



NUTRACEUTICAL ANALYSIS OF AMARANTH OIL, AVOCADO OIL, CUMIN OIL, LINSEED OIL AND NEEM OIL

Ripal Khamar* and YT Jasrai

Department of Botany, University School of Sciences, Gujarat University, Ahmedabad - 380009, Gujarat, India.

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Abstract: Amaranth oil, Avocado oil, Cumin oil, Linseed oil and Neem oil are medicinal herbal oil which is natural source of fatty acids, nutraceuticals and mainly Tocopherol – Vitamin ‘E’. They play important role with various medicinal properties like Anti-oxidant, Cholesterol reducing agent etc. Results suggested that Amaranth oil is reddish brown coloured and have 0.5-2 Acid value, 100-115 Iodine value and 182-190 Saponification value as Biochemical parameters; 20-27% Saturated Fatty acid, 18-25% Monounsaturated fatty acid, 45-55% Polyunsaturated fatty acid and 0% others as Fatty acids profile and 0.3-0.4% Mixed Tocopherols, 0.3-0.4% Phytosterols, 4-6% Squalene, 0% others as Nutraceuticals profile. Avocado oil is greenish brown coloured and its results showed 0.5-2 Acid value, 75-90 Iodine value and 185-190 Saponification value as Biochemical parameters; 12-20% Saturated Fatty acid, 65-70% Monounsaturated fatty acid, 8-12% Polyunsaturated fatty acid and 0% others as Fatty acids profile and 0.5-1.5% Mixed Tocopherols, 0.2-0.5% Phytosterols, 0.5-1.5% Squalene, 0.2-0.5% others as Nutraceuticals profile. Cumin oil is brown coloured and its results showed 1-4 Acid value, 110-120 Iodine value and 190-192 Saponification value as Biochemical parameters; 4-7% Saturated Fatty acid, 64-68% Monounsaturated fatty acid, 28-30% Polyunsaturated fatty acid and 0% others as Fatty acids profile and 0.1-0.2% Mixed Tocopherols, 0% Phytosterols, 0% Squalene, 0% others as Nutraceuticals profile. Flaxseed oil is light yellow coloured and its results showed 1-3 Acid value, 180-182 Iodine value and 198-202 Saponification value as Biochemical parameters; 10-12% Saturated Fatty acid, 18-25% Monounsaturated fatty acid, 65-72% Polyunsaturated fatty acid and 0% others as Fatty acids profile and 0.1-0.2% Mixed Tocopherols, 0.3-0.6% Phytosterols, 0.5-0.7% Squalene, ALA>50% others as Nutraceuticals profile. Neem oil is yellowish brown coloured and its results showed 1-3 Acid value, 70-75 Iodine value and 199-202 Saponification value as Biochemical parameters; 18-25% Saturated Fatty acid, 45-60% Monounsaturated fatty acid, 15-20% Polyunsaturated fatty acid and 0% others as Fatty acids profile and 0.1-0.2% Mixed Tocopherols, 0.2-0.3% Phytosterols, 0% Squalene, 0% others as Nutraceuticals profile. Quantity of mixed tocopherols was high in few refined / non refined herbal medicinal oils so, it can become a commercial source of natural vitamin ‘E’.

Keywords: Medicinal herbal oil, Biochemical profile, Fatty acid Profile, Nutraceutical profile

INTRODUCTION

Vitamin E was discovered in 1922 in green leafy vegetables by Herbert Evans and Katherine Bishop. Because E supported fertility, it was scientifically named tocopherol. This comes from the Greek word *tokos* meaning childbirth, and *phero* meaning to bring forth, and the *ol* ending was added to indicate the alcohol properties of this molecule. In 1936 it was discovered that vitamin E was abundant in wheat germ oil. Two years later, it was chemically synthesized for the first time. The U.S. National Research Council sponsored studies on deficiencies of vitamin E, and based on the results E was designated an essential vitamin. Vitamin E emerged as an essential, fat-soluble nutrient that functions as an antioxidant in the human body. It is essential, because the body cannot manufacture its own vitamin E and foods and supplements must provide it. Since the elucidation of the chemical structure of vitamin E in 1938 by Fehholz and the synthesis of *dl*- α -tocopherol by Karrer in the same year, specific focus was directed on the chemical class of natural compounds that qualify to be vitamin E.

