GC-MS and HPLC-DAD analysis of fatty acid profile and functional phytochemicals in fifty cold-pressed plant oils in Thailand

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ABSTRACT

Cold-pressed oil is one of the healthiest plant extracts, but its use is limited only in some kinds of plants. Therefore, we aimed to investigate some potential cold-pressed oils with attractive fatty acid profiles and high amounts of functional phytochemicals. Fifty cold-pressed plant oils were prepared from various plant materials in Thailand, in which some of them were from uncommon or unattended plant materials. The oils included were nut oils (n = 9), pseudo-cereal oils (n = 9), legume oils (n = 3), amaranth oils (n = 3), marrow seed oils (n = 8), cruciferous seed oils (n = 7), and leafy green seed oils (n = 11). Gas-chromatography mass-spectrometry (GC-MS) and high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD) were employed to analyze fatty acid profile and five functional phytochemicals (e.g., phytosterols, cholecalciferol, and squalene). Saturated fatty acids were detected around 7.87-36.04%, monounsaturated fatty acids 10.17-80.25%, and polyunsaturated fatty acids nondetectable (ND)-78.25%, phytosterols 663-15123 μg g⁻¹, squalene 265-5979 μg g⁻¹, and cholecalciferol ND-1287.75 μg g⁻¹. The study showed chemical characteristic of the analyzed oils: some contained good fatty acid composition and some were rich in functional phytochemical content. Among the obtained oils, marrow seed oils are a good source of phytosterol, cholecalciferol, and linoleic acid. Pseudo-cereal oils are rich in squalene and linolenic acid. Legume oils are rich in phytosterols and oleic acid. Besides, principal component analysis (PCA) was applied to identify the significance of oils that share compositional similarity (e.g., the samples from pseudo-cereal oil were found on the lower side of the PCA space, which separated them from marrow and leafy green seed oils distributed on the upper part of the plot). In summary, the qualitative and quantitative data would provide a good foundation for further application or selection of those plant oils for health purposes.

1. Introduction

Cold-pressed oils are oils obtained by passing plant materials through a screw press or hydraulic press with zero chemicals and no heat treatment, resulting in plant lipophilic extract with high retention of their color, flavor, and nutritive properties and minimum degradation of the functional components [1, 2]. In general, cold-pressed oils contain better nutritional value than commercially refined oils because the chemical and thermal treatments in the refining process such as solvent extraction, degumming, neutralizing with NaOH, bleaching, dewatering and deodorizing can cause degradation and reduction of some essential compounds as a consequence [3]. For instance, sunflower oil could lose up to 60% phytosterols, up to 55% tocoids and up to 60% squalene due to the refining procedure as reported in a study by Gotor and Rhazi [4]. Refining process could increase the acid value, peroxide value and p-anisidine value in rapeseed oil, together with the diminished content of tocopherols, sterols, β-carotene and phenols as previously evidenced [5]. In addition, phytosterols are progressively lost during refining while continuously altering the ratio of free and esterified sterols [6].

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From the perspective of functional compounds, cold-pressed oils are considered as one of the healthiest chemicals-free products commercially available. Lipophilic phytocomponents present in cold-pressed oils such as phytosterols, squalene, and cholecalciferol have been demonstrated to improve physiological and biological activity in human health. Phytosterols have displayed their abilities to reduce the level of cholesterol in the blood, increase metabolic rates, enhance fat burning, and reduce the incidence of some types of cancers [7]. Squalene has some favourable health benefits such as cancer inhibitor, antitumor, antioxidant in the skin, and has been applied in pharmaceutical and cosmetic industries. Cholecalciferol helps regulate and control absorbability of phosphorus and calcium, and, furthermore, it is an effective compound for managing blood sugar levels, thereby controlling diabetic conditions [8, 9]. In addition, nutritional value of plant oils is also evaluated by the amount of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) [10]. Oils rich in MUFAs, such as oleic acid (C18:1), had the potential to lower blood cholesterol levels and reduce the aortic accumulation of oxidized LDL without adversely affecting HDL fraction [11, 12]. PUFAs are essential for the human health and can only be received from a proper diet. The benefits of linoleic acid (C18:2) have been evidenced for cholesterol and triglyceride lowering effects, anti-inflammation, cardioprotection, and diabetes, and cancer prevention [13]. Meanwhile, linolenic acid (C18:3) has been known for its anti-inflammatory, anti-hypertensive, anti-thrombotic activities, and anticancer activities [14].

However, to date, the use of cold-pressed oils is restricted only in some kinds of plants. This is partly due to the difficulty in preparation of cold-press oil, including pre-treatment of plant oil material, shortage of material supply, and lack of knowledge about suitable plant material for oil extraction. In order to promote more use of cold-pressed plant oils, the study on nutritional information of other kinds of plant oils prepared from different plant materials is among one of the many possible solutions.

Therefore, this study is aimed at screening some potential cold-pressed oils with good nutritional properties by preparing a number of cold-pressed oils from various plant materials (total of 50 samples) and analyzing their chemical composition using chromatographic methods. Fatty acid compositions were quantitatively analyzed using gas chromatography mass-spectrometry (GC-MS). Functional phytocomponents, including phytosterols (3 forms), squalene, and cholecalciferol were analyzed using high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD). Principal component analysis (PCA) was applied to identify the significance of oil categories that share chemical similarity to the content of functional compounds and fatty acid composition. In some previous studies, PCA was successfully used to produce a clustering of variables and samples into distinct groups of oil samples such as the variation of fatty acid data on different soybean cultivars [15], and the variation of lipid composition of commercial olive oils [16]. The obtained quantitative data would be useful in the selection of plant materials for cold-press oil production or related applications for therapeutic and health purposes. The obtained quantitative data would be useful in the selection of plant materials for cold-press oil production or related applications for therapeutic and health purposes.

2. Materials and methods

2.1. Chemicals

Fatty acid standards (palmitic (C16:0, purity >97 %), palmitoleic (C16:1, purity >98 %), stearic (C18:0, purity >98 %), oleic (C18:1, purity >85 %), linoleic (C18:2, purity >85 %), linolenic (C18:3, purity >70 %), arachidic (C20:0, purity >98 %), and erucic (C22:1, purity >85 %) acids) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Stigmastanol standard and mixed standards of β-sitosterol (60%) and campesterol (40%), squalene (purity >98 %), and cholecalciferol (purity >98 %) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Methanol and hexane were of HPLC grade, and other reagents were of analytical grade (RCI Labscan Co., Ltd, Bangkok, Thailand).

2.2. Samples and cold extraction

Plant oils were extracted by cold-pressing from different plant parts of the plant material, specifically those which could yield oil. All plant materials were purchased and collected from the agricultural areas in Chiang Mai province (the Northern Thailand, latitudinally and longitudinally extended by 18°47′22.5″ N to 99°00′04.2″ E and 19°29′06.6″ N to 99°01′13.2″ E) (Table 1). Oils derived from those plant samples were classified into 3 main categories, including nut oils, grain oils, and vegetable oils. In the category of nut oils, plant materials were mostly kernel parts (9 samples). In the category of grain oils, subcategories were pseudo-cereals (9 samples), legumes (3 samples) and amaranth (3 samples). The category of vegetable oils contained subcategories of marrow, cruciferous and leafy green seeds (8, 7, and 11 samples, respectively). Before oil extraction, the plant samples were kept in zip-lock bags and stored at or below 4 °C in a refrigerator. In the preparation of oil from plant raw materials, each plant material has different processes depending on the plant type, such as peeling, sun-drying, roasting, grinding, extraction, and bottling as shown in Table 2 with the details are described below.

I) Peeling: to remove the hard shell and obtain kernels.
II) Sun-drying: to reduce the amount of moisture and dry the seeds by pouring the seeds into a tray and then exposed to the sun during 9.00 A.M to 4.00 P.M for 3 days.
III) Roasting: to heat at 160 °C set of the using a heating pan for 5–10 min depends on the type of raw material. The roasting was done until the color changed but not burnt.
IV) Grinding: to down-sizing the sample by pounding with a pestle until the dimension of sample was less than 0.5 cm in size.
V) Extraction: to extract oil from raw materials with cold-press machine.
VI) Bottling: to store the compressed oil into a glass bottle and store it in the refrigerator at below 4 °C.

The cold-press process was operated with a home automatic oil press machine (1500 W, General Equipment Co., Ltd. (Guangzhou, China)). The small or ground plant materials were put into the feeding part, allowing the materials to be pressed with a spiral screw in an extraction cylinder. The screw pressing was continuously driven by a motor, yielding oil as a result of high-pressure pressing and dry solid cake which the oil was excluded. The cold extraction occurred at a temperature of 40–50 °C, and the sample mass of each extraction in this study was between 500-1000 g of each plant material.

