# Compositional and Morphological Characteristics of Cow Cockle (*Saponaria vaccaria*) Seed, a Potential Alternative Crop

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Seeds from cow cockle, an annual weed commonly found in grain fields of North America, were analyzed for their morphological and physical characteristics, proximate chemical composition, mineral constituents, and amino acid, lipid, and fatty acid composition. The results revealed that the composition of whole, mature seed of cow cockle is similar to that of most cereal grains. Dehulled seeds, however, contain over 77% starch with very small (0.5–1.0 μm) granules of uniform size, while the hulls contain about 1% saponins; these seed constituents may find unique applications in the food/feed and cosmetics industries.

## INTRODUCTION

Cow cockle (*Saponaria vaccaria* L.), also known as cowherb, spring cockle, pink cockle, and China cockle, is an annual weed commonly found in grain fields of the northwestern United States and in the prairie provinces of Canada. It was introduced from Europe, where it is said to have been formerly used as a forage plant, the specific name *vaccaria* having been given for its apparent value as a cow feed (Georgia, 1933; Goering et al., 1966). The seed, which is round, 0.24–0.26 mm in diameter, and orange to dull black in color, contains over 60% starch and 15–16% protein and is rich in saponins. Saponins have anti-inflammatory and expectorant properties and have been known to lower plasma cholesterol levels in several mammalian species (Price et al., 1987). The ability of some saponins to lower plasma cholesterol has provoked considerable clinical interest and prompted some workers to propose consumption of saponins as a useful means of dietary management of plasma cholesterol in humans (Oakenfull, 1981).

This investigation was initiated to evaluate the compositional characteristics of cow cockle seed and to establish its potential as a raw material in the feed or food supply.

## MATERIALS AND METHODS

Mature seeds from two dwarf (NC 3, NC 4) and three tall (NC 1, NC 2, NC 5) accessions of cow cockle (*S. vaccaria* L.) grown in 1989 at the Agriculture Canada Research Station, Morden, MB, were used. The seeds were harvested using a Wintersteiger plot combine, cleaned to remove sand and other foreign matter, and stored for 2–3 months at room temperature prior to being dehulled and/or milled and analyzed. Dehulling and fractionation of seeds was done with the tangential abrasive dehulling device (TADD) described by Reichert et al. (1986).

**Morphological and Physical Characteristics.** Surface and interior characteristics of whole and dehulled seed were determined using scanning electron and fluorescence microscopy. The scanning electron microscope, JSM-35C (JEOL-USA, Peabody, MA), was operated at an accelerating voltage of 10 kV. Photomicrographs were taken on Plus-X Kodak film. For the fluorescence microscopy seeds were fixed in 6% glutaraldehyde in 0.025 M potassium phosphate buffer, pH 7.2, and 70% ethanol at 4 °C for 24–48 h. Fixed samples were dehydrated through a series of changes of alcohol, from Methyl Cellosolve, ethanol, 1-propanol to 1-butanol according to the method described by Fulcher and Wong (1980). The samples were then infiltrated with glycol methacrylate (GMA) monomer for 3–5 days prior to polymerization at 60 °C in gelatin capsules. Sections 1–5 μm thick were cut using glass knives in an ultramicrotome (Sorvall Inc., Newton, CT) and were affixed to glass slides for subsequent examination.

Sections were examined with a Zeiss universal research photomicroscope (Carl Zeiss Ltd., Montreal, PQ) equipped for fluorescence analysis as described by Yiu (1986). Photomicro-

