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Characterization of potential health promoting lipids in the co-products of de-flossed milkweed

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Abstract

The floss and oil of the common milkweed (*Asclepias syriaca* L.) seeds are currently used to produce comforters/pillows and skin care products, respectively. As an outcome of these products, copious quantities co-products (pressed seed meal and pod biomass) are disposed of each year despite the presence of potential health benefiting lipids. The objective of this project was to determine the feasibility of developing the lipid fraction from of these co-products for the fast growing dietary human health market. Although certain types of lipids were affected by the extraction solvent used (hexane and diethyl ether) as were overall amounts, analysis of the each extract showed novel lipid profiles with several potential health benefiting agents present at levels comparable to or exceeding those present in other typically consumed dietary oils or food systems (vitamin E, carotenoids, sterols and unsaturated free fatty acids, particularly the both omega 7-fatty acids).

Keywords: milkweed, *Asclepias syriaca* L. co-products, lipids, functional foods, alternative crops

1. Introduction

Alternative crops produced in North America offer growers and processors the opportunity to capitalize on high value technologies and to develop new products. In particular, the National Center for Agriculture Utilization Research has identified the common milkweed (belonging to the *Asclepias* genus) as a highly potential alternative crop. Because most *Asclepias* species are indigenous to North America, the plant grows in many types of agro-regional conditions. Milkweed can also improve the quality of farm life because, as a perennial plant, it is not a labor-intensive crop. Studies completed during and post-World War II has further shown that that milkweed had the greatest potential for commercial marketing than most native plants (Timmons, 1946). Yet, on-going attempts to develop milkweed as an economical viable crop have met with little success (e.g., rubber from the latex of the plant and biodiesel from the seed's oil). Moreover, milkweed has been historically viewed as a noxious weed because it can easily invade other crops due to the floss-covered seeds that are carried by the wind to different areas.

Nonetheless, the milkweed species, *Asclepias syriaca* L., has received industrial crop status because of the silky seed floss that is currently being used to produce hypoallergenic pillows, comforters, and insulating fiber. The de-flossed seeds have in turn been used to develop skin care products and ne-

maticides/pesticides (Harry O'kuru et al., 1999) but large quantities of dried plant biomass (PB) and cold pressed seed meal (SM) end up as co-products that are typically disposed. However, studies completed in our laboratory as well as by other researchers have shown the presence of potentially valuable health benefiting lipids (e.g., specifically the sterols and fatty acids) in the seeds. Considering that functional foods and the dietary supplement industry are expected to grow from 18 billion in 2002 to 30 + billion by 2012 (McCrorie & Bone, 2008), we proposed that the *Syriaca* L. co-products could be potential source of human health ingredients for the niche but fast growing health promoting dietary market and thus add value to this currently underutilized crop. Therefore, the objective of this project was to determine the feasibility of developing these co-products for this market by characterizing for select lipid based components that have been linked to one or more health promoting benefits.

2. Materials and methods

2.1. Preparation of lipid extracts

Samples of dried pod biomass (PB) and seed meal (SM) that had been subjected to a cold pressing process to obtain the oil were obtained from Natural Fibers, Inc., (Ogallala, NE). Lipids were extracted from 105 to 120 g samples ground to fine parti-

cles by recirculating 150–200 ml of hexane or diethyl ether for 3–4 h using a bench-top Soxhlet apparatus. The solvent was collected, removed by a rotovap, and the residue was stored at 4–10 °C until analyzed. Total lipid yields were determined based upon the final weight of the lipid residue respective to the weight of the starting material. The sample was visually inspected for color and phase characteristics.

2.2. Lipid characterization

The lipid classes present in each extract were initially identified and quantitated using thin layer chromatography (TLC). The lipid extract was suspended in hexane at a concentration of 25–30 mg/ml. The samples were spotted onto Whatman silica 60 Å TLC plates (60 general purpose, 20 × 20 cm, 250 µm, Maidstone England) as 10 and 5 µl aliquots. Along with the samples, a cocktail of standards containing cholesterol, fatty acid methyl esters, d- α -tocopherol acetate, and 3 polyoxyethylenesorbitan monooleate, octacosanol, monoacylglycerides of oleic acid, 1,2-diacylglycerols of oleic acid, 1,3-diacylglycerides of oleic acid, and triacylglycerols of oleic acid was spotted on the same TLC plates. The samples/standards were resolved with hexane, diethyl ether, and acetic acid (85:15:2 (v/v/v)). The lipids were visualized by submerging the plate in a 10% cupric sulphate, 8% phosphoric acid solution and charring at 165 °C for 10 min. A Kodak Gel Logic 440 Imaging System interfaced to Kodak ID Image Analysis software (Carestream Health, New Haven, CT) was used to image the developed plate and the simple lipid classes were identified based on their Rf values compared to the standards.