2.3. GC-MS analysis for fatty acid composition

For GC-MS analysis of fatty acid composition, the fatty acids in oil samples were converted to fatty acid methyl esters (FAMEs) according to the method of Ichihara and Fukubayashi [17] before analysis. Briefly, oil samples (0.015 g) were mixed with 0.20 mL of toluene, 1.50 mL of methanol and 0.30 mL of 8.0% (w/v) concentrated hydrochloric acid. The mixture was incubated at 100 °C for 5 min. Then, 1.00 mL of hexane and 1.00 mL of water was added and mixed with the mixture. The hexane layer (upper layer) was separated for analysis. Fatty acid composition in oil samples was analyzed using a 6890 GC/5973 MSD (Agilent, Polo Alto, CA). A HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm) was used, and helium as carrier gas was flowed at 1 mL min⁻¹. The energy of electron impact was 70 eV. The inlet temperature was 280 °C, and injection volume was 1.0 μL with a split ratio of 15:1. The column temperature was set at 165 °C, followed by a 4 °C min⁻¹ oven temperature ramped to 290 °C. The transfer line temperature was 290 °C, and the
The resulting solution was diluted with dichloromethane to make a final volume of 1.00 mL. HPLC-DAD analysis for functional phytochemicals as well as method validation parameters are shown in Table 3. NIST 98 Mass Spectral Library and comparing retention times with their mass spectra in reference spectra in many studies [1, 18]. A portion of oil (0.5 g) was significantly degraded due to high temperature, alkali condition, and its multi-step procedure as evidenced by many studies [1, 18].

Pistacia vera L. was used as an analytical column. The mobile phase was set at 1150 V. Identification was performed by matching their mass spectra with the reference spectra in the NIST 98 Mass Spectral Library and comparing retention times with their mass spectra in reference spectra in many studies [1, 18].

2.5. Statistical analysis

All quantitative determinations were done in triplicates, and the data are presented as the mean ± standard deviation. Statistical analysis was done using one-way ANOVA. Differences at P < 0.05 were considered statistically significant. Principal component analysis (PCA) modeling was calculated based on the algorithm presented [19]. PCA is among the common exploratory data analysis methods in chemometrics. PCA establishes a set of new variables, called principal components (PCs), to represent the main and important variation in data. The mathematical transformation of an original data matrix (X) by PCA can be expressed as the following equation as mentioned in a study of Brereton, R. G. [20].

\[ X = TP + E \]  

(1)

According to equation (1), T is a score matrix, representing the relationship among the studied samples. On the other hand, P is a loading matrix, visualizing the behavior among the parameters. E is a residual matrix, containing the variation which is excluded from the analysis. The constructed calibration curves for the targeted functional phytochemicals as well as method validation parameters are shown in Table 3.
Table 2. Preparation process of sample oil.

| Oils | I) Peeling | II) Sun-drying | III) Roasting | IV) Grinding | V) Extraction | VI) Bottling |
|------|-------------|----------------|--------------|--------------|---------------|--------------|
| Nut/Kernel oil (9 samples) | | | | | | |
| Almond | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Cashew | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Coconut | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Hazelnut | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Macadamia | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Moringa seed | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Sunflower seed | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Pistachio | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Walnut | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Pseudo-cereal oil (9 samples) | | | | | | |
| Black chia seed | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Black sesame | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Brown flaxseed | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Coriander | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Gold flaxseed | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Hairy basil | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Perilla | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| White chia seed | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| White sesame | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Legume oil (3 samples) | | | | | | |
| Inca peanut | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Soybean | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Peanut | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Amaranth oil (3 samples) | | | | | | |
| Green amaranth | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Love-lies-bleeding | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Spiny amaranth | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Marrow seed oil (8 samples) | | | | | | |
| Angled loofah | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Cucumber | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Long skinny eggplant | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Muskmelon | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Pumpkin | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Wax gourd | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Winter melon | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Watermelon | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Cruciferous seed oil (7 samples) | | | | | | |
| Broccoli | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Cauliflower | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Chinese cabbage | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Chinese kale | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Chinese radish | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Curly kale | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Hong Kong Chinese kale | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Leafy green seed oil (11 samples) | | | | | | |
| Chinese flowering cabbage | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Chinese green mustard | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Chinese mustard | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| False Pak Choi (Phukeaw) | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| False Pak Choi (Inthanon) | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Flowering Pak Choi (1) | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Flowering Pak Choi (2) | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Green Pak Choi | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Indian mustard | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Lettuce | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Rat-tailed radish | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
3. Results and discussion

3.1. GC-MS analysis for fatty acid compositions in cold-pressed oils

3.1.1. Analysis of fatty acid composition using GC-MS

GC-MS chromatograms of FAMEs in some cold-pressed oils are shown in Figure 1. Using the method, good separation of FAMEs was obtained as methyl palmitoleate (FAME of palmitoleic acid) was eluted at 7.51 min, methyl palmitate (of palmitic acid) at 7.85 min, methyl linoleate (of linoleic acid) at 10.89 min, methyl oleate (of oleic acid) at 10.98 min, methyl linolenate (of linolenic acid) at 11.04 min, methyl stearate (of stearic acid) at 11.46 min, methyl arachidate (of arachidic acid) at 15.31 min, and methyl erucate (of erucic acid) at 18.75 min. Mass spectra of the detected FAMEs and information of their major fragmented ions were exhibited in Figure 2. According to Figure 1, composition of the fatty acids differed among samples. For example, black chia seed oil contained palmitic, linoleic, oleic, linolenic, stearic, and arachidic acids whilst false Pak Choi (Phukeaw) had palmitic, linoleic, oleic, stearic, erucic and arachidic acids. The result showed that a single run of FAME analysis required only 22 min, suggesting the present method suitable for FAME analysis of a large number of oil samples.

Table 3. Method validation results for determination of fatty acids, phytosterols, squalene, and cholecalciferol.

| Compound            | Regression equation | R²  | Linearity  | LOD (μg mL⁻¹) | LOQ (μg mL⁻¹) | Recovery (%) | Precision (intra-day) |
|---------------------|---------------------|-----|------------|---------------|---------------|--------------|-----------------------|
| Palmitic acid       | y = 432.25 x - 222274 | 0.8217 | valid      | 27.20         | 90.68         | 96.8         | 0.22                  |
| Steric acid         | y = 435.70 x + 166531 | 0.9747 | valid      | 7.39          | 24.62         | 90.4         | 0.76                  |
| Arachidic acid      | y = 522.90 x + 254107 | 0.9469 | valid      | 7.31          | 24.35         | 100.0        | 0.03                  |
| Palmitoleic acid    | y = -530.92 x - 105262 | 0.9763 | valid      | 35.44         | 118.13        | 98.7         | 0.03                  |
| Oleic acid          | y = 435.15 x + 162147 | 0.9867 | valid      | 8.04          | 26.80         | 83.8         | 0.51                  |
| Erucic acid         | y = -435.15 x + 162147 | 0.9867 | valid      | 3.90          | 13.01         | 88.7         | 1.16                  |
| Linoleic acid       | y = 394.34 x - 258209 | 0.9662 | valid      | 10.32         | 34.40         | 92.0         | 0.35                  |
| Linolenic acid      | y = 401.67 x + 131010 | 0.9981 | valid      | 21.88         | 72.92         | 92.6         | 0.63                  |
| Stigmasterol + Campesterol | y = -2.1737 x + 250.45 | 0.9901 | valid      | 0.42          | 1.26          | 99.2         | 2.20                  |
| β-Sitostanol        | y = 0.4587 x + 123.60 | 0.9919 | valid      | 0.36          | 1.04          | 98.6         | 2.02                  |
| Squalene            | y = 1.780 x + 32.819 | 0.9906 | valid      | 0.09          | 0.28          | 100.6        | 1.91                  |
| Cholecalciferol     | y = -14.803 x + 11.001 | 0.9956 | valid      | 0.31          | 0.95          | 99.0         | 0.17                  |

* Linearity assessment was performed by Lack of fit, FIUPAC, and Mandel’s fitting test.
* LOD was limit of detection (signal-to-noise ratio of 3).
* LOQ was limit of quantification (signal-to-noise ratio of 10).
* Percentage of relative standard deviation (%).