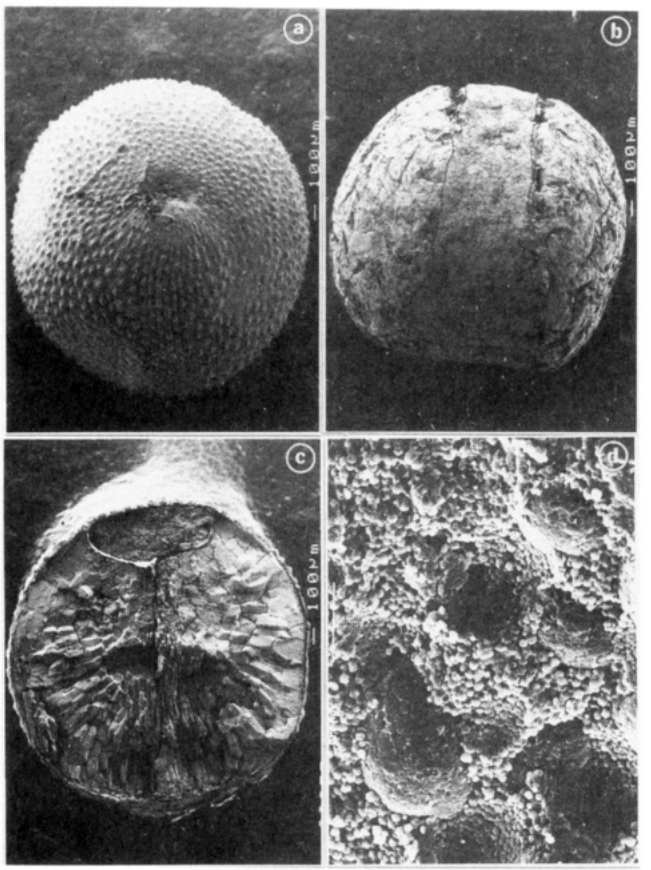


**Figure 1.** Scanning electron micrographs of whole seed (a), dehulled seed (b), cross section of whole seed (c), and starch granule organization in endosperm (d) of cow cockle, *S. vaccaria*. (Figure is reproduced here at 54% of original.)

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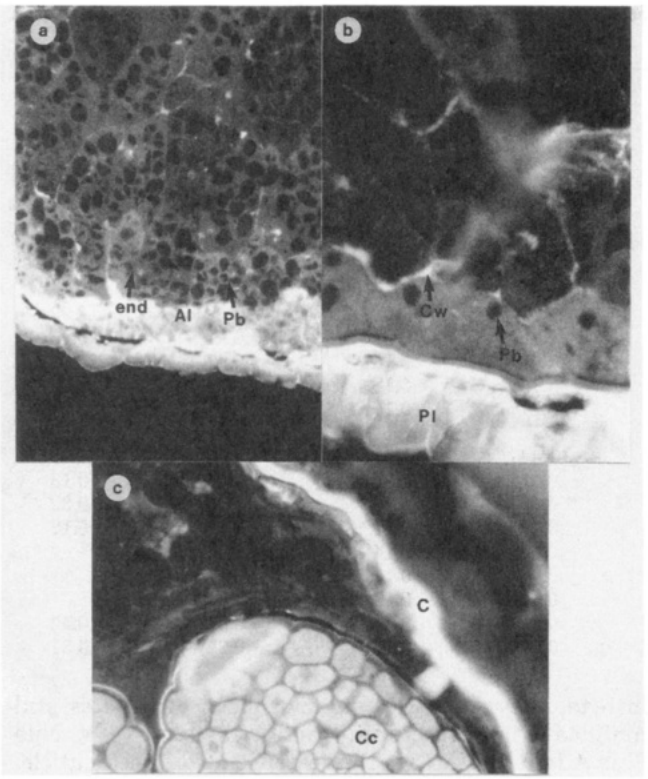


**Figure 2.** Fluorescence micrographs of glycol methacrylate embedded sections of whole seed stained with Calcofluor at 10× magnification (a), ANS at 40× magnification (b), and Nile Blue A at 40× magnification (c). al, aleurone layer; c, cuticle; cc, cotyledonary cells; cw, cell walls; end, starchy endosperm; pb, protein bodies; pl, palisade layer. (Figure is reproduced here at 54% of original.)

