

# *Oxidative properties of Moringa oleifera kernel oil from different extraction methods during storage*

Article

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1 (Title)

2 **Oxidative properties of *Moringa oleifera* kernel oil from different extraction methods**  
3 **during storage**

4  
5 (Running title)

6 **Oxidative properties of *Moringa oleifera* kernel oil during storage**

7  
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20  
21 **Abstract**

22  
23 **BACKGROUND**

24 *Moringa oleifera* (MO) kernel oil is categorized as high-oleic oil which resembles olive oil.

25 However, different from olive, MO trees are largely available in most subtropical and tropical

26 countries. Therefore, in these countries, the benefits of oleic acid can be obtained at a cheaper  
27 price through consumption of MO kernel oil. This study reports on the effect of different  
28 extraction methods on oxidative properties of MO kernel oil during storage for 140 days at 13  
29 °C, 25 °C, and 37 °C.

30

## 31 RESULTS

32 All aqueous enzymatic extraction (AEE)-based methods generally resulted in oil with better  
33 oxidative properties and higher tocopherol retention than the use of solvent. Prior to AEE,  
34 boiling pre-treatment deactivated the hydrolytic enzymes and preserved the oil quality. In  
35 contrast, high pressure processing (HPP) pre-treatment accelerated hydrolytic reaction and  
36 resulted in higher free fatty acids after 140 days at all temperatures. No significant changes  
37 were detected in the oils' iodine values and fatty acid compositions. The tocopherol contents  
38 decreased significantly at both 13 °C and 25 °C after 60 days in the oil from SE method, and  
39 after 120 days in oils from AEE-based methods.

40

## 41 CONCLUSION

42 These findings are significant in highlighting the extraction method resulting in crude MO  
43 kernel oil with greatest oxidative stability in the storage conditions tested. Subsequently, the  
44 suitable storage condition of the oil prior to refining can be determined. Further studies are  
45 recommended in determining the suitable refining processes and parameters for the MO  
46 kernel oil prior to application in variety food products.

47

48 **Keywords** horseradish, drumstick, murungai, seed, lipid, fat

49

## 50 Introduction

51

52 *Moringa oleifera* (MO), also known as horse-radish, kelor, or drumstick tree, are widely  
53 distributed in Pakistan, India, Nigeria, Philippines, Kenya, Caribbean Island, Cambodia,  
54 Malaysia, and Bangladesh. One of the important parts of the tree is its fruit pods which turn  
55 into brown color upon maturation and contain mature brown MO seeds. The kernels inside  
56 the mature seeds contain the edible MO oil, also known as Ben or Behen oil <sup>[1-9]</sup>. This oil is  
57 applicable in perfume industry, hair-care products, in medicinal practices, and act as a good  
58 lubricant for fine machineries <sup>[7]</sup>. As an edible oil, MO oil is generally applied as culinary and  
59 salad oil in Haiti and other countries <sup>[10, 11]</sup>. Tsaknis and Lalas (2002) <sup>[12]</sup> and Abdulkarim et  
60 al. (2007) <sup>[13]</sup> concluded the suitability of MO oil for frying purpose. The fatty acid  
61 composition of MO oil resembles that of olive oil, with high oleic acid content in addition to  
62 the significant tocopherols content <sup>[7, 12, 14]</sup>. These properties contributed to the oil's oxidative  
63 stability <sup>[6]</sup>, and consumption of oleic acids was always related to reduced-risk of developing  
64 coronary heart disease <sup>[13, 15]</sup>.

65 A number of techniques are available for oil extraction from MO kernels, which  
66 include solvent extraction (SE) and aqueous enzymatic extraction (AEE) methods, among  
67 others. The AEE of oil from oil-bearing materials was reviewed by Mat Yusoff et al. (2015)  
68 <sup>[16]</sup> and Rosenthal et al. (1996) <sup>[17]</sup>, while studies specifically on AEE of MO oil have been  
69 reported by Mat Yusoff et al. (2016) <sup>[18]</sup>, Latif et al. (2011) <sup>[19]</sup>, and Abdulkarim et al. (2005,  
70 2006) <sup>[4, 20]</sup>. According to these studies, MO kernel cells contain approximately 35% (w/w)  
71 protein and 40% (w/w) fat content, and the protein is the major component in the MO cell  
72 wall surrounding the oil. These findings proved the need to add a proteolytic enzyme to  
73 hydrolyze the protein component for higher oil release. At the end of an AEE process, two  
74 types of oils are produced – free oil which can be recovered, and emulsified oil in a cream  
75 emulsion which is formed due to the use of water as an extraction medium in the presence of

76 MO kernel protein. The earlier studies only succeeded in extracting up to 70% (w/w) total  
77 MO oil – measured as the mass of oil extracted using enzymes against the total oil extracted  
78 using solvent.

79         Similar issues of lower extracted oil in an AEE process as compared to the use of  
80 solvent were also reported in most studies involving other types of oils as reviewed by Mat  
81 Yusoff et al. (2015) <sup>[16]</sup>. Many studies attempted to overcome this problem by conducting a  
82 pre-treatment in order to assist cells rupture for higher oil release in the following AEE  
83 process. One of the pre-treatments conducted was the use of high-pressure processing (HPP)  
84 on soybean seeds which resulted in 3.20% (w/w) and 1.30% (w/w) higher free oil recovery at  
85 200 MPa and 500 MPa, respectively. In the case of MO kernels, Mat Yusoff et al. (2017) <sup>[21]</sup>  
86 reported that the HPP pre-treatment successfully modified the MO protein structure into a  
87 form of less emulsifying ability, thus smaller amount of oil got emulsified which resulted in  
88 thinner cream emulsion layer and 4.19% (w/w) higher free oil recovery as compared to the  
89 use of AEE alone <sup>[21]</sup>. Additionally, the study also reported 4.98% (w/w) increase in free oil  
90 recovery when the AEE was pre-treated with boiling (100 °C, 5 min) – this boiling pre-  
91 treatment was conducted in earlier studies <sup>[4, 18, 19, 20]</sup> prior to an AEE of MO oil to inactivate  
92 the natural hydrolytic enzymes in the seed kernels <sup>[22]</sup>.

93         Fotouo et al. (2016) <sup>[23]</sup> demonstrated the effect of storage of MO seeds on the  
94 quantity and quality of the MO oil for its potential in biodiesel production. However, the  
95 effect of storage of MO oil at different temperatures on its quality attributes had never been  
96 reported, which is of great importance in determining the shelf life of the oil. Furthermore, to  
97 the best of our knowledge, the study on HPP pre-treatment prior to AEE of MO oil was  
98 conducted for the first time <sup>[21]</sup>, and no study has reported on the quality attributes of the MO  
99 oil extracted from this process.

