

**ENZYME AQUEOUS EXTRACTION OF MORINGA OLEIFERA SEED OILS AND
THEIR EFFECT ON PHYSIOCHEMICAL CHARACTERISTICS AND STABILITY.**

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Abstract

Aqueous extraction is advantageous over conventional solvent extraction methods as the solvent is neither toxic nor presents any risk of fires and explosion. Viscozyme L, Celluclast 1.5L, Nutrase 0.8L and Alcalase 2.4LFG were screened for their efficacies to extract oil from moringa oleifera and canola seeds compared to conventional hexane extraction method. The hydrolytic enzymes exhibited varying extents of effectiveness for oil extractability. The use of ternary enzyme mixtures enhanced the oil extraction yield but it was still significantly ($p < 0.05$) lower than that obtained by hexane extraction. Cellulases, proteases and pectinases are the most types of enzymes used to be most effective for the aqueous enzymatic oil extraction. Samples of oils treated with (65% Alcalase + 25% Neutrase + 10% Viscozyme) showed the lowest accumulation of induction period and peroxide values when incubated at 63°C (Schaal Oven test) indicating non higher oxidative stability compared with those extracted by hexane.

Key words: *Moringa oleifera, Canola oil, hexane, enzyme aqueous extraction, oxidative stability*

INTRODUCTION

Hexane extraction is a mainstay of any successful oil extraction operation and is one of the most commonly used procedures for the preparation and production of oil in the world. Several methods have been developed to extract oil from vegetable oil seeds using aqueous enzymatic methods. Unlike conventional oil extraction methods, these new bioprocesses are performed without the use of presses and organic solvents. The large variations in cell wall ultrastructure and chemical composition (varying proportions of cellulose, hemicelluloses and pectin) of oil-rich plant materials have posed a challenge for the development of aqueous enzymatic oil extraction strategies. Cellulases, proteases and pectinases are the most types of enzymes used to be most effective for the aqueous enzymatic oil extraction (Abdulkarim, et al., (20060 and Moreau, et al. 2007). Although the high cost of enzymes is a major hurdle to the commercialization of aqueous enzymatic oil extraction methods, recent advances in enzyme production technology may soon make the processes economically viable.

MATERIALS AND METHODS**Seed oil samples:**

The two samples of some seeds oils used in this study were moringa oleifera and canola seeds. The canola seed was purchased from the Food Science and Technology Center, Ministry of Agriculture, Giza – Egypt. Moringa oleifera seed was samples purchased from Aswan local market, Egypt. The seed samples were packed into polyethylene bags (bag capacity about 3 – 5 kg seeds) and stored at under frozen conditions at – 20 °C until analysis.

Enzymes:

Four enzymes were used in this study namely Viscozyme L, Celluclast 1.5L, Nutrase 0.8L and Alcalase 2.4LFG. These enzymes were donated by Novozyme A/S, Krogshoejvej 36, Dk-2880 Bagsvaerd, Denemark. They were chosen because of their effectiveness as extraction enzymes and to their industrial utilization fatty foods (**Abdulkarim, et al., (2006)**). Both trade and chemical names of the studied enzymes as well as the optimum conditions of these enzymes activity are shown in Table 1.

Table (1): Trade, chemical names and the optimum conditions of the studied enzymes*:

Code	Enzymes		Optimum conditions*	
	Trade name	Chemical content	T (°C)	pH
A	Alcalase 2.5L	Protease	55	6 – 8.5
B	Celluclast1.5L	Cellulose/xylanase	55	5 – 7
C	Viscozyme L	Pectinase/ xylanase /Bglucanase /cellulase	50	4.5 – 7
D	Neutrase 0.8L	Protease	50	5 – 7

* Source from the supplier (Novozyme A/S, Krogshoejvej 36, Dk-2880 Bagsvaerd, Denemark).

Solvents:

Absolute n-hexane of 99.9% purity, having a boiling point at 70 °C (for 95% evaporation) and specific gravity of 0.656 – 0.665 at 20 o C was used. N-hexane and all chemical products were purchased from El-Nasr Company for Pharmaceutical Chemicals (Qalyubia, Egypt).

Hexane Extraction:

The ground seed material (25g) was fed to a Soxhlet extractor fitted with a 0.5 L round bottom flask and a condenser. The extraction was carried out for 6 h with 0.3 L of n-hexane on a water bath. After extraction, hexane was distilled off under vacuum using a rotary evaporator (Eyela, N-N series, Rikakikai Co, Ltd. Tokyo, Japan) at 45°C and the oil obtained was stored under refrigeration (4 °C), until used for further analyses (**Latif and Anwar, 2009**).

Aqueous enzymatic extraction:

For aqueous enzyme extraction, 15 g each of ground seeds were weighted into different 250 ml conical flasks and 150 ml of distilled water were added to give a ratio of 1:10 (w/v), which is considered to be the best ratio for the oil extraction procedure (**Domingues, et al., 1995**). The samples were gently boiled for 5 min and immediately cooled to room temperature in an ice water bath (Fig. 1). The suspension was then mixed with the enzyme at a 2.0% concentration and incubated at the conditions cited the enzymatic extractions were done ternary mixtures as displayed in Table 2.

