Characterization of Egyptian Moringa oleifera Lipids (Whole Seeds and Kernels)

Salama, M. A.; 1 M. A. Owon; 1 M. F. Osman; 1 Awatif I. Esmail and B. Matthäus 2

1 Food Technology Research Institute, Agriculture Research Center, Egypt.
2 Food Technology Department, Faculty of Agriculture, Kafreshelk University, Egypt.
3 Max Rubner-Institut (MRI) Bundesforschungsinstitut für Ernährung und Lebensmittel, Institut für Sicherheit und Qualität bei GetreideSchützenberg, Detmold, Germany

ABSTRACT

Moringa seeds (Moringa oleifera) are rich in oil contents, the purpose of this study is to extract the oil from the whole seeds and kernels of the Egyptian Moringa by cold and hot extraction methods and to study the effect of extraction method on the composition of fatty acids, tocopherols and triglycerides in extracted oil. The results indicated that the amount of extracted oil by hot extraction were 28.85% and 39.23% and by cold cold extraction were 21.39% and 29.34% for whole seeds and kernels, respectively. The main saturated and unsaturated fatty acids in both were behenic (6.76 – 6.49%) and oleic (66.22 – 65.78%) acids. Palmitic acid was higher in the kernels than in the whole seeds (5.90 – 5.55%), while vaccenic acid was higher in the whole seeds than in the kernels (6.33 – 6.15%), respectively. After cold extraction, stearic and behenic acids increased in whole seeds (4.55 to 5.51%), (6.76 to 7.67%) and kernels (4.45 to 5.52%), (6.49 to 7.85%), respectively. While, oleic, vaccenic, linoleic and gondoic acids decreased by cold extraction in whole seeds and kernels. Tocopherols obtained from kernels were more than whole seeds, α-Tocopherol by hot extraction in the kernels and the whole seeds was 20.92 and 14.61 mg/100g, respectively. Using cold extraction in kernels caused significant decrease in α-Tocopherol (20.92 – 16.82 mg/100g). A significant difference appeared between whole seeds and kernels in the triglycerides (2,3-dioleyl-1-palmititylglycerol) "POO" (15.64% and 15.86%) and (1,2,3 trioleylglycerol) "OOO" (34.22 and 34.32%). After cold extraction in both, triglycerides (1,3-diestearoyl-2-oleylglicerol) "SOS" and (2,3-dioleyl-1- stearoyl) "SOO" increased (1.77 – 2.17% and 9.82 – 11.25% whole seeds) and (1.80 – 2.07% and 9.86 – 11.38% kernels).

Keywords: Moringa oleifera, Oil extraction, Fatty acids, Tocopherols, triglycerides.

INTRODUCTION

Moringa oleifera is a member of family Moringaceae. It is the most famous and utilized species in this family (Morton, 1991). It is widely spread in the western and sub-Himalayan tracts, India, pakistan, Africa (Mughal et al., 1999). Also it is found in central America, Caribbean Islands and North and South of America (Morton, 1991).

It has many traditional names such as horseradish or drumstick. In Egypt they called it "Shagara al Rauwaq" the tree of purification (Von, 1986). Due to its coagulating properties, it is used for water purification (Kalogo et al., 2000; Anwar et al., 2007). The shape of the seeds is triangle or round. These seeds are present inside pods. The kernels surrounded by coat which is easy to remove (Abdulkarim et al., 2005). 400 – 1000 pods and 15000 – 25000 seeds could be taken from each tree per year. The ratio of the kernels to the coat is 75-25% (Jahn, 1988).

Moringa oleifera considers as a vegetable food in some countries (Siddhuraju and Becker, 2003). The young pods are eaten in Indonesia like vegetables and it taste like asparagus (TANCN, 2003). The fried seeds have a taste like peanuts (Qaiser, 1973). The leaves are used by Philippine women mixed with chicken or shellfish soup to increase the production of milk for women (Siddhuraju and Becker, 2003). Oduro et al., (2008) reported that the leaves contained a good amount of iron and calcium. Also, it can be used as an external application for wounds (The Wealth of India, 1962). Sodamade et al., (2013); Govrishankar et al., (2010) reported that the leaves were a good source of Na, Fe, Cu, Zn and Mg. Marrufo et al., (2013) reported that Moringa oleifera leaves contained essential oil which had antimicrobial activities.