3. Results and discussion

3.1. GC-MS analysis for fatty acid compositions in cold-pressed oils

3.1.1. Analysis of fatty acid composition using GC-MS

Figure 1. GC-MS chromatograms of fatty acid methyl esters (FAMEs). (A) Standard fatty acids, (B) moringa seeds oil (C) brown flaxseeds oil, (D) soybean oil, (E) green amaranth seed oil, (F) cucumber seed oil (G) Chinese cabbage seed oil, and (H) False Pak Choi (Phukeaw) seed oil. Peak identification: 1 = methyl palmitoleate, 2 = methyl palmitate, 3 = methyl linoleate, 4 = methyl oleate, 5 = methyl linolenate, 6 = methyl stearate, 7 = methyl arachidate, and 8 = methyl erucate.
Figure 2. MS spectra of FAMEs of analyzed fatty acids found in cold-pressed oils. (A) Methyl palmitoleate, (B) methyl palmitate, (C) methyl linoleate, (D) methyl oleate, (E) methyl linolenate, (F) methyl stearate, (G) methyl arachidate, and (H) methyl erucate.
| Oils                                    | Saturated fatty acids (SFAs) | Monounsaturated fatty acids (MUFAs) | Polyunsaturated fatty acids (PUFAs) | Others |
|----------------------------------------|-----------------------------|-------------------------------------|-------------------------------------|--------|
|                                        | Palmitic acid (C16:0)       | Stearic acid (C18:0)                | Arachidic acid (C20:0)              |        |
|                                        | (C16:0)                     | (C18:0)                             | (C20:0)                             |        |
| Almond                                 | 19.21 ± 0.86                | 2.87 ± 0.56                         | ND                                  |        |
| Cashew                                 | 14.61 ± 0.23                | 8.75 ± 2.74                         | 0.43 ± 0.37                         |        |
| Coconut                                | 21.47 ± 0.05                | 5.56 ± 0.27                         | ND                                  |        |
| Hazelnut                               | 7.95 ± 0.43                 | 2.11 ± 1.52                         | ND                                  |        |
| Macadamia                              | 12.02 ± 0.08                | 2.78 ± 1.27                         | ND                                  |        |
| Moringa seed                           | 13.42 ± 0.32                | 2.83 ± 2.22                         | 0.91 ± 0.81                         |        |
| Sunflower seed                         | 11.30 ± 0.13                | 3.50 ± 0.63                         | ND                                  |        |
| Pistachio                              | 10.73 ± 0.05                | 0.30 ± 0.52                         | ND                                  |        |
| Walnut                                 | 12.50 ± 0.48                | 1.57 ± 0.96                         | ND                                  |        |
| Black chia seed                        | 9.85 ± 0.54                 | 3.25 ± 1.92                         | 0.16 ± 0.16                         |        |
| Black sesame                           | 9.97 ± 0.35                 | 4.48 ± 1.09                         | 0.26 ± 0.10                         |        |
| Brown flaxseed                         | 6.59 ± 0.33                 | 4.65 ± 2.35                         | 0.16 ± 0.11                         |        |
| Coriander                              | 10.72 ± 0.07                | 2.78 ± 0.42                         | 0.40 ± 0.11                         |        |
| Gold flaxseed                          | 8.60 ± 0.18                 | 2.68 ± 0.74                         | 0.59 ± 0.61                         |        |
| Hairy basil                            | 8.66 ± 0.48                 | 3.16 ± 1.93                         | 0.06 ± 0.01                         |        |
| Perilla                                | 10.96 ± 0.17                | 2.64 ± 0.35                         | 0.77 ± 0.46                         |        |
| White chia seed                        | 12.27 ± 0.52                | 3.74 ± 1.29                         | 0.39 ± 0.28                         |        |
| Inca peanut                            | 10.74 ± 0.64                | 2.74 ± 1.49                         | ND                                  |        |
| Soybean                                | 8.84 ± 0.33                 | 2.68 ± 0.78                         | ND                                  |        |
| Peanut                                 | 12.32 ± 0.03                | 5.00 ± 1.59                         | 0.08 ± 0.03                         |        |
| Green amaranth                         | 25.10 ± 0.22                | 2.96 ± 2.23                         | 0.53 ± 0.75                         |        |
| Love-likes-bleeding                    | 11.21 ± 0.34                | 2.04 ± 0.45                         | 0.32 ± 0.08                         |        |
| Spiny amaranth                         | 27.39 ± 0.96                | 3.04 ± 0.87                         | 0.99 ± 0.16                         |        |
| Angled loofah                          | 23.71 ± 0.87                | 5.44 ± 2.43                         | ND                                  |        |
| Cucumber                               | 14.04 ± 0.01                | 7.46 ± 4.32                         | ND                                  |        |
| Long skinny eggplant                   | 16.29 ± 0.03                | 6.00 ± 0.89                         | ND                                  |        |
| Muskmealon                             | 20.10 ± 0.29                | 8.95 ± 0.29                         | ND                                  |        |
| Pumpkin                                | 21.40 ± 0.35                | 3.84 ± 0.82                         | ND                                  |        |
| Wax gourd                              | 20.60 ± 0.15                | 1.59 ± 0.62                         | ND                                  |        |
| Winter melon                           | 21.33 ± 0.77                | 3.05 ± 2.33                         | ND                                  |        |
| Watermelon                             | 16.20 ± 0.14                | 2.39 ± 1.79                         | ND                                  |        |
| Broccoli                               | 8.25 ± 0.01                 | ND                                  | ND                                  |        |
| Cauliflower                            | 30.08 ± 0.83                | ND                                  | ND                                  |        |
| Chinese cabbage                        | 8.19 ± 0.20                 | 0.34 ± 0.03                         | ND                                  |        |
| Chinese kale                           | 14.14 ± 0.49                | 0.56 ± 0.66                         | ND                                  |        |
| Chinese radish                         | 33.50 ± 0.32                | ND                                  | ND                                  |        |
| Curly kale                             | 22.31 ± 0.59                | 4.29 ± 0.72                         | ND                                  |        |
| Hong Kong Chinese kale                 | 9.75 ± 0.41                 | ND                                  | ND                                  |        |
| Chinese flowering cabbage             | 19.37 ± 0.43                | 0.83 ± 0.75                         | ND                                  |        |
| Chinese green mustard                 | 19.98 ± 0.72                | ND                                  | ND                                  |        |
| Chinese mustard                       | 14.80 ± 0.30                | 1.23 ± 0.49                         | ND                                  |        |
| False Pak Choi (Phukeaw)               | 6.08 ± 0.23                 | 1.42 ± 0.82                         | 0.37 ± 0.36                         |        |
| False Pak Choi (Inthanon)              | 6.49 ± 0.21                 | 1.34 ± 0.16                         | 0.09 ± 0.04                         |        |
| Flowering Pak Choi (1)                 | 19.04 ± 0.34                | 5.23 ± 0.37                         | ND                                  |        |
| Flowering Pak Choi (2)                 | 20.37 ± 0.72                | 10.77 ± 0.47                        | 4.90 ± 0.00                         |        |
| Chinese flowering cabbage             | 19.37 ± 0.43                | 0.83 ± 0.75                         | ND                                  |        |
| Chinese green mustard                 | 19.98 ± 0.72                | ND                                  | ND                                  |        |
| Chinese mustard                       | 14.80 ± 0.30                | 1.23 ± 0.49                         | ND                                  |        |
| False Pak Choi (Phukeaw)               | 6.08 ± 0.23                 | 1.42 ± 0.82                         | 0.37 ± 0.36                         |        |
| False Pak Choi (Inthanon)              | 6.49 ± 0.21                 | 1.34 ± 0.16                         | 0.09 ± 0.04                         |        |
| Flowering Pak Choi (1)                 | 19.04 ± 0.34                | 5.23 ± 0.37                         | ND                                  |        |
| Flowering Pak Choi (2)                 | 20.37 ± 0.72                | 10.77 ± 0.47                        | 4.90 ± 0.00                         |        |

(continued on next page)
3.1.2. Composition of fatty acid in fifty cold pressed oils

The fatty acid compositions of the various oil samples are presented in Table 4. The fatty acids were found in ranges from nondetectable to 13.38% for palmitoleic acid (average of 3.52%), 6.08–33.50% for palmitic acid (average of 15.63%), nondetectable to 60.33% for linoleic acid (average of 25.85%), 8.91–78.21% for oleic acid (average of 27.31%), nondetectable to 56.68% for linolenic acid (average of 40.66%), nondetectable to 10.77% for stearic acid (average of 2.96%), nondetectable to 4.90% for arachidic acid (average of 0.28%), and nondetectable to 54.72% for erucic acid (average of 27.00%). Among the fatty acids observed, the major species were palmitic, oleic, linoleic, and linolenic acids. The minor fatty acids were palmitoleic, stearic, arachidic, and erucic acids that were found only in some plant oils. For instance, palmitoleic acid were detected only in 6 plant oils such as macadamia, moringa, pistachio, black sesame, white sesame, and peanut oils. Linolenic acid was mainly found in pseudo-cereal oils and legume oils such as brown flaxseed, perilla and inca peanut oils. Arachidic acid was mostly found in pseudo-cereal and amaranth oils but with relatively low amount of less than 1.00%.