Table (2): Mixed enzymes preparations:

Ternary enzymes mixture types (65:25:10 % w/w)*	
	A + B + C
	A + B + D
	A + C + D
	A + C + B
	A + D + B
	A + D + C

Where: - A, B, C and D Commercial trade name Enzymes as A: Alcalase 2.5L, B: Celluclast 1.5L, C: Viscozyme L, D: Neutrase 0.8L. Enzymes mixture ratios adapted from (**Mehanni, 2010**).

The pH and temperatures were adjusted over night at the optimum conditions for each enzyme treatment in Table 1 according to **Puang Sri, et al., (2005)**. The miscella was collected and filtered. The crude oil was dried over anhydrous sodium sulfate.

The oil recovery was estimated as follows:

Oil recovery % = (weight of oil extracted by aqueous-enzymatic method / total weight of oil extracted by Soxhlet method) x 100.

ANALYTICAL METHODS**Chemical characteristics of investigated oils:****Color:**

The color of oil samples was measured by the color Wesson methods using Lovibond glasses and calibrated (Lovibond and Tintometer model F. Tintometer LTD., Wiles, England). A one inch color cell was used according to AOCS Official Methods **Cc 13- 92 (1998)**.

Refractive index:

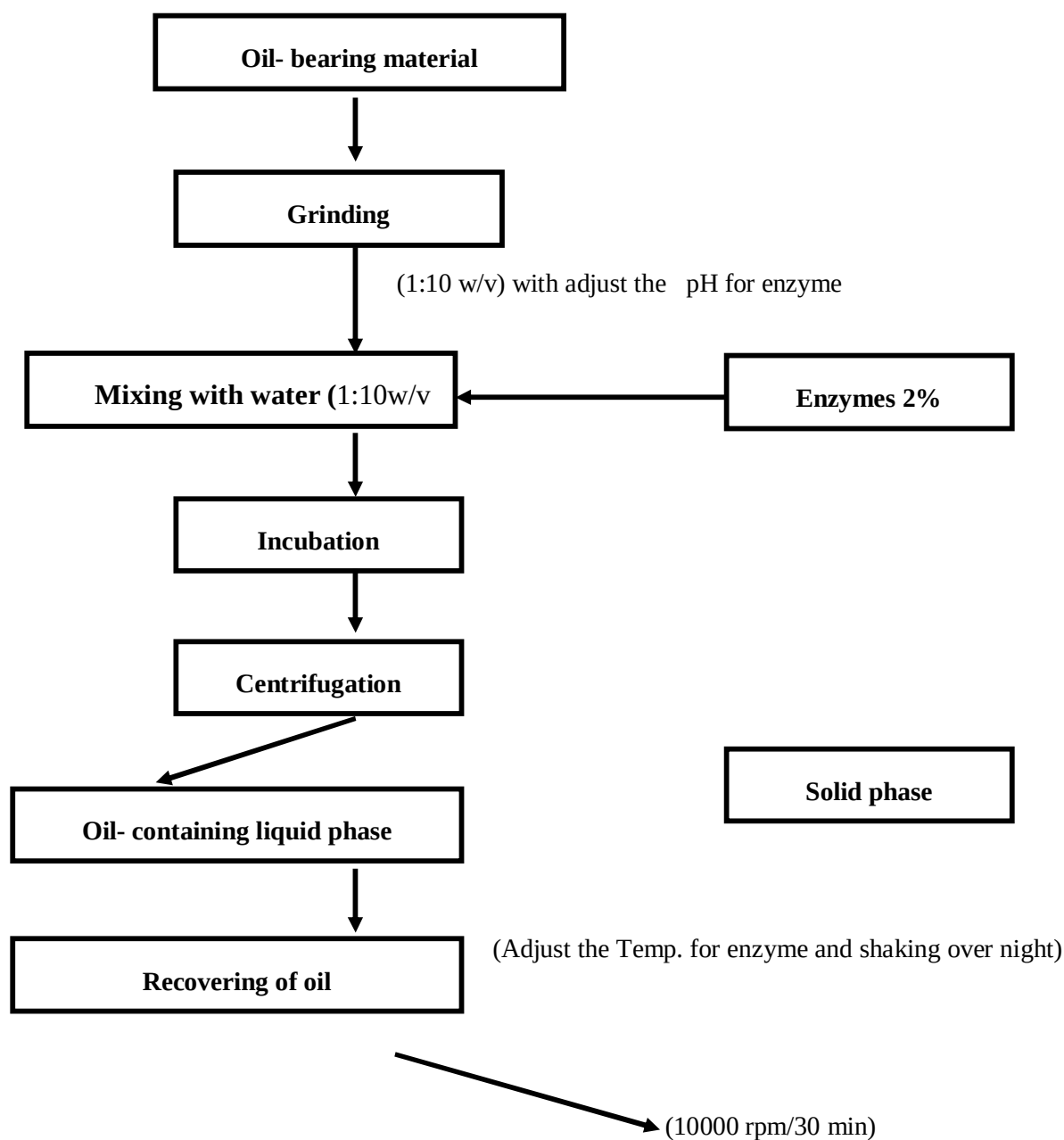
The refractive index (R.I.) was tested according to the method as cited in the AOCS Official method **Cc-25 (1998)**. An automatic refractometer was used and the results were standardized at 25 °C for vegetable oils.

Acid value (AV) and Iodine value (IV) :

The acidity of the investigated samples was examined according to the method described in the AOCS official method **Cd-3d-63 (1998)** and was calculated in terms of free fatty acids percentage as oleic acid.