At present, vitamin E represents a generic term for all tocopherols and their derivatives having the biological activity of RRR- α -tocopherol, the naturally occurring stereoisomer compounds with vitamin E activity. In nature, eight substances have been found to have vitamin E activity: α , β , γ and δ -tocopherol; and α , β , γ and δ -tocotrienol. The current handicap in knowledge of how tocotrienols may be implicated in human health and disease and the significance of filling that void in vitamin E research is discussed.

Amaranth oil is a light to medium colored, clear liquid that is pourable at low temperatures, highly unsaturated with a delicate, agreeable aroma and taste, allowing greater usage versatility. It also provides an excellent resource for omega series fatty acids. Amaranth oil is valued for its ability to add temperature stability at both high and low temperatures. Commercial uses of amaranth oil include foods, cosmetics, shampoos and intermediates for manufacture of lubricants, pharmaceuticals, rubber

*Corresponding Author:

Ripal Khamar,
Department of Botany,
University School of Sciences, Gujarat University,
Ahmedabad - 380009, Gujarat, India.



chemicals, aromatics and surface active agents. As food oil, amaranth oil has a delicate and agreeable taste. Avocado oil functions well as carrier oil for other flavors. It is high in monounsaturated fats and vitamin E. It also enhances the absorption of carotenoids and other nutrients. It has an unusually high smoke point, both unrefined and especially when refined. The smoke point of the unrefined form is 400 °F (204 °C) and the refined form can reach 520 °F (271 °C). Cumin seeds are used in cooking and the oil is used to flavor food and scent cosmetics. Components may have antioxidant, anticancer, antibacterial, and larvicidal effects. Cumin may lower blood sugar, reduce seizures, strengthen bones, and treat the eye. Flaxseed oil is derived from the hard, tiny seeds of the flax plant. Flaxseed oil contains omega-3 fatty acids, a type of fat body needs as much as it needs vitamins. Flaxseed oil has been proposed as a less smelly alternative to fish oil for the prevention of heart diseases. Neem oil is derived by pressing the seed kernels of the neem tree. It is very bitter with a garlic/sulfur smell. A single seed may contain up to 50% oil by weight. Neem oil is excellent moisturizing oil and contains various compounds that have insecticidal and medicinal properties. It is used in making shampoos, toothpaste, soaps, cosmetics, mosquito repellants, creams and lotions, pet products like pet shampoo, etc. Keeping in view the aforesaid facts an extensive study was taken up with the following broad objectives:

1. To analyse Biochemical profile of oils
2. To analyse Fatty acid profile of oils
3. To analyse Nutraceutical profile of oils

MATERIALS AND METHODS

Following refined oils were studied;

1. Amaranth oil
2. Avocado oil
3. Cumin oil
4. Linseed oil
5. Neem oil

(A) Analysis of biochemical profile

The following parameters were studied using standard methods;

1. Colour
2. Acid value
3. Iodine value
4. Saponifiable value

(B) Analysis of fatty acids profile

The following Fatty acids profile was analyzed by Gas chromatography method;

1. Saturated Fatty acid
2. Mono saturated
3. Poly Unsaturated
4. Others

Following steps of Gas chromatography were done;

Chromatographic conditions

Equipment: Varian make 3800-Gas Chromatography or equivalent
Column : DB 225 column, 30 meters x 0.25 mm ID, 0.25µ or equivalent.

Carrier Gas

Nature : Nitrogen
Column flow : 1.2 ml/min
Linear Velocity : 34.8 cm/sec
Purge flow : 3.0 ml/min
Split Ratio : 75

Column Temperature

Start at 180° C and maintain the temperature for 1 minute, raise the temperature from 180°C - 220° C at a rate of 3°C per minute and maintain the temperature at 220° for 2 minutes.

Detector

Nature : FID
Range / Attenuation: 2
Hydrogen gas flow: 30 ml/min
Zero air gas flow : 300 ml/min
Temperature : 300° C

Injector

Temperature : 290° C
Injection volume : 1.0 µL

Data Processor

Set at area percent mode

Preparation of Standard Solution: Weigh about 200 to 250 mg of each C16, C18:0, C18:1, C18:2, C18:3 and C20 separately in a 100 ml flat round bottom flask. Add 5.0 ml 0.5 N Methanolic sodium hydroxide solution, stir for 15 minutes at 50° C temperature. Add 4 ml of boron Tri-fluoride BF₃ and again stir for 10 minutes. Methyl ester formed is extracted in Hexane. Organic hexane layer was washed with hot water and filtered through whatmann filter paper. The filtrate was injected separately and the corresponding retention time of each Individual component was recorded.