100           The main objective of this study was to further explore the differences, particularly in  
101 oxidative properties of MO oil from different extraction methods - the solvent extraction  
102 (SE), aqueous enzymatic extraction (AEE), AEE with boiling pre-treatment (B-AEE), and  
103 AEE with high-pressure processing pre-treatment (HPP-AEE). The MO oils were stored for  
104 140 days at different temperatures of 13 °C, 25 °C, and 37 °C, and changes in the oxidative  
105 properties were evaluated during the storage period in terms of their peroxide value (PV), *p*-  
106 Anisidine value (*p*-AV), total oxidation (TOTOX) value, free fatty acids (FFA), iodine value  
107 (IV), fatty acid composition (FAC), and tocopherol content.

108

## 109 **Materials and Methods**

110

### 111 **Materials**

112

113 Mature MO seeds (PKM1 hybrid) were purchased from Genius Nature Herbs Pvt. Ltd.,  
114 Coimbatore, India. All solvents and enzymes used in this study were purchased from Sigma-  
115 Aldrich Company Ltd., Dorset, UK.

116

### 117 **Statistical Analysis**

118

119 All statistical analyses in this study were done by using Minitab® 14.12.0 Statistical  
120 Software. A one-way analysis of variance (ANOVA) with Tukey's multiple comparison test  
121 (confidence level 95.0%) was applied for the determination of significant differences between  
122 more than two samples (each sample with replicates data). A 2-Sample t-test was used to  
123 determine significant differences between two samples (replicates data for each sample),

124 while a 1-Sample t-test was used when a sample (with replicates data) was statistically  
125 compared with another sample which has one datum only.

126

### 127 **Preparation of *Moringa oleifera* Kernels for Oil Extraction**

128

129 The MO kernels were randomly collected and conditioned at 50 °C for 8 hr followed by  
130 grinding (De'Longhi KG49 Electric Coffee Grinder, Hampshire, UK) and sieving using a  
131 vibratory sieve shaker (Fritsch, Analysette 3E) to  $\leq 710 \mu\text{m}$  particle size. According to Mat  
132 Yusoff et al. (2016) <sup>[18]</sup>, the use of ground-sieved MO kernels at this particle size resulted in  
133 highest amount of MO oil ( $410.3 \text{ g kg}^{-1}$ ) as extracted using hexane. All oil extraction methods  
134 conducted in this study were based on studies done by Mat Yusoff et al. (2016, 2017) <sup>[18, 21]</sup>  
135 and were performed on the ground-sieved MO kernels.

136

### 137 **Solvent Extraction (SE) Method**

138

139 Soxhlet method was used to extract the MO oil with the use of hexane for 6 hr extraction  
140 time. A total of six refluxes were used each time. The hexane was evaporated from the  
141 extracted oil in a round bottom flask of pre-determined weight by using a rotary evaporator  
142 ( $60 \text{ }^\circ\text{C}$ , 10 min), followed by heating in an oven ( $100 \text{ }^\circ\text{C}$ , 15 min). The difference between the  
143 initial (empty) and final (containing the extracted oil) weight of the round bottom flask used  
144 was measured as the oil yield in the meal by normalizing this against the weight of the  
145 kernels taken initially.

146

### 147 **Aqueous Enzymatic Extraction (AEE) Method**

148



149 A mixture of ground-sieved MO kernels and distilled water at 1:4 (w/w) ratio was prepared  
150 and adjusted to pH 6.0. A mixture of 2% (g enzyme / g kernel) of protease (Neutrase 0.8L,  
151 optimum pH 6.8) and cellulase (Celluclast 1.5L, optimum pH 4.8) enzymes at 3:1 (w/w) ratio  
152 was added into the mixture, followed by incubation at 50 °C for 12.5 hr at 300 stroke/min  
153 shaking speed. The incubated mixture was centrifuged at 4000 rev/min for 20 min which  
154 induced separation into four distinct layers of free oil at the top, followed by the cream  
155 emulsion layer, the aqueous phase, and the meal at the bottom. Recovery of the free oil is  
156 explained in section 'Recovery of free oil'.

157

#### 158 **Aqueous Enzymatic Extraction with Boiling Pre-treatment (B-AEE)**

159

160 Similar AEE as in the previous section was conducted, with addition of boiling pre-treatment  
161 in a water bath (100 °C, 5 min), followed by cooling to room temperature prior to adjusting  
162 the mixture's pH, Recovery of the free oil is explained in section 'Recovery of free oil'.

163

#### 164 **Aqueous Enzymatic Extraction with High Pressure Processing Pre-treatment (HPP- 165 AEE)**

166

167 The ground-sieved MO kernels were mixed with distilled water at 1:1 (w/w) ratio and  
168 vacuum sealed in polyethylene bags. According to Mat Yusoff et al. (2017) <sup>[21]</sup>, some  
169 preliminary tests were carried out to determine this solid-to-liquid ratio. The use of smaller  
170 amount of water caused formation of a very thick paste which adhered to the polyester bag,  
171 thus wasted some of the sample. In another way, addition of higher water content resulted in  
172 a very dilute mixture which thus allowed only small amount of ground-sieved MO kernel to  
173 be processed at one time.

174 The mixtures at 1:1 (w/w) ratio were treated with high pressure (Stansted Fluid  
175 Powder Ltd., Stansted, UK) at 50 MPa and 60 °C for 35 min, followed by addition of distilled  
176 water up to 4:1 water/kernel (w/w) ratio for the subsequent AEE as in the earlier section.  
177 Recovery of the free oil is explained in section ‘Recovery of free oil’.

178

### 179 **Recovery of Free Oil**

180

181 The centrifuged mixtures obtained in all the AEE-based methods explained earlier were kept  
182 at -20 °C for 24 h. The solidified oil was transferred to a crucible of pre-determined weight  
183 and heated in an oven at 100 °C for 15 min to ensure complete removal of any aqueous phase  
184 that may present in the recovered oil. The crucible containing the oil was cooled to room  
185 temperature in a desiccant containing silica gel for approximately 10 min before being  
186 weighed. The free oil yield and recovery were calculated as follows:

187

$$188 \text{ Oil yield (\%)} = \frac{[\text{Mass of crucible containing the oil (g)} - \text{Mass of crucible (g)}] \times 100}{\text{Mass of kernels initially taken (g)}}$$

189

$$190 \text{ Free oil recovery (\%)} = \frac{\text{Mass of oil extracted from a given mass of kernel (g)} \times 100}{\text{Mass of oil contained in the kernels initially taken (g)}}$$

191

### 192 **Storage of *Moringa oleifera* Oil**

193

194 MO oil samples extracted using the SE method and all AEE-based methods explained in  
195 previous sections were filled in transparent glass bottles with screw-caps, up to the bottle's  
196 neck in order to minimize the headspace. The bottles were wrapped in aluminium foil and  
197 stored in dark to avoid light exposure. The storage temperatures used in this study were in

198 reference to Pristouri et al. (2010) [24]. At 13 °C, the MO oil was stored to simulate the  
199 temperature of the cellar commercially used for storing olive oil. Storage at 25 °C and 37 °C  
200 were selected for simulating room temperature and elevated ambient temperature normally  
201 occurred during the summer, respectively. All oil samples from different extraction methods  
202 were stored in these temperatures for 140 days, and the analysis of their PV, *p*-AV, TOTOX,  
203 FFA, and tocopherol content were performed on day 0, day 60, day 120, and day 140.  
204 Additionally, differences in the IV and FAC between the extracted oils before (i.e. day 0) and  
205 after the whole storage period (i.e. day 140) were also examined. Determination of these  
206 oxidative properties was performed on each oil sample in triplicate.