However, In a 150 ml Erlenmeyer flask clean and dry, 50 ml of alcohol and few drops of phenolphthalein are added and then neutralized with a solution of sodium hydroxide at 60-65 ° C. 56.4 g of oil dissolved in alcohol and then titrated neutralized with the basic solution until the appearance of a pink color persists. The iodine value defined as a number of grams of iodine required to saturate 100 grams of the oil sample. It was determined by the Hanus methods as described as in the AOCS official method **Cd 1-25 (1998)**.

Fig. (1): Scheme diagram of the aqueous enzymatic oil extraction process adapted from (Mehanni, 2010).



Peroxide Value (PV):

This characteristic was tested according to the AOCS method **Cd8-53 (1998)** and **AOAC (2000)**. The peroxide value was reported as milliequivalents of peroxide per kilogram sample. However, the peroxide value is the amount of active oxygen peroxide content in 1 kg of oil, able to be released under the conditions of the experiment. A 5 g (± 0.05 g) of the weighed sample in an Erlenmeyer flask is added 30 ml of an acetic acid-chloroform (3-2, v / v) to dissolve the oil. 0.5 ml of a saturated solution of KI (5 g / 5 ml water) was added with stirring for 1 minute before standing the solution protected from light. After addition of distilled water (30 ml) and a few drops of starch indicator (end of reaction) the liberated iodine is titrated with stirring a solution of thiosulfate 0.02 N.

Total phospholipids :

Total phospholipids content in oil were calculated by multiplying phosphorus content by a conversion factor of 30 (**Weihrauch and Son, 1983**).

The unsaponifiable matter:

The unsaponifiable matter was separated from the vegetable oil samples after saponification according to the method cited in the **AOCS method Ca6a-40 (1998)**.

Schaal oven test:

The Schaal oven test was used at 63°C (145°F) for evaluating the oxidative stability of the investigated crude oils.

Rancimat method:

Stability tests were performed in duplicate using Metrohm Rancimat 679 (Metrohm Ltd., Herisau, Switzerland) as described by **Taskins, et al. (1999)**. 5.0 ± 0.05 g of each oil was accurately weighed into each of the six reaction vessels, and the following procedure was carried out. The Metrohm Rancimat (Fats & Oils Unit, Food Science & Technology Institute, ARC, Egypt) was switched on until the temperature of the oil batch reached 120°C. Then 60 cm³ of distilled water was placed into each of the six conductivity cells, and the air flow rate was set at 20 L h⁻¹. The temperature was checked to ensure that it had a constant value. The air supply was connected to the tubes containing the oil samples, and the chart recorder was started. The determination continued automatically until the conductivity reached the maximum value and the induction period was read. OSI was mathematically determined as the inflection point of a computer-generated plot of conductivity of distilled water versus time which determined as outlined in the **AOCS Official Methods Cd 12b-92 (AOCS 1998)**.

FATTY ACIDS COMPOSITION

Preparation of fatty acid methyl esters

Fatty acids of standard and samples were converted to methyl ester using hexane solution BF₃ in methane according to **Ackman (1998)** however; fatty acids catalyzed esterification procedures using BF₃ in methanol were adapted from **Christie (1992)**. The sample was placed in a non-polar organic solvent (hexane) and a solution of 12% solution of boron trifluoride (BF₃) in methanol. The subsequent methylic esterification was achieved with boron trifluoride methanol complex at a temperature of 100 °C in 70 min. The fatty acid methyl esters (FAMES) were extracted in n-hexane. The sample was first vortexed for 15 s at room temperature and analyzed by GC.

Identification of fatty acids:

The methyl esters of the fatty acids and standard samples were analyzed by using a GLC Shimadzo 2010. Gas liquid chromatography equipped with a dual flame Ionization detected (F.I.D.). The fatty acids methyl esters (FAME) were analyzed with a gas chromatograph Shimadzu-2010 (Kyoto, Japan) equipped with flame –ionization detector and automatic injection system (AOC-2010i). The analysis was made on a capillary column CP7420 (100 m X 0.25 mm i.d., 0.2 µm film, Varian Inc., Palo Alto, CA). As a carrier gas was used hydrogen and as a make-up gas-nitrogen. A regime of the oven was programmed: initial column temperature 51 °C for min, and a 4-level increasing of the temperature from 170 °C to 240 °C until the process ending. Finally, Fatty acids peaks were performed by comparing the relative retention time of each peak with those of standard samples.

Statistical analysis:

All data were analyzed using a SPSS statistical package. One-way ANOVA was used to determine the significant difference of among the treatments (F-test at 0.05 significance level).

RESULTS AND DISCUSSION**Chemical composition:**

The data cited in table (3) for the chemical analysis of *M. oleifera* and canola seeds from a wild provenance of Egyptian, along with literature values of identical *M. oleifera* and Canola species. Values for the present analysis are given as mean $\pm$ SD of three different *M. oleifera* and canola seeds, analyzed individually in triplicate.