3.1.2.1. Proportion of PUFA:s:MUFA:s:SFAs

In this study, all the oil samples had a large proportion of PUFAs (10.14–62.98%) and MUFAs (22.77–67.34%) but relatively small proportion of SFAs (14.25–26.62%). Table 5 shows compositional percentage of saturated fatty acids (SFAs, including palmitic, stearic, and arachidic acids), MUFAs (including palmitoleic, oleic, and erucic acids), and PUFAs (including linoleic and linolenic acids) in the oil samples. Oils from pseudo-cereals and legumes contained relatively low amount of SFAs as 14.25 and 15.64%, respectively. The oils from pseudo-cereals consisted of high amount of unsaturated fatty acids, especially PUFAs (62.98%), while the oils from kernel, cruciferous seeds, and leafy green seeds were rich in MUFAs (55.68–67.34%). The oils from amaranth and marrow seeds were comparable in ratios of SFAs: MUFAs: PUFA:s (Table 5). The oils from kernel, cruciferous seeds, and leafy green seeds tended to have MUFAs (55.68–67.34%) > PUFAs (10.14–24.69%), but the oils from pseudo-cereal, legumes, amaranths, and marrow seeds had PUFAs (47.23–62.98%) > MUFAs (22.77–31.41%). The UFAs and SFAs ratio of the oil can be used in selection of appropriate oil for the specific application. For instance, oils used for deep-frying should have high content of SFAs as 14.25% and 15.64% and PUFAs > MUFAs (62.98%) to be more stable when heated. Besides the degree of unsaturation, the types of predominant fatty acids were compared (Table 6). The major fatty acids observed in the sample oils were palmitic acid, oleic acid, linoleic acid and linolenic acid. Linolenic acid was abundant in pseudo-cereal oils (33.98%). Linoleic acid was found rich in amaranth and marrow seed oils (49.46% and 47.23%, respectively). The oils from kernels were great in oleic acid (C18:1) as 50.41%. The minor fatty acids were found in pseudo-cereal oils and legume oils such as macadamia, moringa, pistachio, black sesame, white sesame, and peanut oils. Linolenic acid was found in pseudo-cereal and amaranth oils but with relatively low amount of less than 1.00%.

Table 4 (continued)

| Oils                  | Saturated fatty acids (SFAs) | Monounsaturated fatty acids (MUFAs) | Polysaturated fatty acids (PUFAs) |
|----------------------|-----------------------------|-------------------------------------|----------------------------------|
|                      | Palmitic acid (C16:0)       | Stearic acid (C18:0)                | Arachidic acid (C20:0)           |
|                      | Oleic acid (C18:1)          | Linoleic acid (C18:2)               | Linolenic acid (C18:3)           |
| Green Pak Choi       | 24.07 ± 0.55 ND            | ND                                  | ND                               |
| Indian mustard       | 28.12 ± 0.85 ND            | ND                                  | ND                               |
| Lettuce              | 15.36 ± 0.53 2.51 ± 0.15   | 0.01 ± 0.00 ND                      | ND                               |
| Rat-tailed radish    | 18.87 ± 4.50 ND            | ND                                  | ND                               |

ND = non-detectable.

Table 5. Percentage of saturated fatty acids, monounsaturated fatty acids, and polysaturated fatty acids in oil samples.

| Oil                 | Saturated fatty acids (SFAs) | Monounsaturated fatty acids (MUFAs) | Polysaturated fatty acids (PUFAs) |
|---------------------|-----------------------------|-------------------------------------|----------------------------------|
|                     | 19.63                        | 55.68                               | 24.69                            |
| Pseudo-cereal oil   | 14.25                        | 22.77                               | 62.98                            |
| Legume oil          | 15.64                        | 31.41                               | 52.95                            |
| Amaranth oil        | 24.97                        | 25.57                               | 49.46                            |
| Marrow seed oil     | 26.62                        | 26.16                               | 47.23                            |
| Cruciferous seed oil| 22.52                        | 67.34                               | 10.14                            |
| Leafy green seed oil| 22.92                        | 60.32                               | 16.76                            |

Table 6. Average content of fatty acids in each oil group.

| Oil                  | Major fatty acid            | Minor fatty acid                    |
|----------------------|-----------------------------|-------------------------------------|
|                     | Palmitic acid               | Oleic acid | Linoleic acid | Linolenic acid |
|                     | 13.69                       | 50.41     | 23.50        | 1.19          |
|                     | 10.02                       | 21.10     | 29.01        | 33.98         |
|                     | 10.60                       | 30.34     | 36.47        | 16.48         |
|                     | 21.23                       | 15.33     | 47.23        | ND            |
|                     | 19.21                       | 23.99     | 10.14        | ND            |
|                     | 18.03                       | 23.99     | 10.14        | ND            |
|                     | 17.50                       | 23.99     | 16.76        | ND            |

ND = non-detectable.
fatty acids were stearic acid, arachidic acid, palmitoleic acid and erucic acid. Although some minor fatty acids were present in small amounts, it may bear some significant health effect. For instance, erucic acid that were greatly found in cruciferous and leafy green seed oils have been evidenced for it adverse health effects against human organs such as the heart (myocardial lipoidosis) [21].

Furthermore, all cold-pressed oils were individually assessed for health benefits based on their major fatty acid composition (Table 4). Considering legume oils, linolenic acid was found highest in Inca peanut oil (43.42%), and linoleic acid in soybean oil (50.31%). Peanut oil was highest in oleic acid (47.60%). The group of pseudo-cereal oils were relatively high in PUFAs (26.66–78.25%), in which linolenic acid was found highest in brown flaxseed oil (56.68%), and highest linoleic acid in black sesame oil (50.62%). The highest oleic acid was observed in coriander oil (50.86%). In the group of kernel oils, PUFAs was rather low when compared with the two previous groups. The observed PUFA was only linolenic acid in walnut oil (10.73%), but MUFAs were quite abundant as oleic acid content for moringa seed oil and hazelnut oil was 78.21% and 73.19%, respectively. The oils from amaranth and marrow seeds had a comparable decent amount of linoleic acid (39.17–59.24%), but oleic acid was found higher in amaranth oils (24.14–27.07%) than marrow seed oils (8.91–25.39%). In contrast, oils from subcategories of cruciferous seeds and leafy green seeds tended to have mediocre profile of PUFAs (nondetectable-30.35%) except lettuce seed oil (linoleic acid as 60.33%). Erucic acid (C22:1) was observed as a predominant fatty acid in this groups, in which the oils from Chinese mustard seeds, Chinese cabbage seeds, broccoli seeds and false Pak Choi (Inthanon) seeds contained more than 50% of their fatty acid composition.

The fatty acid profiles obtained in this study was compared with some of the same cold-pressed oils that had been reported in the literature [22]. In general, the content of fatty acid profiles from our results was comparable with those in the literature. The oils with both predominantly polyunsaturated linoleic acid and monounsaturated oleic acid were sesame oils (our contents were 49.43–50.62% and 30.06–32.34 %, when the literature showed 46.20% and 38.80% of linoleic acid and oleic acid, respectively), amaranth oils (ours were 44.12–59.24% and 24.14–27.07%, while those of the literature were 40.10–47.30% and 24.50–31.30%), pistachio oil (ours were 40.26% and 43.97%, when those of the literature were 31.33% and 54.59%), and peanut oil (ours were 33.08% and 47.60%, when those of the literature were 20.40–39.10% and 35.70–55.30%). The oils rich in oleic acid were moringa seed oil (our oleic acid content as 78.21%, and that of the literature as 78.21%), but MUFAs were quite abundant as oleic acid content for moringa seed oil and hazelnut oil was 78.21% and 73.19%, respectively. The oils from amaranth and marrow seeds had a comparable decent amount of linoleic acid (39.17–59.24%), but oleic acid was found higher in amaranth oils (24.14–27.07%) than marrow seed oils (8.91–25.39%). In contrast, oils from subcategories of cruciferous seeds and leafy green seeds tended to have mediocre profile of PUFAs (nondetectable-30.35%) except lettuce seed oil (linoleic acid as 60.33%). Erucic acid (C22:1) was observed as a predominant fatty acid in this groups, in which the oils from Chinese mustard seeds, Chinese