207

#### 208 **Determination of Peroxide Value (PV)**

209

210 Peroxide value (PV) of the oil samples was determined in reference to AOCS Official  
211 Method Cd 8-53 (2000) [25] and MPOB Test Method p2.3 (2004) [26] with modification. A  
212 mixture of 5.0 g oil sample and 20 ml glacial acetic acid/chloroform (1.5:1 v/v) was prepared  
213 and swirled until completely dissolved. Excess of saturated potassium iodide solution was  
214 added to the mixture, followed by swirling for 1 min. The mixture was combined with 30 ml  
215 distilled water and few drops of starch indicator, before being titrated with 0.01 N sodium  
216 thiosulphate until the blue-gray color disappeared. The above steps were repeated without  
217 adding the oil sample for blank purpose. The following formula was used to calculate the PV  
218 of the oil sample:

219

$$220 \text{ Peroxide value } \left( \text{mEq} \frac{\text{O}_2}{\text{kg}} \right) = \frac{(V_b - V_s) \times 0.01 \times 1000}{W}$$

221

222  $V_b$  = Titre for blank (ml)

223  $V_s$  = Titre for sample (ml)

224  $W$  = Weight of sample (g)

225 0.01 = Normality of titrant (N)

226 1000 = Unit conversion (g/kg)

227

### 228 **Determination of *p*-Anisidine Value (*p*-AV)**

229

230 *p*-Anisidine value (*p*-AV) of oil samples was slightly modified according to AOCS Official

231 Method Cd18-90 (2000) [25]. An oil sample of 0.5 g was weighed into a 25 ml volumetric

232 flask and topped up with isooctane. The absorbance of the oil-isooctane solution ( $A_1$ ) was

233 determined at 350 nm against isooctane (blank 1) (Cecil CE 1021 UV/Visible

234 Spectrophotometer 1000 series). 5 ml of the oil-isooctane solution was transferred into a 10

235 ml glass bottle (with screw cap), added with 1 ml of anisidine reagent (0.25% w/v anisidine

236 reagent in glacial acetic acid), shook vigorously, and kept in dark for 10 min. Similarly, 5 ml

237 of isooctane in a glass bottle was also added with 1 ml anisidine reagent, shook vigorously,

238 and kept in dark for 10 min (blank 2). The absorbance of the oil-isooctane containing

239 anidisine reagent ( $A_2$ ) was determined at 350 nm against blank 2. The *p*-AV was calculated

240 as follow:

241

$$242 \text{ } p - \text{Anisidine value} = \frac{25 \times (1.2A_2 - A_1)}{W}$$

243

244  $A_1$  = Absorbance of the oil-isooctane solution

245  $A_2$  = Absorbance of the oil-isooctane containing anidisine reagent

246 25 = Volume of which the oil sample is dissolved with isooctane (ml)

247 1.2 = The correction factor for the dilution of the test solution with 1 ml of the anisidine

248 reagent or glacial acetic acid

249 W = Weight of sample taken (g)

250

### 251 **Determination of Total Oxidation (TOTOX) Value**

252

253 Total oxidation (TOTOX) value of the oil samples was determined according to AOCS

254 Official Method Cg 3-91 (2000) [25]. This value takes into account both the PV and *p*-AV of

255 the oil sample and calculated according to the following formula: TOTOX value = 2PV + *p*-

256 AV

257

### 258 **Determination of Free Fatty Acids (FFA)**

259

260 Free fatty acids (FFA) of the oil samples was calculated from its acid value (AV) which was

261 determined in accord to AOCS Official Method Cd 3d-63 (2000) [25]. An oil sample of 0.5 g

262 was added to 50 ml of a mixture of diethyl ether and ethanol (95% v/v) in a 250 ml conical

263 flask. Phenolphthalein was added as an indicator, followed by titration on the whole mixture

264 with 0.1 N potassium hydroxide (KOH) solution. The whole steps were repeated without

265 adding the oil sample for blank purpose. The AV of the oil was calculated as follows:

266

$$267 \text{ Acid value (KOH g}^{-1}\text{)} = \frac{(V_b - V_s) \times 5.61}{W}$$

268

269 V<sub>b</sub> = Titre for blank (ml)

270 V<sub>s</sub> = Titre for sample (ml)

271 W = Weight of sample (g)

272 5.61 = Mass (mg) of KOH in 1 ml of 0.1 N solution

273 Free fatty acids, FFA (%) = Acid value/1.99; where 1.99 is the conversion factor for oleic  
274 acid

275

### 276 **Determination of Iodine Value (IV)**

277

278 Iodine value (IV) of the oil samples was determined according to MPOB Test Method p3:2  
279 (2004) [26] and AOCS Official Method Cd 1d-92 (2000) [25] with slight modification. Earlier  
280 studies revealed IV of MO oil which ranged from 60-70 g I<sub>2</sub> / 100 g [7, 14, 19]. Thus, an oil  
281 sample of 0.2 g was used. The oil was weighed into a conical flask and added with 20 ml  
282 chloroform and 25 ml Wijs reagent. A stopper was placed followed by vigorous shaking, and  
283 the mixture was kept in dark for 30 min. Following this step was addition of 20 ml KI  
284 solution (15% w/v KI in distilled water) and 100 ml distilled water. The mixture was titrated  
285 under vigorous shaking with 0.1 M sodium thiosulphate until the yellow colour due to iodine  
286 has almost disappeared. Few drops of starch were added afterwards, and the titration was  
287 continued until the blue colour just disappeared after very vigorous shaking. The whole steps  
288 were repeated without adding the oil sample for blank purpose. The IV was calculated based  
289 on the following formula:

290

$$291 \text{ Iodine value } \left( \text{g } \frac{\text{I}_2}{100 \text{ g}} \right) = \frac{0.1269 \times 0.1 \times (V_b - V_s) \times 100}{W}$$

292

293 V<sub>b</sub> = Titre for blank (ml)

294 V<sub>s</sub> = Titre for sample (ml)

295 W = Weight of sample (g)

296 0.1 = Normality of titrant (N)

