

In the Name of God



Razi University

Faculty of Chemistry

Department of Applied Chemistry

M.Sc. Thesis

**Determining Optimum Solvent in order to Oil Extraction from
Hempseed by Experimental and Analytical Hierarchy Process**

(AHP) Methods

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Dedicated to:

My Dear Parents

Abstract

Oils and fats after carbohydrates are the second energy sources in human nutrition have great importance. Oil seeds are the most important products containing vegetable oils that important place of agriculture and land use around the world wide culture of these products value is allocated. Edible oils are part of the commodity in our country during recent decades with consistently low coefficient of self have faced. Annually almost 402 thousand tons of oil seed product that rate requirement is that about 15 percent of provider and everything else import of which about 18 billion tomman annually importing oil seeds will cost the country.

Hemp (*Cannabis sativa* L.), an annual herbaceous plant, has been grown agriculturally for many centuries for its fiber and oil. Hemp seed contains approximately 25-35% oil. Hempseed oil, in addition to its nutritional value, has demonstrated positive health benefits, including the lowering of cholesterol and high blood pressure. Hemp oil provides our body with a range of necessary nutrients and helps prevent a variety of common diseases. Hempseed oil has been suggested to be perfectly balanced in regards to the ratio (3:1) of linoleic and linolenic essential fatty acids required for human nutrition. The highly polyunsaturated oil of hempseed has been used also for industrial purpose.

The modern way of processing vegetable oil is chemical extraction, using different solvents, which produces higher yields and is quicker and less expensive.

In this thesis the works is hemp seed oil extraction by various solvents, and examine the effect of time, temperature and mixing on the oil percentage oil. The effect of time has been investigated in the time of 10, 30, 60, 90, 120 minutes for each solvent. Extraction functions for each solvent were repeated in temperatures of 20, 30, 40, and 50°C for one hour. The mixing effect function has been investigated in different engine speeds of 0, 50, 100, 150 rpm at room temperature for one hour.

In this study, nine different solvents (n-Pentane, n-Hexane, n-Heptane, n-Octane, Petroleum ether, Diethyl ether, Methylcyclohexane, Toluene, and Acetone) were used for extracting Hemp oil. Considering the fact that an appropriate solvent to extract oil has different criteria in industry, the Expert Choice software (AHP) was used for the selection of the most appropriate solvent. The obtained results suggest that the petroleum ether and n-hexane are the best solvents for industrial extraction of hemp seed oil.

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Chapter One

Introduction

1.1. Oil Chemistry

Oils constituents are fatty acids. Despite the often confusing debate of "Fat: good or bad?" consumers have caught on to the idea that improper fat intake contributes to a host of - often fatal - illnesses. They've also heard that fats are "the more unsaturated the better". The following sheds some, hopefully not too technical, light on the connection between fat composition and health.

1.1.1. Fatty Acids

Fats and oils have the same chemical structure but a different melting Point, fats are solid at room temperature, and oils are liquid. Chemically they are composed of a glycerol backbone with three fatty acids (FAs) attached. Each type of oil has a characteristic fatty acid composition. Fatty acids are distinguished by their chain length, i.e. the number of carbon atoms, but most important is the distinction between saturated and unsaturated fatty acids.

1.1.1.1. Saturated Fatty Acids

Saturated fatty acids (SAFAs) are straight molecules. All carbon atoms are bonded the same way and with maximum strength. The parallel molecules stick easily to one another and fats high in SaFAs (e.g. animal lards, coconut fat) are solid a room temperature. The human body uses SaFAs primarily for energy storage. Until a few years ago, fats and oils with a high SaFA content had been preferred for foods because their saturated bonds are stable, resist oxidation and thus rancidity. They can be used for frying, and are inexpensive. On the downside, it was discovered that the "sticky" SaFAs contribute strongly to the formation of clots and deposits in our blood vessels, to strokes and other common cardiovascular diseases.

