Changes in Sterols and Fatty Acids of Buckwheat Endosperm and Embryo during Seed Development

Marcin Horbowicz† and Ralph L. Obendorf*

Seed Biology, Department of Soil, Crop and Atmospheric Sciences, Cornell University Agricultural Experiment Station, 619 Bradfield Hall, Cornell University, Ithaca, New York 14853-1901

Buckwheat (*Fagopyrum esculentum* Moench) plants produce a low grain yield because of a low incidence of seed set. As part of a study of seed set and abortion, sterols and fatty acids were extracted from developing embryo and endosperm tissues 6–20 days after pollination (DAP) and analyzed by gas chromatography. The most abundant sterol was β-sitosterol, which made up 70% of the total sterols. Other sterols were campesterol, an unknown, and traces of stigmasterol. Total sterols in embryos were 2.1 ± 0.05 g/kg of dry weight and in endosperm tissues were 0.55 ± 0.02 g/kg at 20 DAP; total fatty acids in embryo and endosperm lipids were 123 ± 8 and 22 ± 3 g/kg, respectively. Linoleic, oleic, and palmitic accounted for 88% of the total fatty acids at 20 DAP. At 6–10 DAP, 60–80% of the total fatty acids were saturated, mostly palmitic acid, but at 12–20 DAP, 65–80% were unsaturated, mostly linoleic and oleic. The rapid transition at 10–12 DAP was associated with rapid embryo growth and a 10-fold increase in storage lipids. Palmitic was 3–5 times more abundant than any other fatty acid at 6 DAP when myristic acid concentration was highest. Long-chain fatty acids ($C_{20:0}$, $C_{22:0}$, and $C_{20:1}$) accumulated late in development to less than 7% of the total fatty acids.

INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Moench) is cultivated in nearly all countries with a temperate climate. In eastern Europe, buckwheat grain is a basic food item in porridges and soups. In Japan, buckwheat is used mostly for manufacturing a noodle, soba, which is prepared from a mixture of buckwheat and wheat flours (Marshall and Pomeranz, 1982).

Buckwheat plants produce a low yield of grain because of a low incidence of seed set (Pomeranz, 1983). The buckwheat seed is actually a fruit, an achene; the dehulled fruit (pericarp removed) is a groat. Only 10–20% of the flowers develop into mature achenes (Ruszkowski, 1986). The causes of low seed set are unknown but have been attributed to high temperature, plant water stress, incompatibility caused by heterostylism, defective reproductive organs, failure of fertilization, and embryo and/or endosperm abortion (Adachi, 1990; Pomeranz, 1983). As part of a study of the changes in composition of developing buckwheat achenes in relation to seed set and abortion, an investigation was undertaken to determine the sterols and fatty acids accumulating during development of the endosperm and embryo, the most important parts of the buckwheat groat.

Although lipids have been analyzed in mature grain (Oka et al., 1972; Dorrell, 1971; Taira et al., 1986; Mazza, 1988; Tauzuki et al., 1991), no information has been published on lipid content and composition during development of buckwheat achenes. Linoleic, oleic, and palmitic are the predominant fatty acids in lipids from mature achenes (Mazza, 1988; Tauzuki et al., 1991). The concentration of total sterols in oil from mature buckwheat is twice that in oil from maize, sorghum, or wheat and 10 times the sterol concentration in soybean oil (Oka et al., 1972). Some recent interests in plant sterols relate to their potential as antivirus agents (Abid Ali Khan et al., 1991), antitumor agents (Yasukawa et al., 1991), and inhibitors of cholesterol uptake (Heinemann et al., 1991). The objectives of the present study were to identify and quantify the sterols and fatty acids during endosperm and embryo development in buckwheat achenes.

* Author to whom correspondence should be addressed.
† Permanent address: Research Institute of Vegetable Crops, 96-100 Skierniewice, Poland.

MATERIALS AND METHODS

Growth of Plants. Mancan buckwheat (*Fagopyrum esculentum* Moench, Minnesota certified seed) plants were grown in greenhouse potting soil mix composed of equal volumes of silty loam soil (pH 7.0) and artificial mix (0.1 m^3 of sphagnum moss, 0.1 m^3 of vermiculite, 0.5 kg of ferrous sulfate, and 1 kg of commercially blended fertilizer, 13-13-13). Three seeds were seeded in 4-L plastic pots and thinned to one plant per pot at first flower. Temperature was controlled at 24 °C day (14 h) and 18 °C night (10 h). Temperature deviations from the set point were less than 5 °C. Natural sunlight was supplemented with 14 h of incandescent light from 1000-W metal halide lamps (Sylvania Metalarc M1000U) at approximately 740 μmol m^{-2} s^{-1}. Plants were thoroughly watered as needed and supplemented with complete fertilizer at weekly intervals.

Pots containing a plant with the pin flower type (short filaments and long styles) were arranged alternately with pots containing a plant with the thrum flower type (long filaments and short styles) to facilitate hand pollination. Tagged flowers were hand pollinated by legitimate cross-pollination, pin by thrum and thrum by pin. Achenes were harvested for chemical analysis at 2- or 4-day intervals from 6 to 20 days after pollination (DAP). Immediately after each harvest, embryo and endosperm tissues were removed from the achenes, weighed, and analyzed separately for sterols and total fatty acids. Endosperm and embryo fractions were extracted immediately upon excision. At later stages of development, a few samples were stored at −85 °C for less than 1 week. Ovule (seed coat) and ovary (pericarp or hull) tissues were discarded. Each replicate sample contained 8–10 achenes, one achene from each of 8–10 plants; three replicate experiments were pooled. Fresh and dry weights were determined on separate samples for the calculation of water concentration and dry matter fraction. Dry matter fraction was used to calculate the dry weight from the fresh weight of samples used for lipid analysis. Because there were no significant replication by treatment differences, the mean ± SEM was calculated for three replicate samples.