Many parts of Moringa tree contain a good amount of amino acids – especially essential amino acids (Amagloh and Benang, 2009). Moringa oleifera root, seeds and leaf protein are characterized of good quality and as such are suitable for animal feeds and human diets (Okereke and Akaninwor, 2013). Anwar et al., (2007) reported that Moringa is an important source of minerals, protein, vitamins, phenolic compounds and beta-carotene.

All parts of this tree have medicinal properties. The Wealth of India, (1962) and Dahot, (1988) reported that it can be used in the treatment of rheumatism, venomous bites and ascesis. Also, it has an effect as antiepileptic, antiyptic, anti-inflammatory, antiulcer effects and antitumor (Cáceres et al., 1992; Singh and Kumar 1999; Morimitsu et al., 2000; Siddhuraju and Becker, 2003).

Ben oil the common name of the oil extracted from Moringa contains high amount of oleic acid. As known mono unsaturated fatty acids (oleic acid) has more oxidative stability than polyunsaturated fatty acids at frying processes and storage. Moringa oil (Ben oil) has more stability during frying than canola, soybean and palm olein oils (Abdulkarim et al., 2007). It smells like peanut (Kleiman et al., 2008). All the main fatty acids in olive oil are found in moringa oil, so it can be used as an alternative to olive oil after some modification (Dahot and Memon, 1985). Tsaknis et al., (1999) said that Moringa oil was used in making perfume and products for hair protection. The most prominent polyunsaturated triglycerides (TAG) in Moringa oil was triolein "OOO" 36.7% (Abdulkarim et al., 2005). Several studies used different methods to extract Moringa oil. Abdulkarim et al., (2005) used hot extraction with different solvents and aqueous enzymatic methods. They found the oil amount extracted by solvent was higher than enzymatic methods. Oleic acid was higher in enzymatic methods than in the solvents (70.00 – 67. 90%). Other study (Tsaknis et al., 1999) extracted the oil from Moringa seeds using three different procedures including cold press, extraction with n-hexane and extraction with a mixture of chloroform/methanol (50:50). Using hexane extracted the highest amount of the oil was 35.70%. Oleic acid by using cold press reached to 75.39% followed by 73.91% by chloroform/methanol (50:50). The properties and the content of the oil can be changed and this may be due to the environmental condition and the species (Ibrahim et al., 1974).
This research aims to study the difference between the oil extracted from *Moringa oleifera* whole seeds and kernels by two methods "hot and cold extraction" by studying fatty acids, tocopherols and triglycerides.

**MATERIALS AND METHODS**

1. **Materials:**
   - The materials used in this investigation were *Moringa oleifera* seeds. These seeds were purchased from Agriculture research center, Sakha, Kaufelsheikh City, Egypt.

2. **Preparation of Moringa oleifera for analysis:**
   - Mature *Moringa oleifera* dried pods were collected from Agriculture research center – Sakha - Kaufelsheikh–Egypt. The pods were opened to collect the seeds from inside. The seeds were dried in the sun. After that the seeds were divided to two portions. The first one we grinded it with the coat, this one called “Whole seeds”. The second one the coat was removed from the seeds before grinding and called it “Kernels”.

3. **Chemicals:**
   - Petroleum ether (40–60) was of analytical grade (>98%; Merck, Darmstadt, Germany). Heptane and tert-butyl methyl ether were of HPLC grade (Merck, Darmstadt, Germany). Tocopherol, tocotrienol standard compounds and Folin-Ciocalteau reagent were purchased from Merck, Germany. Standards fatty acids methyl esters (>98%; Merck, Darmstadt, Germany) onto a Diol phase HPLC column 25 cm × 4.6 mm ID (Merck, Darmstadt, Germany) with a flow rate of 1.3 mL/min. The mobile phase was n-heptane and tert-butyl methyl ether.