1.1.1.2. Unsaturated Fatty Acids

Unsaturated fatty acids (UFAs) contain one (monounsaturated) or several (polyunsaturated) double bonds between adjacent carbon atoms. The resulting curved shape makes oils with a high UFA-content "slicker" and keeps them liquid down to lower temperatures. In contrast to more saturated fatty acids, UFAs serve not as energy sources, but as raw materials for the construction of cell membranes and contribute to cell membrane fluidity [1, 2]. A diet low in UFAs forces the body to use SaFAs for the construction of cell membranes, resulting in cells with stiff, rigid membranes. UFAs also are needed as raw materials for many important messenger and regulator substances in our body (prostaglandins, hormones, neurotransmitters)[2,3]. There are many different UFAs produced in nature, primarily found in plant seeds but also in fish oil. There are two classes of polyunsaturated fatty acids (PUFAs) - omega-6 and omega-3 [4]. All unsaturated fats, heating and cooking causes a percentage of the fat to undergo air oxidation. Polyunsaturated fats such as omega-3s and omega-6s are particularly sensitive to oxidation and must be consumed in relatively fresh foods [5]. Particularly important for the human diet are two of these UFAs, Essential Fatty Acids.

1.1.1.2.1. Essential Fatty Acids

These two essential fatty acids (EFAs) are Linoleic acid (LA, 18:2 *n*-6) and alpha-Linolenic acid (ALA, 18:3 *n*-3). They are called "essential" because our body cannot, as with other FAs, produce them ourselves. Instead, they must be present in our diet. Alpha-Linolenic and Linoleic acids are the main essential unsaturated fatty acids obtained from vegetable oils [6]. Such a 3:1 balance has been claimed optimal for human nutrition [7] and is apparently unique among the common plant oils (Table 1.1) [8]. These fatty acids can be elongated and de-saturated into their longer-chain derivatives [6, 9]. Products of these syntheses include the powerful, short-lived, hormone-like prostaglandins and, coincidentally [3]. Several studies have strongly suggested that these fatty acids are important in relation to the pathogenesis (and prevention) of coronary heart disease and hypertension and during pregnancy and breastfeeding, besides showing a hypocholesterolemic effect when used as food supplements [10-12].

- I. **Linoleic acid** (LA; 18:2, w6, cis-9,12) is a double unsaturated fatty acid common in plants: Evening primrose oil contains up to 70% of its total fatty acid content as

Linoleic acid[8]. Unrefined sun flower oil contains up to 65%, hemp oil up to 60% [8], soybean oil up to 55%, and flax oil up to 30% Linoleic acid. The human body synthesizes another important fatty acid from Linoleic acid. The parent omega-6 fatty acid, Linoleic acid (LA) is de-saturated in the body to form Arachidonic acid and gamma –Linoleic acid [4].

II. Alpha-Linolenic acid (LNA or ALA; 18:3, w3, cis-9, 12, 15), a triple unsaturated fatty acid, is found in algae, crustaceans, and in fish oil. Only a few seeds of higher

Table 1.1. Profile common edible oils (% total fatty acids). Adapted from Erasmus 1993.

	Less healthy/Chemically stable <----> More nutritious/Chemically unstable				
	"Saturated"		"Monounsaturated"	"Polyunsaturated"	
	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1w9)	Linoleic (C18:2w6)	Linolenic (C18:3w3)
Hemp	6-9	2-3	10-16	50-70	15-25
Soy	9	6	26	50	7
Canola	0	7	54	30	7
Wheatgerm	0	18	25	50	5
Safflower	0	12	13	75	0
Sunflower	0	12	23	65	0
Corn	0	17	24	59	0
Cottonseed	0	25	21	50	0
Sesame	0	13	42	45	0
Peanut	0	18	47	29	0
Avocado	0	20	70	10	0
Olive	0	16	76	8	0
Palm	85	0	13	2	0
Coconut	91	0	6	3	0

III. plants have substantial contents of this essential fatty acid: flax (up to 58%), hemp (up to 25%), canola and soybean (up to 7%)[8]. The evidence for the cardio protective nature of omega-3 fatty acids is abundant, and currently available data indicate that patients with known coronary heart disease should consume at least 1 g daily of long-chain omega-3 fatty acids and that individuals without disease should consume at least 250–500 mg daily [13], or 0.6–1% of the total energy intake[14]. It is recommended that the pregnant and nursing woman should take at least 2.6g of omega-3 fatty acids and 100–300 mg of Decosahexaenoic acid (DHA)

daily to look after the needs of her fetus and suckling infant [4]. The parent omega-3 fatty acid, alpha-Linolenic acid (ALA) is de-saturated by microsomal enzyme system through a series of metabolic steps to form Eicosapentaenoic acid (EPA) and Decosahexaenoic acid (DHA) [4]. This suggests conversion of ALA to longer chain omega-3s may be further limited by oxidation of available omega-3 precursors in pre-cooked and/or processed foods [5].