Figure 3. HPLC chromatograms of analyzed cold-pressed oils. (A) Standard solution, (B) pistachio oil, (C) coriander seed oil, and (D) soybean oil, (E) green amaranth seed oil, (F) long skinny eggplant seed oil, (G) cauliflower seed oil, and (H) lettuce seed oil. Peak identification: 1 = cholecalciferol, 2 = stigmasterol + campesterol, 3 = β-sitosterol, and 4 = squalene.
Table 7. Content of bioactive compounds (present as bioactive groups) in each cold-pressed plant oil (μg g⁻¹).

| Nut/Kernel oil (9 samples) | Total Phytosterols | Squalene | Cholecalciferol (Vitamin D₃) | Total Bioactive compounds |
|----------------------------|--------------------|----------|----------------------------|--------------------------|
| 1  | Almond             | 2135 ± 0.35 | 923 ± 0.13 | 29.44 ± 0.10 | 3087        |
| 2  | Cashew             | 705 ± 1.22  | 265 ± 3.08 | ND            | 970         |
| 3  | Coconut            | 2431 ± 1.75 | 702 ± 0.14 | 106.14 ± 0.15 | 3239        |
| 4  | Hazelnut           | 6062 ± 2.27 | 2356 ± 5.14 | 78.03 ± 0.29 | 8496        |
| 5  | Macadamia         | 2400 ± 1.09  | 549 ± 0.18 | 76.58 ± 0.34 | 3026        |
| 6  | Moringa seed       | 2650 ± 0.66  | 671 ± 0.19 | 84.56 ± 0.20 | 3406        |
| 7  | Sunflower seed     | 2999 ± 1.74  | 1537 ± 0.41 | 73.71 ± 0.61 | 4610        |
| 8  | Pistachio          | 1200 ± 0.76  | 2568 ± 0.20 | 11.93 ± 0.23 | 3780        |
| 9  | Walnut             | 2479 ± 0.42  | 1185 ± 0.25 | 48.21 ± 0.46 | 3712        |
| 10 | Black chia seed    | 3684 ± 1.88  | 5625 ± 1.70 | 90.33 ± 0.69 | 9399        |
| 11 | Black sesame       | 2690 ± 1.73  | 1171 ± 0.47 | 650.30 ± 7.10 | 4511        |
| 12 | Brown flaxseed     | 3548 ± 1.52  | 4464 ± 0.14 | 28.58 ± 0.20 | 8041        |
| 13 | Coriander          | 3029 ± 0.92  | 1577 ± 0.14 | 520.83 ± 0.60 | 5127        |
| 14 | Gold flaxseed      | 4328 ± 1.50  | 2485 ± 0.17 | ND            | 6813        |
| 15 | Hairy basil        | 3719 ± 1.61  | 5868 ± 0.28 | 66.37 ± 0.18 | 9653        |
| 16 | Perilla            | 4129 ± 0.36  | 4974 ± 1.18 | 75.19 ± 0.06 | 9178        |
| 17 | White chia seed    | 3903 ± 0.67  | 2278 ± 0.51 | 55.14 ± 0.08 | 6236        |
| 18 | White sesame       | 2565 ± 1.66  | 2611 ± 0.29 | 59.11 ± 1.58 | 5235        |
| 19 | Inca peanut        | 6258 ± 1.23  | 1341 ± 0.15 | 345.04 ± 1.35 | 7944        |
| 20 | Soybean            | 5070 ± 0.75  | 2535 ± 0.45 | ND            | 7605        |
| 21 | Peanut             | 5368 ± 0.43  | 773 ± 1.42  | 124.98 ± 0.21 | 6266        |
| 22 | Green amaranth     | 2326 ± 1.41  | 671 ± 0.15  | 0.63 ± 0.11   | 2998        |
| 23 | Love-lies-bleeding | 2336 ± 1.49  | 788 ± 0.44  | 48.28 ± 0.10  | 3172        |
| 24 | Spiny amaranth     | 2213 ± 1.00  | 725 ± 5.00  | 22.44 ± 0.20  | 2960        |
| 25 | Angled loofah      | 1477 ± 1.41  | 803 ± 0.31  | 1287.75 ± 2.47 | 3568        |
| 26 | Cucumber           | 9814 ± 0.34  | 891 ± 0.42  | 186.39 ± 1.00 | 10891       |
| 27 | Long skinny eggplant | 3397 ± 1.09  | 1801 ± 0.08 | 632.14 ± 4.81 | 5830        |
| 28 | Muskemelon         | 8845 ± 2.57  | 1072 ± 0.44 | 25.18 ± 0.18  | 9942        |
| 29 | Pumpkin            | 6738 ± 0.84  | 516 ± 0.32  | 107.75 ± 1.81 | 7362        |
| 30 | Wax gourd          | 15123 ± 1.60 | 2162 ± 0.34 | 619.02 ± 0.55 | 17904       |
| 31 | Winter melon       | 13138 ± 1.59 | 5979 ± 3.26 | 815.99 ± 0.20 | 19933       |
| 32 | Watermelon         | 11012 ± 0.84 | 3183 ± 0.68 | 98.55 ± 0.09  | 14294       |
| 33 | Broccoli           | 1386 ± 0.66  | 419 ± 0.18  | 16.46 ± 0.10  | 1821        |
| 34 | Cauliflower        | 1365 ± 0.62  | 1174 ± 4.25 | 30.74 ± 0.50  | 2570        |
| 35 | Chinese cabbage    | 1711 ± 1.16  | 2027 ± 1.44 | 102.84 ± 0.42 | 3841        |
| 36 | Chinese kale       | 992 ± 1.02   | 456 ± 0.41  | 41.21 ± 0.09  | 1489        |
| 37 | Chinese radish     | 6666 ± 1.02  | 648 ± 0.43  | 50.67 ± 0.24  | 7365        |
| 38 | Curly kale         | 7504 ± 1.96  | 971 ± 0.15  | 44.85 ± 0.52  | 8520        |
| 39 | Hong Kong Chinese kale | 1435 ± 0.56  | 397 ± 0.74  | 49.64 ± 2.22  | 1882        |
| 40 | Chinese flowering cabbage | 758 ± 2.03  | 268 ± 0.24  | 39.64 ± 0.10  | 1066        |
| 41 | Chinese green mustard | 818 ± 1.29  | 301 ± 1.28  | 3.88 ± 0.23   | 1123        |
| 42 | Chinese mustard    | 749 ± 1.79   | 308 ± 0.70  | 17.39 ± 0.53  | 1074        |
| 43 | False Pak Choi (Phukeaw) | 698 ± 1.59  | 331 ± 0.75  | 32.42 ± 0.18  | 1061        |
| 44 | False Pak Choi (Inthanon) | 663 ± 0.91  | 297 ± 0.16  | 31.60 ± 0.39  | 992         |
| 45 | Flowering Pak Choi (1) | 1318 ± 0.95  | 364 ± 0.10  | 46.36 ± 0.27  | 1728        |
| 46 | Flowering Pak Choi (2) | 1972 ± 0.77  | 445 ± 2.00  | 55.74 ± 0.31  | 2473        |
| 47 | Green Pak Choi     | 944 ± 0.88   | 329 ± 3.67  | 36.29 ± 0.11  | 1309        |
| 48 | Indian mustard     | 909 ± 0.37   | 288 ± 1.71  | 15.07 ± 0.43  | 1212        |
| 49 | Lettuce            | 2550 ± 0.97  | 754 ± 0.67  | 61.10 ± 0.19  | 3365        |
| 50 | Rat-tailed radish  | 7189 ± 1.14  | 750 ± 0.32  | 84.49 ± 0.07  | 8023        |

ND = non-detectable.
### Table 8. Content of individual phytosterol detected in each cold-pressed oil (μg g⁻¹).