Levels of total free fatty acids and triacylglycerides were completed as described by AOCS (American Oil Chemists' Society) (2006) and Vishwanath & Manning (1968), respectively. The results are reported as the mean \pm standard deviation (SD) (in mg oleic acid/g of co-product (free fatty acids) or in mg of olive oil/g of co-product (triacylglycerides) of duplicate or triplicate analyses.

Vitamin E, carotenoid, and Co-enzyme Q10 levels were determined by combining 0.05–0.10 g of lipid extract with 1–2 mL of methanol with mixing for 1–2 h. The methanol phase was passed through a 0.45 µm syringe filter and analyzed for the vitamin E isomers (α -, β -, and γ -tocopherols), carotenoids, and co-enzyme Q10 with a Waters 600 S HPLC (Milford, MA) system interfaced to a Waters Millennium 32 Chromatography Manager workstation. The analytes were resolved with an Agilent Zorbax 300SB C-18 (Santa Clara, CA) column and a mobile phase of methanol, acetonitrile, and triethanolamine (90:10:1) under isocratic conditions at a flow rate of 1 ml/min. A Waters 996 photodiode array detector set at 450, 309, and 295 nm was used to detect the carotene, Co-enzyme Q10, and tocopherols, respectively. These analytes were identified based on their retention times with external standards and quantitated against calibration curves generated from standards. Results are reported as the mean \pm SD in µg of carotene or Co-enzyme Q10/g of co-product of triplicate analyses. Spectral patterns of the individual HPLC bands in the 450 nm window, (other than the carotene bands) were monitored against a calibration curve prepared with lutein standards. Total carotenoid levels were determined by adding the absorbance of the individual bands and expressing the final results as the mean \pm SD in µg lutein/g of co-product of triplicate analyses.

Quantification of sterols (free and esterified) was achieved with gas chromatography (GC) and flame ionization detection (FID). Each co-product lipid extract (10–20 mg) was prepared and analyzed according to published reports (Schneider et al., 2000). Briefly, the samples were injected (1 µl) onto an HP 5890 Series II + GC with a DB-1 column (15 m × 0.25 mm) (J&W

Scientific, Folsom, CA) under the following conditions: initial temperature 190 °C for 1 min, increased to 220 °C at 3 °C/min; injector temperature, 270 °C; flame ionization detector temperature, 300 °C; helium carrier gas; and split ratio of 20:1. External standards of stigmasterol, campesterol, and β -sitosterol were used to identify and quantify the peaks. Results are reported as the mean \pm SD of a sterol/lipid extract (µg/g of co-product) of triplicate analyses.

Fatty acid profiles were determined combining boron trifluoride (14%) in methanol to an extract sample followed by heating to a 100 °C for 30 min in sealed reaction vials. The samples were allowed to cool to room temperature before extraction with 1 ml of hexane and three rinses of 2 ml of water. The hexane fraction and fatty acid standards were resolved with a Hewlett Packard Co. 6890 series GC System Plus + (Wilmington, DE) using a DB-Wax column (30 m × 0.25 mm) by J&W Scientific and detected with a flame ionization detector. Injections were achieved with a split ratio of 10:1 with the temperature set at 185 °C initially for 12 min, the temperature was increased to 210 °C at 10 °C/min and held for 0 min, and then increased to a final temperature of 230 °C at 10 °C/min, which was maintained for the remaining 15 min of the run. Results are expressed as the mean \pm SD of the relative percentage of each fatty acid of triplicate analyses.

2.3. Statistical analysis

Results were analyzed with R: A language and environment for statistical computing software (Vienna, Austria) and/or by StatsGraphic Plus, version 4 (Statpoint Technologies, Inc., Warrenton, VA). The Cockran C test and the Bartlett's test were performed to determine whether the variability between different lipid levels were significantly different at the 95% confidence interval ($p < 0.05$). If not, the Tukey HSD mean separation test was completed to determine whether the means from the lipid data were significantly different at the 95% confidence interval, ($p < 0.05$). Statistical comparisons of the results were reported based on the Tukey HSD mean separation test. The final results were expressed as the mean \pm SD of at least triplicate analyses. Potential outliers were assessed with the Grubbs test and were eliminated at a 5% risk for rejection.

3. Results and discussion

Asclepias syriaca L. is currently used to produce comforters/pillows and skin care products from its seed floss and seed oil, respectively, resulting in large quantities of pressed SM and dried PB that at present are disposed. To determine the feasibility of developing these co-products as sources of dietary human health ingredients, the lipid fraction was analyzed for specific components that have been linked to health promoting benefits. Such applications are particularly conducive to lipid fraction of *Asclepias syriaca* L. as studies have shown that the oil is not contaminated with the potentially dangerous cardiac glycosides common to the milkweed plant (Harry-O'kuru & Abbott, 1997).

