagents were likewise increased by a factor of 10. The final upper phase was concentrated by rotoevaporation (40 °C) to a small, workable volume. Xanthophylls were separated into mono and dihydroxy pigments by AOAC method 43.021b (Association of Official Analytical Chemists, 1975). Total pigment content was determined by AACC method 14-50, (American Association of Cereal Chemists, 1969).

Color reflectance properties of split peas and pea flour–water pastes were determined with an Agtron M-500A spectrophotometer. Split peas were loaded into the glass cells and the reflectance was measured in the blue mode (436 nm) by using the 5 and 30 color standards to set the 0 and 100% transmittance values. Flour–water pastes (9.3 g of flour plus 12.5 g of distilled water) were stirred for 10 min in the glass cells and also measured in the blue mode by using the 10 and 30 color standards to set the 0 and 100% transmittance values.

All analyses are reported on a dry weight basis.

RESULTS AND DISCUSSION

Pea protein contents of farmers' samples (n = 198) collected in 1979 approached a Gaussian distribution

Table I. Seed Characteristics of Dehulled Peas Varying in Protein Content

protein, %	hull,[a] %	1000 seed wt,[b] g
14.5	8.2	127
18.3	7.7	131
24.2	7.9	143
28.5	7.8	138
mean SD[c]	0.07	1

[a] Obtained by manual dissection. [b] Determined on whole peas. [c] Standard deviation. Minimum of duplicate determinations.

Table II. Linear Regression Equations and Correlation Coefficients of Pea Flour Constituents Significantly Correlated with Protein Content

constituent	correlation coefficient (r)[a]	regression equation
starch	−0.99***	$-0.687X^b + 69.7$
neutral detergent fiber	−0.96**	$-0.072X + 5.18$
total α-galactosides	0.91*	$0.072X + 3.45$
sucrose	−0.91*	$-0.094X + 4.66$
verbascose	0.99***	$0.052X + 1.30$
total lipid	−0.99***	$-0.077X + 5.17$
neutral lipid	−0.99***	$-0.067X + 3.40$
polar lipid	−0.99***	$-0.010X + 1.77$
threonine	−0.97**	$-2.30X + 277$
glutamic acid	0.91*	$6.00X + 896$
cystine	−0.98**	$-1.01X + 77.3$
glycine	−0.95**	$-2.51X + 330$
alanine	−0.94*	$-2.70X + 333$
methionine	−0.94*	$-0.84X + 69.8$
lysine	−0.97**	$-2.85X + 522$
arginine	0.97**	$13.5X + 176$
nonprotein nitrogen	−0.94*	$-0.069X + 6.88$
nitrogen solubility index	−0.95**	$-0.515X + 94.9$
zinc	−0.93*	$-0.431X + 49.7$
total pigments	0.93*	$0.905X - 4.67$
carotenoids	0.99***	$0.486X - 3.15$

[a] (*), (**), and (***) = significant at the 10, 5, and 1% level of significance, respectively. [b] X = protein content.

Table III. Complex Carbohydrate, Lipid, and Ash Content of Dehulled Peas Varying in Protein Content

protein, %	starch, %	NDF,[a] %	lipid, % neutral	lipid, % polar	lipid, % total	ash, %
14.5	59.8	4.26	2.47	1.62	4.09	3.3
18.3	56.7	3.67	2.12	1.59	3.71	3.0
24.2	53.8	3.50	1.76	1.53	3.29	2.7
28.5	49.7	3.14	1.51	1.48	2.99	2.8
mean SD[b]	1.1	0.04	0.11	0.03		0.04

[a] Neutral detergent fiber. [b] Duplicate determinations.

(Figure 1). Samples were distributed over a very wide range (13.3–27.1% protein on a dry, whole seed basis) with a mean protein content of 20.7% and a standard deviation of 3.1. Agriculture Handbook 8 (Watt and Merrill, 1963) quotes a value of 24.1% protein in mature dry peas; however, only about 14% of the samples had protein contents greater than or equal to this value. The four samples chosen for detailed chemical analysis were very similar in appearance and contained 14.5, 18.3, 24.2, and 28.5% protein (dry, dehulled seed basis). No green seeds were present in any of the four samples, indicating that they appeared to be harvested at maturity. Hull content and 1000 seed weight did not differ greatly with varying protein content and averaged 7.9% and 135 g, respectively (Table I).