| Oils                        | Phytosterols                  | Total Phytosterols |
|-----------------------------|-------------------------------|--------------------|
|                            | Stigmasterol & Campesterol    | β-Sitosterol        |
| Nut/Kernel oil (9 samples)  |                               |                    |
| Almond                      | 261 ± 0.14                    | 1874 ± 0.40        | 2135 ± 0.35 |
| Cashew                      | 47 ± 0.68                     | 658 ± 0.54         | 705 ± 1.22 |
| Coconut                     | 409 ± 0.17                    | 2022 ± 1.58        | 2431 ± 1.75 |
| Hazelnut                    | 676 ± 0.70                    | 5386 ± 1.91        | 6062 ± 2.27 |
| Macadamia                   | 290 ± 0.72                    | 2110 ± 0.47        | 2400 ± 1.09 |
| Moringa seed                | 285 ± 0.31                    | 2565 ± 0.97        | 2650 ± 0.66 |
| Sunflower seed              | 236 ± 0.25                    | 2763 ± 1.49        | 2999 ± 1.74 |
| Pistachio                   | 137 ± 0.55                    | 1063 ± 0.22        | 1200 ± 0.76 |
| Walnut                      | 106 ± 0.24                    | 2373 ± 0.34        | 2479 ± 0.42 |
| Pseudo-cereal oil (9 samples) |                               |                    |
| Black chia seed             | 922 ± 0.13                    | 3484 ± 1.51        | 3684 ± 1.88 |
| Black sesame                | 74 ± 0.12                     | 1768 ± 1.60        | 2690 ± 1.73 |
| Brown flaxseed              | 621 ± 0.08                    | 3474 ± 1.42        | 3548 ± 1.52 |
| Coriander                   | 496 ± 0.47                    | 2408 ± 0.89        | 3029 ± 0.92 |
| Gold flaxseed               | 387 ± 0.09                    | 3832 ± 1.19        | 4328 ± 1.50 |
| Hairy basil                 | 255 ± 0.13                    | 3332 ± 1.64        | 3719 ± 1.61 |
| Perilla                     | 151 ± 0.08                    | 3874 ± 0.39        | 4129 ± 0.36 |
| White chia seed             | 373 ± 0.18                    | 3752 ± 0.63        | 3903 ± 0.67 |
| White sesame                | 200 ± 0.47                    | 2192 ± 1.48        | 2565 ± 1.66 |
| Legume oil (3 samples)      |                               |                    |
| Inca peanut                 | 83 ± 0.05                     | 6175 ± 1.18        | 6258 ± 1.23 |
| Soybean                     | 1870 ± 0.55                   | 3200 ± 1.09        | 5070 ± 0.75 |
| Peanut                      | 494 ± 0.67                    | 4874 ± 0.58        | 5368 ± 0.43 |
| Amaranth oil (3 samples)    |                               |                    |
| Green amaranth              | 228 ± 0.41                    | 2098 ± 1.16        | 2326 ± 1.41 |
| Love-likes-bleeding         | 348 ± 0.48                    | 1988 ± 1.17        | 2336 ± 1.49 |
| Spiny amaranth              | 282 ± 0.25                    | 1931 ± 1.04        | 2213 ± 1.00 |
| Marrow seed oil (8 samples) |                               |                    |
| Angled loofah               | ND                            | 1477 ± 1.41        | 1477 ± 1.41 |
| Cucumber                    | 480 ± 0.41                    | 9334 ± 0.30        | 9814 ± 0.34 |
| Long skinny eggplant        | 406 ± 0.22                    | 2991 ± 0.91        | 3397 ± 1.09 |
| Muskmelon                   | 977 ± 1.39                    | 7868 ± 2.21        | 8845 ± 2.57 |
| Pumpkin                     | 530 ± 0.38                    | 6208 ± 0.46        | 6738 ± 0.84 |
| Wax gourd                   | 493 ± 0.10                    | 14630 ± 1.70       | 15123 ± 1.60 |
| Winter melon                | 1230 ± 0.24                   | 11908 ± 1.48       | 13138 ± 1.59 |
| Watermelon                  | 604 ± 0.68                    | 10408 ± 1.06       | 11012 ± 0.84 |
| Cruciferous seed oil (7 samples) |                           |                    |
| Broccoli                    | 92 ± 0.21                     | 1294 ± 0.47        | 1386 ± 0.66 |
| Cauliflower                 | 126 ± 0.08                    | 1239 ± 0.69        | 1365 ± 0.62 |
| Chinese cabbage             | 200 ± 0.19                    | 1511 ± 0.97        | 1711 ± 1.16 |
| Chinese kale                | 54 ± 0.07                     | 938 ± 0.97         | 992 ± 1.02 |
| Chinese radish              | 267 ± 0.23                    | 6399 ± 1.22        | 6666 ± 1.02 |
| Curly kale                  | 226 ± 0.06                    | 7278 ± 1.95        | 7504 ± 1.96 |
| Hong Kong Chinese kale      | 122 ± 0.19                    | 1313 ± 0.38        | 1435 ± 0.56 |
| Leafy green seed oil (11 samples) |                       |                    |
| Chinese flowering cabbage   | 46 ± 0.20                     | 712 ± 1.97         | 758 ± 2.03 |
| Chinese green mustard       | 30 ± 0.23                     | 788 ± 1.12         | 818 ± 1.29 |
| Chinese mustard             | 4 ± 0.05                      | 745 ± 1.83         | 749 ± 1.79 |
| False Pak Choi (Phukeaw)     | 48 ± 0.19                     | 650 ± 1.52         | 698 ± 1.59 |
| False Pak Choi (Inthanon)    | 18 ± 0.16                     | 645 ± 1.07         | 663 ± 0.91 |
| Flowering Pak Choi (1)       | 78 ± 0.13                     | 1240 ± 0.90        | 1318 ± 0.95 |
| Flowering Pak Choi (2)       | 172 ± 0.11                    | 1800 ± 0.68        | 1972 ± 0.77 |
| Green Pak Choi              | 54 ± 0.21                     | 890 ± 0.78         | 944 ± 0.88 |
| Indian mustard              | 49 ± 0.13                     | 860 ± 0.47         | 909 ± 0.37 |
| Lettuce                     | 366 ± 0.09                    | 2184 ± 0.90        | 2550 ± 0.97 |
| Rat-tailed radish           | 218 ± 0.10                    | 6971 ± 1.25        | 7189 ± 1.14 |

ND = non-detectable.
chia seed oil (our linolenic acid content as 46.59–49.61%, and that of the literature as 64.00%), and flaxseed oil (our content as 51.22–56.68%, and that of the literature as 54.77%).

Regarding applications of plant oils for health purposes, cold-pressing has demonstrated to yield oils with higher amount of unsaturated fatty acids compared with the oils derived from other preparation methods as reported previously [23]. A study by Ozcan et al. [24] presented that the moringa seed oil obtained from cold-pressing had greater amount of saturated fatty acids (75.49% oleic acid, 1.69% linoleic acid, and 1.87% linolenic acid) than that derived from soxhlet system (73.83, 1.27, and 1.73%, respectively). In a study of chia seed oil, cold-pressing could yield oil with higher content of linolenic acid (67.89%) and linoleic acid

**Figure 4.** Content of bioactive compounds in cold-pressed oils (µg g⁻¹). (A) Total bioactive compounds, (B) total phytosterols, (C) squalene, and (D) cholecalciferol.
(19.07%) compared with the oil obtained from solvent extraction (67.87% and 17.63%, respectively) [25]. Walnut oil obtained by cold-pressing had higher oleic acid content (28.30%) than the oil from supercritical CO2 extraction (23.50%) [26]. A number of studies reported that oils extracted by cold-pressing contained a composition of palmitic acid lower that those derived from other extraction methods [27, 28]. Cold-pressing had higher oleic acid content (28.30%) than the oil from supercritical CO2 extraction (23.50%) [26].

At present, oils with high ratio of MUFA s and PUFA s have been encouraged for the use of health promotion. Similarly, oils with high content of functional compounds have been gaining increasing interest for therapeutic uses. The continuous part of this study were focused on the chemical composition of beneficial lipid-soluble compounds in cold-pressed plant oils.

3.2. HPLC-DAD analysis for functional phytochemicals in cold-pressed oils

3.2.1. Analysis of functional phytochemicals using HPLC-DAD

In this study, HPLC-DAD was employed to separate and quantify some functional compounds in the fifty plant oils. Chromatographic results are shown in Figure 3. Functional phytochemicals were eluted as cholecalciferol (6.33 min), stigmasterol and campesterol (9.92 min), β-sitosterol (10.97 min), and squalene (14.42 min). All targeted compounds were successfully separated within 30 min. The chromatographic result has reconfirmed effectiveness and separability of the reverse-phase column with pentfluorophenyl phase (PFP) for quantification of lipophilic compounds in cold-pressed vegetable oil samples as previously reported [1].