To ensure the recovery of simple lipids with slightly different polarities, each co-product (pressed SM and PB) was extracted with a Soxhlet method using either hexane (HX) or diethyl ether (DE) as the solvent system. As shown in Table 1, statistically higher yields were obtained from the SM compared to the PB even though 10–15% of the lipid had been removed from the former sample using a cold press extraction process. These results were expected as other researchers have reported lipid yields surpassing 20% from unprocessed milkweed seeds (Harry-O'kuru & Carriere, 2002). For both co-products, statistically lower yields were obtained with HX compared to DE. The overall appearance also differed depending on the co-product as well as the extraction solvent

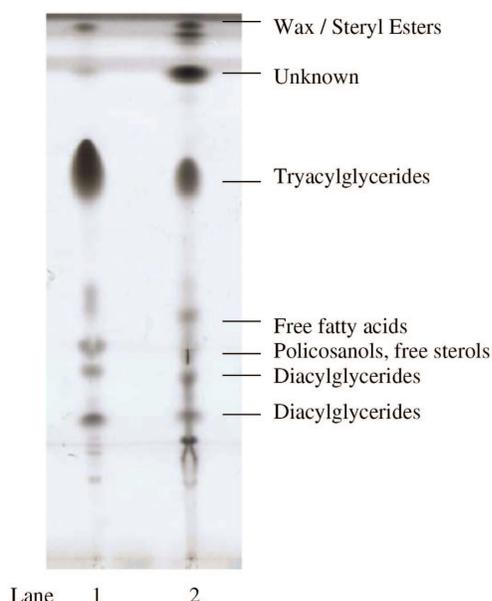


Figure 1. Thin layer chromatography of diethyl ether lipid fraction extracted from *Asclepias syriaca* L seed meal (Lane 1) and pod biomass (Lane 2). Lipids were resolved with a solvent system of hexane:diethyl ether:acetic acid (85:15:2 by vol) and visualized by charring after briefly submerging the plate in 10% cupric sulphate, 8% phosphoric acid.

used (Table 1). Extraction of the SM with both solvents yielded a yellow viscous oil that remained in a liquid state when stored at 4–10 °C whereas a waxy yellow and a waxy green solid were extracted from the PB with HX compared to DE, respectively. The green hue in the PB-DE sample could be due to the presence of chlorophyll but more analyses are required to test this hypothesis.

The overall simple lipid profile for both SM and PB extracts was determined with thin layer chromatography (TLC). From a qualitative perspective, the TLC banding patterns (Figure 1) were similar for a given co-product despite the extraction solvent used (data not shown). As represented by the TLC of the DE extracted SM and PB, several major simple lipid classes were resolved, including triacylglycerides, free fatty acids, policosanols (long chained alcohols), free sterols, diacylglycerides, and steryl esters. Considering that the same amount of sample was analyzed for the SM and PB extracts, the band intensities as well as the banding patterns suggest that the simple lipid classes the co-products differ in composition and total yields (Figure 1). For example, the triacylglyceride band was darker and broader for the SM extract (Lane 1) compared to the PB extract (Lane 2) whereas a multiple banding pattern occurred in the steryl/wax ester region for the latter sample. Most notably, an intense but unknown band was resolved in the PB sample (Lane 2, Figure 1). The R_f value for this band was similar to that resolved for the waxy fraction of the whole grain sorghum, which was identified as long-chain aldehydes (Hwang et al., 2002). More tests are needed to confirm the identity of the unknown TLC band in the PB sample (Lane 2, Figure 1) but the R_f value coupled with the waxy texture of the extract and that

long-chain aldehydes are prevalent in the wax matrices covering all plant organs suggest the presence of aldehydes.

To our knowledge, studies have yet to be reported on the health promoting properties of long-chain aldehydes. However, the reduced form of these compounds, i.e., policosanols, have been linked to multiple health benefits including lower lipid levels in the blood (Gouni-Berthold & Berthold, 2002) albeit these results have recently come under scrutiny (Kasis et al., 2009). For the milkweed co-products, significantly higher levels of policosanols was present in the HX-PB sample compared to the HX-SM with no detectable amounts in either DI-extract (Table 2). As high molecular carbon length weight primary aliphatic (C20–C36) carbon length alcohols derived from plants waxes, it is to be expected that the policosanols were obtained with the hexane process. The policosanols levels in the PB lipid fraction (~322 µg/g of lipids) were comparable to those reported for the sugar cane (~270 µg/g of lipids) (Irmak et al., 2006), which is one of the main sources of policosanols for the commercially available supplements but it must be noted that a different extraction process was used.