Correlation coefficients and regression equations of pea flour constituents, which were significantly correlated with pea protein content, are given by Table II and are discussed in later sections. Since only four samples were analyzed, the statistical significance of correlations was tested at the 1, 5, and 10% level of significance. Minimum r values necessary for significance at these levels are 0.990, 0.950, and 0.900, respectively (degrees of freedom = 2).

Complex Carbohydrates, Lipids, and Ash. Starch content (Table III) was negatively correlated with protein content although an increase in protein content was not fully accounted for by a similar decrease in starch. In wheat a similar relationship between protein and starch content has been found (Hopkins and Graham, 1935). The neutral detergent fiber content, which approximates the sum of hemicellulose, cellulose, and lignin present in the cell wall (Robertson, 1978), was also negatively correlated with pea protein. Neutral, polar, and total lipids were present in smaller amounts and were highly negatively correlated with protein. The variability in the total lipid content with protein was due mainly to the neutral lipid fraction.

Soluble Sugars. The major soluble sugars (Table IV) found in the peas were sucrose and α-galactosides (raffinose, stachyose, and verbascose), confirming the work of Vose et al. (1976) and others. The α-galactosides have been implicated in the production of flatulence in animals and humans (Cristofaro et al., 1974; Fleming, 1981). Verbascose and the total α-galactoside contents were both positively correlated with protein content, whereas sucrose was negatively correlated. Sucrose and α-galactoside contents were highly negatively correlated ($r = -0.99$; $p < 0.01$).

Starch and sucrose contents of the peas were positively correlated ($r = 0.95$; $p < 0.05$). Shallenberger and Moyer (1961) found a negative correlation between sucrose and starch contents during the maturation of peas. This strongly suggests that differences in the protein content of the peas used in this study were not a function of the maturity of the plant.

Total sugar content as measured by the phenol–sulfuric acid method was relatively constant and in fairly good agreement with the sum of the α-galactosides plus sucrose determined by GLC. Raffinose, which is commercially

Table IV. Soluble Sugar[a] Composition of Dehulled Peas Varying in Protein Content

protein, %	sucrose, %	α-galactosides, % raffinose	α-galactosides, % stachyose	α-galactosides, % verbascose	α-galactosides, % total α-galactosides	α-galactosides + sucrose, %	total sugars,[b] %
14.5	3.43	0.73	1.60	2.09	4.42	7.85	8.40
18.3	2.62	0.80	1.92	2.23	4.95	7.57	7.81
24.2	2.65	0.71	1.70	2.51	4.92	7.57	8.03
28.5	1.86	0.70	2.08	2.83	5.61	7.47	7.45
mean SD[c]	0.03	0.01	0.03	0.06			0.06

[a] Soluble in 80% aqueous methanol. [b] Determined by the phenol-sulfuric acid colorimetric procedure. [c] Duplicate determinations.

Table V. Amino Acid Composition[a] of Dehulled Peas Varying in Protein Content

amino acid	mg of amino acid/g of N for protein[b] content, %, of 14.5	18.3	24.2	28.5	SD
aspartic acid	734	719	754	736	11
threonine	246	234	217	215	9
serine	256	260	268	261	8
glutamic acid	994	985	1058	1062	19
proline	238	232	270	251	7
cystine	64	57	53	49	6
glycine	299	278	267	262	5
alanine	299	275	271	256	4
valine	311	298	287	294	14
methionine	57	54	52	44	2
isoleucine	260	264	253	262	8
leucine	420	455	446	464	19
tyrosine	216	185	211	197	10
phenylalanine	299	284	288	287	5
histidine	155	147	148	152	4
lysine	485	464	454	442	8
NH_3	62	57	60	57	2
arginine	387	397	517	559	23
% of total N recovered	84.7	82.6	87.4	87.9	
amino acid score	55	50	48	42	

[a] Duplicate hydrolysates were each analyzed in triplicate except for methionine, where duplicate reaction mixtures were each analyzed in duplicate and for cystine where duplicate hydrolysates were analyzed once. [b] $N \times 6.25$.

