Some Characteristics of Crude Oil Extracted from Roselle
(Hibiscus sabdariffa L.) Seeds Cultivated in Egypt

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Abstract: The physical and chemical properties of crude roselle seed oil as well as its fatty acid composition were estimated and compared with those of corn oil. Campesterol, campestanol, stigmasterol, β-sitosterol and β-sitostanol fractions (the most common phytosterols) of both oils were also determined by GLC. The results indicated that the melting point (−1.1 ± 0.3°C), unsaponifiable matter (1.0 ± 0.2%) and iodine value (109 ± 7) of roselle seed oil were not significantly (P < 0.05) different than those of corn oil (1.0 ± 0.1°C, 1.1 ± 0.2% and 117 ± 5). Therefore, the oil could be classified as semi-dry oil. The oil contained significant amounts of triacylglycerols (69.2 ± 1.6%) and sterols (3.5 ± 0.4%). GLC analysis proved that the linoleic (45.3%), oleic (27.2 ± 0.7%) and palmitic (17.3 ± 0.8%) of roselle seed oil were the predominant fatty acids. Because of the similarity of roselle seed oil to corn oil in chemical composition and lipid fractions, roselle seed oil might provide a new source of edible oil. Further study on the nutritional and physiological value of roselle seed oil is needed.

Key words: roselle seed oil, fatty acid, sterol, triacylglycerol

1 Introduction

There is no doubt that the value of traditional edible oils will increase due to the growth of population all over the world, resulting in an increase in the demand for oil. For that reason, research on increasing oil and fat resources is required to raise the quantity and nutritional value of edible oil. Several excellent attempts are now being made to increase the nutritional value and storage stability of oils by changing their fatty acid composition or removing undesirable characteristics from the seeds in biotechnological studies (1). On the other hand, much attention has been directing to finding new oil sources (2,3).

Roselle (Hibiscus sabdariffa L.) is an annual botanical plant belonging to the Malvasia family cultivated in upper Egypt. The calyces and leaves of the roselle are usually used for making jam, jelly, sauces and pickles (4). The petals of its flowers have been used in Egypt to prepare beverages, which have various important medical purposes (5-7). Various studies have shown that roselle seeds contain much protein and oil (8-10).

Our interest has been directed to evaluating roselle seed oil as an edible oil based on its physical and chemical properties, lipid fractions, fatty acid composition and sterol content compared with corn oil.

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2 Experimental

2.1 Materials

Roselle (Hibiscus sabdariffa L.) seeds were supplied by the Medicinal and Aromatic Research Department, Horticulture Research Station, Agriculture Research Center, Alexandria, Egypt. Corn oil was bought at the retail market in Fukuoka city, Japan. Standard fatty acid methyl esters were purchased from Suplico (Sigma-Aldrich, Japan), and all other chemicals were AR grade.

2.2 Methods

2.2.1 Crude oil extraction

Roselle seeds were carefully cleaned and crushed in an electrical grinder (National Model MX-915C, Japan) at speed 6 for 2 min to pass through a 35 mm (42 mesh) sieve. Crude oil was extracted with petroleum ether (40-60°C) in a soxhlet apparatus according to AOAC(11). After extraction, the rest of the solvent was dried under nitrogen gas. The oil obtained was bright amber in color and had a pleasant spicy aroma.

2.2.2 Determination of physical properties

Specific gravity (SG) at 20°C, melting point (MP) in a micro-melting point apparatus (Yanagimoto, Japan) and refractive index (RI) at 25°C with an Abe refractometer for oils, were determined according to AOAC(11).

2.2.3 Determination of chemical properties

Free fatty acid content (as % oleic), iodine value (Wijs), saponification value and percent of unsaponifiable components present in the oil were determined according to AOAC methods (11). The oil was fractionated by the method of Mangold (12) onto precoated silica gel glass plate 200 x 200 x 0.25 mm (Merck, Darmstadt, Germany). Lipid fractions were identified by comparison of their Rf values with standards. A thin-layer chromatogram was scanned and the percentage of each fraction was calculated.

