

## Quality assessment of *Moringa concanensis* seed oil extracted through solvent and aqueous-enzymatic techniques

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### RESUMEN

#### Evaluación de la calidad del aceite de semilla de *Moringa concanensis* extraído mediante disolvente y adición de enzimas.

En este estudio se compara la composición y la calidad del aceite de semilla de *M. concanensis* extraído mediante enzimas, utilizando tres enzimas comerciales (Natzzyme, Kemzyme, y Feedzyme) con las de un control extraído sin enzimas y con las del aceite extraído con disolvente. El contenido en aceites de las semilla extraídas con enzimas osciló entre 23,54 a 27,46% y fue significativamente más elevado ( $P < 0.05$ ) que el del control sin enzimas (15.41%). Los análisis de los residuos (harinas) no mostraron diferencias significativas ( $P > 0.05$ ) en el contenido de fibra y ceniza para los tres métodos de la extracción. Sin embargo, el contenido proteínico de la harina obtenido por métodos enzimáticos y el control sin enzimas fue significativamente menor ( $P < 0.05$ ) que el de la harina obtenida después de la extracción por disolvente. Las diferencias en el índice de yodo (67.1-68.0 g /100 g of oil), densidad en 24 °C (0,865-0,866 g/mL), índice de refracción a 40 °C (1,4622-1,4627) y fracción insaponificable (0,69-0,76 %) no fueron significativamente diferentes para ninguna de las técnicas de extracción. Las extinciones específicas en 232 y 270 nm, el índice de peróxidos, el índice de *p*-anisidina, la acidez libre y el color de los aceites extraídos con enzimas fueron inferiores a los del aceite obtenido mediante extracción con disolvente. La composición en ácidos grasos de los aceites fue similar en todas los aceites encontrándose sólo pequeñas en los contenidos de ácido esteárico y linoleico. Respecto a los tocoferoles, el contenido en  $\gamma$ -tocoferol fue similar en el control y en las extracciones con enzimas y significativamente más elevado ( $P < 0.05$ ) que en el aceite extraído con disolvente, mientras que el contenido en  $\alpha$ -tocoferol fue superior en el aceite extraído con disolvente.

**PALABRAS-CLAVE:** Aceite de *Moringa concanensis* – Ácidos grasos – Características físico-químicas – Enzimas – Métodos de extracción – Tocoferoles.

### SUMMARY

#### Quality assessment of *Moringa concanensis* seed oil extracted through solvent and aqueous-enzymatic techniques.

The composition and quality of the *M. concanensis* seed oil extracted through an aqueous-enzyme-assisted technique, using three commercial enzyme-mixtures (Natzzyme, Kemzyme, and Feedzyme) was compared to

those of the control-, (without enzymes) and solvent-extracted oils. Aqueous enzyme-extracted *M. concanensis* seed oil content ranged from 23.54 to 27.46% and was significantly ( $P < 0.05$ ) higher than that of the control (15.41%). Analyses of the oilseed residues (meals) revealed no significant ( $P > 0.05$ ) variation in the contents of fiber and ash within the three extraction methods. However, the protein content of the meal obtained through the aqueous-enzyme and control methods was significantly ( $P > 0.05$ ) lower as compared to that of the solvent-extracted. There were no significant ( $P > 0.05$ ) differences in iodine value (67.1-68.0 g of iodine/100 g of oil), density at 24 °C (0.865-0.866 g mL<sup>-1</sup>), refractive index at 40 °C (1.4622-1.4627) and unsaponifiable (0.69-0.76%) matter of the *M. concanensis* seed oils extracted using the three methods. The specific extinctions at 232 and 270 nm, peroxide value, *p*-anisidine, free fatty acid contents and color values of the aqueous-enzyme-extracted oil were found to be lower than that of solvent-extracted oil and thus revealed good quality. The oils extracted through the three methods exhibited no significant ( $P > 0.05$ ) variation in their contents of major fatty acids except stearic (18:0) and linoleic (18:2) acids. The level of  $\gamma$ -tocopherol in enzyme-extracted oil, even though comparable to the control, was significantly ( $P < 0.05$ ) higher than that of solvent-extracted oil. The content of  $\alpha$ -tocopherol in the enzyme-extracted oil was found to be higher than that of the control but lower than that of solvent- extracted oil.