Table VI. Nitrogen Solubility Index and Nonprotein Nitrogen (npn) of Dehulled Peas Varying in Protein Content

protein, %	npn, % of flour N	nitrogen solubility[a] index, % of flour N
14.5	6.02	87.8
18.3	5.47	85.6
24.2	5.06	81.0
28.5	5.04	81.2
mean SD[b]	0.20	1.7

[a] In distilled water. [b] Duplicate determinations.

available, is preferred as a standard rather than glucose, since α-galactosides are the major simple sugars present in peas.

Nitrogen-Containing Compounds. Amino acid analysis (Table V) of the peas showed that threonine, cystine, glycine, alanine, methionine, and lysine (expressed as milligrams of amino acid per gram of N) were negatively correlated with the protein content. Only glutamic acid and arginine showed a positive correlation. Holt and Sosulski (1979) analyzed the amino acid composition of 16 samples of Century field peas and obtained similar correlations for all amino acids except glutamic acid. In addition, they found significant correlations for isoleucine and aspartic acid. Five of the amino acids determined, aspartic acid, threonine, serine, glutamic acid, and arginine, were lower than the range reported by Holt and Sosulski (1979), whereas the remainder were in agreement with their values. The amino acid score based on cystine and methionine ranged from 42 to 55 and was significantly correlated with seed protein content. Holt and Sosulski (1979) reported a mean of 63 and a range of 43–75 for Century peas varying in protein content. A negative correlation between the sulfur-containing amino acids and protein content has also been found by Evans and Boulter (1980) for peas and beans.

The nitrogen solubility index and nonprotein nitrogen content (Table VI) decreased with increasing protein content. Both were significantly correlated with protein content. The major proteins in peas are salt-soluble globulins (Derbyshire et al., 1976). The solubility of the seed N in a suspension of flour in distilled water is a function of the particular proteins in the pea and also the concentration of the water-soluble naturally occurring salts. The correlation coefficients between the N solubility index and potassium (Table VII) and ash contents of the peas were 0.96 and 0.97 (significant at $p < 0.05$), respectively, suggesting that differences in pea mineral content are largely responsible for differences in nitrogen solubility. Nonprotein nitrogen contents were somewhat higher than those reported by Bhatty et al. (1973).

Minerals. The relative amounts of four major elements and seven trace elements in the peas is shown in Table VII. Meiners et al. (1976) reported similar values for K, Mg, P, and Mn in dry dehulled peas. However, Table VII shows approximately twice the Ca content, 4–5 times the Fe, twice the Zn, and somewhat more Cu than those reported by these authors. Lebanese peas have been reported (Kuzayli et al., 1966) to contain 76 ppm of Fe, which is comparable to the value reported in the present study. The elements Mo, Se, and Mn showed the greatest variability within the pea samples (CV = 61.9, 31.3, and 28.9%, respectively), whereas Mg, K, Zn, and B had CV values of less than 10%. Only Zn showed a significant ($p < 0.10$), negative correlation with protein content.

Pigments. Split peas (dehulled) prepared from the low protein content peas appeared very bleached (faded yellow) in color, whereas those from the high protein peas were bright yellow. Reflectance measurements on the split peas and the flour–water pastes prepared from them decreased with increasing protein content (Table VIII), and the correlation coefficients (reflectance measurement on protein content) were −0.98 and −0.94, respectively.

Pigment content determined by the AACC official method was from 2 to 2.5 times higher than that determined by the AOAC method (Figure 2). Both measurements were positively correlated to protein content. The deter-

Table VII. Mineral Content[a] of Dehulled Peas Varying in Protein Content

protein, %	% K	Ca	Mg	P	ppm Fe	Zn	B	Cu	Mn	Mo	Se
14.5	1.11	0.07	0.10	0.45	98	43	4.6	11.5	8.4	0.21	0.54
18.3	1.05	0.08	0.10	0.46	121	43	4.6	9.8	15.6	0.05	0.41
24.2	0.98	0.09	0.10	0.30	82	38	3.8	7.2	10.0	0.07	0.24
28.5	1.01	0.07	0.10	0.36	88	38	4.6	9.1	9.9	0.14	0.49
mean SD[b]	0.01	0.01	0.01	0.004	0.6	0.01		0.1	0.01	0.02	0.02
mean	1.04	0.08	0.10	0.39	97	41	4.4	9.4	11.0	0.12	0.42
CV[c]	5.4	12.4	0	19.4	17.6	7.1	9.1	18.9	28.9	61.9	31.3

[a] Duplicate digests were each analyzed once. [b] Duplicate determinations. [c] Coefficient of variation (percent) of the four samples varying in protein content.