4. **Triglycerides composition:**
   - The triacylglycerol composition was determined by gas chromatography according to method DGF-C-VI 14, (2013).

5. **Statistical analysis:**
   - The data were statistically analyzed by paired samples T test analysis of variance (ANOVA) procedure with SPSS software (Version 16.0, SPSS Inc., Chicago, IL) software (Steel and Torrie, 1980).

6. **Chemical composition:**
   - From Tables (1 and 2), the kernels of *Moringa oleifera* had more amount of protein (40.18%) and ether extract (39.23%) by hot extraction than the whole seeds were (31.30%) and (28.85%), respectively. While by cold extraction the oil amount was 21.39% - 29.34% from the whole seeds and kernels, respectively.

7. **Ash, fibers and carbohydrates were higher in the whole seeds (4.22%, 26.71% and 8.92%) than the kernels (4.04%, 13.96% and 2.59%), respectively. This is due to the presence of the coat with the whole seeds.

8. **This finding is nearly similar with Abiodun et al., (2012) and Rahman et al., (2009) they reported that the moisture, protein and ash from the whole seeds were (4.70 – 7.10%), (28.04 – 31.80%) and (4.10 – 6.30%), respectively. Also, Anwar and Bhangers, (2003) and Compaoré et al., (2011) reported that the protein was 29.36% and 35.37% in the kernels of *Moringa oleifera*.**

<table>
<thead>
<tr>
<th>Table 1. Chemical composition of <em>Moringa oleifera</em> (whole seeds and kernels) (on dry weight basis):</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition</strong></td>
</tr>
<tr>
<td>Moisture</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Ether extract*</td>
</tr>
<tr>
<td>Ash</td>
</tr>
<tr>
<td>Fibers</td>
</tr>
<tr>
<td>Carbohydrates</td>
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</table>

*Hot extraction*

| Table 2. Effect of extraction methods on the oil amount of *Moringa oleifera*:
<table>
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<tbody>
<tr>
<td><strong>Extraction methods</strong></td>
</tr>
<tr>
<td>Hot</td>
</tr>
<tr>
<td>Cold</td>
</tr>
</tbody>
</table>

*The results of the oil amount are in agreement with the results obtained by Bhutada et al., (2016) who reported that the oil yield from Moringa whole seeds using petroleum ether (hot extraction) was 28.60%. According to...*
Kibazohi and Sangwan, (2011), the oil was (24.80%) using hexane (hot extraction) from the Moringa whole seeds. While from Moringa kernels, Rahman et al., (2009) reported that the oil amount was (35.60%) using petroleum ether and (37.50%) using hexane (hot extraction). Also, Anwar and Rashid, (2007) and Da Porto et al., (2016) used hexane (hot extraction) and found the amount from Moringa kernels were 34.80% and 36.33%, respectively. Extraction method and solvent used, climate, time of harvest and plant variety are reasons for the difference in the amount of extracted oil (Abdulkarim et al., 2005).

**Fatty acids composition:**

Table (3) showed that the oil extracted by hot extraction in Moringa whole seeds and kernels contained palmitic acid (5.55% and 5.90%), respectively. While by using cold extraction stearic was (5.51% - 5.52%) and behenic acid was (7.76% - 7.85%) in Moringa whole seeds and kernels, respectively.

The content of the extracted oil by cold extraction for saturated fatty acids was 23.43% and 23.68%, respectively for whole seeds and kernels. While in the case of hot extraction the content of saturated fatty acids was 21.80% and 21.64%, respectively for both whole seeds and kernels. The content of unsaturated fatty acids was 75.15% - 75.42% by using cold extraction in whole seeds and kernels and was 77.54% - 76.81% in whole seeds and kernels, respectively (Table 3).

Using cold extraction caused a slight decrease in palmitic acid in whole seeds and kernels, it reached to 5.15% and 5.16%, respectively. Also, the same effect had happened in the unsaturated fatty acid gondoic (1.88% - 1.91%), respectively. While an increased had happened in stearic acid in both. No significant differences appeared in marginic acid between whole seeds and kernels also by using hot and cold extraction. Between the oil extracted from whole seeds and kernels by hot extraction a differences had appeared in myristic, plamitlic and vaccenic acids. Myristic and plamitic were higher in kernels than whole seeds (0.12 – 0.15%), (5.55 – 5.90%), respectively, while vaccenic was high in whole seeds (6.33 – 6.15%) (Table 3).