297 0.1269 = Mass of iodine in 1 ml of 1 M solution

298

### 299 **Determination of Fatty Acid Composition (FAC)**

300

301 Fatty acid composition (FAC) of MO oil was determined according to Ezech et al. (2016) <sup>[27]</sup>,  
302 Agilent Technologies, and *TraceCERT*® (Supelco®) with slight modification. Gas  
303 Chromatography (GC, Agilent HP 6890) fitted with flame ionization detector (FID) was used  
304 for the analysis, with fused silica capillary column Varian CP-Sil 88 (60 m x 0.25 mm x 0.20  
305 µm) and helium as a carrier gas (flowrate 1.0 ml/min). The oil sample was first converted  
306 into fatty acids methyl esters (FAME) by dissolving 100 mg oil into 10 ml hexane and added  
307 with 100 µl of 2N KOH in methanol (i.e. 11.2 g KOH in 100 ml methanol). The mixture was  
308 vortexed for 30 s, centrifuged, and the clear supernatant at the upper layer was transferred  
309 into an autosampler vial. The injector and detection temperatures were 250 °C and 260 °C,  
310 respectively, while the oven temperature was 230 °C (hold 30 min). The volume of sample  
311 injected was 1 µl with split ratio of 100:1. The standard reference used was the Supelco 37  
312 Component FAME Mix (1x1ml at varied concentrations in dichloromethane). Identification  
313 of the fatty acids was done by comparing retention times with those of standards.

314

### 315 **Determination of Tocopherol**

316

317 Following the method used by Ezech et al. (2016) <sup>[27]</sup> and Costa et al. (2010) <sup>[28]</sup> with slight  
318 modification, the total tocopherols of the oil samples in this study was determined by HPLC-  
319 UV system (Agilent 1200, Manchester, UK). The column used was a Nucleosil C-18-100  
320 reverse phase column (25 cm x 4.6 mm i.d.) with a particle size of 5 µm (Macherey-Nagel,  
321 Duren, Germany), while the mobile phase was a mixture of methanol:tetrahydrofuran:water  
322 (67:27:6 v/v/v) at flowrate of 0.8 ml min<sup>-1</sup>. An oil sample of 0.1 ml was diluted with 1 ml of

323 a mixture of isopropanol:chloroform (75:25 v/v). The mixture was homogenized and 10  $\mu$ l  
324 was injected into the HPLC system at 25 °C and detection wavelength of 292 nm. The types  
325 of tocopherols reported are the  $\alpha$ - and  $\gamma$ -tocopherols, and their standard solutions were  
326 prepared by dissolving in methanol at 0.02-1.0% (v/v) concentrations. Standard calibration  
327 curve was obtained for each type of tocopherol, and identification of the tocopherols in the oil  
328 samples was done by comparing their retention times with that of the standard solutions.

329

## 330 **Results and Discussion**

331

### 332 **Effect of storage condition on peroxide value, *p*-Anisidine value, and total oxidation** 333 **value of *Moringa oleifera* kernel oil**

334

335 In the following discussion, the following terms will be used: SE-oil, AEE-oil, B-AEE-oil,  
336 and HPP-AEE-oil which indicate the MO kernel oil extracted using SE, AEE, B-AEE, and  
337 HPP-AEE methods, respectively.

338       Oxidation of lipids takes place by a free radical chain mechanism which can be  
339 explained in terms of initiation, propagation, and termination processes. These processes  
340 generally comprised of complex sequences and overlapping reactions. Figure 1 revealed  
341 changes in PV in the MO oil samples during storage at different temperatures of (a) 13 °C,  
342 (b) 25 °C, and (c) 37 °C. PV indicates formation of peroxides and hydroperoxides resulted  
343 from propagation reaction. This reaction describes the first oxidation stage involving  
344 formation of hydroperoxides as fundamental primary products. These compounds do not have  
345 significant effect on the oil's flavor deterioration. Propagation is also the most widely  
346 oxidation reaction that takes place in an oil and fat <sup>[29-33]</sup>. According to Figure 1(a), on day 0,  
347 there was no significant difference ( $p > 0.05$ ) between PV of MO kernel oil extracted using



348 different methods (approximately 0.49 mEq O<sub>2</sub>/kg). However, after 140 days storage at 13  
349 °C, PV of the SE-oil, AEE-oil, and HPP-AEE-oil increased significantly ( $p < 0.05$ ) (0.75-  
350 0.98 mEq O<sub>2</sub>/kg). These findings indicated that at 13 °C, oxidation reaction started to take  
351 place in the oil samples after 60 days of storage in SE-oil and AEE-oil. The PV started to  
352 increase later after 120 days in HPP-AEE oil, while the PV of B-AEE oil remained  
353 unchanged. At 25 °C, Figure 1(b) shows significant increase ( $p < 0.05$ ) in PV of SE-oil from  
354 day 0 (0.49 mEq O<sub>2</sub>/kg) to day-120 (2.19 mEq O<sub>2</sub>/kg) as compared to other oil samples. At  
355 37 °C (Figure 1(c)), faster increase in PV was observed in the SE-oil. On day-60, the PV of  
356 SE-oil at 25 °C was 0.99 mEq O<sub>2</sub>/kg, and the PV was significantly higher ( $p < 0.05$ ) at 37 °C;  
357 1.97 mEq O<sub>2</sub>/kg. Furthermore, on day-120, the PV of SE-oil at 25 °C was 2.19 mEq O<sub>2</sub>/kg,  
358 and the PV was significantly higher ( $p < 0.05$ ) at 37 °C; 2.96 mEq O<sub>2</sub>/kg. These findings  
359 revealed the significant effect of higher storage temperature in increasing the oxidation rate  
360 of SE-oil as compared to other oil samples.

361 After 120 days at both 25 °C and 37 °C, the PV of SE-oil started to decrease which  
362 indicated the start of initiation reaction. In this reaction, decomposition of hydroperoxides  
363 into free radicals took place which is an endothermic reaction promoted by the higher  
364 temperatures of 25 °C and 37 °C. Moreover, this thermal oxidation of unsaturated lipids is  
365 normally an autocatalytic reaction and is metal-catalyzed. The SE-oil was a crude oil which  
366 most likely contained trace metals. These trace metals are very difficult to be eliminated, thus  
367 they may act as potent catalysts which catalyzed the initiation reaction <sup>[33]</sup>. Figure 1 (a-c) also  
368 shows that the B-AEE-oil exhibited higher oxidative stability upon 140 storage days at 13 °C,  
369 since its PV did not increase at this temperature as compared to other oil samples. On the  
370 other hand, the maximum PV of HPP-AEE-oil was 0.75 mEq O<sub>2</sub>/kg under all storage  
371 condition.