Reagents. High-purity solvents were used in all analyses. Methanol, chloroform, and hydrochloric acid were obtained from

0021-8561/92/1440-0745$03.00/0

Mallinckrodt. n-Hexane was obtained from Baker and isooctane from Aldrich. Plant sterol standards were obtained from Matreya Inc. Other reagents including dihydrocholesterol, fatty acid methyl esters, 14% boron trifluoride–methanol reagent, trimethylsilylimidazole, potassium hydroxide, sodium chloride, and anhydrous sodium sulfate were purchased from Sigma.

Extraction of Sterols and Fatty Acids. Sterols and fatty acids were extracted with potassium hydroxide–methanol solution which simultaneously combined extraction and saponification steps (Horbowicz and Kosson, 1983). Tissues were homogenized in 3 mL of methanol containing 20 μg of dihydrocholesterol and 50 μg of methyl heptadecanoate ($C_{17:0}$) as internal standards. After grinding, 1 mL of 5% (w/v) potassium hydroxide in methanol was added, and the mixture (4 mL) was heated at 80 °C (water bath) for 1 h to facilitate extraction and saponification. After the mixture cooled to 22 °C, 4 mL of a saturated solution of sodium chloride were added, and the mixture was extracted two times, each with 2 mL of hexane. The hexane and methanol–water layers were separated by centrifugation at 1000*g* for 5 min, and the hexane fractions were pooled. Hexane was evaporated under a stream of nitrogen, and the residue from the nonsaponified fraction was analyzed for sterols.

The methanol–water layer (containing the saponified materials) was acidified with 1 mL of concentrated hydrochloric acid and extracted two times, each with 2 mL of hexane. Hexane and water layers were separated by centrifugation. Hexane fractions were pooled, and hexane was evaporated in a stream of nitrogen. The residue (saponified fraction) was analyzed for total fatty acids.

Analysis of Sterols. Extracted sterols were silylated at 70 °C (aluminum heating block) for 0.5 h in 200 μL of silylation mixture (trimethylsilylimidazole/chloroform, 1:1 v/v) in tightly capped silylation vials (0.3-mL volume, Supelco). Instead of pyridine, chloroform was used to increase the solubility of sterols. After the mixture cooled to 22 °C, 2 μL of each sample was injected (split mode) into a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionization detector and a Hewlett-Packard 3396A integrator. Sterols were analyzed on a DB-1701 capillary column (30-m length, 0.25-mm i.d., and 0.25-μm film thickness; J&W Scientific) operated isothermally at 275 °C. Temperatures of the injector port and detector were 275 and 325 °C, respectively. The carrier gas was helium at 1.0 mL/min, split 1:100. The detector gases were hydrogen at 30 mL/min, air at 300 mL/min, and nitrogen (auxiliary gas) at 30 mL/min.

Analysis of Fatty Acids. Total extracted fatty acids were converted to methyl esters (Metcalfe et al., 1966) in 0.5 mL of 14% boron trifluoride–methanol reagent in tightly capped vials (1-mL volume) at 70 °C for 0.5 h. After the mixture cooled to 22 °C, 0.5 mL of saturated sodium chloride solution was added, and the fatty acid methyl esters were extracted in 0.4 mL of isooctane. The isooctane fraction was transferred to a clean, tightly capped vial. Anhydrous sodium sulfate (100–200 mg) was added to remove traces of water and to protect the methyl esters from hydrolysis.

Fatty acids were analyzed on a DB-1701 capillary column (described above) operated at a programmed oven temperature of 200 °C for 20 min, adjusted to 225 °C at 10 °C/min, and then held at 225 °C for 20 min. Temperature of the injector (split mode) was 220 °C, and the flame ionization detector was operated at 250 °C. The carrier gas was helium at 0.6 mL/min, split 1:90. The detector gases were hydrogen at 30 mL/min, air at 300 mL/min, and nitrogen (auxiliary gas) at 30 mL/min.

Calculations. Quantities of sterols and fatty acids were determined by extrapolation from standard curves; the ratios of the area of peaks for known sterols or fatty acids to the area of the peak for the internal standard (dihydrocholesterol for sterols or heptadecanoic acid methyl ester for fatty acids) were plotted against known quantities of each sterol (2–25 μg of campesterol and 5–50 μg of β-sitosterol) or fatty acid [5–100 μg each of palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), and behenic ($C_{22:0}$) acids and 5–20 μg of myristic ($C_{14:0}$), palmitoleic ($C_{16:1}$), arachidic ($C_{20:0}$), and eicosenoic ($C_{20:1}$) acids] to establish the standard curves. Total lipids were calculated by summation of total fatty acid and sterol contents without correction for the glycerol component of the acylglycerols.