Preparation of Sample: Weigh about 200 to 250 mg of sample in 100 ml flat bottom flask. Add 5.0 ml 0.5N methanolic sodium hydroxide solution and stir for 15 minutes at 50° C. Add 4 ml of boron trifluoride (BF₃) and again stir for 10 minutes. Methyl ester formed than extracted in hexane. Organic hexane layer was than washed with hot water and filtered through whatmann filter paper. Filtrate was injected. Corresponding area percent was recorded.

(C) Analysis of nutraceutical profile:

The following nutraceutical were analysed using GC method;

1. Mixed Tocopherol content
2. Phytosterols
3. Other nutraceutical

Internal Standard Solution: Weigh accurately 750 mg Hexadecyl hexadecanoate in 250 ml volumetric flask, dissolve it in a mixture of 2 parts of pyridine and 1 part of propionic anhydride and make the volume up to the mark with the same, having concentration 3.0 mg/ml internal standard. (If necessary stir the solution for 10 minutes so that the crystal of internal standard gets completely dissolved).

Standard Preparation: Weigh accurately 100-120 mg of mixed tocopherol standard in 100 ml Erlenmeyer flask having 19/38 standard ground glass neck. Pipette 15 ml internal standard solution and reflux it for 15 minutes under cooled water condenser.

Assay Preparation: Weigh accurately about 300-400 mg of test sample in 100 mL Erlenmeyer flask having 19/38 standard ground glass neck. Pipette 15 mL internal standard solution and reflux it for 15 minutes under cooled water condenser.

Chromatographic Conditions

Column	: CP Sil 5 CB make Varian, 30 m x 0.25 mm, 0.25µ
Column type	: Capillary Column
Column flow Rate	: 1.2 ml/min
Initial Column Temp	: 285 °C
Hold time (1)	: 35 minutes
Temperature Raise	: 3.0 °C
Final column Temp.	: 295 °C
Hold time (2)	: 1 minute
Total Time	: 39.33 min.
Detector	: Flame Ionization detector
Detector Temperature	: 300 °C
Injector Temperature	: 290 °C
Split Ratio	: 100

System Suitability: Inject blank and record the chromatogram, inject internal standard and record the retention time of internal standard, inject standard preparation and record the retention time of d-alpha tocopherol and internal standard. Disregard any peak obtained due to blank. The test is valid if and only if the resolution between internal standard and d-alpha-Tocopherol is more than 2.0.

Procedure: Inject standard preparation twice and record the chromatogram, inject sample preparation in duplicate and record the chromatogram. Calculate ratio of each alpha, beta plus gamma and

delta tocopherol against internal standard in the same way calculate ratio of each individual sterol viz. campesterol, stigmasterol and sitosterol in both standard and sample preparation and calculate percentage of each tocopherol and sterol derivative using formula:

$$\text{Calculation: Assay} = \frac{Ws \times \text{Purity} \times Ru}{Wu \times 100 \times Rs}$$

Where,

Ws = Weight of standard taken

Wu = Weight of sample taken

Ps = Purity of individual alpha, beta plus gamma and delta tocopherol, campesterol, stigmasterol and sitosterol

Ru = Ratio of the peak response of individual alpha, beta plus gamma and delta tocopherol and individual sterols to the peak response of IS in sample preparation

Rs = Ratio of the peak response of individual alpha, beta plus gamma and delta tocopherol and the individual sterols to the peak response of IS in standard preparation

RESULTS AND DISCUSSION

Results of studied oils suggested that Amaranth oil is reddish brown coloured and its results showed 0.5-2 Acid value, 100-115 Iodine value and 182-190 Saponification value as Biochemical parameters; 20-27% Saturated Fatty acid, 18-25% Monounsaturated fatty acid, 45-55% Polyunsaturated fatty acid and 0% others as Fatty acids profile and 0.3-0.4% Mixed Tocopherols, 0.3-0.4% Phytosterols, 4-6% Squalene, 0% others as Nutraceuticals profile (Table 1). The total lipid content in amaranth oil was 0.33% (Haytowitz and Matthews, 1984) and linolenic, palmitic, linoleic and oleic acids were the major fatty acids (Fernando and Bean, 1984; Lakshminarayana et al., 1984).