Quantitative analysis of the major fatty acid-containing groups showed that triacylglycerides were the most prevalent lipid class for all extracts but the PB samples contained lower levels compared to the SM (Table 2), as also evidenced by the TLC results (Figure 1). Moreover, total free fatty acids quantities were generally lower for the PB compared to the SM (Table 2). Further analysis of the fatty acid profiles showed a high degree of unsaturation for the DE- and HX-SM extracts (Table 3), which accounts for their viscous oily state at temperatures of 4–10 °C. Chain lengths ranged C16 to C18 while C20 and higher fatty acids were detected at negligible percentages. The most abundant fatty acid was linoleic acid (C18:2) for both the HX- and DE-SM extracts followed by oleic acid. Linoleic acid is an important non-essential nutrient that has low-density lipoprotein lowering properties, as reported by the early work (Hegsted et al., 1965). Researchers have also demonstrated that oleic acid is beneficial for reducing blood pressure (Terés et al., 2008) and is a potential anti-proliferative agent against breast cancer (Menendez et al., 2005).

The DE and HX-SM extracts also contained relatively high levels of the omega-7 monounsaturated fatty acid, palmitoleic acid (16:1) (Table 3). As a minor but novel fatty acid present as a glyceride in human adipose tissue, reports recently showed that palmitoleic acid acts as a hormone that communicates to distant organs for regulating systemic homeostasis (Cao et al., 2008). In another study examining the effects of a palmitoleic acid rich diet on young Japanese women, an overall reduction in weight gain and body mass index occurred that was accompanied by a significant decrease in total cholesterol and low-density lipoprotein (LDL)-cholesterol levels (Hiraoka-Yamamoto et al., 2004). Dietary sources that contain relatively high levels of palmitoleic acid include the oils of the macadamia nuts (25–30%) and sea buckthorn (25%) but substantially lower levels are present in typically consumed oils (0.0–1.5%) (Kamal-Elden & Andersson, 1997).

For the common milkweed, the HX-SM/PB samples contained similar fatty acids profiles compared to their DE-extracted counterparts (Table 2). However, comparison between

Table 1. Yields and appearance of lipids extracted from each *Asclepias syriaca* L. co-product with either hexane or diethyl ether.

Co-product	Lipid yields (hexane) (%)	Lipid yields (%) (Diethyl ether) (%)	Appearance* (hexane)	Appearance (diethyl ether)
Seed Meal	10.1 ± 0.1 ^a	13.2 ± 0.6 ^b	Viscous yellow liquid	Viscous yellow liquid
Pod Biomass	4.0 ± 0.2 ^c	5.0 ± 0.0 ^d	Waxy yellow solid	Waxy green solid

Results are expressed as the mean ± SD of three analyses. Values with different superscript letters are statistically different ($p < 0.05$).

* At room temperature.

Table 2. Simple lipid classes present in each *Asclepias syriaca* L. co-product extracted with either hexane or diethyl ether.

Lipid class	Seed meal (hexane)	Seed meal (diethyl ether)	Pod biomass (hexane)	Pod biomass (diethyl ether)
β-Carotene (μg/g)	†-	-	8.2 ± 1.3 ^a	2.7 ± 0.8 ^b
Carotenoids (μg/g)	32.3 ± 2.5	28.6 ± 6.1	33.7 ± 2.5	37.8 ± 2.3
Co-enzyme Q10 (μg/g)	-	-	-	-
Free fatty acids (mg/g)	4.7 ± 0.4 ^a	6.4 ± 0.2 ^b	3.7 ± 0.6 ^{ac}	2.9 ± 0.6 ^c
Policosanols (μg/g)	7.8 ± 0.6 ^a	-	12.8 ± 0.7 ^b	-
**Sterols (μg/g)	542.4 ± 29.5 ^a	686.6 ± 90.1 ^a	60.3 ± 22.3 ^b	230.3 ± 33.6 ^c
*Vitamin E (μg/g)	65.9 ± 10.4 ^a	73.9 ± 4.8 ^a	28.5 ± 3.9 ^b	29.2 ± 3.8 ^b
Triacylglycerides (mg/g)	143.0 ± 43.4 ^a	130.9 ± 63.2 ^a	26.3 ± 20.3 ^b	29.8 ± 15.7 ^b

Results expressed as the mean ± standard deviation (wt of lipid/wt of co-product) of two–three analyses. Values with different superscript in the same row are statistically different ($p < 0.05$). Reported as the summation; * of α, γ, δ-tocopherol, or as ** of free or esterified sterols.

† Lipid class was not detected.