2.2.4 Fatty acid composition

Fatty acid composition of the oil was performed by converting about 5 mg oil containing 200 μg pentadecanoic acid (C15:0) as an internal standard to their fatty acid methyl esters with a mixture of methanol:sulfuric (32:1v/v). The fatty acid methyl esters derived from the oil were analyzed in a Shimadzu GC-14A Gas Chromatograph on a 30-meter (0.32 mm) glass capillary column coated with OMEGA WAX 320 equipped with an FID detector. The chromatographic conditions were: injection port and detector temperatures were 240°C and 260°C, respectively. The temperature program was 50 to 220°C at 5°C/min and 30 min (final period) at 220°C. An electronic laboratory data system, Shimadzu C-R 5A Chromatopac (chart speed 10 mm/min), was used. The injection volume was 1 μl (fatty acid methyl esters). Identification of fatty acid methyl esters was based on comparison of retention times of unknown peaks to authentic fatty acid methyl esters. The quantity of each fatty acid methyl ester was estimated by comparison with the known amounts of pentadecanoic acid methyl ester. Fatty acids composition was expressed as the weight percentage (%) of total fatty acid methyl ester.

2.2.5 Determination of sterols

Approximately 300 nmol (251 μg) of 5-α-cholestan as an internal standard was added to the n-hexane extract of unsaponifiable components. The mixture was esterified with TMS reagent (BSTFA+1%TMCS, Supelico, Cat.no., 33149-U). After transesterification, the sterols were analyzed in a GC-4 CNPF Shimadzu Gas Chromatograph on a 3 mm x 2 m stainless steel column packed with Gas Chrom Q 60-80 mesh as support coated with 3% Silicon OV-17 equipped with a flame ionization detector (FID) in air and helium at 1.8 and 0.6 kg/cm², respectively. The carrier gas was nitrogen at 1.8 kg/cm². The injection and detection temperature was 300°C and the column temperature was 260-270°C. Sterol fractions were identified by comparing their relative retention times (RRT) with β-sitosterol as a reference (β-sitosterol RRT=1) (13)

2.3 Statistical Analysis

Values were the means and standard deviation for three replicates. Statistical analysis was carried out according to Snedecor and Cochran (14).

3 Results and Discussion

3.1 Physical and Chemical Properties

As shown in Table 1, the specific gravity of corn oil (0.922 ± 0.008 g/cm³) was significantly (P < 0.05) lower than that of roselle seed oil (0.956 ± 0.001 g/cm³). The refractory index (RI) of corn oil (1.4720 ± 0.01) was considerably higher than that of roselle seed oil (1.4592 ± 0.00), but no significant (P < 0.05) difference was detected between the melting points (MP)
Some Characteristics of Crude Oil Extracted from Roselle

Table 1  Some Physical and Chemical Properties of Roselle Seed and Corn Oilsa.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Roselle seed oil</th>
<th>Corn oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Gravity (g/cm³)</td>
<td>0.956 ± 0.001a</td>
<td>0.922 ± 0.008b</td>
</tr>
<tr>
<td>Refractive Index</td>
<td>1.459 ± 0.0003a</td>
<td>1.4720 ± 0.0009b</td>
</tr>
<tr>
<td>Melting Point (°C)</td>
<td>-1.10 ± 0.30e</td>
<td>1.00 ± 0.10f</td>
</tr>
<tr>
<td>Free Fatty Acid (as % oleic)</td>
<td>2.10 ± 0.10e</td>
<td>0.13 ± 0.01h</td>
</tr>
<tr>
<td>Iodine Value (Wijs)</td>
<td>109.00 ± 7.00e</td>
<td>117.00 ± 5.00e</td>
</tr>
<tr>
<td>Saponification Number</td>
<td>165.00 ± 5.00f</td>
<td>190.00 ± 1.00g</td>
</tr>
<tr>
<td>Unsaponifiable Matters (%)</td>
<td>1.00 ± 0.20e</td>
<td>1.10 ± 0.20e</td>
</tr>
</tbody>
</table>

a) Data are mean ± SD for triplicate analysis. *Values within row with similar superscript letters are not significantly different P < 0.05.

Table 2  Percentage of Lipid Fractions in Roselle Seed and Corn Oils Separated by TLCa.