3.2.2. Content of the functional phytochemicals in the fifty cold pressed oils

Contents of cholecalciferol, phytosterols, and squalene present in each oil are individually reported in Tables 7 and 8. Presents the amount of each bioactive compound group and the sum value. The total amounts of the bioactive compounds were found in the range of 970–19933 μg g⁻¹, in which an average content was 5402 μg g⁻¹. As a result, oils from marrow seeds had the highest content of all the active compounds (19933 μg g⁻¹), and was 368.99% higher than the average value. Among the marrow seed oils analyzed, the oils that had the highest amount of bioactive compounds were winter melon (19933 μg g⁻¹), wax gourd (17904 μg g⁻¹), and watermelon (14294 μg g⁻¹). Oils from pseudo-cereals and legumes were also relatively high in the total content (comparable to 178.69 % and 147.06% of the average value, respectively) compared to those of the other categories. Since the functional phytochemicals are comprised of many chemical compounds that are diversified in structures and physiological roles, each group of compounds was then discussed for its content and availability among oil samples. Average contents of each specific compounds among categories of oils were compared and exhibited in Figure 4.

3.2.2.1. Content of phytosterols. Plant sterols, generally known as phytosterols, have gained much attention in reducing the serum cholesterol level in humans, as well as the risk of heart disease. In addition, phytosterols have anti-inflammatory, anti-bacterial, and anti-tumor properties [29]. Considering phytosterols content (Figure 4B), the oils from marrow seeds and legumes were of the highest values (8693 μg g⁻¹ and 5565 μg g⁻¹ respectively) with an average content of 3903 μg g⁻¹. The oils from leafy green seeds were lowest in phytosterols (1688 μg g⁻¹). Regarding individual oil, the oils rich in phytosterols were wax gourd seed oil (15123 μg g⁻¹), winter melon seed oil (13138 μg g⁻¹), and watermelon seed oil (11012 μg g⁻¹). In this study, β-sitosterol, campesterol, and stigmasterol were analyzed because they are the most abundant form in nature. In general, β-sitosterol is more abundant than stigmasterol and campesterol. Among the samples analyzed, the highest β-sitosterol content was detected in wax gourd seed oil (14630 μg g⁻¹), winter melon seed oil (11908 μg g⁻¹), and watermelon seed oil (10408 μg g⁻¹). All of them were subcategorized in marrow seed oils suggesting a close relationship between their chemical composition (phytosterols) and taxonomy. However, stigmasterol and campesterol were found relatively high in soybean oil (1870 μg g⁻¹) and winter melon seed oil.

Figure 5. Principle component analysis of the cold-pressed oils for the content of fatty acids and bioactive compounds in the function of PC1 and PC2, score plot (n = 50) for oil samples in 7 groups of nut/kernel, pseudo-cereal, legume, amaranth, marrow seed, cruciferous seed, and leafy green seed. The code for the oils is a number that is counted from the order of the oils written in Table 7.
(1230 μg g⁻¹) (Table 8). Our findings are in agreement with a study by Vlahakis et al. [30], who reported the phytosterol content of soybean oil ranging between 2350 and 4050 μg g⁻¹ that were comprised of β-sitosterol for 1250–2360 μg g⁻¹, campesterol for 620–1310 μg g⁻¹, and stigmasterol for 470–770 μg g⁻¹.

3.2.2.2. Content of squalene. Squalene is a hydrocarbon intermediate in the biosynthesis of phytosterols in plants, and it is widely used for applications such as skin moisturizers, vaccines or carriers for active lipophilic molecules [31]. In this study, squalene was detected in the range of 265–5979 μg g⁻¹. The high amount of squalene was found in marrow seed and pseudo-cereals oils, in which the oils from wintermelon seeds (5979 μg g⁻¹), hairy basil seeds (5868 μg g⁻¹), and black chia seeds (5625 μg g⁻¹) had the greatest amount of squalene (Figure 4C). On the other hand, squalene was least found in oils of leafy green seeds (403 μg g⁻¹). However, there is a report by Bozorov et al. [32] claiming that squalene content in oils obtained by extraction is greater than that obtained by cold-pressing. This implies that squalene content in the sample oils might be increased if extraction solvent was employed for extraction. However, it seems that many studies have emphasized that the quantitation of plant squalene is mostly done from materials rather than oils. For example, Ryan et al. [33] studied the content of squalene from some selected plant materials such as seeds, grains, and legumes, and found that pumpkin seed was the most abundant source of squalene (as high as 890 μg g⁻¹). The results contradict with ours because in our study oils obtained from grains and legume (such as sesame, soybean and peanut) contained higher amount of squalene than those of pumpkin. The difference would be partly due to difference in samples (grain versus oil) and in extraction efficiency of two different methods (solvent extraction versus cold pressing).

3.2.2.3. Content of cholecalciferol. Cholecalciferol, also known as vitamin D₃, is an important nutrient for calcium homeostasis and optimal bone health. It is found in some foods and can be taken to prevent vitamin D deficiency and related diseases, including altered phosphorus metabolism which could lead to renal osteodystrophy and increased mortality due to cardiovascular disease [34]. Recently, a number of investigations have focused on quantification of precursors and metabolites of vitamin D in plant lipids to be used for human nutrition [35]. Regarding Figure 4D, the oils from marrow seeds had remarkably high content of cholecalciferol as 471.60 μg g⁻¹, and an average value of 153.30 μg g⁻¹. The oils rich in cholecalciferol were angled loofha seed oil (1287.75 μg g⁻¹), wintermelon seed oil (815.99 μg g⁻¹), and long skinny eggplant seed oil (632.14 μg g⁻¹). Cholecalciferol in the other groups were in range of non-detectable to 650.30 μg g⁻¹. Although there have been some reports evidencing vitamin D precursors and metabolites in plants [36], to our understanding this is the first study to report high contents of cholecalciferol in marrow seed oils, suggesting marrow seeds to be another good source of vitamin D from foods.

The content of functional phytochemicals derived from this study was compared with those reported in the literature of cold-pressed oil [22]. On the contrary to fatty acid composition, the obtained phytochemical content was rather different than that reported in the literature. Regarding phytosterol content, sesame oil had comparable amount of β-sitosterol (ours was 1768–2192 μg g⁻¹ and that of the literature was 1900–2600 μg g⁻¹) and campesterol together with stigmasterol (ours was 74–200 μg g⁻¹ and that of the literature was 310–420 μg g⁻¹). Total phytosterol content in amaranth oils (2213–2336 μg g⁻¹), chia seed oils (3684–5903 μg g⁻¹), pistachio oil (1200 μg g⁻¹), and coriander oil (3029 μg g⁻¹) were lower than those reported in the literature (19300, 6653–7776, 5586 and 9180 μg g⁻¹, respectively). However, the phytosterol content in sunflower seed oil (3000 μg g⁻¹), pumpkin seed oil (6736 μg g⁻¹), hazelnut oil (6066 μg g⁻¹), peanut oil (5368 μg g⁻¹), moringa seed oil (2650 μg g⁻¹), walnut oil (2479 μg g⁻¹), soybean oil (5070 μg g⁻¹), macadamia oil (2400 μg g⁻¹) and muskmelon seed oil (8845 μg g⁻¹) were greater than those reported in the literature (2400–2600, 1806, 1096, 220–1200, 1344, 1236, 2970, 740 and 3250 μg g⁻¹, respectively). On the other hand, squalene content in the obtained cold-pressed oils were compared with those reported in the literature. Squalene content in sunflower seed oil (1537 μg g⁻¹), hazelnut oil (2356 μg g⁻¹) and macadamia oil (549 μg g⁻¹) was greater than those previously reported (150–200, 186 and 23 μg g⁻¹, respectively), while squalene content in pumpkin seed oil (516 μg g⁻¹) was lower than that in the literature (3520 μg g⁻¹). Since the analyzed functional phytochemicals are secondary metabolites in plants, they can be easily influenced by the type, variety, and environmental conditions, including fertilizers, farm management, harvesting conditions, and agroclimatic properties [37]. For instance, a study by Roche et al. [38] showed a large variability of phytosterol among sixteen sunflower inbred lines and hybrids (1780–3040 ppm). A delay of sowing and increased temperatures during seed formation could improve the sterol content by up to 35%. Therefore, in order to obtain high quality plant oils, breeding programs as well as cultivation conditions of the plant should be carefully considered and manipulated.