**Table I. Proximate Composition and Selected Mineral Profile of Cow Cockle Whole Seed (ws), Dehulled Seed (ds), and Hulls (h)**

| | accession | | | | | | |
|---|---|---|---|---|---|---|---|
| | NC 1 | | | NC 2 | NC 3 | NC 4 | NC 5 |
| assay (dwb) | ws | ds | h | ws | ws | ws | ws |
| moisture, % | 11.3 | 10.4 | 6.5 | 12.1 | 10.9 | 11.4 | 10.9 |
| protein,[a] % | 16.2 | 12.9 | 21.6 | 15.2 | 15.9 | 16.3 | 16.3 |
| crude fiber, % | 10.8 | 2.2 | 40.6 | 11.5 | 11.9 | 10.4 | 10.6 |
| ash, % | 2.6 | 1.0 | 6.9 | 2.5 | 2.8 | 2.5 | 2.8 |
| fat, % | 3.4 | 1.8 | 4.7 | 2.4 | 2.1 | 2.0 | 2.0 |
| starch, % | 64.1 | 77.4 | 20.0 | 56.0 | 56.5 | 60.0 | 65.5 |
| Ca, % | 0.355 | 0.056 | 1.54 | 0.333 | 0.403 | 0.385 | 0.360 |
| Mg, % | 0.192 | 0.103 | 0.299 | 0.193 | 0.211 | 0.201 | 0.181 |
| P, % | 0.374 | 0.238 | 0.473 | 0.415 | 0.426 | 0.392 | 0.368 |
| K, % | 0.390 | 0.212 | 0.749 | 0.501 | 0.415 | 0.384 | 0.314 |
| Cu, ppm | 5.2 | 6.5 | 1646.0 | 6.1 | 5.9 | 6.2 | 4.9 |
| Fe, ppm | 282.9 | 98.2 | 897.2 | 400.4 | 843.1 | 274.3 | 209.9 |
| Zn, ppm | 70.7 | 68.4 | 1267.0 | 60.6 | 64.7 | 79.4 | 71.9 |
| Mn, ppm | 60.1 | 16.3 | 206.4 | 54.5 | 62.8 | 70.8 | 63.1 |
| Na, ppm | 27.1 | 23.4 | 247.6 | 14.8 | 11 | 20.3 | 5.6 |

[a] N × 6.25.

graphs were obtained using 35-mm Kodak Ektachrome 400 daylight film. Sections were photographed after one of the following staining procedures. GMA-embedded sections were stained with 0.01% (w/v) aqueous solutions of 1-anilino-8-naphthalenesulfonic acid (ANS) (Sigma Chemical Co., St. Louis, MO), Nile Blue A (Eastman Kodak Co., Rochester, NY), and Calcofluor (Polysciences Inc., Warrington, PA) for 1–2 min for storage proteins, lipids, and cell walls, respectively.

**Proximate Analyses.** Seed and seed fractions were ground in a Wiley mill equipped with a 60-mesh screen. Analyses for moisture, nitrogen, fat, fiber, ash, and mineral content were carried out according to standard procedures (AOAC, 1980). Starch was assayed by a modification of the enzymatic semi-micro method of Banks et al. (1970) as described by Biliaderis et al. (1979).