Table (3): Physical properties and proximate chemical composition of *M. oleifera* seed and canola seed

Property	Oil seed samples*	
	<i>M. oleifera</i> seed	Canola seed
Moisture (%)	7.60 $\pm$ 0.15 ^c	6.11 $\pm$ 0.12 ^{ab}
Ash (%) **	4.53 $\pm$ 0.22 ^a	4.23 $\pm$ 0.11 ^a
Oil (%) **	33.30 $\pm$ 1.64 ^b	36.59 $\pm$ 1.09 ^a
Crude Protein (%) (N x 6.25) **	30.14 $\pm$ 1.28 ^b	22.55 $\pm$ 1.22 ^a
Fiber (%) **	7.20 $\pm$ 0.13 ^c	9.34 $\pm$ 1.11 ^b
Total carbohydrates (%) ***	17.23 $\pm$ 1.12 ^a	21.22 $\pm$ 1.15 ^c

(*)Each figure in this table is a mean of three determinations. Mean $\pm$ SD, Means within the same row followed by the same superscript letters, are not significant ($P > 0.05$) different.

(**) Data was calculated on wet weight basis.

(***) Total carbohydrates were calculated by difference.

Oil extraction yield

In the initial phase of this study, moringa oleifera and canola seed oils were extracted with the conventional solvent hexane compared to aqueous enzymatic oil extraction (AEOE). Results in table 4 demonstrated that the conventional (non-enzymatic processes) resulted in oil yields recovery ranging from 66.05 to 68.26 % and 70.11 to 70.87 % for moringa and canola seed, respectively. Generally the highest oil yield was obtained with hexane. However, the maximum oil yield (33.30 %) was achieved when moringa seed was extracted with hexane. On the other hand, the results of oil yield and recovery among the combined ternary AEOE treatments were cited in Table 4. It was obvious that the use of ternary enzyme mixtures enhanced the oil extraction yield but it was still significantly ($p < 0.05$) lower than that obtained by solvent (hexane) extraction. Results clearly show that the different enzyme mixtures used in the present study exhibited varying extents of effectiveness towards improving the oil yield. This might be attributed to the different compositions of the enzyme mixtures tested. Enzyme mixtures with combined activity provide better results in agreement with those reported by **Rosenthal et al., (2001)**. It is obvious that the maximum oil recovery from moringa oleifera (68.26%) was achieved when a mixture of 65% Alcalase: 25% Celluclast: 10% Viscozyme was applied followed by that of 65% Alcalase: 25% Celluclast: 10% Neutrase. The last mixture was found to be superior for the extraction of canola seed oil corresponding to 70.48 % oil recovery. **McGlone et al., (1986)** observed a maximum yield of coconut oil (80%) when polygalacturonase, α -amylase and protease were used in combination. According to **Abdulkarim et al., (2006)** AEOE proteases were found to be superior to α -amylase, pectinase and cellulase in extracting oil from Moringa oleifera seeds, but a mixture of these four enzymes was even more effective. In addition, Womeni et. al., (2008) found that AEOE carbohydrases blend (cellulase, arabanase, β -glucanase, hemicellulase and xylanase) was more effective rather than protease or pectinase used individually. In rapeseed oil extraction AEOE pectinase, when used alone, was more effective than cellulase, β -glucanase and xylanase, but was less effective than the combination of these 4 enzymes (**Zhang et al., 2007 and Xie, 2009**).

From these results, it could be concluded that the use of ternary AEOE enzymes treatments was more effective and had high extractability of oils with moringa oleifera seed and canola seed. This increase of oil extraction yield was due to the varied for enzymes and its effects on the seed components like protein, starch and cellulose. These results are in accordance with those reported by **Rosenthal et al., (2001)**; **Hanmoungjai et al., (2002)**; **Dzondo-Gadet et al., (2004)** and **Sawan et al., (2007)**.

Physicochemical characteristics of the extracted oils:

The effects of the aforementioned six ternary enzyme mixtures and hexane on the physicochemical characteristics of moringa oleifera and canola seed oils were compared in Tables 5 and 6. When these enzymes were applied together, they did not have any determining effects on the iodine value and the refractive index of the extracted oils compared to the conventional hexane extraction procedure.

Free fatty acids and peroxide values are valuable measure of oil quality. Using this new protocol, these values were considerably low than oils extracted by hexane. This result is of special interest which is in agreement with previous studies (**Ranalli et. al., 1999**). Mild extraction conditions in an aqueous medium at lower temperature could explain the lower FFA and peroxide values of the oils extracted by aqueous enzyme mixtures reaction.

Table (4): Oil recovery (%) extracted by different ternary enzyme mixtures*:

Ternary mixtures	Oil recovery (%) **	
	moringa oleifera seed	Canola seed
A+B+C (65:25:10 w/w/w)*	68.26 ^d	70.38 ^b
A+B+D (65:25:10 w/w/w)*	67.79 ^c	70.48 ^b
A+C+D (65:25:10 w/w/w)*	66.05 ^b	71.87 ^a
A+C+B (65:25:10 w/w/w)*	67.44 ^d	70.23 ^d
A+D+B (65:25:10 w/w/w)*	66.13 ^c	70.34 ^d
A+D+C (65:25:10 w/w/w)*	67.54 ^a	70.11 ^c

(*) A,B,C and D Enzymes are: A=Alcalase 2.5L, B=Celluclast 1.5L, C=Viscozyme L, and D=Neutrase 0.8.

(**) Oil recovery are expressed as relative to hexane extraction (hexane extraction = 100%). Each figure in this table is a mean of three determinations. Mean values within the same column followed by the same superscript letters, are not significant ($P > 0.05$) different.