Recovery of Lipids. After removal of the pericarp from dry mature achenes, the dehulled buckwheat groats were ground in a Waring blender to pass a 0.2-mm sieve. Three samples of buckwheat meal, each 200 mg, were fortified with 200 μg of β-sitosterol, 200 μg of stigmasterol, 200 μg of campesterol, 1 mg of tripalmitin, 1 mg of triolein, and 1 mg of trilinolenin. Total lipids in these three samples, and also in a duplicate set of three 200-mg samples without added lipids, were extracted according to the method of Horbowicz and Kosson (1983) as described above. The percent recoveries of the added sterols and fatty acids as methyl esters were determined. In addition, total lipids were extracted from three 1-g samples of buckwheat meal, without added lipids, according to the method of Folch et al. (1957). Results of the two methods were compared statistically by Student's *t*-test, $\alpha = 0.05$ and 0.01, for levels of sterols and fatty acid methyl esters.

RESULTS

Extraction of sterols and fatty acids using the simultaneously combined extraction and saponification steps of Horbowicz and Kosson (1983) provided results similar to those for total lipid extraction according to the method of Folch et al. (1957) (Table I). No significant difference was observed in the level of extracted sterols between the methods. Only traces of stigmasterol were detected by both methods. Likewise, fatty acid methyl esters were not different, or slightly higher, for $C_{16:0}$, $C_{18:1}$, and $C_{18:3}$ when extracted according to the Horbowicz and Kosson (1983) method (Table I). Eighty-two to 86% of the added sterols and 81–84% of the fatty acids from added tripalmitin, triolein, and trilinolenin were recovered with the Horbowicz and Kosson (1983) method (Table I). The uniform recovery of all lipids indicates that the lipid extraction and saponification procedures used in the present study did not discriminate against individual sterols or fatty acids by differential extraction or degradation. The simplicity, ease of use, and repeatability make the Horbowicz and Kosson (1983) method suitable for analysis of total lipids in developing buckwheat endosperm and embryo.

Endosperm and Embryo Growth. Pericarp (hull) tissues increased rapidly in weight, length, and width between 2 and 6 DAP (data not shown). Fresh and dry weights of endosperm tissues increased from 6 until 16 DAP (Figure 1). Fresh weight of embryo tissues was maximum at 12 DAP, and dry weight of the embryo was maximum at 16 DAP. Water concentration in the endosperm was 850 g/kg of fresh weight at 6 DAP, 752 g/kg at 8 DAP, 561 g/kg at 10 DAP, 466 g/kg at 12 DAP, 220 g/kg at 16 DAP, and 192 g/kg at 20 DAP; corresponding values for the embryo were 750, 697, 590, 548, 416, and 203 g/kg of fresh weight. The decline in water concentration reflected the accumulation of dry matter in endosperm and embryo tissues. Upon cessation of dry matter accumulation, transport to the embryo and endosperm had ceased, and the tissues desiccated rapidly. At 16 DAP, 80% of the dry matter was in the endosperm and 20% was in the embryo.

Sterols. β-Sitosterol, campesterol, and an unknown (presumed to be a sterol) were tentatively identified in developing buckwheat endosperm and embryo tissues. Retention time for the unknown sterol was longer than that for β-sitosterol. Stigmasterol was present in trace amounts as noted by a small peak between campesterol and β-sitosterol. β-Sitosterol, which was the most abundant sterol in developing endosperm and embryo tissues, represented about 70% of all sterols (Figure 2). The amount of each sterol increased continuously during endosperm development (Figure 2A,C). In embryo tissues, however, the amount and concentration of sterols reached

Table I. Comparison of Sterols and Fatty Acid Methyl Esters after Lipid Extraction of Mature, Dehulled Groats According to the Method of Folch et al. (1957) with Those Obtained According to the Method of Horbowicz and Kosson (1983) and Recovery of Added Sterols and Fatty Acids According to the Horbowicz and Kosson (1983) Method

	Folch et al.[a] [mg/kg ± SD (CV)]	Horbowicz and Kosson[b] [mg/kg ± SD (CV)]	LSD 0.05	LSD 0.01	recovery,[c] % ± SD (CV)
sterols					
β-sitosterol	667 ± 95.8 (14.4)	753 ± 47.7 (6.3)	NS	NS	83.6 ± 3.3 (3.9)
campesterol	89 ± 10.1 (11.3)	97 ± 12.8 (13.2)	NS	NS	82.2 ± 2.5 (3.1)
stigmasterol					86.1 ± 5.4 (6.3)
fatty acids					
$C_{16:0}$	3.7 ± 0.17 (4.7)	4.8 ± 0.36 (7.5)	0.6	NS	84.0 ± 4.1 (4.9)
$C_{16:1}$	0.8 ± 0.17 (21.6)	0.8 ± 0.15 (19.1)	NS	NS	
$C_{18:0}$	0.4 ± 0.07 (17.5)	0.4 ± 0.10 (25.0)	NS	NS	
$C_{18:1}$	12.8 ± 1.06 (8.3)	17.0 ± 0.50 (2.9)	1.9	3.1	81.3 ± 5.8 (7.1)
$C_{18:2}$	14.0 ± 0.60 (4.3)	17.9 ± 0.75 (4.2)	1.5	2.6	
$C_{18:3}$	1.4 ± 0.20 (14.3)	1.7 ± 0.12 (6.8)	NS	NS	80.9 ± 5.2 (6.4)