Also the results in Table (3) showed that the content of oleic acid from the oil extracted by hot extraction was 66.22% and 65.78% in whole seeds and kernels, respectively and vaccenic acid was 6.33% and 6.15% in both in comparison with cold extraction. This high amount of oleic acids makes Moringa oil preferable in nutrition and cooking (Abdulkarim et al., 2005). Also, it can be used in frying due to its oxidative stability (Petukhov et al., 1999). This finding is in agreement with the results obtained by Abdulkarim et al., (2005), Nzikou et al., (2009) and Tsaknis et al., (1999) they found that the oleic acid in Moringa oil was 70.00%, 74.93% and 73.60%, respectively.

Table 3. Fatty acids composition of Moringa oleifera (whole seeds and kernels ) by hot and cold extraction (%):

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Whole seeds</th>
<th>Kernels</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hot</td>
<td>Cold</td>
<td>Hot</td>
<td>Cold</td>
<td></td>
</tr>
<tr>
<td>Myristic C_{14:0}</td>
<td>0.12±0.021a</td>
<td>0.09±0.006</td>
<td>0.15±0.023a</td>
<td>0.09±0.000</td>
<td></td>
</tr>
<tr>
<td>Palmitic C_{16:0}</td>
<td>5.55±0.255a</td>
<td>5.15±0.046</td>
<td>5.90±0.139a</td>
<td>5.16±0.030</td>
<td></td>
</tr>
<tr>
<td>Margaric C_{17:0}</td>
<td>0.08±0.012</td>
<td>0.09±0.000</td>
<td>0.08±0.010</td>
<td>0.08±0.006</td>
<td></td>
</tr>
<tr>
<td>Stearic C_{18:0}</td>
<td>4.55±0.066a</td>
<td>5.51±0.066a</td>
<td>4.45±0.112a</td>
<td>5.52±0.010</td>
<td></td>
</tr>
<tr>
<td>Arachidic C_{20:0}</td>
<td>3.35±0.119</td>
<td>3.63±0.036</td>
<td>3.21±0.046</td>
<td>3.72±0.020</td>
<td></td>
</tr>
<tr>
<td>Behenic C_{22:0}</td>
<td>6.76±0.419a</td>
<td>7.67±0.139a</td>
<td>6.49±0.082</td>
<td>7.85±0.030</td>
<td></td>
</tr>
<tr>
<td>Lignoceric C_{24:0}</td>
<td>1.39±0.060</td>
<td>1.29±0.015</td>
<td>1.36±0.023a</td>
<td>1.26±0.006</td>
<td></td>
</tr>
<tr>
<td>TSFA</td>
<td>21.80</td>
<td>23.43</td>
<td>21.64</td>
<td>23.68</td>
<td></td>
</tr>
<tr>
<td>Palmitoleic C_{16:1}</td>
<td>1.42±0.066</td>
<td>1.36±0.006</td>
<td>1.42±0.040</td>
<td>1.43±0.015</td>
<td></td>
</tr>
<tr>
<td>Elaidic C_{18:1}</td>
<td>0.24±0.050a</td>
<td>0.16±0.020a</td>
<td>0.26±0.029a</td>
<td>0.37±0.000</td>
<td></td>
</tr>
<tr>
<td>Oleic C_{18:1}cis</td>
<td>66.22±0.172</td>
<td>65.16±0.016</td>
<td>65.78±0.154</td>
<td>64.84±0.172</td>
<td></td>
</tr>
<tr>
<td>Vaccenic C_{18:1}tr</td>
<td>6.33±0.031a</td>
<td>5.78±0.010</td>
<td>6.15±0.045a</td>
<td>6.07±0.015</td>
<td></td>
</tr>
<tr>
<td>Linoleic C_{18:2}</td>
<td>0.63±0.006a</td>
<td>0.56±0.010a</td>
<td>0.62±0.010a</td>
<td>0.55±0.006</td>
<td></td>
</tr>
<tr>
<td>Arachidonic C_{18:3}</td>
<td>0.18±0.032</td>
<td>0.16±0.000</td>
<td>0.17±0.000</td>
<td>0.16±0.006</td>
<td></td>
</tr>
<tr>
<td>Gondoic C_{20:1}</td>
<td>2.37±0.075a</td>
<td>1.88±0.025a</td>
<td>2.29±0.012a</td>
<td>1.91±0.010a</td>
<td></td>
</tr>
<tr>
<td>Erucic C_{22:1}</td>
<td>0.15±0.029</td>
<td>0.09±0.006</td>
<td>0.12±0.015</td>
<td>0.09±0.000</td>
<td></td>
</tr>
<tr>
<td>TUSFA</td>
<td>77.54</td>
<td>75.15</td>
<td>76.81</td>
<td>75.42</td>
<td></td>
</tr>
<tr>
<td>The sum</td>
<td>99.34</td>
<td>98.58</td>
<td>98.45</td>
<td>99.10</td>
<td></td>
</tr>
</tbody>
</table>