It is interesting also to note that as alone worked; the mixture of enzymes allows reduction of the level of phospholipids and color substances as evident from the total Lovibond color parameter. The low phospholipids content was probably not due to the enzyme treatment, but rather the washing effect of the water during the aqueous-enzymatic process. With regard to the unsaponifiable matters content, it appears that the oils extracted by the different ternary enzyme mixtures were significantly ($p < 0.05$) higher than those extracted by means of hexane. However, the highest unsaponifiable matters content was recorder for the oil extracted by a mixture of 65% Alcalase: 25% Celluclast: 10% Viscozyme which represented also the highest oil extraction yield.

The fatty acid composition of the extracted seed oils

The fatty acid profiles of moringa oleifera seed and canola seed oils extracted with some selected ternary enzyme mixtures are displayed in **Tables 7 and 8**. Tables show the FA composition of the oil extracted using enzymes mixtures and hexane. Oleic acid was the most prominent FA, ranging from 76.03 to 76.30 % for enzymes-extracted samples, at 76.03 % for the hexane extracted oil, respectively. Other prominent FAs were palmitic (5.64 to 5.89%), stearic (4.30 to 4.67%) and behenic (4.49 to 4.91%) for moringa seed oils. There was no significant difference ($P > 0.05$) in the major FA of the enzyme-extracted oil compared hexane trails. The FA composition was unaffected by the type of enzyme used in the extraction. The high oleic acid content of the oil may qualify it as high oleic-type oil which can be compared to other high oleic oils such as high oleic canola (55.79%). In general, there were no significant differences ($p < 0.05$) in the amounts of the major fatty acids among the oils extracted via the combined ternary AEOE treatments when compared with each other or with the n-hexane extracted oil **Dominguez et al., 1995 and 1996**). This demonstrated that the use of enzymes in the extraction process in combinations had no incidence on the composition of fatty acids of the oils. A similar behavior was reported by other authors (**Dominguez et al., 1994; Rosenthal et al., 1996; Sengupta and Bhattacharyya, 1996 and Latif et al., 2008**).

Table (5): Effect of different ternary enzyme mixtures and hexane on the physicochemical characteristics of the extracted moringa oleifera oil*

Characteristics**	moringa oleifera						
	Ternary enzyme mixture						Hexane
	A+B+C	A+B+D	A+C+D	A+C+B	A+D+B	A+D+C	
Free fatty acids (F.F.A.) (% as oleic acid)	0.33 ^b	0.30 ^b	0.32 ^{ab}	0.30 ^b	0.33 ^{ab}	0.29 ^b	0.25 ^a
Iodine value (I.V.)	66.20 ^a	66.22 ^a	66.38 ^a	66.27 ^a	66.16 ^a	66.28 ^a	66.14 ^a
Peroxide value (P.V.) (meq. Peroxide/kg oil)	1.28 ^b	1.25 ^b	1.21 ^c	1.22 ^b	1.20 ^c	1.22 ^{bc}	1.29 ^a
Refractive index (25°C)	1.4571 ^a	1.4561 ^a	1.4568 ^a	1.4567 ^a	1.4578 ^a	1.4560 ^a	1.4574 ^a
Total Lovibond color (Y+10R)	148.24 ^b	142.50 ^b	146.63 ^b	148.12 ^b	143.50 ^b	142.62 ^b	202.22 ^a
Phospholipids (mg/100g oil)***	322.1 ^b	323.2 ^b	319.2 ^b	318.3 ^b	315.2 ^b	316.1 ^b	782.2 ^a
Unsaponifiable matter (%)	0.88 ^{ab}	0.87 ^b	0.80 ^a	0.87 ^b	0.86 ^b	0.87 ^a	0.80 ^c

(*) Enzymes commercial trade names are: A= Alcalase 2.5L, B= Celluclast 1.5L, C= Viscozyme L, and D= Neutrase 0.8L. (Ternary enzymes mixture extraction (65:25:10 w/w/w)).

(**) Each figure in this table is a mean of three determinations. Mean values for each oil group within the same row followed by the same superscript letters, are not significant ($P > 0.05$) different.

(***) Phospholipids content = phosphorous content X 30 (Weihrauch and Son, 1983).

Table (6): Effect of different ternary enzyme mixtures and hexane on the physicochemical characteristics of the extracted canola seed oil*

Characteristics**	Canola seed oil						
	Ternary enzyme mixture						Hexane
	A+B+C	A+B+D	A+C+D	A+C+B	A+D+B	A+D+C	
Free fatty acids (F.F.A.) (% as oleic acid)	0.59 ^c	0.56 ^c	0.55 ^b	0.56 ^c	0.54 ^{bc}	0.53 ^c	0.61 ^a
Iodine value (I.V.)	119.26 ^a	119.11 ^a	119.32 ^a	119.35 ^a	119.22 ^a	119.24 ^a	119.53 ^a
Peroxide value (P.V.) (meq. Peroxide/kg oil)	1.12 ^{ab}	1.13 ^{ab}	1.28 ^b	1.13 ^{ab}	1.16 ^b	1.18 ^b	1.19 ^a
Refractive index (25 °C)	1.4690 ^a	1.4688 ^a	1.4691 ^a	1.4690 ^a	1.4689 ^a	1.4692 ^a	1.4691 ^a
Total Lovibond color (Y+10R)	48.69 ^b	51.02 ^b	49.41 ^b	51.04 ^b	51.11 ^b	50.22 ^b	76.52 ^a
Phospholipids (mg/100g oil)***	212.1 ^b	218.2 ^b	215.0 ^b	226.2 ^b	218.15 ^b	222.32 ^b	629.9 ^a
Unsaponifiable matter (%)	1.12 ^b	1.13 ^c	1.26 ^{ab}	1.25 ^a	1.20 ^b	1.16 ^{bc}	1.12 ^d

(*) Enzymes commercial trade names are: A= Alcalase 2.5L, B= Celluclast 1.5L, C= Viscozyme L, and D= Neutrase 0.8L. (Ternary enzymes mixture extraction (65:25:10 w/w/w)).