1.1.1.2.2. Other Unsaturated Fatty Acids

Plants also contain various levels of omega-3 fatty acids as 'precursors' mainly in the form of alpha-Linolenic acid (ALA). Other dietary long chain fatty acids are the omega-6 fatty acids. Linoleic acid (LA) is the main omega-6 'precursor' in plant/vegetable oils. Fig (1.1) shows the fatty acids of major interest in the diet where n-3 indicates omega-3 and n-6 indicates omega-6 (Fig1.1.A-B) [15].

- I. Gamma-Linolenic acid (GLA) and Stearidonic acid (SDA):** SDA n-3 and GLA n-6 are the same length with 18 carbons each. However, GLA is un-saturated at the n-6 position providing signaling and metabolic specificity between GLA and SDA [15]. Formation of these two compounds in humans occurs via action of the enzyme *delta*-6-desaturase on the essential Linoleic and Linolenic acids, respectively (fig1.2) [2].

Delta-6-desaturase converts LNA to SDA at a faster rate than its conversion of LA to GLA. However, it is LNA that is more often missing from modern diets. In addition, the surfeit of LA (combined with the low LNA levels) usually found in those with the usual health-conscious "polyunsaturated" diet may impair delta-6desaturase conversion of both LA and the little LNA present [1, 2, 15]. For those whose levels of SDA are low due only to diet, supplementation with LNA is sufficient to restore the necessary balance [15]. However, this enzymatic activity can be weak or lacking due to hereditary defects or be provoked by alcoholism, physiological stress, and some degenerative diseases, especially in the elderly. If impairment of this enzyme is the problem, dietary supplementation with GLA and (in more severe cases) SDA can compensate for this deficiency. Unfortunately, very few foods contain GLA or SDA, and available concentrated supplements of these fatty acids are expensive [16]. Evening primrose (*Oenothera biennis* L.) and borage (*Borago officinalis* L.) seed oils are popularly consumed to provide GLA, although the best natural source of both fatty acids has been thought to be black currant (*Ribes*

nigrum L.) seed oil(table 1.2).[8,16]. However, daily consumption of this oil in amounts sufficient to provide an optimum daily intake of LA and LNA may provide too much GLA, allowing the accumulation of a metabolic excess of Arachidonic acid (AA)and, thereby, possibly promoting inflammation, thrombosis or immune-suppression [16].

SDA may function as an important human dietary component of hempseed oil, even in amounts lower 1% . This is because SDA formation in the human body is