<table>
<thead>
<tr>
<th>Lipid Fraction</th>
<th>Roselle seed oil</th>
<th>Corn oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polar Lipids</td>
<td>1.9 ± 0.1a</td>
<td>4.0 ± 0.8b</td>
</tr>
<tr>
<td>Monoacylglycerol</td>
<td>2.8 ± 0.2a</td>
<td>0.6 ± 0.2b</td>
</tr>
<tr>
<td>1,2 &amp; 2,3 Diacylglycerols</td>
<td>6.8 ± 0.4a</td>
<td>2.6 ± 0.2b</td>
</tr>
<tr>
<td>Free Sterols</td>
<td>3.5 ± 0.4a</td>
<td>3.7 ± 0.4a</td>
</tr>
<tr>
<td>Unknown</td>
<td>9.3 ± 0.5</td>
<td>—</td>
</tr>
<tr>
<td>Free Fatty Acids</td>
<td>3.5 ± 0.4a</td>
<td>1.4 ± 0.4b</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>69.2 ± 1.6e</td>
<td>84.0 ± 1.0e</td>
</tr>
<tr>
<td>Sterol Esters</td>
<td>2.5 ± 0.4a</td>
<td>2.9 ± 0.1e</td>
</tr>
</tbody>
</table>

a) Data are mean ± SD for triplicate analysis. *Values within row with similar superscript letters are not significantly different P < 0.05.

of the two types of oils. The low MP of roselle oil made it a previously favorite household food oil (15). Similar results concerning corn oil were reported previously (16).

Percentage of free fatty acid in roselle seed oil was 2.10 ± 0.10%, significantly (P < 0.05) higher than that of corn oil (0.13 ± 0.01%). This may be due to the nature of roselle seeds, whereas many oil-bearing seeds such as olive, palm and rice bran contain high acidity oils (17). In contrast, the saponification number (SN) of roselle seed oil (165 ± 5) was significantly lower than that of corn oil (190 ± 1). Consequently, the chain length of fatty acids in roselle seed oil was normally longer than that of those in corn oil. As for the iodine value (IV) and percentage of unsaponifiable components (%USM), no significant differences were detected between the two oils. Therefore, roselle seed oil, like corn oil, could be classified as a semi-dry oil type (18).

Itoh et al (13) reported that the SN, IV and %USM of corn oil were 195.9, 105.3 and 1.3%, respectively.

3.2 Lipid Fractions

The data in Table 2 show that roselle seed oil contains 7 known lipid classes besides one unknown lipid which comprised about 9% of total lipids. According to Mangold (12), this fraction could be 1,3 diacylglycerol. The percentage value of triacylglycerol fraction in roselle seed oil was 69.2 ± 1.6%. In addition, it contained significantly (P < 0.05) higher amounts of free fatty acids (3.5 ± 0.4%) than corn oil (1.4 ± 0.4%), and there were no significant (P < 0.05) differences between them in free sterols and sterol esters fractions. The phospholipid fraction represented 1.9%. It was reported that phospholipid content in Malvaceae ranged from 1.2 to 1.9% (19). The data showed that triacylglycerol was the main lipid fraction of roselle seed oil as it involved considerable amounts of sterols and sterol esters. These results confirm the resultants in Table 1, since roselle seed oil contained much more free fatty acids and a similar percentage of unsaponifiable components than corn oil.
3.3 Fatty Acid Composition and Sterols

One of the criteria for the determination of fat quality is the content of essential fatty acids such as linoleic (C18:2n-6) and linolenic (C18:3n-3). Since humans require some of these fatty acids in the diet to prevent fatty acid deficiency, and diseases such as skin lesions, poor hair growth and low growth rate (20). The GLC analysis of fatty acid methyl esters derived from roselle seed and corn oils as well as roselle seed oil lipid fractions is shown in Table 3. The data show that linoleic (C18:2), oleic (C18:1) and palmitic (C16:0) are the major (> 5%) fatty acids in both roselle seed and corn oils. The total percentages of these fatty acids in roselle seed and corn oils were 89.8% and 93.5%, respectively. These results are in agreement with earlier studies (10,21,22). Plant oils were 89.8% and 93.5%, respectively. The percentage of total polyunsaturated fatty acids in roselle seed oil was about 78.2% which was 3.7 times that of the saturated ones. Therefore, it could be concluded that roselle seed oil is a good source of mono- and polyunsaturated fatty acids which are effective in reducing both LDL and HDL cholesterol in serum (23,24). Furthermore, it is clear that none of the low molecular weight fatty acids (less than C13) were present, which enhances the oil stability (16). The calculated peroxidizability index (25) of crude roselle seed oil was 5645, decreased to 48 in its triacylglycerol fraction, whereas it was 57 in corn oil. As a result, either crude roselle seed oil or its triacylglycerol might be more resistant to oxidation rancidity than corn oil.