Table 3. Compositional profiles of fatty acids extracted from each *Asclepias syriaca* L co-product with either hexane or diethyl ether.

Fatty acid	Seed meal (hexane) (%)	Seed meal (diethyl ether) (%)	Pod biomass (hexane) (%)	Pod biomass (diethyl ether) (%)
C16:0 (Palmitic)	8.4 ± 1.2 ^a	7.7 ± 0.3 ^a	23.6 ± 8.6 ^b	11.9 ± 0.3 ^{ab}
C16:1 (Palmitoleic)	16.0 ± 0.6 ^a	17.0 ± 0.4 ^a	2.9 ± 1.3 ^b	5.0 ± 0.0 ^b
C16:2 (Hexadecadienoic)	1.0 ± 0.0 ^a	1.0 ± 0.0 ^a	3.5 ± 0.0 ^b	3.7 ± 0.1 ^b
C18:0 (Stearic)	2.0 ± 0.0	1.8 ± 0.0	2.4 ± 0.4	4.6 ± 3.6
C18:1 (Vaccenic)	11.8 ± 0.3	11.1 ± 0.5	14.8 ± 3.2	17.6 ± 1.1
C18:1 (Oleic)	21.4 ± 0.4 ^a	22.4 ± 0.5 ^a	6.6 ± 3.0 ^b	9.7 ± 0.3 ^b
C18:2 (Linoleic)	34.6 ± 0.4 ^{ab}	34.6 ± 0.3 ^{ab}	39.8 ± 6.0 ^{ab}	46.0 ± 2.4 ^b
C18:3 (Linolenic)	2.1 ± 0.8 ^a	1.6 ± 0.1 ^a	8.6 ± 3.8 ^b	4.1 ± 0.0 ^{ab}

Results expressed as the mean ± standard deviation (in relative% fatty acid) of duplicate or triplicate analyses. Values with different superscript in the same row are statistically different ($p < 0.05$).

Table 4. Compositional profiles of sterols of each *Asclepias syriaca* L. co-product extracted with either hexane or diethyl ether.

Sterol class	Seed meal (hexane) (%)	Seed meal (diethyl ether) (%)	Pod biomass (hexane) (%)	Pod biomass (diethyl ether) (%)
Campesterol	33.7 ± 0.2 ^a	28.4 ± 0.7 ^b	†-	17.5 ± 1.9 ^d
Stigmasterol	31.3 ± 0.1 ^a	13.2 ± 0.1 ^b	100.0 ± 0.0 ^c	17.2 ± 0.5 ^d
β-Sitosterol	35.0 ± 0.1 ^a	58.3 ± 0.6 ^b	-	67.1 ± 1.6 ^d

Results expressed as the mean ± standard deviation (in relative% sterol) of duplicate or triplicate analyses. Values with different superscript in the same row are statistically different ($p < 0.05$).

† Sterol was not detected.

the SM and PB extracts showed differences in several fatty acids. The PB generally contained significantly higher levels of palmitic and linolenic acids but lower levels of oleic and palmitoleic acids. Interestingly, all of the extracts contained vaccenic acid, another omega-7 monounsaturated fatty acid, at moderately high but significantly similar percentages. *cis*-vaccenic acid has already been identified as a major fatty acid of the milkweed seeds (Turner & McKeon, 2002) but to our knowledge has yet to be reported for PB. Vaccenic acid is absent in most dietary systems but primary sources include ruminant/milk fat and sea buckthorn oil as *trans*- and *cis*-isomers, respectively (Destailats et al., 2005; Kamal-Elden & Andersson, 1997). Research using animal model systems has shown that a diet enriched with *trans*-vaccenic acid presented with lower risk factors for heart disease and obesity (Wang et al., 2008). These results were attributed in part to vaccenic acid's ability to reduce chylomicron production, which is considered to play a critical role in the onset of metabolic disorders. While other studies have linked *trans*-vaccenic acid to anticarcinogenic properties (Banni et al., 2001), related research on *cis*-vaccenic acid remains limited.

Sterol compositional analysis (free and esterified) of PB and SM co-products showed that most of the extracts contained predominantly β-sitosterol followed by campesterol, and stigmasterol for most of the extracts with the notable exception of the HX-PM (Table 4). Awad & Fink (2000) demonstrated that β-sitosterol, and possibly stigmasterol and campesterol, in-

creased apoptotic activity cells by affecting the sphingomyelin cycle, and the release of ceramide. Other studies have shown that phytosterols and their esters are effective in lowering total and low-density lipoprotein cholesterol in blood by inhibiting the absorption of cholesterol in the small intestine (Ostlund, 2004). As shown by Table 2, the highest levels of sterols were obtained from the SM using either DE or HX. Although the total levels were not significantly different between the two SM extracts, the sterol profiles were affected by the solvent system as the DE-SM contained higher relative percentages of β-sitosterol compared to the HX-SM but the latter sample contained higher stigmasterol and campesterol (Table 4). In addition, different compositional profiles were obtained for the PB extracts depending on the solvent system used (Table 4) but the DE-PB contained higher total levels compared to the HX-PB sample (Table 2). Nonetheless, the milkweed sterol levels were 25–95% lower than other typically consumed seeds and nuts (Phillips et al., 2005).