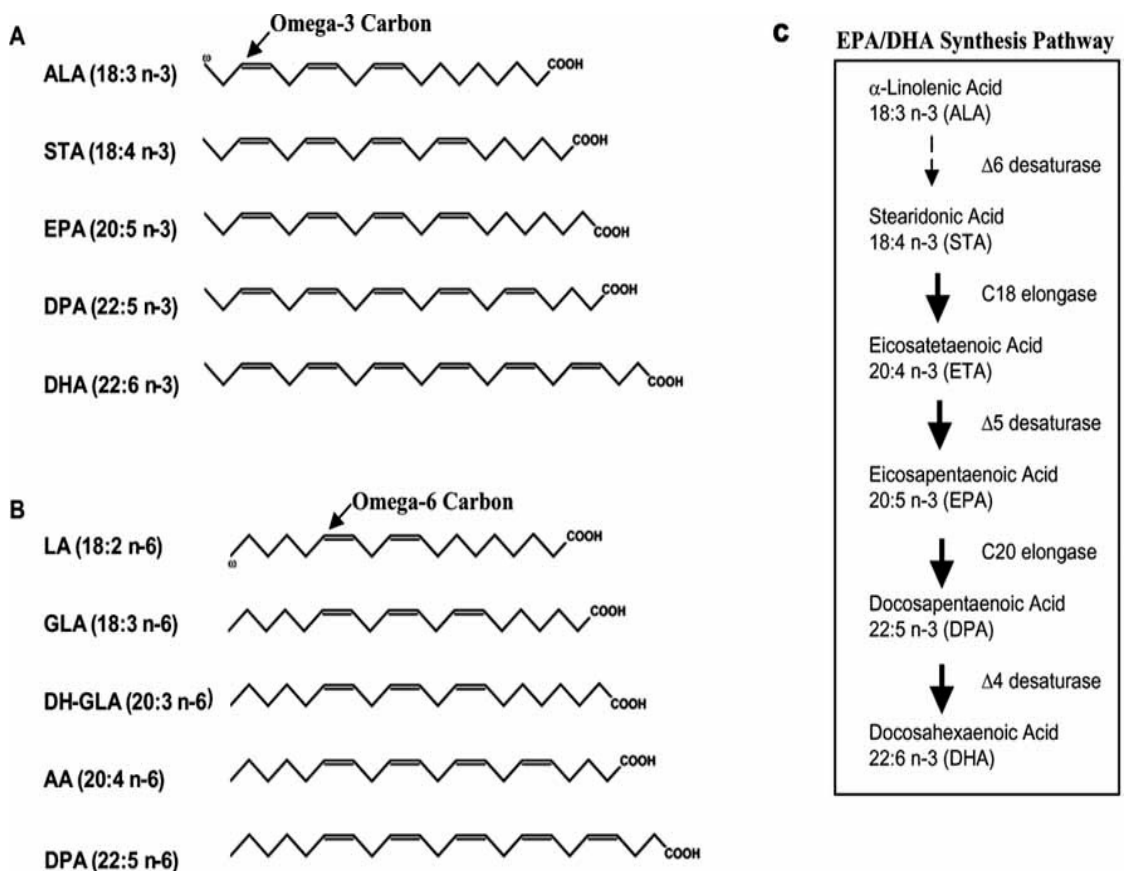


Figure 1.1. Omega-3 and Omega-6 fatty acid structures and EPA/DHA synthesis pathway. A) Omega-3 fatty acids include ALA (alpha-Linolenic acid), STA (Stearidonic acid), EPA (Eicosapentaenoic acid), DPA n-3 (Docosapentaenoic acid n-3), DHA (Docosahexaenoic acid). B) Omega-6 fatty acids include LA (Linoleic acid), GLA (gamma-Linolenic acid), DH-GLA (Dihomo-gamma-Linolenic acid), AA (Arachidonic acid), DPA n-6 (Docosapentaenoic acid n-6) . The conventional designations are as follows # of carbons and # of double bonds with the first double bond from the end, the omega, stated as n-3 or n-6; thus for DPA n-3 the fatty acid is given as (22:5 n-3) and for DPA n-6 it is given as (22:5 n-6). Additionally, the arrows in A and B point to the omega-3 and omega-6 carbon, respectively. C) The EPA/DHA synthesis pathway is given with fatty acids and respective enzymes for each step. The omega-6 synthesis of LA (18:2 n-6) to DPA n-6 (22:5 n-6) uses the same enzymes.

faster than that of GLA (assuming adequate LNA levels), so not much of the former supplement is needed until SDA/GLA processing becomes quite inhibited. However,

unlike black currant seed oil, the SDA/GLA content of hemp seed oil is probably low enough to avoid excessive dietary intake for those not needing such supplements, while providing optimum daily levels of the essential fatty acids. The presence of SDA in the hemp seed oil in amounts approaching 2%, combined with its 4% GLA content, also suggests this variety as a possible raw-material source for the economical manufacturing of isolated SDA/GLA supplements [2].

