Smooth and Wrinkled Peas. 2. Distribution of Protein, Lipid, and Fatty Acids in Seed and Milling Fractions

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The yield, protein and lipid distribution, and fatty acid composition of fractions from roller-milling of smooth and wrinkled pea seeds were compared with the composition of hand-dissected germ, whole cotyledon, and hull and pea cotyledon layers removed by abrasion. When peas were abraded to remove up to 65% of the kernel, there was a gradual decrease in protein and lipid; the protein and lipid concentrations in the residual 35% were only about 75% and 50%, respectively, of that in the original seed. Both protein and lipid concentrations decreased from shorts, to reduction flour, to break flour, and to bran. Linoleic acid was the main fatty acid. In smooth peas, oleic acid was higher in germ lipid than in the cotyledon lipid. Hull lipids contained the highest concentrations of total saturated fatty acids, primarily palmitic acid. Decreasing concentrations of stearic acid and oleic acid and increasing concentrations of linoleic acid were recorded in lipids of successive cotyledon layers of abraded smooth peas. There were significant correlations ($p < 0.05$) between total lipid and stearic acid, oleic acid, and linoleic acid of cotyledon layers of smooth seeds.

INTRODUCTION

Peas (*Pisum* spp.) contribute substantially to both human and animal nutrition since they are considered a good source of protein. Pea lipids have received relatively little attention even though their contents in seeds can affect the stability of seeds and pea flour during storage and suitability for processing (Colonna and Mercier, 1983).

Whole pea seeds contain 0.8–6.1% (Savage and Deo, 1989), 1–4% (Colonna and Mercier, 1983; Reichert and MacKenzie, 1982) or 1.1–2.8% (Exler et al., 1977; Welch and Griffiths, 1984) lipids. The lower levels, generally, represent free lipids and the higher concentrations "total lipids" extracted by a variety of solvents or solvent mixtures. The protein concentration in different parts of the pea seed can range from 3.1–3.8% in the hull to 14.5–34.1% in the cotyledon, and the lipid contents can range between 0.4% and 0.6% in the hull and between 1.1% and 3.3% in the cotyledon (Savage andDeo, 1989; Singh et al., 1968). The cotyledon contributes to about 95% of the seed protein and to about 90% of the seed lipid (Adsule et al., 1989). The protein and lipid contents in the germ are much higher than in the cotyledon and in the hull, albeit the germ comprises only about 1% of the kernel weight in peas (Adsule et al., 1989) and can be as high as 2.3% in seeds of other legumes (Singh et al., 1968). The lipid content is independent of the protein content (Reichert and MacKenzie, 1982). Approximately 43–60% of the total lipid content of the pea seed is represented by a neutral lipid fraction (Reichert and MacKenzie, 1982; Hoover et al., 1988). The distribution of lipid classes varies with the cultivar, location, climate, season, and environmental conditions (Patte et al., 1982).

Total lipid content is higher in wrinkled than in smooth peas (Colonna et al., 1980; Coxon and Davies, 1982). The amount of unsaturated fatty acids in lipids of pea seeds is higher (79.2–86.2% of total) than the amount of saturated fatty acids (Exler et al., 1977; Welch and Griffiths, 1984). Linoleic acid is the main fatty acid of pea lipids. Among the fatty acids in pea lipids (C16:0, C18:0, C18:1, C18:2, C18:3), only palmitic acid was significantly correlated with total lipid content (Welch and Griffiths, 1984).

In fractions obtained by pin-milling and air-classifying of pea seed flours the high-protein fraction was also rich in lipids (Vose et al., 1976; Tyler et al., 1981). Studies of Wright et al. (1984) indicated also fractionation of total lipids between coarse and fine pin-milled fractions, most noticeably at high classifying speeds; this was not reflected, however, in differences in either lipid class or fatty acid composition.