[a] After the pericarp was removed, mature dehulled groats were ground to pass a 0.2-mm sieve. Total lipids were extracted from three replicate 1-g samples of buckwheat meal using the method of Folch et al. (1957). Data are expressed as mean ± SD for the three independent extractions. The coefficient of variability (CV) is expressed as a percent of the mean. [b] Total lipids were extracted from three replicate 200-mg samples of buckwheat meal according to the method of Horbowicz and Kosson (1983). The least significant difference (LSD by Student's *t*-test) for comparing the methods is given for $\alpha = 0.05$ and 0.01. [c] Another three 200-mg samples of buckwheat meal were fortified with 200 μg of β-sitosterol, 200 μg of stigmasterol, 200 μg of campesterol, 1 mg of tripalmitin, 1 mg of triolein, and 1 mg of trilinolenin. Total lipids in the three samples were extracted according to the method of Horbowicz and Kosson (1983), and the percent recoveries of added sterols and fatty acids are expressed as mean ± SD. CV is expressed as a percent of the mean.

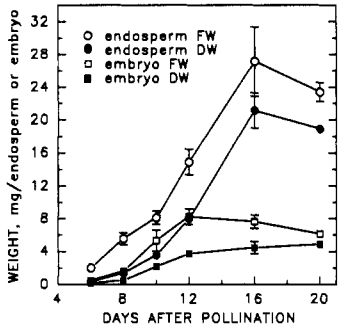

Figure 1. Fresh weight (FW) and dry weight (DW) of endosperm and embryo tissues in relation to days after pollination during buckwheat achene development. Symbols represent the mean ± SEM, if error bars exceed the size of the symbol, for three replications of each treatment.

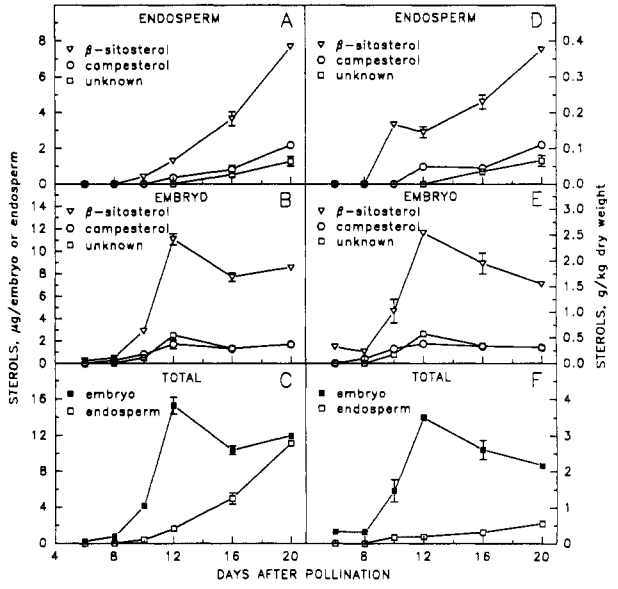

Figure 2. β-Sitosterol, campesterol, an unknown, and total sterols in developing endosperm and embryo tissues expressed as amount (A–C) or concentration (D–F) of each sterol in endosperm (A, D) or embryo (B, E) tissues or as total sterols (C, F) in relation to days after pollination. The amount of the unknown was calculated using the standard curve for β-sitosterol. Symbols represent the mean ± SEM, if error bars exceed the size of the symbol.

a maximum at 12 DAP (Figure 2B,C). The endosperm contained 11 μg and the embryo contained 12 μg of total sterols at 20 DAP. The concentration of sterols in endosperm tissues increased from 0.1 to 0.5 g/kg of dry weight between 10 and 20 DAP (Figure 2D,F). In contrast, the concentration of β-sitosterol and total sterols in embryo tissues increased rapidly between 8 and 12 DAP (Figure 2E,F), corresponding to the period of rapid increase in fresh and dry weights (Figure 1). The concentration of total sterols in embryo tissues, which exceeded the concentration in endosperm by 4 times, attained 2.1 g/kg of dry weight at 20 DAP compared to 0.5 g/kg in the endosperm (Figure 2F).

Fatty Acids. Ten fatty acids were tentatively identified with good resolution and sensitivity in lipids extracted from embryos at 20 DAP. Linoleic ($C_{18:2}$), oleic ($C_{18:1}$), and palmitic ($C_{16:0}$) were the most abundant fatty acids at 20 DAP in both the embryo (Figure 3A,D) and the endosperm (Figure 4A,D). Other identified unsaturated fatty acids were eicosenoic ($C_{20:1}$), linolenic ($C_{18:3}$), and palmitoleic ($C_{16:1}$) in embryo (Figure 3B,E) and endosperm (Figure 4B,E) tissues; other saturated fatty acids were stearic ($C_{18:0}$), behenic ($C_{22:0}$), arachidic ($C_{20:0}$), and myristic ($C_{14:0}$) for both embryo (Figure 3C,F) and endosperm (Figure 4C,F) tissues. Palmitic acid was the predominant fatty acid at 6–10 DAP in both embryo and endosperm tissues (Figures 3A and 4A). Of the other fatty acids in buckwheat achenes, linolenic and myristic were at highest concentrations at 6 DAP in embryo tissues (Figure 3E,F) and palmitoleic, linolenic, and myristic were at highest concentrations at 6 DAP in the endosperm (Figure 4E,F).