It has been postulated that consumption of plant sterols leads to a reduction in the absorption of dietary cholesterol (26). In addition, they play an important role in protecting against colon, breast and prostate cancer (27). The most common plant sterols are β-sitosterol, campsterol and stigmasterol (28). As shown in Table 4 crude roselle seed oil contained 559 mg β-sitosterol per 100 g oil which was higher than that found corn oil (458 mg/100 g). These amounts were higher than those detected in peanut and unrefined olive oils (29). β-sitosterol has an inhibitory effect on colon cancer in both humans and laboratory animals (30). It was noticed that the amounts of campesterol, stigmasterol and β-sitostanol (107, 50.6 & 39.9 mg/100 g) in roselle seed oil were comparable to those detected in corn oil (115, 45.7 & 42.4 mg/100 g). Although the percentage of campesterol is the lowest among the three main plant sterols, it is more quickly and easily absorbed than sitosterol (31).

### Table 3 Fatty Acid Composition of Roselle Seed and Corn Oils (% of total fatty acid)*

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Corn oil</th>
<th>Roselle oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FFA1</td>
<td>MG2</td>
</tr>
<tr>
<td></td>
<td>4.59±0.61</td>
<td>7.13±0.23</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.24±0.03</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.52±0.12</td>
<td>0.19±0.70</td>
</tr>
<tr>
<td>C16:0</td>
<td>13.15±1.03</td>
<td>17.27±0.77</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.34±0.01</td>
<td>0.64±0.04</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.05±0.10</td>
<td>3.63±0.12</td>
</tr>
<tr>
<td>C18:1</td>
<td>29.44±2.15</td>
<td>27.22±0.67</td>
</tr>
<tr>
<td>C18:2</td>
<td>50.87±0.98</td>
<td>45.29±0.59</td>
</tr>
<tr>
<td>C18:3</td>
<td>4.85±0.50</td>
<td>40.68±0.5</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.41±0.04</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>C20:1(n-6)</td>
<td>—</td>
<td>0.21±0.00</td>
</tr>
<tr>
<td>C22:1</td>
<td>—</td>
<td>1.69±0.44</td>
</tr>
<tr>
<td>C22:2(n-6)</td>
<td>—</td>
<td>1.22±0.01</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.31</td>
<td>0.59</td>
</tr>
</tbody>
</table>

*Values means ± SD for triplicates analysis. 1) Free fatty acids. 2) Monoacylglycerol. 3) Diacylglycerol. 4) Triacylglycerol. 5) Sterol esters.
Table 4  Sterol Fractions in Crude Roselle Seed and Corn oils. (mg / 100 g oil)\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>roselle seed oil</th>
<th>corn oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campesterol</td>
<td>107.0 ± 6.1</td>
<td>115.3 ± 7.3</td>
</tr>
<tr>
<td>Campestanol</td>
<td>trace</td>
<td>8.5 ± 0.7</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>50.6 ± 2.5</td>
<td>45.7 ± 3.6</td>
</tr>
<tr>
<td>(\beta)-Sitosterol</td>
<td>559.3 ± 8.5</td>
<td>458.0 ± 9.1</td>
</tr>
<tr>
<td>(\beta)-Sitostanol</td>
<td>32.9 ± 6.5</td>
<td>42.4 ± 1.5</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values are mean ± SD of triplicat analysis

4 Conclusion

The fatty acid compositions of roselle seed and corn oils were analogous to many patterns of conventional edible oils (32-34) and their chemical and physical characteristics were similar, so that it could be predicted that roselle seed oil may be used as an edible oil. The oil contains large amounts of unsaponifiable components and is rich in phytosterols. Further studies on the nutritional value of the oil is necessary.

References