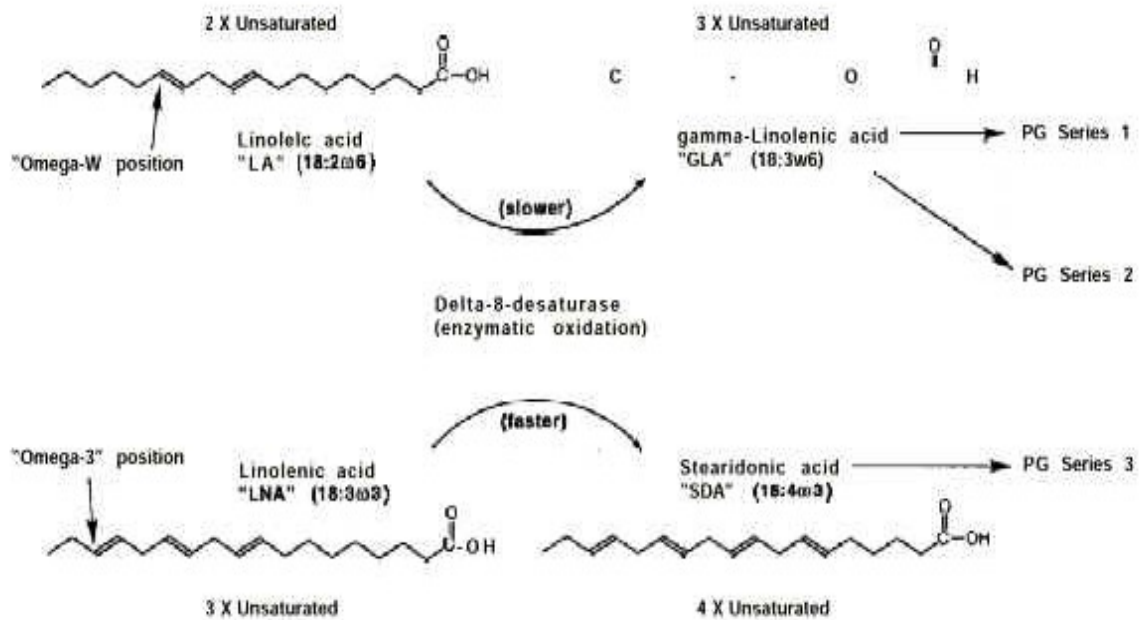


Figure 1.2. Metabolic pathways for the production of prostaglandins from fatty acids. Not show is additional metabolic steps after the production of GLA and SDA.

Table 1.2. Oil profiles of major GLA sources (% total fatty acids).Adapted from various sources.

	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1w9)	Linoleic (C18:2w6)	Linolenic (C18:3w3)	gamma-Linolenic (C18:3w6)
Hemp	6-9	2-3	10-16	50-70	15-25	1-6
Evening	4-12	1-7.5	4-12	65-72	0	3-15
Primrose	6-7	1-2	9-11	45-60	12-15	15-19
Black Currant	~11	~4	~16.5	~37	<1	~23
Borage	9-12	1-2	20-40	18-20	0	20-40

The potential physiological effects of GLA have been extensively investigated only recently. In the body, GLA is normally derived from LA and serves as an intermediary for the formation of longer-chain fatty acids and Eicosanoids. Eicosanoids are short-lived hormone-like substances which fulfill numerous vital roles, ranging from control of inflammation processes and vascular tone to initiation of contractions during delivery. The metabolic conversion of LA to GLA is slow in mammals. Further, it has been suggested that due to stress, ageing or pathology (e.g., hypertension, diabetes, etc.), formation of a sufficient amount or balance of Eicosanoids may be impaired. This problem may be relieved by direct GLA supplementation [17-18] although caution is warranted since overconsumption could be harmful [16]. Its alleviating action on psoriasis, atopic eczema, and mastalgia are already well documented and GLA preparations are now frequently prescribed for the treatment of the latter two disorders. GLA has also been under investigation for its beneficial effects in cardiovascular, psychiatric and immunological disorders [17-19].

If a favorable response to GLA supplementation does not occur, additional application of Stearidonic acid (SDA) may be indicated, since the same enzyme (δ -6-desaturase) that converts LA to GLA is also responsible for converting LNA to SDA. However, relatively few people suffer from a defect in this enzyme compared to the nearly universal lack of adequate LNA levels in the diet [7].

- II. Eicosapentaenoic acid (EPA) and Arachidonic acid (AA):** EPA n-3 and AA n-6 are the same length with 20 carbons each. However, EPA is unsaturated at the n-3 position providing signaling and metabolic specificity between AA and EPA. AA is converted into inflammatory compounds, but the unique n-3 bond means EPA is converted into dramatically different bioactive products. In addition, EPA may compete directly with AA for many of the same enzymes during the inflammatory process [20].
- III. Docosahexaenoic acid (DHA) and Docosapentaenoic acid (DPA):** DPA n-6 and DHA n-3 are the same length with 22 carbons each. The other important omega-3, DHA n-3, is unsaturated at n-3 and is significant for affecting triglyceride levels, by as yet unknown mechanisms [21]. DHA is also incorporated into membrane phospholipids in the brain [22].

In fact, EPA/DHA omega-3 status may be one of the single most important nutritional requirements left unaddressed in nutritional medicine, but emphasis on omega-3 supplementation for health is rapidly increasing [23]. At the same time