372 Earlier studies also reported higher PV of MO oil extracted using solvents (0.94-1.83  
373 mEq O<sub>2</sub>/kg) as compared to cold-pressed oil (0.11-0.36 mEq O<sub>2</sub>/kg) [7, 12]. According to  
374 O'Brien (2009) [29], the quality of SE-oil may be lower than that of pressed oil due to  
375 simultaneous extraction of non-triglycerides and other undesirable minor components in the  
376 former case. Therefore, in this study, the SE-oil exhibited higher PV as compared to other  
377 extraction methods. The non-triglycerides and other minor components include fatty acids,  
378 phosphatides, sterols, tocopherols, hydrocarbons, colorants, pigments, vitamins, sterol  
379 glucosides, protein fragments, glycolipids, traces of pesticides, trace metals, resinous, and  
380 mucilagenous materials [29, 34]. A product with PV of 1-5 mEq O<sub>2</sub>/kg is categorized as  
381 exhibiting low oxidation rate, followed by PV of 5-10 mEq O<sub>2</sub>/kg as moderate oxidation rate,  
382 while a product with PV of higher than 10 mEq O<sub>2</sub>/kg is considered as having high oxidation  
383 rate [30, 31]. Moreover, according to Codex (1999) [35], the maximum PV for refined oil is 10  
384 mEq O<sub>2</sub>/kg, while for cold pressed and virgin oils, the maximum PV is 15 mEq O<sub>2</sub>/kg.  
385 Despite the high PV of SE-oil, all values in all storage conditions were less than 3 mEq  
386 O<sub>2</sub>/kg. Thus, in terms of PV, the MO kernel oil samples from SE and enzymatic extraction  
387 methods are categorized as oil samples with low oxidation rate within the storage conditions  
388 used.

389 With reference to Figure 1(c) at 37 °C, the PV of SE oil started to decrease after 120  
390 days which indicated decomposition of primary oxidation products into secondary oxidation  
391 products as explained earlier, besides indicating the faster oxidation reaction in the SE-oil as  
392 compared to other oil samples. This phenomenon is reinforced by the sudden increase in the  
393 *p*-AV of SE oil up to 10.72±1.41. The *p*-AV represents the formation of secondary oxidation  
394 products in the form of 2-alkenals and 2,4-alkadienals. In this same storage condition, the *p*-  
395 AV of other oil samples remained as low as 0.70-1.44. Latif et al. (2011) [19] reported  
396 approximately similar *p*-AV of MO kernel oil extracted using solvent and enzymes which

397 ranged from 1.60-1.92. An oil is considered as having good quality if its *p*-AV is less than  
398 10.0 according to Rossell (1989) <sup>[36]</sup>, or less than 2.0 according to Subramaniam et al. (2000)  
399 <sup>[37]</sup>. Therefore, to conclude, SE-oil is considered unacceptable after 120 days of storage at 37  
400 °C due to the high *p*-AV, and TOTOX value of 16.64.

401

#### 402 **Effect of storage condition on free fatty acids of *Moringa oleifera* kernel oil**

403

404 FFA is responsible for the off-flavor and off-odor in fats and oils products <sup>[29, 38]</sup>. Prolong  
405 storage time causes decomposition and oxidation of secondary oxidation products into FFA  
406 as tertiary oxidation product <sup>[31]</sup>. In crude vegetable oils, improper-stored or field-damaged  
407 seeds contribute to abnormally high FFA level. Lipases and other enzymes in seeds and fruits  
408 are activated in the presence of moisture which initiates a hydrolysis reaction, causing  
409 formation of FFA <sup>[29]</sup>.

410 Figure 2 shows the FFA (as oleic acid) of oil samples stored at different temperatures  
411 of (a) 13 °C, (b) 25 °C, and (c) 37 °C. On day 0, the SE-oil exhibited significantly higher (*p* <  
412 0.05) FFA (2.02±0.14%) than the B-AEE-oil (1.09±0.32%). Abdulkarim et al. (2005) <sup>[4]</sup> also  
413 reported higher FFA in SE-oil (2.48%) as compared to B-AEE-oil (1.13%). The higher FFA  
414 in SE-oil may be due to simultaneous extraction of other non-triglycerides and minor  
415 components by the solvent which also contributed to higher PV as explained earlier.

416 Additionally, on day 0, the B-AEE-oil exhibited nearly 4 times lower FFA  
417 (1.09±0.32%) than the AEE-oil (3.85±0.26%). This finding proved the importance of boiling  
418 pre-treatment on the ground MO kernels to inactivate hydrolytic enzymes prior to oil  
419 extraction. Along the storage period at all temperatures, the FFA in B-AEE-oil remained at  
420 low level of 0.96-1.71%, while the FFA in AEE-oil decreased to 1.34-2.08%. These values  
421 were significantly lower (*p* < 0.05) as compared to the FFA in HPP-AEE-oil which

422 significantly increased ( $p < 0.05$ ) from day 0 to day 140 at all temperatures of 13 °C  
423 (6.66±0.19%), 25 °C (7.19±0.32%), and 37 °C (5.96±0.12%). These significantly higher FFA  
424 in HPP-AEE-oil samples were most likely due to possible presence of minute moisture  
425 content in the oil.

426 According to O'Brien (2009) <sup>[29]</sup>, presence of moisture in combination with high  
427 pressure may results in acceleration of hydrolytic reaction, therefore resulted in higher FFA  
428 as compared to other enzymatic extraction methods in this study. Increase in FFA was also  
429 observed in the SE-oil along the storage period, yet was still lower (2.47-3.62%) than that of  
430 HPP-AEE-oil samples.

431 Codex (1999) <sup>[35]</sup> indicates maximum FFA level in oil in terms of its AV which is 0.6  
432 mg KOH / g for refined oils, 4.0 mg KOH / g for cold pressed and virgin oils, and 10.0 mg  
433 KOH / g for virgin palm oils. In this study, highest FFA was observed in HPP-AEE-oil on  
434 day 140: 13.26 mg KOH / g (13 °C), 14.30±0.64 mg KOH / g (25 °C), and 11.86 mg KOH / g  
435 (37 °C). The B-AEE-oil exhibited lowest AV of below 4.0 mg KOH / g throughout the  
436 storage conditions (1.34-3.40 mg KOH / g). These findings further highlighted the significant  
437 effect of boiling pre-treatment in inactivating the hydrolytic enzymes, prevents enzymatic  
438 hydrolysis, and thus preserving the oil's oxidative stability.

439

#### 440 **Effect of storage condition on iodine value and fatty acid composition of *Moringa*** 441 ***oleifera* oil**

442

443 There was no significant difference ( $p > 0.05$ ) in IV of all oil samples from all extraction  
444 methods on day 0 (58-65 g I<sub>2</sub> / 100 g) and after 140 days (54-60 g I<sub>2</sub> / 100 g) at both 25 °C  
445 and 37 °C. Abdulkarim et al. (2005) <sup>[4]</sup> also reported similar IV of SE-oil (65.4 g I<sub>2</sub> / 100 g)  
446 and B-AEE-oil (66.1 g I<sub>2</sub> / 100 g). Additionally, there was no difference in IV between SE-oil

447 (66.6-66.8 g I<sub>2</sub> / 100 g) and cold-pressed oil (66.8 g I<sub>2</sub> / 100 g) in a study done by Tsaknis et  
448 al. (1999) <sup>[7]</sup>. These findings indicated that the MO oil did not undergo severe changes in  
449 degree of unsaturation within the storage conditions used, despite the production of oxidation  
450 products in certain oil samples as explained earlier.