In this investigation, we analyzed the distribution of protein, lipid, and fatty acids in different parts of smooth and wrinkled seeds, and different layers of the pea cotyledon were analyzed. Pea parts–fractions were obtained by hand dissection, abrasion, and roller-milling.

MATERIALS AND METHODS

Two smooth pea cultivars, green-seeded cv. SS Alaska and yellow-seeded cv. Latah, and one wrinkled cultivar, cv. Scout, were fractionated by three methods:

(a) For hand dissection seeds were soaked in distilled water for 3 h at room temperature (about 21 °C) and separated into hull, germ, and cotyledon, and the seed parts were freeze-dried.

(b) The seed layers were abraded by application of the Strong-Scott barley pearler (Taylor et al., 1939). Successive layers, 10%, 20%, and 35% (by weight) of cotyledon, were removed by adjusting the time of seed abrasion. The three fractions plus the residual 35% were analyzed. Due to the irregular shape of wrinkled seeds, only smooth cotyledon layers could be abraded in a consistent and reproducible manner.

(c) Milling of whole seeds (1-kg samples) was performed on a Bühler roller mill (Uzwil, Switzerland) to obtain seven fractions: first and second break flours (1B, 2B), first, second, and third reduction flours (1R, 2R, 3R), shorts, and bran. The terms break and reduction flours refer to streams from corrugated and smooth rolls of the experimental mill, respectively.

Chemical Analyses. Protein (N × 6.25) was analyzed as nitrogen on a Leco instrument (Leco Corp., St. Joseph, MI) equipped with a thermoconductivity detector. Free lipids were extracted with petroleum ether, the extracts were evaporated to constant weight under vacuum [AACC, 1983 (Method 30–25)],

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Table 1. Weight,[a] Free Lipid, and Protein Distribution in Hand-Dissected Parts of Pea Seeds

part of seed	% as is								
	cv. SS Alaska (smooth green)			cv. Latah (smooth yellow)			cv. Scout (wrinkled)		
	wt	lipid	protein	wt	lipid	protein	wt	lipid	protein
cotyledon	88.4	1.09	22.4	88.9	0.51	28.5	85.0	1.83	25.7
germ	0.9	6.17	42.5	0.7	4.41	46.8	1.0	7.45	44.2
hull	10.8	0.20	3.6	10.4	0.26	4.4	14.0	0.25	3.5

[a] Corrected for recovery.

Table 2. Free Lipid and Protein Distribution[a] of Pea Cotyledon Fractions from Abrasion Studies

part of cotyledon[b] (% by wt)	% as is			
	cv. SS Alaska (smooth green)		cv. Latah (smooth yellow)	
	lipid	protein	lipid	protein
10	1.80 a	26.4 a	1.00 a	33.2 a
20	1.32 b	23.3 b	0.70 b	31.1 b
35	1.22 c	20.9 c	0.61 c	28.1 c
residual 35	1.00 d	18.7 d	0.46 d	25.5 d

[a] Results followed by the same letter within a column are not significantly different ($p < 0.05$). [b] Layers of cotyledon starting from outside to inside of seed.

and moisture was determined by oven-drying for 1 h at 130 °C [AACC, 1983 (Method 44-15A)].

Gas Chromatography. Lipid extract (approximately 100 mg) was butylated by gentle boiling in a 0.5 N butanolic potassium hydroxide solution for 2 min. The mixture was cooled to 25 °C, 3 mL of 12.5% boron trifluoride–butanol reagent (Supelco, Inc., Bellefonte, PA) was added, and the mixture was gently boiled for 3 min. After the mixture cooled to 25 °C, fatty acid butyl esters (FABEs) were extracted from the reaction mixture with hexane (Iverson and Sheppard, 1977).