**KEY-WORDS:** Enzyme-assisted aqueous – Extraction methods – Fatty acids – *Moringa concanensis* oil – Oxidative stability – Physico-chemical characteristics – Tocopherols

### 1. INTRODUCTION

Moringaceae, a monogeneric family, belonging to the genus *Moringa* is characterized by a number of species such as *Moringa peregrina* (*M. peregrina*), *M. oleifera*, *M. arabica*, *M. stenopetala*, *M. concanensis*, *M. ovalifolia*, *M. hildebrandtii*, *M. arborea*, *Moringa rivae* and *Moringa ruspoliana* etc. (Tsaknis et al., 1999; Olson, 2003; Aregheore 2002). Almost all *Moringa* species, native to India are now distributed in many tropical and sub-tropical parts of the world like India, Pakistan, Asia Minor, Africa and Arabia (Mughal et al., 1999, Anwar and Bhangar 2003).

The *Moringa* plant is well-regarded as an important food commodity equipped with high-value nutritious properties (Anwar et al., 2007). Different parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for

various ailments in the indigenous medicine practices of South Asia, which included the cure of inflammation, cardiovascular, gastrointestinal, hematological and hepatorenal disorders (Singh *et al.*, 1999; Morimitsu *et al.*, 2000; Siddhuraju and Becker, 2003). The seeds of the *Moringa* are considered to exhibit antipyretic and antimicrobial activities, used for water purifying and are also a good source of non-desiccating oil, commercially known as 'Ben oil' (Anwar *et al.*, 2007).

In Pakistan *Moringa* is characterized by two species; *Moringa oleifera* (*M.oleifera*) and *M. concanensis* (*M.concanensis*). The former is widely cultivated across the country; however, the latter is rarely found and only confined to a remote area of Tharparkar, Sindh (Manzoor *et al.*, 2007, Anwar 2003). The species, *Moringa concanensis* has recently been characterized with regard to its seed oil potential. Its oil is high in oleic acid and resembles in context of fatty acid composition with seed oils of other *Moringa* species, which includes the *M. stenopetala*, *M. peregrina* and *M. oleifera* (Manzoor *et al.*, 2007). High-oleic oils are gaining importance, especially for replacing polyunsaturated vegetable oils (Corbett, 2003) and are reported to exhibit good oxidative stability during frying (Anwar *et al.*, 2007).

Conventionally, *n*-hexane is used as a solvent for the extraction of oils from vegetable oil seeds. However, the environmental problems, safety issues and health concerns have prompted the need to find some environmentally-friendly alternatives to hexane (Bhattacharjee *et al.*, 2006). The U.S. Environmental Protection Agency provided incentives for developing alternative methods of edible oil extraction and guidelines for hexane emissions by vegetable oil extraction sources (EPA, 2001). The new tendency to avoid the use of toxic organic solvents in large installations has renewed interest in alternative extraction processes (involving the use of water, alcohol aqueous solutions and supercritical fluids) (Johnson and Lusas, 1983).

A number of aqueous (Rhee *et al.*, 1972; Shi *et al.*, 1998), and aqueous enzymatic extraction (Hanmoungjai *et al.*, 2001; Rosenthal *et al.*, 1996; Rosenthal *et al.*, 2001; Sharma *et al.*, 2002) methods have been developed for the extraction of oil seeds. Shi *et al.* (1998), reported an aqueous method that resulted in an 80% yield of peanut oil from peanuts. Karlovic *et al.* (1994) investigated an aqueous enzymatic method for the extraction of corn germs using cellulase which resulted in 80% oil yield.

The main purpose of using the enzymes during aqueous oil extraction is to hydrolyze the structural polysaccharides which form the cell wall of oilseeds or the proteins which form the cell and lipid body membrane. The aqueous enzyme-assisted extraction process removes phospholipids from the oil so that there is no need for degumming, thereby, reducing the overall cost of processing of the oil to the final product (Christensen, 1991). The major disadvantage of using enzymes for oil seed extraction, although safer, is that

they are expensive and offer quite lower oil yield as compared to solvent extraction. Nevertheless, the other technical benefits surrounding enzyme-assisted aqueous oil extraction can make this technique viable.

Currently, much effort is being devoted to develop and introduce commercial enzymes for aqueous enzymatic oil extraction. The present work was therefore, an attempt to evaluate the potential of three commercial enzymes for extraction of oil from *M. concanensis* seeds by applying the aqueous enzymatic technique. The physico-chemical characteristics of the aqueous enzyme-extracted *M. concanensis* seed oil were also compared to that of conventional solvent extracted and control oils.