3.3. Statistical analysis of principal component analysis (PCA)

PCA was applied after the data was pretreated by standardization to exploratorily analyze the pattern in the oil samples. Figure 5 demonstrates the PCA score plots where the samples were labeled according to the categories of oils. It was observed that the plant oil samples from the same categories were located nearby each other. For example, most of the samples from pseudo-cereal oil were found on the lower side of the PCA space. The samples from the categories of marrow seed oil had higher PC2 scores, and tended to locate on the upper part of the score plots. A dense cluster in the middle right area of the PCA space were plots of samples from legume oils, amaranth oils, pseudo-cereal oils, and cruciferous and leafy green seed oils. The plots of the pseudo-cereal oils are quite distinct that some plots are distributed outside the main cluster, which is located around the middle low of the PCA plot. For instance, the plots of coriander seed oil (coded 13), black sesame oil (coded 11), and white sesame oil (coded 18) are scattered because these oils did not contain linolenic acid. Black sesame oil contained the highest content of linoleic acid and cholecalciferol in their group. White sesame oil was found high content of linoleic and oleic acids, and the coriander seed oil had very high cholecalciferol content and the highest content of oleic acid. Samples in a category of nut/kernel oil had a relatively high and wide range of oleic acid content as 24.02–78.21%, contributing to the dispersion of some plots from this group. Most of the plots from cruciferous seed oils and leafy green seed oils are found in the middle right area of the PCA space. This might be partly because the oils from these two groups contain rather a high composition of oleic and erucic acids. The latter was also found in some kernel oils including coconut and macadamia oils. The plots of amaranth oils are distributed in the same area of legume oils, which share the same range of oleic and linoleic acids content. For the cluster of samples from marrow seed oils, a group with relatively high contents of phytosterols and cholecalciferol, a lot of dispersion in PCA plot is observed because wax gourd seed oil (coded 30) and winter melon seed oil (coded 31) contained the highest amount of phytosterols. Their plots are scattered to the upper right area of the PCA plot. The PCA plot (Figure 5) suggests a similarity pattern for the analyzed samples that would be useful in performed to highlighting the dissimilarity of the oil profiles and PCA can clearly distinguish between different categories of plant material in cold-pressed oil. Taken together, oil samples were evaluated in terms of both fatty acid composition and functional phytochemicals. In this work, superior oils refer to oils that contain larger fraction of unsaturated fatty acids and high amount of those functional compounds. Accordingly, characteristics of oils from different categories of plants are summarized. Marrow seed oils are a good source of total bioactive, phytosterol, cholecalciferol and linoleic acid, in which winter melon seed oil and wax gourd seed oil are
the most preferable ones. Pseudo-cereal oils are superior oils for high squalene and linolenic acid content, where perilla oil and hairy basil oil are the lead in this group. Legume oils are rich in phytosterols, where peanut oil, as well as soybean oil, are a good choice in this group. In contrast, oils from kernel, amaranth, cruciferous seeds, and leafy green seeds are rather moderate or inferior when considering the amounts of unsaturated fatty acids and the targets bioactive compounds present.

On the basis of the result from this study, some plant oil materials are suggested to be a good source for oil extraction with high nutritional value regarding fatty acid composition and functional phytochemicals. However, quality and stability of oil is another aspect to be concerned with, considering human consumption. If the oil is inappropriately prepared, handled and stored, its quality is diminished. For instance, triacylglycerol in oil can be hydrolyzed under improper circumstance, yielding free fatty acids and glycerol which can increase the rancidity of the oil. The oils with rancidity and off-flavor can be toxic and ultimately pose health risks. In general, quality and stability of oil can be accessed by mean of chemical properties including peroxide value (PV), acid value (AV), and oxidative stability index (OSI). The parameters are subjected to be studied together with optimization of oil extraction after plant oil materials are selected for further application.

Despite the content of beneficial phytochemicals being investigated and discussed in this study, some plants materials (such as oil seeds) contain natural anti-nutritional factors that can be toxic or reduce digestibility of oil nutrition on their consumption. For example, anti-nutrition substances in oil seeds include trypsin inhibitor, goitrogens, sponin, lectin, aflatoxin, allergens, and allergens [39]. Therefore, in order to safely consume those oils for health purpose, it is important to clarify the type and amount of those anti-nutrition substances in the targeted plant materials and remove these harmful substances by employing proper material control and related manufacturing processes.

On the basis of our results, the analyzed 50 cold-pressed oils were further classified according to their edibility, applicability and generalizability.

Regarding edibility, the analyzed oils can be categorized into three categories.

I) Oils containing large amounts of erucic acid. Erucic acid is a monounsaturated omega-9 fatty acid that has been evidenced for its long-term health risk such as myocardial lipodosis especially in children up to ten years [21]. The oils in this groups include Chinese mustard seed oil, Chinese cabbage seed oil, broccoli seed oil, false Pak Choi (Inthanon) seed oil, green Pak Choi seed oil, false Pak Choi (Phukeaw) seed oil, cauliflower seed oil, Chinese kale seed oil, flowering Pak Choi (1) seed oil, Hong Kong Chinese kale seed oil, Indian mustard seed oil, Chinese radish seed oil, Chinese green mustard seed oil, curly kale seed oil, rat-tailed radish seed oil, flowering Pak Choi (2) seed oil, and Chinese flowering cabbage seed oil.

II) Oils containing low amounts of erucic acid, or containing anti-nutrient substance that needs proper control on material handling and oil manufacturing process. The oils in this group include

- black chia seed oil, white chia seed oil, moringa seed oil, hazelnut oil, pistachio oil, hairy basil oil, green amaranth oil, love-lies-bleeding oil, spiny amaranth oil, inca peanut oil, cashew oil, almond oil, muskmelon seed oil, pumpkin seed oil, coriander oil, macadamia oil, watermelon seed oil, lettuce seed oil, wax gourd seed oil, winter melon seed oil, angled loofah seed oil, cucumber seed oil, and long skinny eggplant seed oil.

III) Oils generally containing no harmful substances. The oils in this group include black sesame oil, white sesame oil, sunflower seed oil, soybean oil, peanut oil, walnut oil, brown flaxseed oil, gold flaxseed oil, coconut oil, and perilla oil.

On the other hand, the oils can be categorized into four groups based on their usability as follows.

I) Oils with limited sources of raw materials but high nutritional value. The strategic application of oils in this group is mainly as an ingredient of cosmetic or high value products. The oils in this group are black chia seed oil, white chia seed oil, moringa seed oil, hazelnut oil, pistachio oil, hairy basil oil, green amaranth oil, love-lies-bleeding oil, spiny amaranth oil, inca peanut oil, cashew oil, almond oil, coriander oil, macadamia oil, angled loofah seed oil, cucumber seed oil, long skinny eggplant seed oil, watermelon seed oil, lettuce seed oil, curly kale seed oil, Chinese kale seed oil, Hong Kong Chinese kale seed oil, broccoli seed oil, and cauliflower seed oil.

II) Oils with raw material being treated as agricultural by-product or waste but containing good nutritional value. Raw materials from this group can be processed into cold-pressed oils or other value-added products as it could increase their economic value and reduce unnecessary waste. The oils in these groups are wax gourd seed oil, winter melon seed oil, muskmelon seed oil, and pumpkin seed oil.

III) Oils generally suitable for consumption or health purpose. There is no shortage supply of plant oil materials in this group, and the oils are black sesame oil, white sesame oil, sunflower seed oil, soybean oil, peanut oil, walnut oil, brown flaxseed oil, gold flaxseed oil, coconut oil, and perilla oil.

IV) Oils not suitable for consumption or health purpose. The oils that contain large amount of hazardous substance such as erucic acid. The oils in this group are include Chinese mustard seed oil, Chinese cabbage seed oil, false Pak Choi (Inthanon) seed oil, green Pak Choi seed oil, false Pak Choi (Phukeaw) seed oil, flowering Pak Choi (1) seed oil, Indian mustard seed oil, Chinese radish seed oil, Chinese green mustard seed oil, rat-tailed radish seed oil, flowering Pak Choi (2) seed oil, and Chinese flowering cabbage seed oil.

4. Conclusion

In summary, total fifty different cold-pressed oils were prepared in this study. The oils were analyzed for their fatty acid composition and the functional compounds using GC-MS and HPLC-DAD, respectively. The two chromatographic techniques allowed a good separation and quantification of the targeted compounds. A great diversification of fatty acid composition and bioactive compounds was demonstrated. Most oils had the greatest proportion of PUFAs, followed by SFAs and MUFAs, respectively. Considering the bioactive compounds, phytosterols were found in the highest amount, followed by squalene, and cholecalciferol, respectively. PCA was also performed to analyze the variation in identified cold-pressed oil profiles and it can be used to group oil with similar composition. Oils from marrow seeds, pseudo-cereals, and legumes tended to have superior chemical properties compared to oils from kernel, amaranth, cruciferous seeds, and leafy green seeds. In conclusion, the qualitative and quantitative data would be useful for application or selection of those plant oils in food and pharmaceutical industries.

Declarations

Author contribution statement

Jikunyna Yuenyong: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Piramon Pokkanta: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Nuttatida Phuangsaigai, Sila Kittiwachana, Sugunya Mahatheeranont: Analyzed and interpreted the data.
Phumon Sookwong: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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