Analysis of the samples for vitamin E showed significantly higher levels in the SM co-product (~70 μg/g of product) relative to the PB (~30 μg/g of product) regardless of the solvent extraction system used (Table 1). α-Tocopherol was the most abundant tocopherol present in all the extracts followed by γ tocopherol (Table 5). δ-Tocopherol was not present in either the HX- or DE-PB extracts but these samples contained significantly higher levels of α-tocopherol with respect to the SM samples. Relative to many other seed oils, the milkweed

Table 5. Compositional profiles of vitamin E (tocopherol isomers) of each *Asclepias syriaca* L. co-product extracted with either hexane or diethyl ether.

Tocopherol isomer	Seed meal (hexane) (%)	Seed meal (diethyl ether) (%)	Pod biomass (hexane) (%)	Pod biomass (diethyl ether) (%)
α -Tocopherol	45.3 \pm 2.0 ^a	39.9 \pm 2.5 ^a	72.7 \pm 4.6 ^b	76.2 \pm 2.3 ^b
γ -Tocopherol	31.5 \pm 1.1 ^a	30.3 \pm 1.8 ^{ab}	27.2 \pm 4.6 ^{ab}	23.8 \pm 2.3 ^b
δ -Tocopherol	23.1 \pm 1.3 ^a	29.8 \pm 3.7 ^b	†-	-

Results expressed as the mean \pm standard deviation (in relative% tocopherol) of triplicate analyses. Values with different superscript in the same row are statistically different ($p < 0.05$).

† Tocopherol was not detected.

co-products contained higher tocopherol levels in $\mu\text{g/g}$ of oil, including sesame seed ($\sim 600 \mu\text{g/g}$), linseed ($\sim 600 \mu\text{g/g}$), rapeseed ($\sim 340 \mu\text{g/g}$), and sunflower, ($700 \mu\text{g/g}$), (Kamal-Elden & Andersson, 1997). For example, ~ 680 and $570 \mu\text{g/g}$ of lipid (data not shown in Table 1) were obtained from the HX- and DE-SM fractions, respectively, whereas lower amounts were obtained from the DE-PB ($\sim 585 \mu\text{g/g}$ of lipid) and the HX-PB extract ($\sim 725 \mu\text{g/g}$ of lipid) extracts. Vitamin E intake has been linked to the prevention of multiple diseases, including cardiovascular disease, cancer, diabetes, etc., due in part to its antioxidative properties. Many studies have indicated that γ -tocopherol is more efficacious in delivering antioxidative as well as non-antioxidative health benefits compared to the other tocopherols (Jiang et al., 2001). Although α -tocopherol was the most abundant vitamin E isomer in the milkweed co-products, the pressed SM co-product contained higher or comparable γ tocopherol to other typically consumed seed oils (Kamal-Elden & Andersson, 1997).

Carotenoids (the oil based pigments in photosynthetic organisms) also act as potent antioxidants by deactivating free radicals and singlet oxygen. Deficiency of dietary carotenes is a major cause of premature death in developing nations but elevated consumption may be harmful to certain populations (Alija et al., 2004). Other types of carotenoids have been shown to exert multiple health benefits including enhancing the immune system function (Bendich, 1988) and inhibiting the development of certain types of cancers (Nishino, 1998). Based upon these benefits, the milkweed co-products were examined for β -carotene and for total carotenoid levels (excluding the β -carotene). As listed in Table 2, β -carotene was present in only the PB samples, which in turn was affected by the extraction solvent with higher quantities obtained with HX compared to DE. Conversely, each co-product contained other carotenoids at levels that were not significantly different regardless of co-product or extraction solvent used (Table 2). Comparison with the literature further showed that the milkweed co-products are good sources of carotenoids with quantities higher than many seeds and plants (Holden et al., 1999).

Co-enzyme Q10 is another ubiquitous oil soluble vitamin that has been studied in regard to heart health, anti-ageing, neurological health, anti-cancer, diabetes prevention, etc., (Dhanasekaran & Ren, 2005). Although meat and marine based sources have been identified as containing the highest amounts of co-enzyme Q10 plant-based systems, such as spinach, broccoli, peanuts, wheat germ and whole grains, have also been shown to contain co-enzyme Q10 but at significantly lower levels. The milkweed co-product extracts were thus evaluated for co-enzyme Q10 but the vitamin was not detected in any sample (Table 2).