(**) Each figure in this table is a mean of three determinations. Mean values for each oil group within the same row followed by the same superscript letters, are not significant ($P > 0.05$) different.

(***) Phospholipids content = phosphorous content X 30 (Weihrauch and Son, 1983).

Table (7): Effect of different ternary enzyme mixtures and hexane on the fatty acid composition of moringa oleifera seed oil*

Relative % of fatty acids** (wt. of total fatty acids)	moringa oleifera seed oil						
	Ternary enzyme mixture						Hexane
	A+B+C	A+B+C	A+B+C	A+B+C	A+B+C	A+B+C	
Saturated fatty acid(SFA)							
Palmitic C _{16:0}	5.81 ^a	5.88 ^a	5.64 ^a	5.89 ^a	5.82 ^a	5.72 ^a	5.82 ^a
Stearic C _{18:0}	4.42 ^a	4.30 ^a	4.31 ^a	4.58 ^a	4.64 ^a	4.67 ^a	4.53 ^a
Arachidic C _{20:0}	2.63 ^a	2.76 ^a	2.85 ^a	2.63 ^a	2.62 ^a	2.63 ^a	2.48 ^a
Behenic C _{22:0}	4.91 ^a	4.69 ^a	4.60 ^a	4.49 ^a	4.61 ^a	4.67 ^a	4.64 ^a
Total SFA	17.71 ^a	17.63 ^a	17.40 ^a	17.59 ^a	17.69 ^a	17.69 ^a	17.47 ^a
Unsaturated fatty acids (UFA)							
Palmitoleic C _{16:1}	3.90 ^a	3.97 ^a	3.80 ^a	3.86 ^a	3.90 ^a	3.60 ^a	3.80 ^a
Oleic C _{18:1}	76.10 ^a	76.20 ^a	76.30 ^a	76.15 ^a	76.12 ^a	76.21 ^a	76.03 ^a
Gadoleic C _{20:1}	1.40 ^a	1.40 ^a	1.60 ^a	1.42 ^a	1.41 ^a	1.60 ^a	1.80 ^a
Erucic C _{22:1}	-	-	-	-	-	-	-
Linoleic C _{18:2n-6}	0.90 ^a	0.80 ^a	0.90 ^a	0.98 ^a	0.88 ^a	0.90 ^a	0.90 ^a
Linolenic C _{18:3n-3}	-	-	-	-	-	-	-
Total UFA	82.30 ^a	82.37 ^a	82.6 ^a	82.41 ^a	82.31 ^a	82.31 ^a	82.53 ^a
TUFA : TSFA	4.65 ^a	4.67 ^a	4.75 ^a	4.69 ^a	4.65 ^a	4.65 ^a	4.72 ^a
C _{18:2} : C _{18:1}	0.012 ^a	0.010 ^a	0.012 ^a	0.013 ^a	0.012 ^a	0.012 ^a	0.012 ^a

(*) Enzymes commercial trade names are: A= Alcalase 2.5L, B= Celluclast 1.5L, C= Viscozyme L, and D= Neutrase 0.8L. (Ternary enzymes mixture extraction (65:25:10 w/w/w)).

Table (8): Effect of different ternary enzyme mixtures and hexane on the fatty acid composition of canola seed oil*

Relative % of fatty acids** (wt. of total fatty acids)	Canola seed oil						
	Ternary enzyme mixture						Hexane
	A+B+C	A+B+C	A+B+C	A+B+C	A+B+C	A+B+C	
Saturated fatty acids (SFA)							
Palmitic C _{16:0}	5.94 ^a	5.91 ^a	5.90 ^a	5.94 ^a	5.93 ^a	5.95 ^a	5.91 ^a
Stearic C _{18:0}	2.49 ^a	2.50 ^a	2.52 ^a	2.51 ^a	2.43 ^a	2.44 ^a	2.45 ^a
Arachidic C _{20:0}	0.67 ^a	0.62 ^a	0.65 ^a	0.62 ^a	0.63 ^a	0.60 ^a	0.62 ^a
Behenic C _{22:0}	-	-	-	-	-	-	-
Total SFA	9.10 ^a	9.03 ^a	9.07 ^a	9.07 ^a	8.99 ^a	8.99 ^a	8.98 ^a
Unsaturated fatty acids (UFA)							
Palmitoleic C _{16:1}	-	-	-	-	-	-	-
Oleic C _{18:1}	55.89 ^a	55.80 ^a	55.79 ^a	56.20 ^a	55.71 ^a	55.73 ^a	55.79 ^a
Gadoleic C _{20:1}	1.52 ^a	1.54 ^a	1.50 ^a	1.54 ^a	1.56 ^a	1.53 ^a	1.51 ^a
Erucic C _{22:1}	0.32 ^a	0.30 ^a	0.29 ^a	0.29 ^a	0.39 ^a	0.38 ^a	0.34 ^a
Linoleic C _{18:2n-6}	25.97 ^a	26.06 ^a	25.87 ^a	25.48 ^a	25.98 ^a	25.92 ^a	26.07 ^a
Linolenic C _{18:3n-3}	7.20 ^a	7.27 ^a	7.48 ^a	7.42 ^a	7.37 ^a	7.45 ^a	7.31 ^a
Total UFA	90.9 ^a	90.97 ^a	90.93 ^a	90.93 ^a	91.01 ^a	91.01 ^a	91.02 ^a
TUFA : TSFA	9.99 ^a	10.07 ^a	10.03 ^a	10.03 ^a	10.12 ^a	10.12 ^a	10.14 ^a
C _{18:2} : C _{18:1}	0.46 ^a	0.47 ^a	0.46 ^a	0.45 ^a	0.47 ^a	0.47 ^a	0.47 ^a