**Table II. Amino Acid Composition of Cow Cockle Whole Seed**

| amino acid[a] | NC 1 | NC 2 | NC 3 | NC 4 | NC 5 | wheat flour[b] | soy flour[b] |
|---|---|---|---|---|---|---|---|
| aspartic acid | 9.4 | 9.7 | 10.3 | 9.8 | 9.7 | 4.1 | 11.7 |
| threonine | 4.1 | 4.1 | 4.2 | 4.2 | 4.2 | 2.5 | 3.6 |
| serine | 5.4 | 5.4 | 5.4 | 5.5 | 5.6 | 4.4 | 4.9 |
| glutamic acid | 17.1 | 16.3 | 17.4 | 17.8 | 17.0 | 33.1 | 18.6 |
| proline | 4.3 | 4.3 | 4.0 | 4.0 | 4.1 | 11.5 | 5.2 |
| glycine | 8.8 | 8.6 | 9.0 | 8.9 | 8.9 | 3.5 | 4.0 |
| alanine | 4.3 | 4.5 | 4.4 | 4.4 | 4.4 | 2.8 | 4.1 |
| cystine | 1.7 | 1.2 | 1.4 | 1.5 | 1.4 | 2.2 | 1.1 |
| valine | 5.2 | 5.6 | 5.4 | 5.4 | 5.5 | 4.1 | 5.2 |
| methionine | 1.9 | 1.7 | 1.9 | 2.0 | 1.9 | 1.6 | 1.2 |
| isoleucine | 3.7 | 3.7 | 3.8 | 3.8 | 3.9 | 3.5 | 4.7 |
| leucine | 6.2 | 6.6 | 6.4 | 6.3 | 6.4 | 6.7 | 7.7 |
| tyrosine | 4.6 | 4.8 | 4.6 | 4.8 | 4.8 | 3.2 | 3.4 |
| phenylalanine | 4.8 | 5.0 | 5.1 | 4.9 | 4.9 | 4.8 | 5.1 |
| histidine | 2.7 | 2.7 | 2.8 | 2.7 | 2.7 | 2.1 | 2.5 |
| lysine | 5.3 | 5.6 | 5.6 | 5.6 | 5.5 | 1.9 | 5.8 |
| arginine | 11.0 | 10.5 | 11.3 | 11.2 | 10.9 | 3.7 | 7.3 |

[a] In g/100 g of protein. [b] From Friedman and Levin (1989).

**Table III. Lipid Composition of Cow Cockle Seed (Percent of Total)**

| component | whole seed | dehulled seed |
|---|---|---|
| triglycerides | 70.4 | 72.6 |
| free fatty acids | 0.7 | 0.6 |
| diglycerides | | |
| 1,3 | 1.6 | 1.5 |
| 1,2 | 1.4 | 1.3 |
| monoglycersides | | 1.4 |
| phosphatidic acid | 0.1 | 0.2 |
| phosphatidylserine | 3.1 | 2.4 |
| phosphatidylinositol | 7.0 | 7.6 |
| phosphatidylethanolamine | 5.0 | 1.5 |
| phosphatidylcholine | 9.7 | 9.2 |
| lysophosphatidylethanolamine | 0.4 | 1.1 |
| lysophosphatidylcholine | 0.6 | 0.6 |

**Amino Acid Composition.** Amino acids were measured in samples hydrolyzed with HCl (6 N) for 22 h at 110 °C with a Technicon amino acid analyzer. Cysteine and methionine were determined separately as cysteic acid and methionine sulfone after performic acid oxidation as described by Moore (1963). Results are averages of duplicate determinations.

**Lipid and Fatty Acid Composition.** Lipids were extracted with hot butanol saturated with water, extracts combined and evaporated to dryness, and lipids dissolved in chloroform (Morrison et al., 1980). Individual lipid components were quantified using thin-layer chromatography with a flame ionization detector, Iatroscan, Model TH-10 (Iatron, Tokyo). The lipid extracts were applied once, and multiple developments on the individual rod coated with sintered silica gel (Chromarods SIII, Iatroscan, Iatron) were performed. Each group of lipids was developed in suitable solvent mixtures and separated components were quantified by an FID detector. Neutral lipids were separated first with chloroform/dichloroethane/acetone/acetic acid (10:59:1.4:0.3 v/v) mixture, while polar lipids were left at the application point. A second development was performed for polar lipids with chloroform/methanol/water/acetic acid (50:27:3:0.5 v/v) solvent mixture, and again separated components were quantified (TLC-FID, Iatroscan). Components were identified by cochromatography and comparison of retention data on the authentic standards. Quantification was performed by individual calibration for each component analyzed (Przybylski and Eskin, 1992). For fatty acid composition of lipid classes, the lipid extract was separated in neutral, polar, and free fatty acids using a solid-phase extraction cartridge, Sep-Pak, with aminopropyl packing (Przybylski and Eskin, 1991). Classes of the lipids were obtained by sequential elution with chloroform/2-propanol (2:1) for neutral lipids, diethyl ether containing 2% acetic acid to elute free fatty acids, and methanol to remove polar lipids. Fatty acid composition was determined by capillary gas chromatography with fused silica column (25 m × 0.25 mm i.d.) coated with SP-2340 (Supelco, Oakville, ON). The column temperature was isothermal at 190 °C, and the carrier gas was hydrogen. The