Eighty percent of the total fatty acids in embryo tissues accumulated between 10 and 12 DAP, the majority being unsaturated fatty acids (Figure 5). The embryo had a 2–3 times higher concentration of total fatty acids than the endosperm at 8–10 DAP and a 4–10 times higher concentration at 12–20 DAP (Figure 5E). At 6–10 DAP, 60–80% of the total fatty acids were saturated. At 12–20 DAP, however, 65–80% were unsaturated in both the embryo and the endosperm (Table II). The transition from predominantly saturated fatty acids to predominantly unsaturated fatty acids between 10 and 12 DAP (Table II) corresponded to the period of most rapid accumulation of stored lipids, particularly in the embryo, which had an increase in the concentration of total fatty acids from 15 ± 3 to 123 ± 8 g/kg of dry weight (Figure

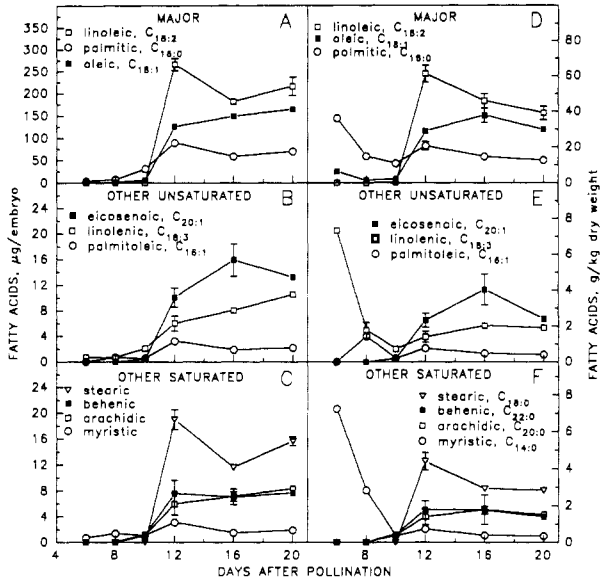

Figure 3. Relationship of content (A–C) and concentration (D–F) of major (A, D), other unsaturated (B, E), and other saturated (C, F) fatty acids to days after pollination during embryo development. Symbols represent the mean ± SEM, if error bars exceed the size of the symbol.

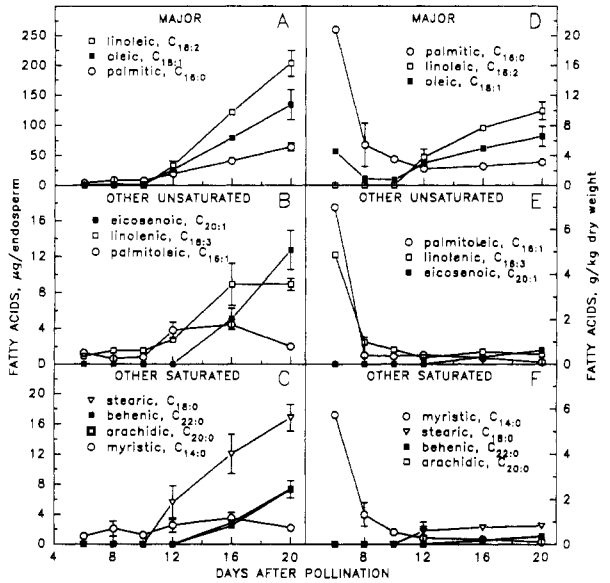

Figure 4. Relationship of content (A–C) and concentration (D–F) of major (A, D), other unsaturated (B, E), and other saturated (C, F) fatty acids to days after pollination during endosperm development. Symbols represent the mean ± SEM, if error bars exceed the size of the symbol.

5). Total lipids increased 10-fold between 10 and 12 DAP (Table III); at 12 DAP more than 80% of the total lipids were in the embryo (Figures 2C and 5C). Total lipids increased to 20 DAP, but the concentration of total lipids in buckwheat groats (endosperm plus embryo) decreased after 12 DAP when expressed on a dry weight basis (Table III).

DISCUSSION

The present work is the first documentation of the dynamic changes in composition of fatty acids and sterols during endosperm and embryo development in buckwheat achenes. Our results are consistent with the lipid analyses reported for mature buckwheat grain (Oka et al., 1972; Dorrell, 1971; Taira et al., 1986; Mazza, 1988; Tauzuki et al., 1991). Of the total sterols in mature buckwheat grain,

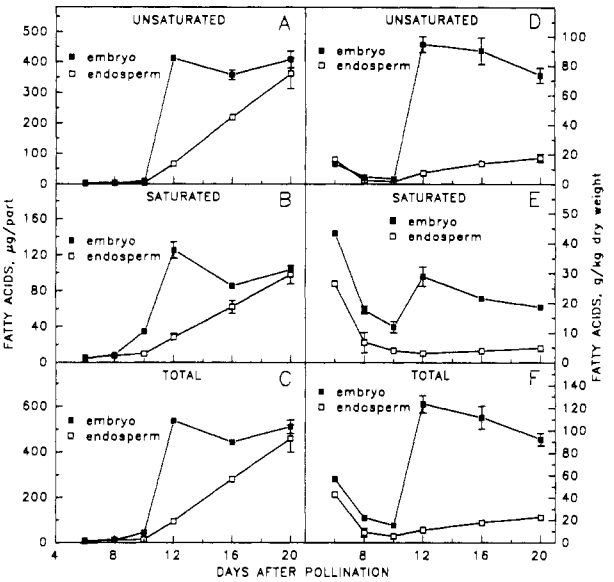

Figure 5. Relationship of content (A–C) and concentration (D–F) of unsaturated (A, D), saturated (B, E), and total (C, F) fatty acids to days after pollination during embryo and endosperm development. Symbols represent the mean ± SEM, if error bars exceed the size of the symbol.