Table VIII. Color of Dehulled, Split Peas, and Flour-Water Pastes Prepared from Peas Varying in Protein Content

protein, %	color,[a] % split peas	color,[a] % paste
14.5	47.5	76.6
18.3	37.2	70.1
24.2	31.8	59.8
28.5	21.7	31.9
mean SD[b]	1.1	2.0

[a] Measured at 436 nm on the Agtron M-500 spectrophotometer. [b] Minimum of triplicate determinations.

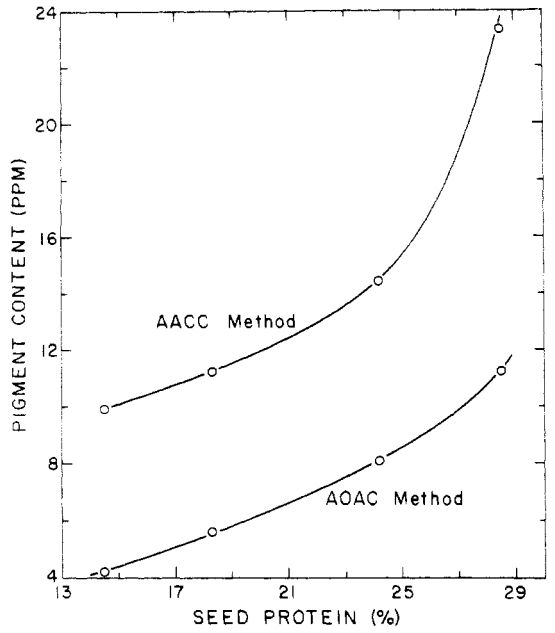

Figure 2. Pigment content of dehulled peas varying in protein content determined by the AOAC method (total carotenes plus total xanthophylls) and by the AACC method (total pigment).

mination by the AACC method was considered to measure the total pigment content. It measures all pigments soluble in water-saturated butanol, which includes carotenoid pigments as well as more polar compounds such as the flavonoids. The AOAC method measures only carotenoid pigments by first eliminating the more polar pigments by phase separation (hexane and water mainly) followed by column chromatography. The carotenoid content correlated well with reflectance measurements of the split peas ($r = -0.98$; $p < 0.05$) and flour–water pastes ($r = -0.98$; $p < 0.05$). Total pigment content also correlated well with these reflectance measurements. These results show that the color quality of peas can be measured by using either reflectance or spectrophotometric methods. The reflectance method is considerably faster, however.

Xanthophylls were the predominant carotenoids in these peas (Table IX). Variation in the monohydroxy pigments accounted for most of the difference in carotenoid content between high and low protein content peas. The dihydroxy pigments and carotenes were present in very low concentrations.

CONCLUSIONS

Field peas showed an unusually high range in seed protein content. Starch accounted for most of the difference in protein content, while the remainder consisted of lipid, neutral detergent fiber (NDF), soluble sugars, and ash. Starch, lipid, NDF, sucrose, nonprotein nitrogen, nitrogen solubility index, and zinc content were negatively correlated with pea protein content, whereas total α-galactosides, verbascose, total pigments, and carotenoid pigments were positively correlated. Six amino acids (expressed as milligrams of amino acid per gram of N) were negatively correlated to protein content, whereas only two were positively correlated. The intensity of the yellow pigmentation, as measured by both reflectance and extraction procedures, increased with increasing protein content.

Table IX. Characterization of Carotenoids in High and Low Protein Content Dehulled Peas

protein, %	xanthophylls, ppm mhp[a]	xanthophylls, ppm dhp[b]	carotenes, ppm
14.5	3.84	0.19	0.12
28.5	10.29	0.35	0.30

[a] Monohydroxy pigments. [b] Dihydroxy pigments.

These results suggest that only peas within a narrow range of protein content be used if uniform products are to be achieved by dry- or wet-milling technologies. To ensure that field peas supply nutritionally adequate quantities of protein, work is required to define those agronomic conditions which are conducive to generating higher protein content peas. To ensure that higher protein content peas are nutritionally adequate, peas should be selected for higher content of methionine and cystine.