## 2. EXPERIMENTAL

### 2.1. Materials

The seeds of *Moringa concanensis* were assayed from the vicinity of Tharparker, Sindh, Pakistan. All reagents (analytical and HPLC) used for analysis were from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland), unless stated otherwise. Pure standards of tocopherols [DL- $\alpha$ -tocopherol, (+)- $\delta$ -tocopherol, (+)- $\gamma$ -tocopherol] and FAMES (fatty acid methyl esters) were obtained from Sigma Chemical Co. (St. Louis, MO). Three commercial enzyme-mixtures: Kemzyme (mainly alpha-amylase, beta-glucanase, cellulase-complex, hemicellulase-complex, protease and xylanase activities), Natuzyme (mainly cellulose, xylanase, phytase, alpha-amylase, pectinase activities), Feedzyme (mainly xylanase beta-glucanase cellulase and hemicellulase activities) were of Kemin Europa N.V., Herentals, Belgium (Vet Pharma Trading Company, Gujranwala, Pakistan); Bioproton Pty Ltd, Brisbane, Australia (M/S Ghazi Brothers, Karachi, Pakistan); and Agil, Reading, England (Better Traders International, Faisalabad, Pakistan), respectively.

### 2.2. Oil extraction

#### 2.2.1. Aqueous enzyme-assisted extraction

For aqueous enzyme extraction, ground seeds (25g) were mixed with distilled water (150 ml) at a ratio of 1:6 w/v for the method followed by Abdulkarim *et al.* (2005). The mixture was boiled for 5 min and allowed to cool down to room temperature. The pH was then adjusted to the optimal pH for each enzyme with 0.5 N NaOH and 0.5N HCl. Two percent (w/w) E/S (enzyme/substrate) ratio of each of the three enzyme-mixtures (Kemzyme, Natuzyme, and Feedzyme) were added and the mixture incubated at 45 °C for 24 h with constant shaking at 120 rpm. The oil was recovered after centrifugation (8000 rpm, 20 °C) for 20 minutes using a centrifuge machine (Sigma, 3K 30, Osterode am Harz, Germany) (Abdulkarim *et al.*, 2005). The wet

meal was mixed and dried overnight in a vacuum oven (VOC-300 SD; EYELA, Tokyo, Japan) at 85-90°C. The dry meal was ground and analyzed for ash, fiber, and protein contents. The Control samples were also proceeded under the same set of conditions, except enzyme addition.

### 2.2.2. Solvent extraction

After the removal of seed hull and other impurities, the seeds (150 g) were crushed and then fed to a Soxhlet extractor fitted with a 1.0 L round-bottom flask and a condenser. The extraction was executed on a water bath for 6 h with 0.5 L of *n*-hexane. The solvent was distilled off under vacuum in a rotary evaporator (Eyela, N-N Series, Rikakikai Co. Ltd. Tokyo, Japan) at 45 °C. The oil obtained from both extractions was stored under refrigeration (4 °C), until used for further analyses.

## 2.3. Analysis of the oilseed residues/ meals

After oil extraction (both by enzyme and solvent extraction), the meals were analyzed for protein, fiber, and ash contents. Protein content was determined according to the AOAC (1990) method 954.01. Fiber content was estimated according to an ISO (1977) method 5983. A finely ground sample (2.5 g) of meal was weighed and freed from fat by extraction with 15-mL *n*-hexane. The test portion was boiled with sulfuric acid (0.255 mol L<sup>-1</sup>), followed by separation and washing of the insoluble residue. The residue was then boiled with sodium hydroxide (0.313 mol L<sup>-1</sup>), followed by separation, washing, and drying. The dried residue was weighed and ashed in a muffle furnace (TMF-2100, Eyela, Tokyo, Japan) at 600 °C, and the loss of mass was determined.

Ash content was determined according to an ISO (1977) method 749. Two grams of the test portion were taken and carbonized by heating on a gas flame. The carbonized material was then ashed in an electric muffle furnace (TMF-2100, Eyela, Tokyo, Japan) at 550 °C until a constant mass was achieved.