451         These outcomes are also reflected by insignificant changes in FAC of the oil samples  
452 (Table 1(a-d)) at all storage temperatures. All oil samples consist of up to 76% oleic acid  
453 (C18:1) which contributes to the oil's oxidative stability and is related to reduced risk of  
454 developing coronary heart disease <sup>[13, 15]</sup>. Additionally, the oil samples consist of up to 6.60%  
455 behenic fatty acid (C22:0) in all storage conditions, thus suits its other names as Ben or  
456 Behen oil as described in the Introduction.

457

#### 458 **Effect of storage condition on $\alpha$ -tocopherol content in *Moringa oleifera* oil**

459

460 Figure 3 shows the  $\alpha$ -tocopherol content in oil samples stored at different temperatures of (a)  
461 13 °C, (b) 25 °C, and (c) 37 °C. On day 0, highest  $\alpha$ -tocopherol content was discovered in B-  
462 AEE-oil (31.17±3.52 mg/l) which was insignificantly different ( $p > 0.05$ ) with the AEE-oil  
463 (28.04±1.26 mg/l) and HPP-AEE-oil (28.77±1.05 mg/l). As compared to these enzymatic  
464 extraction methods, significantly lower ( $p < 0.05$ )  $\alpha$ -tocopherol content was observed in SE-  
465 oil (23.33±0.99 mg/l). In a study done by Tsaknis et al. (1999) <sup>[7]</sup> using MO seed kernels of  
466 Kenya origin, the  $\alpha$ -tocopherol content in the oil samples were similar in the case of solvent  
467 (98-105 mg/kg) and cold press (101.46 mg/kg) methods. With the use of MO seed kernels of  
468 Bangladesh origin, Rahman et al. (2009) <sup>[14]</sup> also revealed as high as 121-154 mg/kg  $\alpha$ -  
469 tocopherol content in the oil extracted using different types of solvents. In another study done  
470 by Tsaknis and Lalas (2002) <sup>[12]</sup> on seed kernels of India origin, the SE-oil contained higher  
471  $\alpha$ -tocopherol (15.38 mg/kg) as compared to cold-pressed oil (5.06 mg/kg). To summarize,

472 regardless of the extraction methods, the  $\alpha$ -tocopherol contents reported in this present study  
473 on day 0 (23.33-31.17 mg/l) and those reported by Tsaknis and Lalas (2002) <sup>[12]</sup> (5.06-15.38  
474 mg/kg) were far too low than that of reported by Tsaknis et al. (1999) <sup>[7]</sup> (98-105 mg/kg) and  
475 Rahman et al. (2009) <sup>[14]</sup> (121-154 mg/kg). These findings highlighted variations in the MO  
476 seed kernels of different origins which resulted in different oil properties. Besides  $\alpha$ -  
477 tocopherol, earlier studies reported presence of  $\gamma$ - and  $\delta$ -tocopherols in MO kernel oils  
478 extracted using solvents, enzymes, cold press, and supercritical fluid extraction method <sup>[6, 7, 12,</sup>  
479 <sup>14]</sup>, yet the values varied significantly. In this study, the tocopherols reported are the  $\alpha$ - and  $\gamma$ -  
480 tocopherols only, due to low amount of  $\delta$ -tocopherol detected.

481        Presence of higher oxidation products in SE-oil as indicated by increased in its PV, *p*-  
482 AV, and TOTOX as compared to enzymatic extraction methods was reflected by significant  
483 decrease ( $p < 0.05$ ) in the oil's  $\alpha$ -tocopherol content during storage. On day 60, the lowest  $\alpha$ -  
484 tocopherol content was detected in SE-oil at 37 °C (18.14±1.24 mg/l) as compared to 13 °C  
485 (29.51±0.75 mg/l) and 25 °C (25.60±2.24 mg/l). Greatest effect of storage temperature took  
486 place on day 120 where the  $\alpha$ -tocopherol content in SE-oil decreased with temperature  
487 increased from 13 °C (27.81±0.89 mg/l) to 25 °C (7.89±0.14 mg/l). On day 140, the  $\alpha$ -  
488 tocopherol content in SE-oil was not significantly affected ( $p > 0.05$ ) by the storage  
489 temperatures, yet highest  $\alpha$ -tocopherol content was detected at 37 °C in AEE-oil (31.22±1.73  
490 mg/l), B-AEE-oil (28.79±3.56 mg/l), and HPP-AEE-oil (32.86±0.56 mg/l) as compared to  
491 storage at lower temperatures of 13 °C (14-19 mg/l) and 25 °C (14-16 mg/l). The reason  
492 behind this finding is not yet been understood.

493

494 **Effect of storage condition on  $\gamma$ -tocopherol content in *Moringa oleifera* oil**

495

496 Figure 4 shows the  $\gamma$ -tocopherol content in oil samples stored at different temperatures: (a) 13  
497 °C, (b) 25 °C, and (c) 37 °C. In this study, all MO oil samples exhibited lower  $\gamma$ -tocopherol  
498 content as compared to  $\alpha$ -tocopherol. On day 0, all extraction methods resulted in oil samples  
499 with approximately similar  $\gamma$ -tocopherol content: 14.74±1.29 mg/l (SE), 12.79±1.26 mg/l  
500 (AEE), 15.32±1.57 mg/l (B-AEE), and 13.84±0.97 mg/l (HPP-AEE). Differently, Tsaknis et  
501 al. (1999) [7] reported higher  $\gamma$ -tocopherol content in cold-pressed oil (39.54 mg/kg) than that  
502 of SE-oil (27.90-33.45 mg/kg) with the use of MO seed kernels of Kenya origin. Tsaknis and  
503 Lalas (2002) [12] also reported higher  $\gamma$ -tocopherol content in cold pressed-oil (25.40 mg/kg)  
504 as compared to SE-oil (4.47-5.52 mg/kg) from MO seed kernels of India origin. In a study  
505 done by Rahman et al. (2009) [14] using seed kernels of Bangladesh origin, different types of  
506 solvents resulted in oil samples with approximately similar  $\gamma$ -tocopherol content (62.2-77.4  
507 mg/kg). To conclude, similar with  $\alpha$ -tocopherol, the  $\gamma$ -tocopherol content varied in MO oil  
508 samples from seed kernels of different origins and is also dependent on extraction methods  
509 used.

510 On day 140, the  $\gamma$ -tocopherol content in all oil samples was significantly higher ( $p <$   
511 0.05) at 37 °C (12.48-16.07 mg/l) as compared to lower storage temperatures of 13 °C (7.76-  
512 9.61 mg/l) and 25 °C (7.58-8.20 mg/l). The reasons for this sudden increment was not  
513 identified. In the SE-oil, this trend was different from that of  $\alpha$ -tocopherol which did not  
514 change upon different storage temperatures on day 140.