Table 1: Analysis of Biochemical profile, Fatty acids profile and Nutraceuticals profile of Amaranth seed oil

Biochemical profile	Colour	Reddish brown
	Acid value	0.5-2
	Iodine value	100-115
	Saponifiable value	182-190
Fatty acids profile	Saturated fatty acids (%)	20-27
	MonoUnsaturated fatty acids (%)	18-25
	PolyUnsaturated fatty acids (%)	45-55
	Others (%)	-
	Mixed Tocopherols (%)	0.3-0.4
Nutraceuticals profile	Phytosterols (%)	0.3-0.4
	Squalene (%)	4-6
	Others (%)	-

Avocado oil is greenish brown coloured and its results showed 0.5-2 Acid value, 75-90 Iodine value and 185-190 Saponification value as Biochemical parameters; 12-20% Saturated Fatty acid, 65-70% Monounsaturated fatty acid, 8-12% Polyunsaturated fatty acid and 0% others as Fatty acids profile and 0.5-1.5% Mixed Tocopherols, 0.2-0.5% Phytosterols, 0.5-1.5%

Squalene, 0.2-0.5% others as Nutraceuticals profile (Table 2). The avocado is a cultivated fruit in which the oil is a main component on a dry weight basis. The tree grows in a number of countries, including India, Egypt, Mexico, and the United States. The high oil content (15%–30%) and fatty acid composition depend on many factors such as variety, geographical location, climate conditions, and stage of fruit development. Avocado oil is obtained by either solvent extraction or centrifugal separation. Avocado oil is highly unsaturated with 75%–80% oleic acid, 7%–10% linoleic acid and only trace levels of stearic acid (Werman and Neeman, 1987). The values for avocado mesocarp fatty acid composition are the averages from four cultivars grown in the Mediterranean region. *cis*-Vaccenic acid (1%–2%) has been identified and is reported as the sum of vaccenic and oleic acids because it coelutes with oleic acid. The fatty acid composition has been shown to change with maturation. Thirty-six weeks after flowering, the oleic - vaccenic acid levels increased steadily, whereas all other major fatty acids decreased. The three minor fatty acids - palmitoleic, stearic and linolenic acids either decreased or remained constant (Ratovohery *et al.*, 1988).

Table 2: Analysis of Biochemical profile, Fatty acids profile and Nutraceuticals profile of Avocado seed oil

Biochemical profile	Colour	Greenish brown
	Acid value	0.5-2
	Iodine value	75-90
	Saponifiable value	185-190
Fatty acids profile	Saturated fatty acids (%)	12-20
	MonoUnsaturated fatty acids (%)	65-70
	PolyUnsaturated fatty acids (%)	8-12
	Others (%)	-
Nutraceuticals profile	Mixed Tocopherols (%)	0.5-1.5
	Phytosterols (%)	0.2-0.5
	Squalene (%)	0.5-1.5
	Others (%)	0.2-0.5

Cumin oil is brown coloured and its results showed 1-4 Acid value, 110-120 Iodine value and 190-192 Saponification value as Biochemical parameters; 4-7% Saturated Fatty acid, 64-68% Monounsaturated fatty acid, 28-30% Polyunsaturated fatty acid and 0% others as Fatty acids profile and 0.1-0.2% Mixed Tocopherols, 0% Phytosterols, 0% Squalene, 0% others as Nutraceuticals profile (Table 3). Einafshar, *et al.*, 2012 had evaluated the antioxidant activities of the essential oil and crude methanolic extract of cumin seed (*Cuminum cyminum*). Total phenolics and tocopherols contents, reducing power, and the oxidative/oil stability index were assessed. Bettaieb, *et al.*, 2010 were investigated *Cuminum cyminum* L. roots, stems and leaves, and flowers for their essential oils, total phenolics, flavonoids, and tannins contents, individual

phenolic compounds, and antioxidant activities. The essential oil was investigated by gas chromatography and gas chromatography mass spectrometry, whereas identification and quantification of individual target polyphenolic compounds was performed by reversed-phase high-performance liquid chromatography. Aruna, *et al.*, 2006 evaluated the effect of *Cuminum cyminum* on lipid peroxidation induced by ethanol and preheated sunflower oil. Hepatotoxicity, assessed by the activities of plasma aspartate transaminase, alkaline phosphatase and γ -glutamyl transferase was apparent in rats fed alcohol and preheated oil as compared with control rats on a normal diet. Treatment with cumin significantly reversed the metabolic trends associated with alcohol and preheated sunflower oil, bringing liver and kidney activities close to normal levels, indicating antioxidant properties of cumin. Ramadan, *et al.*, 2012 were evaluated Cold-pressed black cumin seed oil and cumin seed oil for their fatty acid profiles, phytosterol and tocopherol contents, antiradical properties and inhibition of microbial growth. The main fatty acids in black cumin seed oil were linoleic followed by oleic and palmitic acids. Petroselinic acid (C18:1n-12) was the main fatty acid in cumin seed oil, while linoleic acid was the second major unsaturated acid. Six sterol compounds were measured in both oil, wherein the sterol marker was β -sitosterol. α -Tocopherol constituted 45% of tocopherols in black cumin seed oil, while β -tocopherol was the main component in cumin seed oil.