## 2.4. Analysis of extracted oil

### 2.4.1. Physical and chemical parameters of oil

Iodine value, refractive index, density, unsaponifiable matter, peroxide and saponification values of the enzyme extracted and the control *M. concanensis* seed oils were determined by various standard AOCS (1997) methods. The color of the oil was determined by a Lovibond tintometer (Tintometer Ltd., Salisbury, Wiltshire, United Kingdom) using a 1-inch cell. Specific extinctions at 232 and 270 nm were determined using a spectrophotometer (U-2001, Hitachi Instruments, Inc., Tokyo, Japan). Samples of oil were diluted with iso-octane, and spectra were

recorded at 232 and 270 nm, and  $\epsilon^{1\%}_{1\text{cm}}(\lambda)$  calculated following the standard IUPAC (1987) method. *para*-Anisidine values were also determined following an IUPAC (1987) method.

### 2.4.2. Fatty acid (FA) composition

FAMES were prepared according to the IUPAC (1987) method 2.301 and were analyzed on a Shimadzu (Kyoto, Japan) gas chromatograph, model 17-A, fitted with a methyl lignocerate coated film thickness 0.20  $\mu\text{m}$ ) SP-2330 polar capillary column (30 m  $\times$  0.32 mm; Supelco Inc., Supelco Park Bellefonte, PA), and a flame ionization detector (FID). Oxygen-free nitrogen was used as a carrier gas at a flow rate of 3.0 mL min<sup>-1</sup>. Other conditions were as follows: initial oven temperature, 180 °C; ramp rate, 5 °C min<sup>-1</sup>; final temperature, 220 °C; injector temperature, 230 °C; detector temperature, 250 °C; and temperature hold, 2 min before and 10 min after the run. A sample volume of 1.5  $\mu\text{L}$  was injected. FAMES were identified by comparing their relative and absolute retention times to those of authentic standards. A data-handling program, Chromatography Station for Windows (CSW32), was used for quantification. The FA composition was reported as a relative percentage of the total peak area.

### 2.4.3. Tocopherol content

Tocopherols ( $\alpha$ ,  $\gamma$ , and  $\delta$ ) were analyzed using an HPLC following the CPFA (Current Protocols in Food Analytical Chemistry) (Wrolstad, 2003) methods. 0.1 g oil sample and 0.05 g ascorbic acid were weighed accurately into a 16  $\times$  125-mm test tube. Five milliliters of 90.2% ethanol and 0.5 ml of 80% aqueous KOH solution were added to the test tube and vortexed for 30 sec. The test tube was flushed with nitrogen, capped and incubated in a water bath (70 °C) for 30 min with periodical vortexing. The tubes were placed in an ice bath for 5 min then 3 ml deionized water and 5 ml *n*-hexane were added and vortexed for 30 sec followed by centrifugation for 10 min at 1000  $\times$  g (room temperature). The upper hexane layer was transferred to another test tube. The aqueous layer and the residue were re-extracted by repeating the same procedure. The upper hexane layers from both the extractions were combined and evaporated to dryness under nitrogen streaming. One milliliter of mobile phase was added to the tube and vortexed 30 sec to re-dissolve the extract and then transferred to an HPLC sample vial. A 20- $\mu\text{L}$  sample was injected into a Supelcosil LC-Si column (250  $\times$  4.6 mm, Supelco Inc., Supelco Park, Bellefonte, USA). A mobile phase of ethyl acetate/acetic acid/hexane (1:1:198, v/v/v) was used at the rate of 1.5 mL min<sup>-1</sup>. Detection was monitored at 295 nm. Tocopherols were identified by comparing their retention times with those of pure standards of  $\alpha$ -,  $\gamma$ -, and  $\delta$ - tocopherols, and were quantified on the basis of peak area of the unknowns with those of



pure standards (Sigma Chemical Co.). Quantification was based on an external standard method. A D-2500 Hitachi Instruments, Inc., Tokyo, Japan Chromatointegrator model with a built-in computer program for data handling was used for quantification.

## 2.5. Statistical analysis

Three *M. concanensis* seed samples for each of the three enzyme treatments were analyzed individually in triplicate. Data were reported as means  $\pm$  SD ( $n = 3 \times 3$ ). One way ANOVA was used to determine significant differences between groups, considering a level of significance of less than 5% ( $P < 0.05$ ) by using the statistical software STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA).