4. Conclusions

In summary, this study confirmed the presence of several potential health-benefiting simple lipid components in both the pressed SM and PB co-products. In particular, the fatty

acid data showed a unique compositional profile that could in combination with the other lipid components deliver optimal but different health benefits compared to other types of lipids. In addition, it is the SM co-product will more readily lend itself as an ingredient source for human health promoting dietary foods or supplements due to the presence of higher lipid levels. More research is needed to develop and optimize extraction processes that do not involve volatile solvents and to directly link specific lipid components in the common milkweed to scientifically supported health benefits.

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References

- Alija et al., 2004 ◀ A. J. Alija, N. Bresgen, O. Sommerburg, W. Siems and P. M. Eckl, Cytotoxic and genotoxic effects of β -carotene breakdown products on primary rat hepatocytes, *Carcinogenesis* **25** (2004), pp. 827–831.
- AOCS, 2006 ◀ AOCS (American Oil Chemists' Society). (2006). AOCS Official method Ca 5a-40. Free fatty acid. In *Official method and recommended practices of the American Oil Chemists' Society*. 5th ed. Champaign, Illinois, USA.
- Awad & Fink, 2000 ◀ A. B. Awad and C. S. Fink, Phytosterols as anticancer dietary components: Evidence and mechanism of action, *Journal of Nutrition* **130** (2000), pp. 2127–2130.
- Banni et al., 2001 ◀ S. Banni, E. Angioni, E. Murru, G. Carta, M. Melis and D. Bauman et al., Vaccenic acid feeding increases tissue levels of conjugated linoleic acid and suppresses development of premalignant lesions in rat mammary gland, *Nutrition and Cancer* **41** (2001), pp. 91–97.
- Bendich, 1988 ◀ A. Bendich, Carotenoids and the immune response, *Journal of Nutrition* **119** (1988), pp. 112–115.
- Cao et al., 2008 ◀ H. Cao, K. Gerhold, J. R. Mayers, M. M. Wiest, S. M. Watkins and G. S. Hotamisligil, Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism, *Cell* **134** (2008), pp. 933–944.
- Destaillets et al., 2005 ◀ R. Destaillets, E. Buyukpamukcu, P. -A. Goulay, F. Dionisi and F. Giuffrida, Vaccenic and rumenic acids, a distinct feature of ruminant fats, *Journal of Dairy Science* **88** (2005), p. 449.
- Dhanasekaran & Ren, 2005 ◀ M. Dhanasekaran and J. Ren, The emerging role of coenzyme Q-10 in aging, neurodegeneration, cardiovascular disease, cancer and diabetes mellitus, *Current Neurovascular Research* **2** (2005), pp. 447–459.
- Gouni-Berthold & Berthold, 2002 ◀ I. Gouni-Berthold and H. K. Berthold, Policosanol: Clinical pharmacology and therapeutic significance of a new lipid-lowering agent, *American Heart Journal* **143** (2002), pp. 356–365.
- Harry-O'kuru & Abbott, 1997 ◀ R. E. Harry-O'kuru and T. P. Abbott, Cardenolide analysis of cold-pressed milkweed oil, *Industrial Crops and Products* **7** (1997), pp. 508–530.
- Harry O'kuru et al., 1999 ◀ R. E. Harry O'kuru, H. Mojtahedi, S. F. Vaughan, P. F. Dowd, G. S. Santo and R. A. Holser et al., Milkweed seedmeal: A control for Meloidogyne chitwoodi on potatoes, *Industrial Crops and Products* **9** (1999), pp. 145–150.