Oka et al. (1972) found 85.5% to be β-sitosterol, 10.5% campesterol, and 4% stigmasterol. In contrast, our results show only traces of stigmasterol and a significant accumulation of an unknown during deposition of reserves in addition to β-sitosterol and campesterol. β-Sitosterol may have important roles in plant disease resistance (Abid Ali Khan et al., 1991) and human health (Yasukawa et al., 1991; Heinemann et al., 1991). Total lipids in developing achenes make up 1–2% of endosperm dry weight but 9–12% of embryo dry weight. Pericarp (hull) tissues increase rapidly in weight, length, and width between 2 and 6 DAP. Ovary tissues (including the endosperm and embryo) increase rapidly in length and width between 6 and 10 DAP with a rapid increase in fresh weight after 6 DAP and a rapid increase in dry weight after 8 DAP (unpublished data). The embryo attains maximum fresh weight at 12 DAP, but the endosperm continues to increase in fresh and dry weights until 16 DAP. The embryo rapidly increases in dry weight between 10 and 12 DAP, when more than half of the total lipids in mature achenes are accumulated.

Other species also show a similar rapid increase in lipid biosynthesis. Seeds of *Crambe abyssinica* do not accumulate significant oil until 10–12 DAP, at which time the oil content increases rapidly (Gurr et al., 1972). Rapeseed (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) begin rapid biosynthesis of triacylglycerols at 14 DAP (Murphy and Cummins, 1989; Stokes et al., 1986). In developing soybean (*Glycine max* [L.] Merrill) seeds, the rapid increase in fatty acid biosynthesis and accumulation of triacylglycerol correlates with the amount and activity of acyl carrier protein (Ohlrogge and Kuo, 1984) and the increase in number of lipid bodies (Adams et al., 1983).

Biosynthesis and accumulation of specific fatty acids change during development of buckwheat achenes. In the present study, palmitic is the predominant fatty acid early in endosperm and embryo development. As the storage lipids begin to accumulate in the achene, sterols and palmitic acid accumulate initially followed by stearic, oleic, linoleic, and linolenic acids. The long-chain fatty acids, eicosenoic, arachidic, and behenic, are synthesized last. These long-chain fatty acids are probably incorporated into triacylglycerols with highest concentrations in the

Table II. Fatty Acid Methyl Ester Composition of Buckwheat Embryo and Endosperm Fractions as a Function of Days after Pollination

FAME	% of total fatty acids N days after pollination (DAP)					
	6 DAP	8 DAP	10 DAP	12 DAP	16 DAP	20 DAP
embryo						
$C_{14:0}$	12.9	12.9 ± 1.2[a]	2.4 ± 0.3	0.6 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
$C_{16:0}$	63.3	66.8 ± 0.3	70.5 ± 3.1	16.4 ± 1.3	13.1 ± 0.1	13.7 ± 0.5
$C_{16:1}$	–[b]	6.4 ± 0.2	1.3 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
$C_{18:0}$	–	–	–	3.6 ± 0.3	2.6 ± 0.1	3.1 ± 0.1
$C_{18:1}$	11.0	6.1 ± 0.2	12.9 ± 3.0	23.5 ± 0.8	33.7 ± 0.6	32.3 ± 0.6
$C_{18:2}$	–	–	1.9 ± 0.5	49.6 ± 2.4	41.1 ± 0.1	42.3 ± 1.6
$C_{18:3}$	12.9	7.8 ± 1.3	4.7 ± 0.2	1.1 ± 0.2	1.8 ± 0.1	2.1 ± 0.1
$C_{20:0}$	–	–	2.4 ± 0.2	1.1 ± 0.3	1.6 ± 0.2	1.6 ± 0.1
$C_{20:1}$	–	–	1.1 ± 0.1	1.9 ± 0.3	3.6 ± 0.4	2.6 ± 0.1
$C_{22:0}$	–	–	2.8 ± 0.1	1.4 ± 0.4	1.6 ± 0.3	1.5 ± 0.1
saturated	76.1	79.7 ± 0.9	78.2 ± 3.2	23.3 ± 1.8	19.3 ± 0.7	20.3 ± 0.8
unsaturated	23.9	20.3 ± 0.9	21.8 ± 3.2	76.7 ± 1.8	80.7 ± 0.7	79.7 ± 0.8
endosperm						
$C_{14:0}$	13.3	14.9 ± 0.5	9.1 ± 1.6	2.5 ± 0.8	1.3 ± 0.2	0.5 ± 0.0
$C_{16:0}$	48.4	56.5 ± 8.1	60.8 ± 3.5	20.3 ± 1.4	14.6 ± 1.2	14.1 ± 0.4
$C_{16:1}$	16.3	5.0 ± 1.2	5.9 ± 0.4	3.8 ± 0.7	1.6 ± 0.2	0.4 ± 0.0
$C_{18:0}$	–	–	–	5.3 ± 1.9	4.2 ± 0.8	3.7 ± 0.1
$C_{18:1}$	10.7	10.9 ± 2.1	12.9 ± 6.5	26.3 ± 0.9	28.2 ± 2.3	29.0 ± 1.7
$C_{18:2}$	–	–	–	32.6 ± 5.5	43.3 ± 0.1	44.4 ± 1.0
$C_{18:3}$	11.3	12.7 ± 4.2	11.3 ± 1.0	2.7 ± 0.3	3.1 ± 0.8	2.0 ± 0.1
$C_{20:0}$	–	–	–	–	1.0 ± 0.1	1.6 ± 0.1
$C_{20:1}$	–	–	–	–	1.8 ± 0.4	2.8 ± 0.1
$C_{22:0}$	–	–	–	–	0.9 ± 0.1	1.6 ± 0.1
saturated	61.7	71.4 ± 7.6	69.9 ± 5.1	28.2 ± 2.6	22.0 ± 2.0	21.4 ± 0.6
unsaturated	38.3	28.6 ± 7.6	30.1 ± 5.1	65.5 ± 6.2	78.0 ± 2.0	78.6 ± 0.6