Table IV. Fatty Acids of Lipid Fractions from Whole and Dehulled Cow Cockle Seed (Percent of Total)

| | whole seed | | | | dehulled seed | | | |
|---|---|---|---|---|---|---|---|---|
| fatty acid | total lipids | neutral lipids | free fatty acids | polar lipids | total lipids | neutral lipids | free fatty acids | polar lipids |
| 14:0 | 0.23 | 0.16 | 0.83 | 0.34 | 0.23 | 0.26 | 0.73 | 0.34 |
| 15:0 | 0.25 | 0.23 | 0.16 | 0.33 | 0.25 | 0.21 | 0.46 | 0.23 |
| 16:0 is | 0.11 | 0.17 | | | | | 0.11 | 0.16 |
| 16:0 | 12.71 | 9.60 | 15.32 | 13.77 | 11.77 | 10.96 | 16.43 | 10.97 |
| 16:1 *n*-2 | 0.16 | 0.18 | 0.45 | 0.12 | 0.15 | 0.18 | 0.55 | 0.22 |
| 17:0 anteiso | 0.38 | 0.36 | 0.25 | 0.32 | 0.35 | 0.36 | 0.35 | 0.43 |
| 17:0 | 0.12 | 0.18 | 0.57 | 0.13 | 0.12 | 0.16 | 0.35 | 0.13 |
| 18:0 iso | 1.18 | 1.15 | 0.65 | 1.03 | 1.31 | 1.35 | 0.83 | 1.14 |
| 18:0 | 1.35 | 1.53 | 11.32 | 2.72 | 1.23 | 1.13 | 7.32 | 2.92 |
| 18:1 *n*-11 | 0.32 | 0.41 | 0.11 | 0.45 | 0.35 | 0.31 | 0.21 | 0.25 |
| 18:1 *n*-9 | 36.72 | 38.68 | 38.16 | 28.72 | 34.43 | 35.14 | 36.41 | 27.93 |
| 18:2 *n*-6 | 42.63 | 43.42 | 23.43 | 36.63 | 46.13 | 46.18 | 30.81 | 49.93 |
| 19:0 | 0.11 | 0.14 | | | 0.21 | 0.15 | | |
| 18:3 *n*-6 | 0.13 | 0.11 | | | 0.12 | 0.13 | | |
| 18:3 *n*-3 | 1.74 | 1.68 | 4.72 | 1.35 | 1.44 | 1.48 | 1.32 | 1.05 |
| 20:0 | 0.21 | 0.23 | 0.54 | 0.17 | 0.21 | 0.25 | 0.57 | 0.13 |
| 20:1 *n*-9 | 0.63 | 0.74 | 1.15 | 0.26 | 0.65 | 0.76 | 0.93 | 0.32 |
| 22:0 | 0.13 | 0.15 | 0.23 | 0.27 | 0.17 | 0.11 | 0.43 | 0.12 |
| 22:1 *n*-9 | 0.12 | 0.14 | 0.34 | | 0.12 | 0.14 | 0.34 | |
| 22:2 *n*-6 | 0.13 | 0.10 | | | 0.14 | 0.15 | | |
| 22:3 *n*-6 | 0.14 | 0.11 | | | 0.16 | 0.19 | | |
| 24:0 | 0.24 | 0.26 | 1.18 | 0.27 | 0.34 | 0.23 | 0.53 | 0.42 |
| 24:1 *n*-9 | 0.13 | 0.15 | 0.26 | 3.12 | 0.12 | 0.17 | 0.32 | 3.31 |

injector and detector temperatures were held at 240 °C. The lipid samples were esterified with methanol–HCl. To calculate fatty acid composition, response factors published by Ackman and Sipos (1964) were applied.