(*) Enzymes commercial trade names are: A= Alcalase 2.5L, B= Celluclast 1.5L, C= Viscozyme L, and D= Neutrase 0.8L. (Ternary enzymes mixture extraction (65:25:10 w/w/w)).

(**) Each figure in this table is a mean of three determinations. Mean values for each oil group within the same row followed by the same superscript letters, are not significant (P> 0.05) different.

Oxidative stability of the extracted oils:**Schaal oven test**

The conventional Schaal oven test was applied in this study in order to evaluate the effect of enzyme-assisted oil extraction on the oxidative stability. Samples of moringa oleifera seed oil and canola seed oils treated with (65 % Alcalase + 25 % Neutrase + 10% Viscozyme) were incubated at 63 °C compared with samples of the corresponding oils extracted by hexane. Changes in peroxide values were recorded periodically. Induction period also was tested by rancimat.

Peroxide value:

Peroxide value (PV) is the measurement of hydroperoxide products which are produced as a result of primary oxidation of oils (**McGinely, 1991**). Therefore, the concentration of peroxide is a valuable measure of the early stage of oxidation. The changes in PV during accelerated temperature storage (63° C) are shown in **Fig. 2** for moringa oleifera seed oil and canola seed oil, respectively. The results of this study show that the peroxide formation increased notably with storage time as a result of the autoxidation of double bonds of unsaturated fatty acids in oils. Such increase in peroxide values had been reported by **Liu et al., (1991)** and **Neff et al., (1997)**. Crude moringa oleifera seed oil was more stable and more resistant to oxidation over the duration of storage time (**Fig. 2**). On the other hand, crude canola seed oil displayed the fastest hydroperoxide formation in the early stages of oxidation due to its linoleic and linolenic acid contents (**Fig. 3**). In general, depending on the extraction method, the rate of increasing in PV can be possibly arranged in descending order as follows: hexane > EAOE

In all cases, the rate of PV accumulation during this storage was lower in the enzymatic extracted oils indicating better stability that related to the higher availability of such bioactive components of cell wall degrading enzymes during seed extraction (**Latif et al., 2007, Latif and Anwar, 2009**).

Rancimat method:

The oxidative stability of crude moringa seed oil obtained by the different extraction techniques was also evaluated by the Rancimat method at the temperature of 120° C based on the conductimetric determination of volatile degradation of secondary products of oxidation. The Rancimat induction period (IP) values are presented in Table 9. It was clear that the oil extracted by the conventional hexane method had the lowest total antioxidant activity; this confirmed by the low induction time (7.24 h). In addition, the hexane extracted oil showed significantly ($p < 0.05$) higher oxidative stability oil extracted with enzyme. Therefore, taking into account the amount of the unsaponifiable fraction, the stability of the oils was dependent on a complex interaction among their endogenous components correspond to the shelf life of the extracted crude oil.

Table (9): Effect of different extraction methods on the oxidative stability of crude moringa seed oil as determined by Rancimat.

Type of extraction	Induction period (h)*
Hexane	7.24 ^a
Aqueous enzyme (65%Alcalase+25%Neutrase+10%Viscozyme)	9.8 ^b

* Each figure in this table is a mean of three determinations. Mean values within the same column followed by a different letters are significant at ($P < 0.05$).