[a] Mean ± SE of the mean. [b] Not detected.

Table III. Relationship of Days after Pollination to Content and Concentration of Total Lipids in Buckwheat Groats (Endosperm plus Embryo)

days after pollination	total lipids[a] in endosperm plus embryo	
	content, μg/groat ± SEM	concn, g/kg of dry weight ± SEM
6	14 ± 1[b]	29.7 ± 3.5[b]
8	25 ± 8	15.4 ± 4.5
10	62 ± 1	9.9 ± 1.5
12	648 ± 18	53.9 ± 2.5
16	739 ± 25	38.1 ± 2.5
20	994 ± 90	34.9 ± 0.6

[a] Total lipids in groats were calculated by summation of total fatty acid and sterol contents of both endosperm and embryo tissues without correction for the glycerol component of acylglycerols. Lipids in seed coat and pericarp tissues were not determined. [b] Mean ± SEM for three replicate samples of 8–10 groats.

embryo. In mature buckwheat achenes, long-chain fatty acids are present in higher concentrations in neutral and free lipids but in lower concentrations in phospholipids (Mazza, 1988). A particulate fraction from developing seeds of *Lunaria annua* L. and *Sinapis alba* L. synthesizes long-chain monounsaturated fatty acids which are readily incorporated into triacylglycerols (Fehling et al., 1990). Milling fractions of mature buckwheat vary in concentrations of long-chain fatty acids (Tauzuki et al., 1991).

The degree of unsaturation and the amount of total lipid may be altered by temperature during seed development. A decrease in temperature during seed maturation resulted in less total lipid and lower concentrations of oleic, arachidic, behenic, and eicosenoic acids but higher concentrations of linoleic and linolenic acids in mature buckwheat achenes (Taira et al., 1986). The long-chain fatty acids were reduced only slightly and no change in myristic, palmitic, or stearic acids was induced by temperature (Taira et al., 1986). Because temperature fluctuations are minor in the present study, the marked changes in fatty acid composition during endosperm and embryo development of buckwheat are developmentally induced.

In buckwheat, the level of linolenic acid was 11% of the total fatty acids in the endosperm and 13% in the embryo at 6 DAP (Table II). In developing wheat seeds (Stokes et al., 1986), linolenic accounted for 25% of the total fatty acids at 8 days after anthesis. The higher concentration of linolenic in wheat is associated largely with polar lipids in the green pericarp–testa fraction (Stokes et al., 1986). In the present work, care was taken to exclude the green maternal tissues of the buckwheat pericarp and testa–nucellus fractions during separation of the endosperm and embryo fractions. However, the isolated endosperm fractions of wheat contained higher linolenic acid concentration than buckwheat throughout seed development but declined in both during storage lipid accumulation. Because the Horbowicz and Kosson (1983) extraction and saponification procedure did not discriminate specifically against linolenic acid (Table I), the endosperm fractions of wheat and buckwheat are probably truly different in linolenic acid composition.

Long-chain fatty acids ($C_{20:0}$, $C_{22:0}$, and $C_{20:1}$) in buckwheat achenes accumulate to less than 7% of the total fatty acids from 10 to 20 DAP in the embryo and from 16 to 20 DAP in the endosperm but were not detected earlier. During early development of the endosperm and embryo, when abortion is likely to occur, the short-chain fatty acids ($C_{16:0}$, $C_{16:1}$, and $C_{14:0}$) and saturated fatty acids are predominant. At 6 DAP, the concentration of palmitic acid is 3 (endosperm) to 5 (embryo) times higher than that of any other fatty acid. At the earliest stages of seed formation and before major accumulation of storage lipids, the short-chain fatty acids may have a potential role in stress-related reductions in seed set and increases in embryo and/or endosperm abortion. However, clean separation of endosperm and embryo fractions from buckwheat achenes before 6 DAP is not feasible by hand in the present study.

ACKNOWLEDGMENT

Contribution from the Department of Soil, Crop and Atmospheric Sciences, Cornell University Agricultural

Experiment Station, and published as SCAS Department Series Paper 1722. Funded by grants from MINN-DAK Growers, Ltd., The Birkett Mills, Japan Buckwheat Millers Association, and Kasho Co. Ltd. M.H. was supported in part by a grant from the Alfred Jurzykowski Foundation, Inc. We thank William J. Cox for his contributions.

LITERATURE CITED

Abid Ali Khan, M. M.; Jain, D. C.; Bhakuni, R. S.; Zaim, M.; Thakur, R. S. Occurrence of Some Antiviral Sterols in *Artemisia annua*. *Plant Sci.* **1991**, *75*, 161–165.