## 3. RESULTS AND DISCUSSION

The oil content (23.54-27.46%) of aqueous enzyme-assisted *M. concanensis* seed was significantly ( $P < 0.05$ ) higher than that of the control (15.41%), but lower than that extracted with hexane (38.40%) (Table 1). The oil concentration was highest (27.46%) in the Kemzyme treated seed samples, whereas the seeds treated with Feedzyme afforded the lowest oil content (23.54%). The increase in oil yield in the present aqueous

enzyme-assisted extraction of *M. concanensis* seed as compared to the control might be attributed to the enzyme activity. The multiple activity complexes and enzyme mixtures are especially effective for degradation of seed cell walls, due to their synergistic action thus making more oil available for extraction (Sineiro *et al.*, 1998). The analysis of the meals revealed the protein content (19.06-20.17%) of the *M. concanensis* seed treated with enzymes to be significantly ( $P < 0.05$ ) lower than that of the control and solvent-extracted seeds. This decrease may be due to the extraction of some protein in the aqueous and the emulsion phases. Fiber and ash contents in the enzyme treated oil seeds ranged from 5.94 to 6.12, and 6.98 to 7.17%, respectively and were almost comparable to those of the control and solvent-extracted and showed no significant ( $P > 0.05$ ) variations.

Table 2 shows the data for various physico-chemical parameters of the enzyme-, solvent-extracted and the control- *M. concanensis* seed oils. There were no significant ( $P > 0.05$ ) differences in the iodine value (67.1-68.0 g of iodine/100 g of oil), density at 24 °C (0.865-0.866 g mL<sup>-1</sup>), refractive Index at 40 °C (1.4622-1.4627) and unsaponifiable matter (0.69-0.71%) of the *M. concanensis* seed oils extracted through three extraction methods. The saponification (173-175 mg of KOH/g), of the aqueous enzyme-extracted *M. concanensis* seed oil was found to be comparable to the control but

Table 1  
Proximate composition of *Moringa concanensis* seeds<sup>A</sup>

Constituent (%)	Enzyme-extracted				
	Solvent-extracted	Natuzyyme	Kemzyme	Feedzyme	Control
Oil content	38.40 $\pm$ 0.97 <sup>a</sup>	25.34 $\pm$ 0.59 <sup>bc</sup>	27.46 $\pm$ 0.64 <sup>b</sup>	23.54 $\pm$ 0.43 <sup>c</sup>	15.41 $\pm$ 0.28 <sup>d</sup>
Protein content	30.25 $\pm$ 0.86 <sup>a</sup>	20.17 $\pm$ 1.03 <sup>c</sup>	19.45 $\pm$ 0.80 <sup>c</sup>	19.06 $\pm$ 1.05 <sup>c</sup>	25.41 $\pm$ 0.84 <sup>b</sup>
Fiber content	6.15 $\pm$ 0.12 <sup>a</sup>	6.09 $\pm$ 0.10 <sup>a</sup>	6.12 $\pm$ 0.08 <sup>a</sup>	5.94 $\pm$ 0.11 <sup>a</sup>	6.10 $\pm$ 0.09 <sup>a</sup>
Ash content	7.20 $\pm$ 0.11 <sup>a</sup>	7.17 $\pm$ 0.13 <sup>a</sup>	6.98 $\pm$ 0.09 <sup>a</sup>	7.00 $\pm$ 0.12 <sup>a</sup>	7.12 $\pm$ 0.10 <sup>a</sup>

<sup>A</sup> Values are means  $\pm$  SD, calculated as percentage on dry seed weight basis for three *Moringa concanensis* seed samples for each enzyme analyzed individually in triplicate.

Mean values in the same row followed by the same superscript letters are not significantly different ( $P > 0.05$ ).

Table 2  
Physico-chemical properties of *Moringa concanensis* seed oils<sup>A</sup>