Fig (2): Changes in PV of moringa oleifera seed oil stored at 63 °C

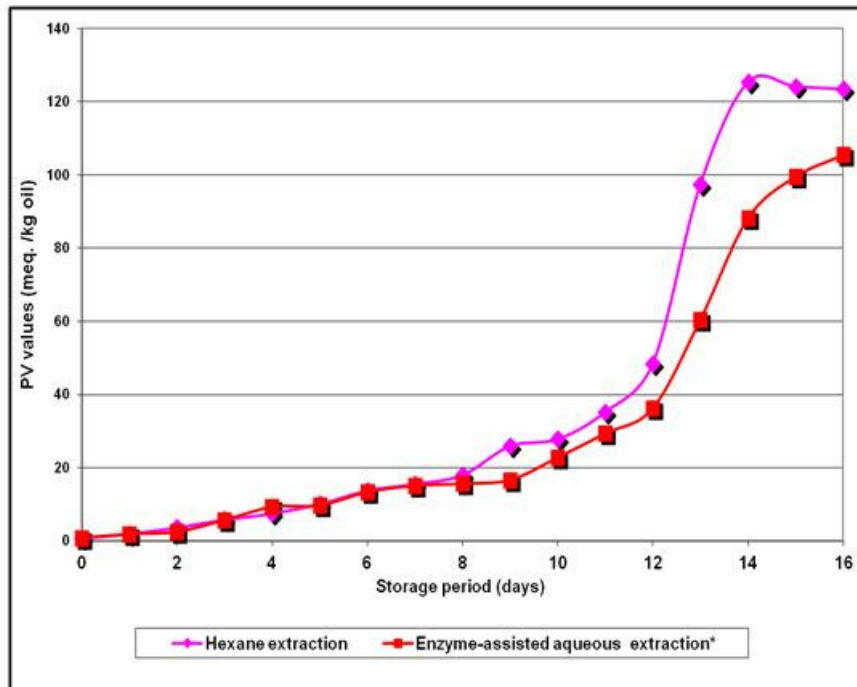
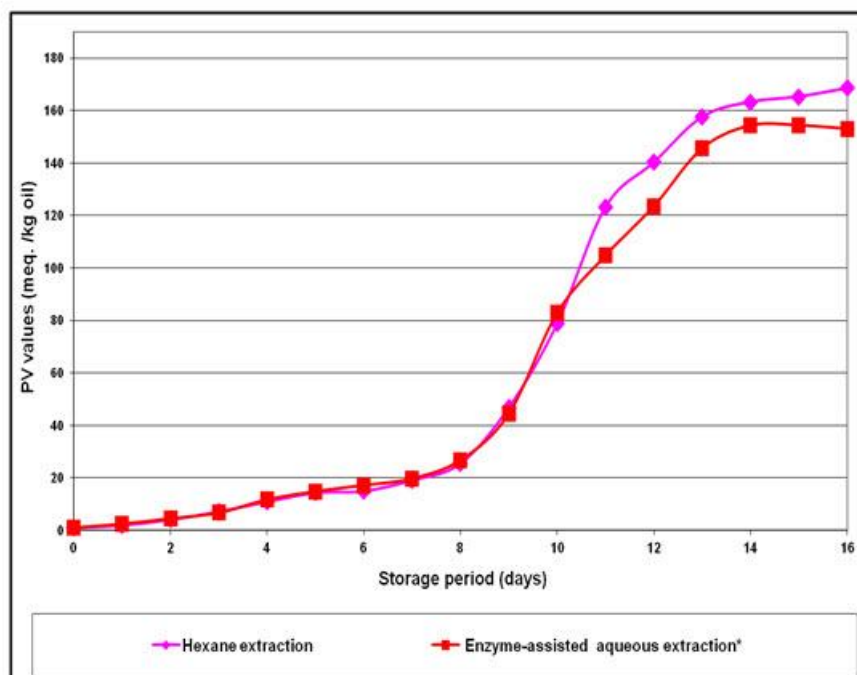


Fig (3): Changes in PV of canola seed oil stored at 63 °C



CONCLUSIONS

In this study four enzymes, Viscozyme L, Celluclast 1.5L, Nutrase 0.8L and Alcalase 2.4LFG were screened for their efficacies to extract oil from moringa seed oil and canola seed by ternary enzyme mixtures compared to conventional hexane extraction methods.

The use of ternary enzyme mixtures enhanced the oil extraction yield but it was still significantly ($p < 0.05$) lower than that obtained by solvent (hexane) extraction. The maximum oil recovery (68.26%) from moringa seed was achieved when a mixture of 65% Alcalase: 25% Celluclast: 10% Viscozyme was applied followed by that of 65% Alcalase: 25% Viscozyme: 10% Neutrase. The last mixture was found to be superior for the extraction of canola seed oil corresponding to 71.87% oil recovery. No improvements were obtained on the quality characteristics of oils extracted by the different ternary enzyme combinations compared to that conventional hexane in trials. Furthermore, the use of ternary enzyme combinations had no incidence on the composition of fatty acids. Samples of oils treated with (65 % Alcalase + 25 % Neutrase + 10% Viscozyme) showed the lowest accumulation of peroxide values when incubated at 63 °C (Schaal Oven test) indicating higher oxidative stability compared with those extracted by hexane. The oxidative stability of crude moringa seed oils obtained by the Rancimat method revealed that the aqueous-enzymatic method had significantly ($p < 0.05$) longer Rancimat induction period than those extracted by hexane. The data obtained of the economical study in this investigation revealed that the enzyme-aided aqueous extraction process was considerably more expensive than the traditional solvent based procedure. It has been shown that enzymes, when used under optimal conditions, significantly enhance the recovery of oil compared to traditional aqueous extraction methods. Generally, enzyme-extracted oils had lower acid values and color intensities. Also because the enzymatic extraction process was carried out in an aqueous medium, phospholipids and other water-soluble gums are separated from the oil so that there is no need for degumming which reduces the overall cost of the refining process. Using the enzyme-extraction process also eliminated loss of oil as compared to aqueous extraction methods because of inadequacies of the latter. It is safer to use enzymes rather than solvents that are hazardous and difficult to dispose after use. This will be useful as an indication of the potentially economical for the industrial application.

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