Table 3: Analysis of Biochemical profile, Fatty acids profile and Nutraceuticals profile of Cumin seed oil

Biochemical profile	Colour	Brown
	Acid value	1-4
	Iodine value	110-120
	Saponifiable value	190-192
Fatty acids profile	Saturated fatty acids (%)	4-7
	MonoUnsaturated fatty acids (%)	64-68
	PolyUnsaturated fatty acids (%)	28-30
	Others (%)	-
Nutraceuticals profile	Mixed Tocopherols (%)	0.1-0.2
	Phytosterols (%)	-
	Squalene (%)	-
	Others (%)	-

Flaxseed oil is light yellow coloured and its results showed 1-3 Acid value, 180-182 Iodine value and 198-202 Saponification value as Biochemical parameters; 10-12% Saturated Fatty acid, 18-25% Monounsaturated fatty acid, 65-72% Polyunsaturated fatty acid and 0% others as Fatty acids profile and 0.1-0.2% Mixed Tocopherols, 0.3-0.6% Phytosterols, 0.5-0.7% Squalene, ALA>50% others as Nutraceuticals profile (Table 4). Worldwide production of linseed oil does not generally rank among the top ten major vegetable oils; however, its production as an edible oil product has

increased in recent years, with the development of linseed varieties producing oils with modified fatty acid compositions. Linseed oil production in the United States was estimated to be 335 million pounds for 2005–2006 (USDA, 2006). Malcolmson et al. (1998) evaluated the oxidative and photooxidative stability of solin oil under accelerated storage conditions. In the presence of light, solin oil was more stable to peroxide development and flavor volatiles than sunflower oil. In the absence of light, solin oil was more stable to peroxide development but was less stable to painty odor development than sunflower oil. Regardless, the high concentrations of 18:2 in linola and solin oils make them particularly susceptible to oxidation when compared with most other vegetable oils. The addition of natural antioxidants - carotene and quercetin improved the oil stability (Łukaszewicz et al., 2004), as did a blend of alpha-tocopherol, ascorbyl palmitate, citric acid, ascorbic acid and ethoxylated ethylene glycol (Rudnik et al., 2001).

Table 4: Analysis of Biochemical profile, Fatty acids profile and Nutraceuticals profile of Flax seed oil

Biochemical profile	Colour	Light yellow
	Acid value	1-3
	Iodine value	180-182
	Saponifiable value	108-202
Fatty acids profile	Saturated fatty acids (%)	10-12
	MonoUnsaturated fatty acids (%)	18-25
	PolyUnsaturated fatty acids (%)	65-72
	Others (%)	-
	Mixed Tocopherols (%)	0.1-0.2
Nutraceuticals profile	Phytosterols (%)	0.3-0.6
	Squalene (%)	0.5-0.7
	Others (%)	ALA > 50

Neem oil is yellowish brown coloured and its results showed 1-3 Acid value, 70-75 Iodine value and 199-202 Saponification value as Biochemical parameters; 18-25% Saturated Fatty acid, 45-60% Monounsaturated fatty acid, 15-20% Polyunsaturated fatty acid and 0% others as Fatty acids profile and 0.1-0.2% Mixed Tocopherols, 0.2-0.3% Phytosterols, 0% Squalene, 0% others as Nutraceuticals profile (Table 5). Similar work on oils, DODs and fixed oils / butters is carried out by our research group on different oils and distillates (Khamar & Jasrai, 2014 a, 2014 b, 2014 c).

Table 5: Analysis of Biochemical profile, Fatty acids profile and Nutraceuticals profile of Neem seed oil

Biochemical profile	Colour	Yellowish brown
	Acid value	1-3
	Iodine value	70-75
	Saponifiable value	199-202
Fatty acids profile	Saturated fatty acids (%)	18-25
	MonoUnsaturated fatty acids (%)	45-60
	PolyUnsaturated fatty acids (%)	15-20
	Others (%)	-
	Mixed Tocopherols (%)	0.1-0.2
Nutraceuticals profile	Phytosterols (%)	0.2-0.3
	Squalene (%)	-
	Others (%)	-

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