515 At 13 °C, the  $\gamma$ -tocopherol content in all oil samples from all extraction methods  
516 decreased significantly ( $p < 0.05$ ) after 120 days. Similarly, at 25 °C, the  $\gamma$ -tocopherol content  
517 in oil samples from enzymatic extraction methods decreased after 120 days, while in SE-oil,  
518 the  $\gamma$ -tocopherol content started to decrease earlier which was after 60 days. At 37 °C,  
519 insignificant ( $p > 0.05$ ) decrease in  $\gamma$ -tocopherol content in SE-oil was observed, which was  
520 different from significant decrease ( $p < 0.05$ ) in the case of  $\alpha$ -tocopherol in the same storage

521 condition. In overall at 37 °C, the storage time imparted no significant changes in the  $\gamma$ -  
522 tocopherol content in oil samples from all extraction methods.

523

## 524 **Conclusions**

525

526 In most MO oil samples, changes in oxidative properties and tocopherol contents started to  
527 take place after 120 days of storage, and the rate of changes increased with increased in  
528 temperature. The SE-oil underwent greater oxidative deterioration as compared to other AEE-  
529 based oils. The SE-oil was not in good quality after 120 days at 37 °C, while it is still  
530 acceptable during storage at 13 °C up to 140 days of storage. The AEE-based oils exhibited  
531 approximately similar oxidative properties throughout the whole storage conditions, except in  
532 the case of HPP-AEE-oil which exhibited high FFA content after 120 days, even at as low as  
533 13 °C storage temperature. This may be due to the high pressure applied which caused  
534 acceleration of hydrolytic reaction. On the other hand, the boiling pre-treatment was  
535 necessary to inactivate the hydrolytic enzymes in the seed kernels for better oil quality during  
536 storage. Thus, to conclude, within the storage conditions tested, B-AEE-oil exhibited greatest  
537 oxidative properties, followed by the AEE-oil, HPP-AEE-oil, and the SE-oil. No significant  
538 changes occurred in IV of all oil samples, indicating no changes in their degree of  
539 unsaturation throughout the storage condition. After 140 days at 37 °C, the concentration of  
540 both  $\alpha$ - and  $\gamma$ -tocopherols in all oil samples were nearly two times higher than their  
541 concentrations at lower temperatures, and the reasons for this finding is not yet discovered.  
542 Both the boiling and HPP pre-treatments did not significantly affect the tocopherol contents  
543 of the MO oil. Moreover, the AEE-based methods resulted in oils with better oxidative  
544 properties as compared to the use of solvent. This advantage assists in minimizing refinery  
545 loss and therefore should further be explored.



546

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553

## 554 **References**

555

556 [1] Anwar F and Rashid U, Physico-chemical characteristics of *Moringa oleifera* seeds and  
557 seed oil from a wild provenance of Pakistan. Pak J Bot **39(5)**:1443-1453 (2007).

558

559 [2] Mani S, Jaya S, Vadivambal R. Optimization of solvent extraction of *Moringa* (*Moringa*  
560 *oleifera*) seed kernel oil using response surface methodology. Food Bioprod Proc  
561 **85(C4)**:328-335 (2007).

562

563 [3] Anwar F, Zafar SN, Rashid U. Characterization of *Moringa oleifera* seed oil from drought  
564 and irrigated regions of Punjab, Pakistan. Gras Y Ace **57(2)**:160-168 (2006).

565

566 [4] Abdulkarim SM, Long K, Lai OM, Muhammad SKS, Ghazali HM, Some physico-  
567 chemical properties of *Moringa oleifera* seed oil extracted using solvent and aqueous  
568 enzymatic methods. Food Chem **93**:253-263 (2005).

569

570 [5] Anwar F and Bhangar MI, Analytical characterization of *Moringa oleifera* seed oil grown  
571 in temperate regions of Pakistan. J Agr Food Chem **51**:6558-6563 (2003).

572

573 [6] Lalas S, Tsaknis J. Characterization of *Moringa oleifera* seed oil variety "Periyakulum 1".  
574 J Food Comp Anal **15**:65-77 (2002).

575

576 [7] Tsaknis J, Lalas S, Gergis V, Dourtoglou V, Spiliotis V. Characterization of *Moringa*  
577 *oleifera* variety Mbololo seed oil of Kenya. J Agr Food Chem **47**:4495-4499 (1999).

578

579 [8] Morton JF. The horseradish tree, *Moringa pterigosperma* (Moringaceae)-A boon to arid  
580 lands? Econ Bot **45**:318-333 (1991).

581

582 [9] The Wealth of India, in *A Dictionary of Indian Raw Materials and Industrial Products*;  
583 *Raw Materials (Vol. VI, L-M)*. Council of Scientific and Industrial Research, New Delhi,  
584 India, pp. 425-429 (1962).

585

586 [10] Lowell JF. *Moringa oleifera*: Natural nutrition for the tropics. Church World Service,  
587 Dakar Senegal (1999).

588  
589 [11] Price ML, Ramachandran C, Peter KV, Gopalakrishnan PK. *Vegetables from a tree!*  
590 ECHO News (1986).  
591  
592 [12] Tsaknis J and Lalas S. Stability during frying of *Moringa oleifera* seed oil variety  
593 "Periyakulum 1". J Food Compos Anal **15**:79-101 (2002).  
594  
595 [13] Abdulkarim SM, Long K, Lai OM, Muhammad SKS, Ghazali HM, Frying quality and  
596 stability of high-oleic *Moringa oleifera* seed oil in comparison with other vegetable oils.  
597 Food Chem **105**:1382-1389 (2007).  
598  
599 [14] Rahman IMM, Barua S, Nazimuddin M, Begum ZA, Azizur Rahman M, Hasegawa H.  
600 Physicochemical properties of *Moringa oleifera* Lam. seed oil of the indigenous-cultivar of  
601 Bangladesh. J Food Lipids **16**: 540-553 (2009).  
602  
603 [15] Anwar F, Hussain AI, Iqbal S, Bhangar MI, Enhancement of the oxidative stability of  
604 some vegetable oils by blending with *Moringa oleifera* oil. Food Chem **103**:1181-1191  
605 (2007).  
606  
607 [16] Mat Yusoff M, Gordon MH, Niranjana K. Aqueous enzyme assisted oil extraction from  
608 oilseeds and emulsion de-emulsifying methods: A review. Trends in Food Science and  
609 Technology **41**:60-82 (2015).  
610  
611 [17] Rosenthal A, Pyle DL, Niranjana K. Aqueous and enzymatic processes for edible oil  
612 extraction. Enz Microb Tech **19**:402-420 (1996).  
613  
614 [18] Mat Yusoff M, Gordon MH, Ezech O, Niranjana K. Aqueous enzymatic extraction of  
615 *Moringa oleifera* oil. Food Chem **211**:400-408 (2016).  
616  
617 [19] Latif S, Anwar F, Hussain AI, Shahid M. Aqueous enzymatic process for oil and protein  
618 extraction from *Moringa oleifera* seed. Eur J Lipid Sci Tech **113**:1012-1018 (2011).  
619  
620 [20] Abdulkarim SM, Lai OM, Muhammad SKS, Long K, Ghazali HM, Use of enzymes to  
621 enhance oil recovery during aqueous extraction of *Moringa oleifera* seed oil. J Food Lipids  
622 **13**:113-130 (2006).  
623  
624 [21] Mat Yusoff M, Gordon MH, Ezech O, Niranjana K. High pressure pre-treatment  
625 of *Moringa oleifera* seed kernels prior to aqueous enzymatic oil extraction. Innovative Food  
626 Sci Emerg Tech **39**:129-136 (2017).  
627  
628 [22] Zhang SB, Wang Z, Xu SY. Optimization of the aqueous enzymatic extraction of  
629 rapeseed oil and protein hydrolysates. J Am Oil Chem Soc **84**:97-105 (2007).  
630  
631 [23] Fotouo-MH, du Toit ES, Robbertse PJ. Effect of storage conditions on *Moringa oleifera*  
632 Lam. seed oil: Biodiesel feedstock quality. Ind Crops Prod **84**:80-86 (2016).  
633  
634 [24] Pristouri G, Badeka A, Kontominas M. Effect of packaging material headspace, oxygen  
635 and light transmission, temperature and storage time on quality characteristics of extra virgin  
636 olive oil. Food Cont **21**:412-418 (2010).  
637