Constituent	Enzyme-extracted				
	Solvent-extracted	Natuzyyme	Kemzyme	Feedzyme	Control
Refractive Index (40 °C)	1.4644 $\pm$ 0.002 <sup>a</sup>	1.4627 $\pm$ 0.003 <sup>a</sup>	1.4625 $\pm$ 0.002 <sup>a</sup>	1.4622 $\pm$ 0.003 <sup>a</sup>	1.4624 $\pm$ 0.002 <sup>a</sup>
Density (24 °C g/ mL)	0.866 $\pm$ 0.04 <sup>a</sup>	0.866 $\pm$ 0.02 <sup>a</sup>	0.865 $\pm$ 0.06 <sup>a</sup>	0.865 $\pm$ 0.01 <sup>a</sup>	0.865 $\pm$ 0.03 <sup>a</sup>
Saponification value (mg KOH/g oil)	180 $\pm$ 3.43 <sup>a</sup>	174 $\pm$ 2.59 <sup>a</sup>	175 $\pm$ 4.32 <sup>a</sup>	173 $\pm$ 3.86 <sup>a</sup>	176 $\pm$ 4.79 <sup>a</sup>
Acidity (% as oleic acid)	0.32 $\pm$ 0.01 <sup>a</sup>	0.19 $\pm$ 0.02 <sup>b</sup>	0.17 $\pm$ 0.02 <sup>b</sup>	0.20 $\pm$ 0.01 <sup>b</sup>	0.18 $\pm$ 0.03 <sup>b</sup>
Iodine value (g of I/ 100g oil)	66.7 $\pm$ 1.28 <sup>a</sup>	67.6 $\pm$ 1.59 <sup>a</sup>	68.0 $\pm$ 1.70 <sup>a</sup>	67.1 $\pm$ 2.15 <sup>a</sup>	67.2 $\pm$ 1.98 <sup>a</sup>
Unsaponifiable matter (% w/w)	0.76 $\pm$ 0.02 <sup>a</sup>	0.71 $\pm$ 0.01 <sup>a</sup>	0.69 $\pm$ 0.02 <sup>a</sup>	0.70 $\pm$ 0.04 <sup>a</sup>	0.67 $\pm$ 0.03 <sup>a</sup>
Color (1-in. cell)					
Red units	2.1 r $\pm$ 0.04 <sup>a</sup>	1.2 r $\pm$ 0.03 <sup>b</sup>	1.3 r $\pm$ 0.05 <sup>b</sup>	1.2 r $\pm$ 0.07 <sup>b</sup>	1.2 r $\pm$ 0.04 <sup>b</sup>
Yellow units	19 y $\pm$ 0.38 <sup>a</sup>	14 y $\pm$ 0.43 <sup>b</sup>	14 y $\pm$ 0.35 <sup>b</sup>	12 y $\pm$ 0.51 <sup>c</sup>	14 y $\pm$ 0.49 <sup>b</sup>

<sup>A</sup> Values are means  $\pm$  SD, of three *Moringa concanensis* seed oils analyzed individually in triplicate.

Mean values in the same row followed by the same superscript letters are not significantly different ( $P > 0.05$ ).

slightly lower than that of the solvent-extracted oils. The contents of free fatty acids (% as oleic acid) (0.17-0.20) and color (1.2-1.3 r + 12-14 y) of the enzyme-extracted *M. concanensis* seed oils were found to be well in line with that of the control (0.18, and 1.2 r + 14 y respectively) but significantly lower ( $P < 0.05$ ) than that of the solvent-extracted oil (0.32, and 2.1 r + 19 y respectively).

The results of different oxidation parameters of the control, enzyme-, and solvent-extracted *M. concanensis* seed oils are shown in Table 3. The specific extinctions at 232 and 270 nm, which revealed the oxidative deterioration and purity of the oils (Anwar *et al.*, 2006a), of enzyme-extracted *M. concanensis* seed oils ranged from 2.88 to 2.94 and 0.57 to 0.62, respectively. The content of conjugated diene and triene of the enzyme-extracted oils was found to be comparable to that of the control (2.95, 0.60), but significantly ( $P < 0.05$ ) lower than that of solvent-extracted oil (3.17, 0.67). The peroxide and *p*-anisidine values of enzyme-extracted *M. concanensis* seed oil (1.08-1.22 meq/kg and 1.61-1.65 respectively) were comparable to that of the control (1.24 meq/kg and 1.59 respectively), however, these were significantly ( $P < 0.05$ ) lower than that of the solvent-extracted oil (1.73 meq/kg and 1.82, respectively). A noticeably lower level of the values of different oxidation parameters and thus good oxidative stability exhibited in the present analysis of enzyme-extracted *M. concanensis* seed oil as compared to the solvent-extracted oil might be attributed to the extraction technique applied. The

conventional vegetable oil seed extraction process is performed by means of organic solvents, where the high operational temperature might affect the oil quality and its composition, particularly, with regard to the oxidation state. No previously reported data on the oxidation parameters of enzyme-extracted *M. concanensis* seed oil is available with which to compare the results of our present analysis.