Adachi, T. How to Combine the Reproductive System with Biotechnology in Order to Overcome the Breeding Barrier in Buckwheat. *Fagopyrum* **1990**, *10*, 7–11.

Adams, C. A.; Norby, S. W.; Rinne, R. W. Ontogeny of Lipid Bodies in Developing Soybean Seeds. *Crop Sci.* **1983**, *23*, 757–759.

Dorrell, G. G. Fatty Acid Composition of Buckwheat Seed. *J. Am. Oil Chem. Soc.* **1971**, *48*, 693–696.

Fehling, E.; Murphy, D. J.; Mukherjee, K. D. Biosynthesis of Triacylglycerols Containing Very Long Chain Monounsaturated Acyl Moieties in Developing Seeds. *Plant Physiol.* **1990**, *94*, 492–498.

Folch, J.; Lees, M.; Sloan Stanley, G. H. A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. *J. Biol. Chem.* **1957**, *226*, 479–509.

Gurr, M. I.; Blades, J.; Appleby, R. S. Studies on Seed-oil Triglycerides. The Composition of *Crambe abyssinica* Triglycerides During Seed Maturation. *Eur. J. Biochem.* **1972**, *29*, 362–368.

Heinemann, T.; Kullak-Ublick, G.-A.; Pietruck, B.; Von Bergmann, K. Mechanisms of Action of Plant Sterols on Inhibition of Cholesterol Absorption. Comparison of Sitosterol and Sitostanol. *Eur. J. Clin. Pharmacol.* **1991**, *40* (*Suppl. 1*), S59–S63.

Horbowicz, M.; Kosson, R. Fatty Acids Content in Dry Bean Seeds. *Bromat. Chem. Toksykol.* **1983**, *16*, 9–12.

Marshall, H. G.; Pomeranz, Y. Buckwheat: Description, Breeding, Production, and Utilization. *Adv. Cereal Sci. Technol.* **1982**, *5*, 157–210.

Mazza, G. Lipid Content and Fatty Acid Composition of Buckwheat Seed. *Cereal Chem.* **1988**, *65*, 122–126.

Metcalfe, L. D.; Schmitz, A. A.; Pelka, J. R. Rapid Preparation of Fatty Acid Esters from Lipids for Gas Chromatographic Analysis. *Anal. Chem.* **1966**, *38*, 514.

Murphy, D. J.; Cummins, I. Biosynthesis of Seed Storage Products During Embryogenesis in Rapeseed, *Brassica napus*. *J. Plant Physiol.* **1989**, *135*, 63–69.

Ohlrogge, J. B.; Kuo, T.-M. Control of Lipid Synthesis During Soybean Seed Development: Enzymic and Immunochemical Assay of Acyl Carrier Protein. *Plant Physiol.* **1984**, *74*, 622–625.

Oka, Y.; Kiriyama, S.; Yoshida, A. Sterol Composition of Cereals, Potatoes, Seeds, Nuts and Pulses. Part II. Sterol Composition of Japanese Vegetable Foodstuffs. *J. Jpn. Soc. Food Nutr.* **1972**, *25*, 543–549.

Pomeranz, Y. Buckwheat: Structure, Composition, and Utilization. *CRC Crit. Rev. Food Sci. Nutr.* **1983**, *19*, 213–258.

Ruszkowski, M. Productivity of Buckwheat. In *Buckwheat Research 1986*, Proceedings of the 3rd International Symposium on Buckwheat, Pulawy, Poland, Part I; Institute of Soil Science and Plant Cultivation; Laboratory of Science Publisher: Pulawy, Poland, 1986; pp 78–98.

Stokes, D. N.; Galliard, T.; Harwood, J. L. Changes in the Lipid Composition of Developing Wheat Seeds. *Phytochemistry* **1986**, *25*, 811–815.

Taira, H.; Akimoto, I.; Miyahara, T. Effects of Seeding Time on Lipid Content and Fatty Acid Composition of Buckwheat Grains. *J. Agric. Food Chem.* **1986**, *34*, 14–17.

Tauzuki, W.; Ogata, Y.; Akasaka, K.; Shibata, S.; Suzuki, T. Fatty Acid Composition of Selected Buckwheat Species by Fluorometric High-performance Chromatography. *Cereal Chem.* **1991**, *68*, 365–369.

Yasukawa, K.; Takido, M.; Matsumoto, T.; Takeuchi, M.; Nakagawa, S. Sterol and Triterpene Derivatives from Plants Inhibit the Effects of a Tumor Promoter, and Sitosterol and Betulinic Acid Inhibit Tumor Formation in Mouse Skin Two-stage Carcinogenesis. *Oncology* **1991**, *48*, 72–76.

Received for review August 27, 1991. Revised manuscript received January 10, 1992. Accepted January 31, 1992.

Registry No. β-Sitosterol, 83-46-5; campesterol, 474-62-4; stigmasterol, 83-48-7; palmitic acid, 57-10-3; palmitoleic acid, 373-49-9; stearic acid, 57-11-4; oleic acid, 112-80-1; linoleic acid, 60-33-3; linolenic acid, 463-40-1; myristic acid, 544-63-8; arachidic acid, 506-30-9; eicosenoic acid, 506-31-0; behenic acid, 112-85-6.