- Harry-O'kuru & Carriere, 2002** ◀ R. E. Harry-O'kuru and C. J. Carriere, Synthesis, rheological characterization and constitutive modeling of polyhydroxy triglycerides derived from milkweed oil, *Journal of Agricultural and Food Chemistry* **50** (2002), pp. 3214–3221.
- Hegsted et al., 1965** ◀ D. M. Hegsted, R. B. McGandy, M. L. Myers and F. Stare, Quantitative effects of dietary fat on serum cholesterol in man, *American Journal of Clinical Nutrition* **17** (1965), pp. 281–295.
- Hiraoka-Yamamoto et al., 2004** ◀ J. H. Hiraoka-Yamamoto, K. Ikeda, H. Negishi, M. Mori, A. Hirose and S. Sawada *et al.*, Serum lipid effects of a monounsaturated (palmitoleic) fatty acid-rich diet based on macadamia nuts in healthy, young Japanese women, *Clinical and Experimental Pharmacology and Physiology* **31** (2004), pp. S37–S38.
- Holden et al., 1999** ◀ J. A. Holden, A. L. Eldridge, G. R. Beecher, S. B. Buzzard, C. S. Davis and L. W. Douglas *et al.*, Carotenoid content of US foods: An update of the database, *Journal of Food Composition and Analysis* **12** (1999), pp. 169–196.
- Hwang et al., 2002** ◀ K. T. Hwang, S. L. Cuppett, C. L. Weller, M. A. Hanna and R. Shoemaker, Aldehydes in grain sorghum wax, *Journal of the American Oil Chemists Society* **79** (2002), pp. 529–533.
- Irmak et al., 2006** ◀ E. Irmak, N. T. Dunford and J. Milligan, Policosanol contents of beeswax, sugar cane and wheat extracts, *Food Chemistry* **95** (2006), pp. 312–318.
- Jiang et al., 2001** ◀ Q. Jiang, S. Christen, M. K. Shigenaga and B. Ames, Gamma-tocopherol, the major form of vitamin E in the US diet deserves more attention, *American Journal of Clinical Nutrition* **74** (2001), pp. 714–722.
- Kamal-Elden & Andersson, 1997** ◀ A. Kamal-Elden and R. Andersson, A multivariate study of the correlation between tocopherol content and fatty acid composition in vegetable oils, *Journal of the American Oil Chemists Society* **74** (1997), pp. 375–380.
- Kasis et al., 2009** ◀ A. N. Kasis, E. S. Kubow, E. Peter and J. H. Jones, Sugar cane policosanols do not reduce LDL oxidation, *Lipids* **44** (2009), pp. 391–396.
- McCrorie & Bone, 2008** ◀ V. McCrorie and D. Bone, Fantastic future for functional foods, *Prepared Foods* **177** (2008), p. 70.
- Menendez et al., 2005** ◀ J. A. Menendez, L. Vellon, R. Colomer and R. Lupu, Oleic acid, the main monounsaturated fatty acid of olive oil, suppresses Her-2/*neu* (*erbB-2*) expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptin™) in breast cancer cells with Her-2/*neu* oncogene amplification, *Annals of Oncology* **16** (2005), pp. 359–371.
- Nishino, 1998** ◀ H. Nishino, Cancer prevention by carotenoids, *Mutation Research* **402** (1998), pp. 159–163.
- Ostlund, 2004** ◀ R. E. Ostlund Jr., Phytosterols and cholesterol metabolism, *Current Opinion in Lipidology* **15** (2004), pp. 37–41.
- Phillips et al., 2005** ◀ K. M. Phillips, D. M. Ruggio and M. Ashraf-Khorassani, Phytosterol composition of nuts and seeds commonly consumed in the United States, *Journal of Food Agriculture and Chemistry* **53** (2005), pp. 9436–9445.
- Schneider et al., 2000** ◀ C. L. Schneider, R. L. Cowles, C. L. Stuefer-Powell and T. P. Carr, Dietary stearic acid reduces cholesterol absorption and increases endogenous cholesterol excretion in hamster fed cereal based diets, *Journal of Nutrition* **130** (2000), pp. 1232–1283.
- Timmons, 1946** ◀ F. L. Timmons, Studies of the distribution and floss yield of common milkweed (*Asclepias Syriaca* L.) in Northern Michigan, *Ecology* **27** (1946), pp. 212–225.
- Terés et al., 2008** ◀ S. Terés, G. Barceló-Coblijn, M. Benet, R. Alvarez, R. Bressani and Je Halver *et al.*, Oleic acid content is responsible for reduction in blood pressure induced by olive oil, *Proceedings of the National Academy of Sciences of the United States of America* **105** (2008), p. 13811.
- Turner & McKeon, 2002** ◀ C. Turner and T. McKeon, The use of immobilized *Candida Antarctica* lipase for simultaneous super critical extraction and in-situ methylation of cis-vaccenic acid in milkweed seeds, *Journal of American Oil Chemist Society* **79** (2002), pp. 473–478.
- Vishwanath & Manning, 1968** ◀ M. S. Vishwanath and J. A. Manning, The determination of triglycerides in plasma and tissues, *Clinical Chemistry* **14** (1968), pp. 156–161.
- Wang et al., 2008** ◀ Y. Wang, M. M. Jacome-Sosa, R. R. Megan, S. D. Goruk, J. J. Reaney and D. R. Glimm *et al.*, Trans-11 vaccenic acid reduces hepatic lipogenesis and chylomicron secretion in JCR: LA-cp rats, *Journal of Nutrition* **139** (2008), pp. 2045–2049.