The FABEs were analyzed with a Varian 2700 GC chromatograph with a stainless steel column (1.8 m × 2 mm i.d.) containing 10% SP2330 impregnated on 100–120-mesh Chromosorb W-AW and a flame ionization detector. Nitrogen carrier gas flow rate was 20 mL/min. The temperature of the injector and the detector was 250 °C. The column temperature was 150 °C for 1 min, increased to 200 °C at the rate of 20 °C/min, and was maintained at 200 °C for 20 min to complete elution of FABEs. Individual fatty acid esters were identified on the basis of standard retention times (Sigma Chemical Co., St. Louis, MO), and quantity was estimated on the basis of peak areas of known concentrations of the standards (AOCS, 1989).

All analyses were done at least in duplicate; the results were computed and evaluated by the SAS Institute (1985) system.

RESULTS AND DISCUSSION

Lipid and Protein Distribution in the Seed. Distribution of free lipids (petroleum ether-extractable) and protein in hand-dissected seed parts, cotyledon, germ, and hull of green-seeded smooth pea cv. SS Alaska, yellow-seeded smooth cv. Latah, and wrinkled cv. Scout, is summarized in Table 1. Both lipids and proteins of the three cultivars were highest in the germ. The cotyledon of smooth seeds contained 6–8 times less lipid than the germ. The lowest concentration of lipids and proteins was in the hull. A similar lipid distribution in seed parts was reported by Welch and Griffiths (1984). Differences in protein contents between cotyledon and germ were not as high as differences in lipid content. Lipid and protein contents in cotyledon layers from abraded peas showed significant differences in concentration of both components between outer and inner cotyledon layers (Table 2). The outer 10% cotyledon layer contained almost twice the amounts of lipid than the inner 35% layer. Such a distribution of lipids and proteins in the cotyledon makes it possible to obtain abrasion fractions with different concentrations of lipids and proteins. The lipid contents of seven fractions from roller-milling of smooth and wrinkled seeds confirmed this assumption (Table 3). Significant differences in lipid concentration among shorts, reduction flours, and break flours were found. A similar trend in protein distribution in milling fractions was observed (Table 4); it was most conspicuous and significant for the cv. SS Alaska. The shorts were the high-lipid and high-protein fractions, and the break flours were the low-lipid and low-protein fractions. The lipid content of the major milling fraction (1R) for the three cultivars was about the same as the lipid content of the whole seed (Tables 3 and 4).

Table 3. Free Lipid Contents[a] of Roller Milling Fractions of Peas[b]

flour fraction	free lipid (%)		
	cv. SS Alaska (smooth green)	cv. Latah (smooth yellow)	cv. Scout (wrinkled)
whole peas	1.10 d	0.53 e	1.72 d
flour fractions			
1B[c]	0.94 g	0.57 cd	1.72 d
2B[c]	0.97 f	0.55 de	1.59 e
1R[d]	1.04 e	0.52 e	1.75 d
2R[d]	1.20 c	0.59 c	1.81 c
3R[d]	1.25 b	0.64 b	1.93 b
shorts	1.86 a	0.99 a	2.39 a
bran	0.41 h	0.24 f	0.47 f

[a] As is basis, %. [b] Results followed by the same letter within a column are not significantly different ($p < 0.05$). [c] Break flours. [d] Reduction flours.

Table 4. Yield and Protein Content of Milling Fractions

fraction	yield (%)			protein (%)[d]		
	cv. SS Alaska[a]	cv. Latah[b]	cv. Scout[c]	cv. SS Alaska[a]	cv. Latah[b]	cv. Scout[c]
whole seed	100.0	100.0	100.0	19.1	30.5	27.0
break 1	4.0	4.1	6.4	20.5	32.5	25.3
break 2	6.3	7.1	6.9	22.5	33.1	25.7
reduction 1	39.0	37.9	36.8	24.4	33.9	27.9
reduction 2	8.0	11.2	10.5	25.8	35.1	28.4
reduction 3	7.7	11.1	12.8	25.6	35.5	28.4
shorts	10.4	5.9	10.4	29.7	36.8	29.1
bran	11.4	12.6	12.3	9.7	11.2	10.3

[a] Smooth green. [b] Smooth yellow. [c] Wrinkled. [d] Dry matter basis.