**Saponin Content.** The saponins were extracted and quantified according to the method of Lalitha et al. (1987). The ground samples were defatted with petroleum ether in a Soxhlet extractor for 36 h. The defatted samples were extracted with 60% (v/v) aqueous ethanol, and the extract was concentrated under reduced pressure at 35–40 °C into a syrupy liquid. The liquid thus obtained was extracted several times with diethyl ether in a separating funnel, until the coloring matter was removed. The syrupy mass was diluted to about 500 mL with water and extracted four or five times with butanol in the ratio of 5:1 (v/v). The pooled butanol extracts were evaporated under reduced pressure and dried over anhydrous phosphorus pentoxide. A solution of the powder thus obtained taken in 50–100 mL of dry methanol was poured into large excess quantities of ether (500–600 mL), and the precipitated saponins were centrifuged out and dried over $P_2O_5$ to constant weight.

## RESULTS AND DISCUSSION

Seeds of five accessions of cow cockle were very similar in appearance and morphology. Whole mature seeds were round, 0.24–0.26 mm in diameter, and weighed an average of 8.5 mg each. Parts a and b of Figure 1 show the size, shape, and surface characteristics of the seed before and after dehulling. A cross section through the seed (Figure 1c) reveals the three main parts: the embryo or germ, the endosperm, and the seed coat or hull. On a weight basis, the hull constituted 10–12%, the embryo 16–18%, and the endosperm 70–74% of the seed. The endosperm consisted of densely packed, small, round, and uniform starch granules (Figure 1d). Deposited starch granules in the endosperm are found as single entities within the cells or as compound structures of spherical or oblong aggregates.

When stained with Calcofluor, the endosperm cell walls showed a strong fluorescence (Figure 2a,b). The subaleurone endosperm cell walls were considerably thicker than the inner endosperm walls. Intracellular components were visible, and lamellar structure was also observed in the walls. Cow cockle storage proteins occurred primarily as protein bodies within various tissues of the seed. Their presence using ANS demonstrated that most of the protein bodies were spherical (Figure 2a). However, some of them were no longer recognizable as individual structures. Instead, they appeared as amorphous aggregates still confined within structurally well-defined cell walls. Nile Blue A induced intense fluorescence in the seed cuticle, in the aleurone cell, and in most of the starchy endosperm (Figure 2c). The structures of the oil bodies were confined within individual cells.

Table I shows moisture, protein, fiber, ash, fat, starch, and mineral content of whole seed from five accessions of cow cockle and corresponding values for dehulled seed and hull fraction of NC 1 seed. The protein content of 15.2–16.3% for whole seed is greater than that of cereals but much lower than that of oilseeds such as canola and soyabean (Duke and Atchley, 1986). The table also shows that fat, crude fiber, and starch contents of whole seed of cow cockle are similar to those of wheat and rye (Friedman and Levin, 1989). Protein, crude fiber, ash, and fat contents of hull fraction were higher than those of dehulled seed, but the reverse was true for starch content. The starch of cow cockle seeds consists of 18% amylose and has a gelatinization temperature of 67–69 °C. The rather small and uniform size of the starch granules may prove useful for specific applications of this starch in the cosmetics industry.