Table 4 shows the fatty acid composition of the control, enzyme-, and solvent-extracted *M. concanensis* seed oils. The contents of oleic acid (18:1) and palmitic (16:0) in the aqueous enzyme-extracted oils ranged from 68.64 to 68.78% and 10.83 to 10.91%, respectively. Other prominent fatty acids like; behnic (22:0), arachidic (20:0) and stearic (18:0) acids in aqueous enzyme-extracted oils were 7.39 to 7.48%, 3.71 to 3.83% and 2.68 to 2.87% respectively, followed by palmitoleic (16:1), gadoleic (20:1), and linoleic (18:2) acids from 2.50 to 2.57%, 1.82 to 1.90%, and 1.59 to 1.65%, respectively. There was no significant ( $P > 0.05$ ) difference in the amounts of the major fatty acids, except stearic (18:0) and linoleic (18:2) acids in the control, enzyme-, and solvent-extracted *M. concanensis* seed oils. The results of the present investigation of the fatty acid profiles of *M. concanensis* seed oils are in agreement with those of Abdulkarim *et al.* (2005) who showed no significant variation in the contents of major fatty acids in the enzyme-, and solvent-extracted *M. oleifera* seed oils.

Table 5 shows the contents of different tocopherols in the control, enzyme-, and solvent-extracted *M. concanensis* seed oils. The levels of

Table 3  
Determination of the oxidative state of *Moringa concanensis* seed oils<sup>A</sup>

Constituent	Solvent-extracted	Enzyme-extracted			Control
		Natuzyme	Kemzyme	Feedzyme	
Conjugated diene $\epsilon^{1\%}_{1\text{cm}}$ ( $\lambda 232$ )	3.17 ± 0.24 <sup>a</sup>	2.92 ± 0.22 <sup>b</sup>	2.88 ± 0.15 <sup>b</sup>	2.94 ± 0.23 <sup>b</sup>	2.95 ± 0.19 <sup>b</sup>
Conjugated triene $\epsilon^{1\%}_{1\text{cm}}$ ( $\lambda 270$ )	0.67 ± 0.02 <sup>a</sup>	0.59 ± 0.01 <sup>bc</sup>	0.62 ± 0.01 <sup>b</sup>	0.57 ± 0.02 <sup>c</sup>	0.60 ± 0.01 <sup>bc</sup>
Peroxide value (m eq/kg of oil)	1.73 ± 0.14 <sup>a</sup>	1.22 ± 0.13 <sup>b</sup>	1.12 ± 0.09 <sup>c</sup>	1.08 ± 0.11 <sup>c</sup>	1.24 ± 0.13 <sup>b</sup>
<i>p</i> -anisidine	1.82 ± 0.06 <sup>a</sup>	1.65 ± 0.04 <sup>b</sup>	1.61 ± 0.08 <sup>b</sup>	1.62 ± 0.11 <sup>b</sup>	1.59 ± 0.09 <sup>b</sup>

<sup>A</sup> Values are means ± SD, of three *Moringa concanensis* seed oils analyzed individually in triplicate. Mean values in the same row followed by the same superscript letters are not significantly different ( $P > 0.05$ ).

Table 4  
Fatty acid composition (g/100g) of *Moringa concanensis* seed oils<sup>A</sup>

Fatty Acid	Solvent-extracted	Enzyme-extracted			Control
		Natuzyme	Kemzyme	Feedzyme	
C <sub>16:0</sub>	11.92 ± 0.21 <sup>a</sup>	10.88 ± 0.19 <sup>a</sup>	10.91 ± 0.24 <sup>a</sup>	10.83 ± 0.17 <sup>a</sup>	11.20 ± 0.21 <sup>a</sup>
C <sub>16:1</sub>	2.44 ± 0.05 <sup>a</sup>	2.57 ± 0.06 <sup>a</sup>	2.53 ± 0.04 <sup>a</sup>	2.50 ± 0.03 <sup>a</sup>	2.68 ± 0.07 <sup>a</sup>
C <sub>18:0</sub>	3.64 ± 0.18 <sup>a</sup>	2.87 ± 0.12 <sup>b</sup>	2.68 ± 0.14 <sup>b</sup>	2.84 ± 0.16 <sup>b</sup>	2.86 ± 0.13 <sup>b</sup>
C <sub>18:1</sub>	67.30 ± 1.72 <sup>a</sup>	68.72 ± 1.85 <sup>a</sup>	68.64 ± 2.50 <sup>a</sup>	68.78 ± 1.95 <sup>a</sup>	68.07 ± 2.11 <sup>a</sup>
C <sub>18:2</sub>	1.77 ± 0.01 <sup>a</sup>	1.62 ± 0.02 <sup>bc</sup>	1.65 ± 0.02 <sup>bc</sup>	1.59 ± 0.01 <sup>c</sup>	1.70 ± 0.02 <sup>b</sup>
C <sub>20:0</sub>	3.65 ± 0.08 <sup>a</sup>	3.75 ± 0.11 <sup>a</sup>	3.83 ± 0.09 <sup>a</sup>	3.71 ± 0.06 <sup>a</sup>	3.79 ± 0.07 <sup>a</sup>
C <sub>20:1</sub>	1.79 ± 0.05 <sup>a</sup>	1.85 ± 0.07 <sup>a</sup>	1.90 ± 0.09 <sup>a</sup>	1.82 ± 0.06 <sup>a</sup>	1.74 ± 0.08 <sup>a</sup>
C <sub>22:0</sub>	7.58 ± 0.18 <sup>a</sup>	7.48 ± 0.15 <sup>a</sup>	7.39 ± 0.12 <sup>a</sup>	7.46 ± 0.14 <sup>a</sup>	7.34 ± 0.16 <sup>a</sup>