(1) Means that there are significant differences between whole seeds and kernels (hot extraction) at ≤0.05.
(a) Means that there are significant differences between whole seeds (hot and cold extraction) at ≤0.05.
(b) Means that there are significant differences between kernels (hot and cold extraction) at ≤0.05.

Moringa oleifera whole seeds and kernels contained a good amount of behenic acid (6.76 – 6.49%), respectively. Behenic acid using hot extraction of whole seeds and kernels (6.76 – 7.67%) increased in cold extraction of kernels (7.85%) and whole seeds (7.67%). Behenic acid result was similar to the one which reported by Abdulkarim et al., (2005), Rashid et al., (2008) and Nguyen et al., (2011) which reached (5.80%), (7.00%), (7.01%) and (6.71%), respectively. In skincare, behenic acid is most commonly used to provide soothing relief for dry and sensitive skin (Banov et al., 2014). The obtained results of vaccenic acid was in similar to the result reported Al Juhaimi et al., (2016) who found vaccenic acid was (6.00%) in Moringa seeds. Some health benefits for vaccenic acid were reported by Field et al., (2009). Significant difference occurred in myristic and plamonic acids between whole seeds and kernels (0.12 – 0.15%) and (5.55 – 5.90%), respectively.

**Tocopherols:**

Moringa oleifera (whole seeds and kernels ) has a good amount of tocopherols and this give more protection and stability during storage and manufacturing processes for Moringa oil (Tsaknis et al., 1999). The kernels had more amounts of tocopherols by hot extraction than the whole seeds 20.92 – 14.61 mg/100g α-tocopherol, 1.01 –
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0.94 mg/100g β-tocopherol, 5.77 – 5.31 mg/100g γ-tocopherol and 1.10 – 1.01 mg/100 g δ-tocopherol, respectively (Table 4).

Almost these results were near to Rahman et al., (2009) who used light petroleum ether for extracting the oil form the Moringa kernels and found that α-tocopherol, γ-tocopherol and δ-tocopherol were 12.1, 6.4, 5.77 mg/100g, respectively. Also, Anwar and Bhanger, (2003) determined α-tocopherol, γ-tocopherol and δ-tocopherol from Moringa kernels but they used n-hexane and they found the results were 13.44, 9.37 and 4.80 mg/100g, respectively. However, from whole seeds and by using n-hexane, Tskakis et al., (1999) reported that α-tocopherol, γ-tocopherol and δ-tocopherol were (9.82, 2.79 and 7.11 mg/kg), respectively.