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LITERATURE CITED

American Association of Cereal Chemists "Approved Methods of the AACC"; AACC: St. Paul, MN, 1969.
American Oil Chemists Society "Official and Tentative Methods", 3rd ed.; AOCS: Champaign, IL, 1976.
Association of Official Analytical Chemists "Official Methods of Analysis", 10th ed.; Horwitz, W., Ed.; AOAC: Washington, DC, 1975.
Banks, W.; Greenwood, C. T.; Muir, D. D. *Staerke* **1970**, *22*, 105.
Bhatty, R. S.; Sosulski, F. W.; Wu, K. K. *Can. J. Plant Sci.* **1973**, *53*, 651.
Cristofaro, E.; Mottu, F.; Wuhrmann, J. J. In "Sugars in Nutrition"; Sipple, H. L.; McNutt, K. W., Eds.; Academic Press: New York, 1974.
Derbyshire, E.; Wright, D. J.; Boulter, D. *Phytochemistry* **1976**, *15*, 3.
Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350.
Eppendorfer, W. H.; Bille, S. W. *Plant Soil* **1974**, *41*, 33.
Evans, I. M.; Boulter, D. *J. Sci. Food Agric.* **1980**, *31*, 238.
Finlayson, A. J.; MacKenzie, S. L. *Anal. Biochem.* **1976**, *70*, 397.
Fleming, S. E. *J. Food Sci.* **1981**, *46*, 794.
Food and Agriculture Organization *FAO Nutr. Meet. Rep. Ser.* **1973**, *No. 52*, 62.
Gottschalk, W.; Mueller, H. P.; Wolff, G. *Egypt. J. Genet. Cytol.* **1975**, *4*, 453.
Gupta, S. K.; Stewart, J. W. B. *Schweiz. Landwirtsch. Forsch.* **1975**, *14*, 153.
Holl, F. B.; Vose, J. R. *Can. J. Plant Sci.* **1980**, *60*, 1109.
Holt, N. W.; Sosulski, F. W. *Can. J. Plant Sci.* **1979**, *59*, 653.
Hopkins, C. Y.; Graham, R. P. *Can. J. Res.* **1935**, *12*, 820.
Kuzayli, M. V.; Cowan, J. W.; Sabry, Z. I. *J. Sci. Food Agric.* **1966**, *17*, 82.
MacKenzie, S. L. *J. Chromatogr.* **1977**, *130*, 399.
McLean, L. A. M.Sc. Thesis, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1972.
McLean, L. A.; Sosulski, F. W.; Youngs, C. G. *Can. J. Plant Sci.* **1974**, *54*, 301.
Meiners, C. R.; Derise, N. L.; Lau, H. C.; Crews, M. G.; Ritchey, S. J.; Murphy, E. W. *J. Agric. Food Chem.* **1976**, *24*, 1126.
Reichert, R. D., submitted for publication in *J. Food Sci.*, 1982.

Robertson, J. B. In "Topics in Dietary Fiber Research"; Spiller, G. A.; Amen, R. J., Eds.; Plenum Press: New York, 1978.
Robertson, R. N.; Highkin, H. R.; Smydzuk, J.; Went, F. W. *Aust. J. Biol. Sci.* **1962**, *15*, 1.
Shallenberger, R. S.; Moyer, J. C. *J. Agric. Food Chem.* **1961**, *9*, 137.
Singh, B.; Campbell, W. F.; Salunkhe, D. K. *Am. J. Bot.* **1972**, *59*, 568.
Slinkard, A. E. "Production, Utilization and Marketing of Field Peas"; Crop Development Centre, University of Saskatchewan: Saskatoon, Saskatchewan, Canada, 1972; Annual Report No. 1.
Stewart, J. W. B.; Moir, J. O.; Racz, V. J. "Recent Work on Copper and Molybdenum in Pastures and Soils in Saskatchewan"; University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1979; Agricultural Science Proceedings, Publication No. 403.
Vose, J. R.; Basterrechea, M. J.; Gorin, P. A. J.; Finlayson, A. J.; Youngs, C. G. *Cereal Chem.* **1976**, *53*, 928.
Watt, B. K.; Merrill, A. *U.S., Dep. Agric., Agric. Handb.* **1963**, *No. 8*.