<sup>A</sup> Values are means ± SD, of three *Moringa concanensis* seed oils analyzed individually in triplicate. Mean values in the same row followed by the same superscript letters are not significantly different ( $P > 0.05$ ).

Table 5  
Comparison of tocopherol in *Moringa concanensis* seed oils<sup>A</sup>

Tocopherols (mg kg <sup>-1</sup> )	Solvent-extracted	Enzyme-extracted			Control
		Natuzyme	Kemzyme	Feedzyme	
α	79.40 ± 2.36 <sup>b</sup>	74.29 ± 2.67 <sup>c</sup>	82.05 ± 2.49 <sup>a</sup>	72.76 ± 2.71 <sup>c</sup>	69.27 ± 2.10 <sup>c</sup>
γ	11.38 ± 0.48 <sup>c</sup>	18.83 ± 0.56 <sup>ab</sup>	16.93 ± 0.75 <sup>b</sup>	21.22 ± 0.82 <sup>a</sup>	17.56 ± 0.53 <sup>b</sup>
δ	29.61 ± 1.21 <sup>b</sup>	30.50 ± 1.72 <sup>b</sup>	29.27 ± 1.18 <sup>b</sup>	35.49 ± 1.52 <sup>a</sup>	30.78 ± 1.34 <sup>b</sup>

<sup>A</sup> Values are means ± SD, of three *Moringa concanensis* seed oils analyzed individually in triplicate. Mean values in the same row followed by the same superscript letters are not significantly different ( $P > 0.05$ )

α-, γ-, and δ-tocopherol in the enzyme-extracted oils were 72.76-82.05, 16.93-21.22, and 29.27-35.49 mg kg<sup>-1</sup>, respectively. The content of α-tocopherol, which exhibits the highest vitamin E activity (Anwar *et al.*, 2006a), in the Kemzyme-extracted oil, was found to be higher, whereas, Feedzyme-, and Natuzyme-extracted oils showed significantly ( $P < 0.05$ ) lower values than that of solvent-extracted oil. The concentration of δ-tocopherol, with potent antioxidant activity (Anwar *et al.*, 2006a), in the Natuzyme-, and Kemzyme-extracted oils (30.50 and 29.27 mg kg<sup>-1</sup>, respectively) was in close agreement with the control, and solvent-extracted oil (30.78 and 29.61 mg kg<sup>-1</sup>, respectively). However, a significantly ( $P < 0.05$ ) higher content of δ-tocopherol was observed in Feedzyme-extracted oil (35.49 mg kg<sup>-1</sup>). The level of γ-tocopherol in enzyme-extracted oils (16.93-21.22 mg kg<sup>-1</sup>), was significantly higher than that of solvent-extracted (11.38 mg kg<sup>-1</sup>) oil. In context of δ-tocopherol concentration, Kemzyme-extracted (16.93 mg kg<sup>-1</sup>) oil was noted to have close resemblance to that of the control (17.56 mg kg<sup>-1</sup>). No previous reports on the tocopherol contents of enzyme-extracted *M. concanensis* seed oils are available in the literature which with the data of present analysis could be compared.

It could be concluded from the results of the present investigation that aqueous enzymatic treatment enhanced the oil yield of *M. concanensis* seeds with respect to the control although the yield of solvent extraction was not reached. The oil extracted through this approach revealed improved oxidative stability without exhibiting any considerable change in the contents of major fatty acids. Furthermore, the aqueous phase obtained through this extraction method seems to be a potential source of edible protein, and may be utilized for value-addition in beverages and other food commodities.

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