- 638 [25] Association of Official Analytical Chemists - AOAC. *Official methods of analysis of*  
639 *AOAC International*, 17th edn. Washington (2000).  
640  
641 [26] Malaysian Palm Oil Board - MPOB Test Method, Malaysia (2004).  
642  
643 [27] Ezeh O, Gordon MH, Niranjana K. Enhancing the recovery of tiger nut (*Cyperus*  
644 *esculentus*) oil by mechanical pressing: Moisture content, particle size, high pressure and  
645 enzymatic pre-treatment effects. *Food Chem* **194**:354-361 (2016).  
646  
647 [28] Costa PA, Ballus CA, Teixeira-Filho J, Godoy HT. Phytosterols and tocopherols content  
648 of pulps and nuts of Brazilian fruits. *Food Res Int* **43(6)**:1603-1606 (2010).  
649  
650 [29] O'Brien RD. *Fats and Oils: Formulating and Processing for Applications*, 3rd edn. CRC  
651 Press, Boca Raton (2009).  
652  
653 [30] Diana Moigradean, Poiana MA, Gogoasa I. Quality characteristics and oxidative  
654 stability of coconut oil during storage. *J Agro Proc Tech* **18(4)**:272-276 (2012).  
655  
656 [31] deMan JM (ed.) Lipids, in *Principles of Food Chemistry*, 3rd edn, ed. by deMan JM.  
657 Springer, New York, pp. 33-107 (1999).  
658  
659 [32] Keeney M. Secondary degradation products, in *Lipids and Their Oxidation*, ed. by  
660 Schultz HW, Day EA, Sinnhuber RO. AVI Publishing Co., Westport CT (1962).  
661  
662 [33] Frankel, EN. *Lipid Oxidation*, 2nd edn, Woodhead Publishing Limited, Cambridge  
663 (2012).  
664  
665 [34] Weidemann LH. Degumming, refining and bleaching soybean oil. *J Am Oil Chem Soc*  
666 **58**:159-165 (1981).  
667  
668 [35] Codex Section 2. Codex Standards for Fats and Oils from Vegetable Sources, Codex  
669 Standard for Named Vegetable Oils (CODEX-STAN 210 - 1999). Available:  
670 <http://www.fao.org/docrep/004/y2774e/y2774e04.htm>  
671  
672 [36] Rossell JB. Measurement in rancidity, in *Rancidity in Food*, ed. by Allen JC and  
673 Hamilton RJ. Elsevier, London, pp. 22-51 (1989).  
674  
675 [37] Subramaniam R, Nandinim KE, Sheila PM, Gopalakrishna AG, Raghavarao KSMS,  
676 Nakajima M, Kimura T, Maekawa T. Membrane processing of used frying oil. *J Am Oil*  
677 *Chem Soc* **77(3)**:323-328 (2000).  
678  
679 [38] Noor N, Augustin MA. Effectiveness of antioxidants on the stability of banana chips. *J*  
680 *Sci Food Agri* **35(7)**:805-812 (1984).

681  
682

### 683 **Table Caption**

684

685 **Table 1** Fatty acid composition (%) of *Moringa oleifera* oil from (a) solvent (hexane)  
686 extraction method (SE), (b) aqueous enzymatic extraction (AEE) method, (c) aqueous  
687 enzymatic extraction method with boiling pre-treatment (B-AEE), and (d) aqueous enzymatic

688 extraction method with high pressure processing pre-treatment (HPP-AEE) on day 0 and after  
689 140 days of storage at different temperatures. nd, not detected

690

691

## 692 **Figure Captions**

693

694 **Fig. 1.** Effect of different extraction methods on the peroxide values of *Moringa oleifera*  
695 kernel oil stored for 140 days at different temperatures of (a) 13 °C; (b) 25 °C; and (c) 37 °C.  
696 SE, solvent extraction; AEE, aqueous enzymatic extraction; B-AEE, aqueous enzymatic  
697 extraction with boiling pre-treatment; HPP-AEE, aqueous enzymatic extraction with high  
698 pressure processing pre-treatment.

699

700 **Fig. 2.** Effect of different extraction methods on the free fatty acids of *Moringa oleifera*  
701 kernel oil stored for 140 days at different temperatures of (a) 13 °C; (b) 25 °C; and (c) 37 °C.  
702 SE, solvent extraction; AEE, aqueous enzymatic extraction; B-AEE, aqueous enzymatic  
703 extraction with boiling pre-treatment; HPP-AEE, aqueous enzymatic extraction with high  
704 pressure processing pre-treatment.

705

706 **Fig. 3.** Effect of different extraction methods on the alpha tocopherol content in *Moringa*  
707 *oleifera* kernel oil stored for 140 days at different temperatures of (a) 13 °C; (b) 25 °C; and  
708 (c) 37 °C. SE, solvent extraction; AEE, aqueous enzymatic extraction; B-AEE, aqueous  
709 enzymatic extraction with boiling pre-treatment; HPP-AEE, aqueous enzymatic extraction  
710 with high pressure processing pre-treatment.

711

712 **Fig. 4** Effect of different extraction methods on the gamma tocopherol content in *Moringa*  
713 *oleifera* kernel oil stored for 140 days at different temperatures of (a) 13 °C; (b) 25 °C; and  
714 (c) 37 °C. SE, solvent extraction; AEE, aqueous enzymatic extraction; B-AEE, aqueous  
715 enzymatic extraction with boiling pre-treatment; HPP-AEE, aqueous enzymatic extraction  
716 with high pressure processing pre-treatment.

717

718

719