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Isolation and Analysis of Carrot Constituents: Myristicin, Falcarinol, and Falcarindiol

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Carrot roots are blended with dichloromethane in the presence of an antioxidant, and the resulting suspension is centrifuged. An aliquot of the clear extract is removed, concentrated, dissolved in hexane, and fractionated by gravity-flow adsorption chromatography on silica gel (70–325 mesh). Carotenoid pigments are removed with hexane, and myristicin (1-allyl-3,4-methylenedioxy-5-methoxybenzene), falcarinol (3-hydroxyheptadeca-1,9-diene-4,6-diyne), and falcarindiol (3,8-dihydroxyheptadeca-1,9-diene-4,6-diyne) are eluted with 45:55 ethyl ether–hexane. After a 20-fold concentration, these compounds are analyzed directly by gas–liquid chromatography (GLC) on SE-52 and OV-17 columns (80–250 °C at 4 °C/min) with methyl palmitate as an internal standard. Recoveries of added myristicin, falcarinol, or falcarindiol (15 ppm) were about 95%. Analyses by this method support previous estimates of falcarinol levels in carrots (40 ppm). Falcarindiol, however, was found in higher concentrations (80 ppm) than reported previously (6 ppm) for whole carrots (Bentley et al., 1969).

In 1967, Crosby and Aharonson (1967) reported that extracts of carrot root were toxic to an indicator organism, *Daphnia magna* Straus. The purified toxicant that they isolated produced "neurotoxic symptoms upon injection into mice"; the LD_{50} was estimated to be 100 mg/kg. In addition to this toxicant (falcarinol; 3-hydroxyheptadeca-1,9-diene-4,6-diyne), other related acetylenes have been reported [falcarindiol, acetylfalcarindiol, and falcarinolone (Bentley et al., 1969)], as well as a hallucinogen, myristicin (Wulf et al., 1978).

Carrot root is believed to contribute 13.9% of our national intake of provitamin A, 0.6% of vitamin B_6, and 0.6% of magnesium (Senti and Rizek, 1974). Thus, it is worthy of close attention to properly assess the impact of deleterious constituents that may attend genetic or environmentally induced changes in the carrot.

If the components to be assayed are volatile, they can be removed from the plant material by steam distillation and purified in the same step by extracting the distillate with an organic solvent (Wulf et al., 1978). Unfortunately, carrot roots have many volatile components that complicate analysis of minor toxicants. The analytical method described herein employs a solvent, dichloromethane, to extract myristicin, falcarinol, and falcarindiol from carrots, along with other plant constituents. The toxicants are then purified by column chromatography and measured by gas–liquid chromatography. These components also can be isolated by scaling up the extraction procedure and isolating the desired compounds by gravity-flow column chromatography.

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EXPERIMENTAL SECTION

Plant Materials. Carrot roots, *Daucus carota*, were bought in commercial markets, grown at NRRC, or grown at various field locations directed by Department of Horticulture, University of Wisconsin, Madison.

Thin-Layer Chromatography of Carrot Components. Commercial precoated, silica gel 60, 0.25-mm plates (E. M. Reagents, MC/B Manufacturing Chemists, Inc., Cincinnati, OH 45212) were developed with (1) ether–hexane (20:80) or with (2) ether–benzene–chloroform (10:30:60). Individual spots were detected by spraying with sulfuric acid and heating at 130 °C for about 3 min. Polyacetylenic compounds such as falcarinol and falcarindiol react immediately after spraying to form chocolate brown spots. Carotenoid pigments also react immediately at room temperature to form a dark spot.

Chromatography Equipment and Conditions. Gravity-flow chromatography was accomplished with 70–325-mesh silica gel (E. M. Reagents) for analytical separations and for initial preparative separations; for final purification steps, 70–230-mesh silica gel 60 (E. M. Reagents) was used. Gas–liquid chromatography was performed with a Bendix 2600 instrument (flame ionization detectors); injector temperature was 240 °C and detector temperature was 260 °C, with helium carrier gas at 10–20 mL/min, air at 500–600 mL/min, and hydrogen at