Table 4. Tocopherols composition of Moringa oleifera (whole seeds and kernels) by hot and cold extraction mg/100g:

<table>
<thead>
<tr>
<th>Tocopherols</th>
<th>Moringa oleifera Whole seeds</th>
<th>Moringa oleifera Kernels</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol</td>
<td>Hot: 14.61±1.497*</td>
<td>Cold: 20.92±0.710**</td>
</tr>
<tr>
<td>α-Tocotrienol</td>
<td>0.18±0.015*</td>
<td>0.22±0.029</td>
</tr>
<tr>
<td>β-Tocopherol</td>
<td>0.94±0.076</td>
<td>1.01±0.035</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>5.31±0.235</td>
<td>5.77±0.278b</td>
</tr>
<tr>
<td>Plastochromanol 8</td>
<td>0.20±0.171a</td>
<td>0.25±0.223b</td>
</tr>
<tr>
<td>δ-Tocopherol</td>
<td>1.01±0.092a</td>
<td>1.10±0.174</td>
</tr>
</tbody>
</table>

(*) Means that there are significant differences between whole seeds and kernels (hot extraction) at ≤0.05.
(a) Means that there are significant differences between whole seeds (hot and cold extraction) at ≤0.05.
(b) Means that there are significant differences between kernels (hot and cold extraction) at ≤0.05.

The results of γ-Tocopherol, Plastochromanol 8 (Plastochromanol-8 is an analogue of γ-tocotrienol with a much longer side-chain). Plastochromanol-8 was first found in leaves of the rubber tree (Hevea brasiliensis) from 50 years ago (Whittle et al., 1965). It has been found in many other plants including rapeseed and maize (Dunphy et al., 1966), but usually at lower levels than of the tocopherols and δ-tocopherol in whole seeds and kernels after cold extraction changed. Data in Table (4) indicated that a decreasing happened to γ-tocopherol and δ-tocopherol in whole seeds and kernels (5.31 – 3.35 mg/100g), (5.77 – 4.27 mg/100g) and (1.01 – 0.41 mg/100g), (1.10 – 0.52 mg/100g), respectively, while plastochromanol 8 increased in cold extraction (0.20 – 0.25 mg/100g) than hot extraction (0.10 – 0.25 mg/100g), respectively. No significant differences occurred in α-tocopherol between hot and cold extraction of the whole seeds, while in the kernels a differences occurred in α-tocopherol (20.92 – 16.82 mg/100g) in hot and cold extraction, respectively (Table 4).

Triglycerides profile:

As shown in the Table (5) eleven triglycerides were detected in the order of (OOO, POO, SLO, SOO, PLS, PLO, POP, POS, PLL and PLP) (O = oleic acid, L = linoleic acid, P = palmitic acid and S = stearic acid). and those accounts for 85% of total triglycerides. The highest triglyceride was (OOO) in the whole seeds and kernels (34.22 – 34.32%) followed by “POO” (15.64 – 15.86%), “SLO” (10.25 – 10.45%) and “SOO” (9.82 – 9.86%). A difference occurred in the triglycerides “POO” and “OOO” between whole seeds and kernels. These results were near to Abdulkarim et al., (2005) who reported that “OOO” and “SOO” were (36.70 – 11.40%) in the whole seeds.

Using cold extraction (whole seeds) made a difference in the triglycerides “SOS” and “SOO” (1.77 – 2.17%) and (9.82 – 11.25%), respectively. This increase may be due to the increase of stearic acid in the whole seeds after cold extraction (4.55 - 5.51%). Also, due to the increase reported in stearic acid after cold extraction in kernels, the triglycerides “POS” and “SOO” increased (2.07 – 2.32%) and (9.87 – 11.38%), respectively. However the decreasing occurred in “PLP” (1.38 – 0.83%) was due to the decrease in palmitic acids after cold extraction in the kernels (5.90 – 5.16%).

Table 5. Triglycerides profile of Moringa oleifera (whole seeds and kernels) by hot and cold extraction (%):
CONCLUSION

Removing the coat from the whole seeds and extracting oil from the kernels increased the oil amount. This coat can be used in several uses. Cold extraction method caused an increase in the TSFA in the whole seeds and kernels, while the TUSFA decreased in both. Tocopherols were higher in the kernels oil than the whole seeds oil but cold extraction caused a decrease in tocopherol in both. Also, triolen "OOO" and "POO" were higher in the kernels oil. Due to the increase of stearic acid after cold extraction in whole seeds and kernels this makes an increase in the triglycerides "SOO and SOS".

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