The concentrations of lipids and proteins in the bran (Tables 3 and 4) were higher than in the hull (Table 1); some germ tissue apparently was present in the milled bran fraction. Yields of specific milling fractions were similar in the smooth and wrinkled seeds. On the basis of the data in Tables 2 and 3, one can conclude that most of the high-lipid and -protein shorts is derived from the outer layer, break flours from the inner layer, and reduction flours from the intermediate part of the cotyledon. Since the wrinkled cv. Scout and the smooth cv. SS Alaska had similar patterns of lipid and protein distribution in milling fractions, it is postulated that lipid and protein distribution in the cotyledon layers of wrinkled seeds (not analyzed by abrasion due to the irregular shape of seeds) is similar to that in smooth seeds.

Fatty Acid Composition. Linoleic acid (C18:2) was the main fatty acid of all seed parts, cotyledon layers, and milling fractions (Tables 5–7). The cotyledon fatty acids were on the average about 57% linoleic acid. Lower concentrations of C18:2 were in the germ and in the hull. Both linoleic acid and linolenic acid (C18:3) were lowest in lipids of the hull of the three cultivars SS Alaska, Latah, and Scout. The hull lipids, on the other hand, were highest in oleic acid (C18:1). Due to the significantly high

Table 5. Fatty Acid Composition of Lipids in Hand-Dissected Pea Tissues

tissue	g/100 g of total fatty acids						
	C16:0	C18:0	TSAT[a]	C18:1	C18:2	C18:3	TUNS[b]
Cv. SS Alaska (Smooth, Green)							
cotyledon	9.6	3.5	13.1	15.5	58.9	13.0	87.4
germ	11.7	3.3	15.0	18.3	53.8	13.1	85.2
hull	23.5	9.7	33.2	23.2	37.6	6.0	66.8
Cv. Latah (Smooth, Yellow)							
cotyledon	9.5	3.4	12.9	14.4	57.2	14.8	86.4
germ	10.3	3.6	13.9	19.0	53.6	13.8	86.4
hull	16.6	3.5[c]	20.1	23.1	52.4	3.1	78.6
Cv. Scout (Wrinkled)							
cotyledon	10.0	3.5	13.5	22.4	54.9	8.3	85.6
germ	9.5	3.3	12.8	23.6	49.8	13.8	87.2
hull	26.4	9.5	35.9	25.2	34.0	6.5	65.7
LSD ($p < 0.05$)	2.18	2.11		2.02	4.57	2.08	

[a] TSAT, total saturated fatty acids. [b] TUNS, total unsaturated fatty acids. [c] Incompletely resolved peak.

Table 6. Fatty Acid Composition in Lipids of Pea Layers Separated by Abrasion

part of cotyledon (% by wt)	g/100 g of total						
	C16:0	C18:0	TSAT[a]	C18:1	C18:2	C18:3	TUNS[b]
Cv. SS Alaska (Smooth, Green)							
10	11.7	4.4	16.1	19.3	52.6	12.0	83.9
20	11.5	4.1	15.6	17.7	57.1	10.3	85.1
35	11.5	3.9	15.4	15.5	59.4	9.1	84.0
residual 35	9.8	3.7	13.5	13.8	62.9	9.0	85.7
LSD (0.05)	2.19	0.62		0.69	3.79	3.92	
Cv. Latah (Smooth, Yellow)							
10	10.1	3.3	13.4	17.9	52.9	16.0	86.8
20	10.1	2.8	12.9	14.9	56.2	15.1	86.2
35	9.9	2.4	12.3	13.4	58.8	14.4	86.6
residual 35	10.1	2.0	12.1	10.4	62.6	14.7	87.7
LSD (0.05)	3.18	0.59		0.54	2.98	2.64	

[a] TSAT, total saturated fatty acids. [b] TUNS, total unsaturated fatty acids.