The content of minerals was also much higher in the hull fraction of the seed than in the dehulled seeds. The data show that copper, zinc, sodium, calcium, manganese, and iron were 317, 18, 5.4, 4.3, 3.4, and 3.2 times more concentrated in the hull than in the whole seed. Of all minerals, calcium was the most abundant, ranging from 56 mg/100 g in the dehulled seeds to 1540 mg/100 g in the hulls. In the whole seed, calcium ranged between 330 and 403 mg/100 g, and the values for this element were higher than those of most other higher plants (Duke and Atchley, 1986). The levels of phosphorus and potassium in whole seeds of cow cockle were comparable to those of wheat, but the levels of iron were lower than those encountered in most cereals and legumes.

The amino acid compositions of the five cow cockle accessions are shown in Table II, together with the amino acid compositions of defatted soy flour and commercial wheat flour reported by Friedman and Levin (1989). The data show that the amino acid pattern of cow cockle whole seeds, especially essential amino acids, falls between those of a legume seed such as soy and a cereal such as wheat. All accessions used in this study had relatively high

essential and nonessential amino acids, with the exception of proline, which was lower than the contents reported for wheat flour and soy flour. The content of the sulfur amino acids, cystine and methionine, was relatively high in all accessions of cow cockle, and the values for both amino acids meet the provisional requirements established by the Food and Agricultural Organization of the United Nations (FAO, 1973).

The major components of the lipids of whole and dehulled seeds were triglycerides (Table III). Minor fractions of the total lipids were diglycerides, free fatty acids, and, in dehulled seed, monoglycerides. Phosphatidylcholine and phosphatidylinositol were the major phospholipids of whole and dehulled seed, with also high contributions of phosphatidylethanolamine in whole seed. Other components included phosphatidylserine, lysophosphatidylethanolamine, and lysophosphatidylcholine (Table III).

The major fatty acids of all lipid fractions of whole and dehulled seed of cow cockle were linoleic acid, oleic acid, and palmitic acid (Table IV). These three fatty acids represented over 89% of the fatty acids in neutral, polar, and total lipids of whole and dehulled seeds and 77 and 89% of the free fatty acid fractions of the whole and dehulled seed, respectively. Linoleic acid content was slightly higher and oleic acid content slightly lower in the total, neutral, and polar lipids of dehulled seed compared to their content in whole seed.

Mazza (1988) found linoleic, oleic, and palmitic acids to be the major fatty acids of total lipids, free lipids, neutral lipids, glycolipids, and phospholipids of dehulled buckwheat. Similarly, Price and Parsons (1975) found that in the total lipids of wheat, barley, corn, oats, rye, sorghum, and triticale linoleic acid is the predominant fatty acid followed by oleic and palmitic acids. Thus, the lipid composition of cow cockle is very similar to that reported for buckwheat and other cereal grains.

The amounts of saponins in whole seeds, dehulled seeds, and hulls of cow cockle were $0.64 \pm 0.02$, $0.45 \pm 0.01$, and $0.96 \pm 0.04$ g/100 g of dry matter, respectively. There are no reported literature values for saponin contents of cow cockle seed, although Abubakirov and Amanmuradov (1964) isolated vaccaroside from seeds of *S. vaccaria* from the USSR and showed it to be the β-D-glucopyranuronoside of gypsogenin. The reported literature values for saponin contents of other plant tissues are highly variable and dependent on the extraction and quantitation method used (Oakenfull, 1981; Price et al., 1987). The saponin content of the seeds used in this study, determined by a gravimetric method, is comparable to reported saponin levels of legumes (Oakenfull, 1981).

## CONCLUSIONS

The overall composition of cow cockle seed is similar to that of most cereal grains. The contents of protein, crude fiber, ash, fat, mineral, and saponins are much higher in the hulls than in dehulled seeds. Dehulled seeds, however, contain over 77% starch, which has unique physical properties and may find useful applications in the food and cosmetic industries. The shape and size of cow cockle seed are similar to those of canola seed. This indicates that cow cockle seed can be planted, harvested, and processed using existing equipment. The results of this study indicate that this underutilized seed has potential as a source of starch and saponins. Further work is in progress in our laboratories to fully characterize the starch and saponins and to assess the agronomic potential of cow cockle.

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