Table 7. Fatty Acid Composition of Milling Fractions of Pea Seeds

fraction	g/100 g of total						
	C16:0	C18:0	TSAT[a]	C18:1	C18:2	C18:3	TUNS[b]
Cv. SS Alaska (Smooth, Green)							
whole seed	10.2	4.1	14.3	17.5	58.8	10.6	86.9
break flours	9.0	4.0	13.0	15.8	59.5	11.3	86.6
reduction flours	10.9	4.3	15.2	16.6	58.9	10.3	85.8
shorts	10.7	4.2	14.9	18.9	56.3	9.3	84.5
bran	12.3	4.5	16.8	17.4	56.2	9.7	83.3
LSD ($p < 0.05$)	1.60	0.64		0.73	1.69	2.34	
Cv. Latah (Smooth, Yellow)							
whole seed	9.3	4.2	13.5	15.2	57.1	14.3	86.6
break flours	7.1	4.8	11.9	14.9	58.8	14.2	87.9
reduction flours	8.3	4.5	12.8	14.5	57.8	14.9	87.2
shorts	8.9	3.6	12.5	17.2	56.6	14.1	87.9
bran	13.3	4.4	17.7	15.8	52.7	12.2	80.7
LSD ($p < 0.05$)	1.28	1.89		1.82	5.75	1.63	
Cv. Scout (Wrinkled)							
whole seed	11.6	5.3	16.9	23.1	51.7	7.9	82.7
break flours	10.3	3.6	13.9	21.9	56.8	7.6	86.3
reduction flours	11.2	3.4	14.7	22.8	54.8	8.3	85.8
shorts	10.8	8.8	19.6	23.5	47.4	9.8	80.7
bran	11.3	4.8	16.1	23.1	51.2	10.3	84.6
LSD ($p < 0.05$)	1.51	2.81		1.59	2.57	2.05	

[a] TSAT, total saturated fatty acids. [b] TUNS, total unsaturated fatty acids.

concentration of palmitic (C16:0) in the hull of the three cultivars, total saturated fatty acids were considerably higher in the hull than in the cotyledon and the germ. Welch and Griffiths (1984) reported that total saturated fatty acids represented about 50–60% of the hull total fatty acids. The fatty acid compositions of lipids in the cotyledon and germ of the three analyzed cultivars were similar; fatty acids in hull lipids differed considerably among tissues and cultivars. Data in Table 5 show that cotyledon layers differ not only in protein and lipid contents but also in fatty acid composition of lipids. The patterns of fatty acids in lipids of cv. SS Alaska and cv. Latah were similar: (a) a decrease in concentrations of C18:0, C18:1, and C18:3 and an increase in C18:2 in the cotyledon from the outer to the inner layers; (b) concentrations of C16:0 changed little and inconsistently; and (c) concentrations of C18:0 and C18:1 were positively correlated and of C18:2 negatively correlated with total lipid contents ($p < 0.05$).

Fatty acid composition of lipids in milling fractions of smooth and wrinkled seeds is illustrated in Table 7. The fatty acid analysis was performed on combined reduction flours (1R + 2R + 3R) and combined break flours (1B + 2B). Significant differences (or trends) in fatty acid composition of milling fractions were found. It seems that decreasing concentration of linoleic and linolenic acids in break flours, reduction flours, and shorts as well as increasing contents of oleic acid is a reflection of differences in contents of fatty acids in various cotyledon layers. Similar differences in cv. Latah were found for oleic acid and linoleic acid of reduction flours and shorts. Data on fatty acid composition in Table 7 confirm the earlier conclusion that the milling fractions shorts, reduction flours, and break flours originate, respectively, with the outer, intermediate, and inner layers of the cotyledon. Thus, it is possible to accomplish a shift in protein and lipid contents and composition by abrasion or milling on a roller mill. The shift can be confirmed and explained by comparison with results obtained on analyzing hand-dissected pea tissues.

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