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Analysis of Bioactive Compounds Inraw (Dried), Autoclaving, Boiling and Roasted Linum Usitatissimumseed Using Gas Chromatography and Mass Spectroscopy

KEYWORDS: Linum usitatissimum,GC/MS, Bioactive compounds, Fatty acids

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ABSTRACT *In this study, the bioactive components of Linum usitatissimum seeds have been evaluated using GC/MS. The chemical compositions of Linum usitatissimum seeds were investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the plant was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of raw (dried), autoclaving, boiling and roasted Linum usitatissimum seed revealed the existence of biologically active compounds.The maximum peak area represented in the raw, roasted and autoclaved linseeds were 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z) - (88.38%) (C19H32O2) with retention time of 15.03. This compound is called as Alpha-Linolenic acid. The maximum peak area represented in the boiled linseeds was Hexadecanoic acid, methyl ester (88.33%) (C17H34O2) with retention time of 12.92. This compound is called as Palmitic acid.The results of this study offer a platform of using Linum usitatissimum seed as nutrition.*

INTRODUCTION

Plant and plant products are an important part of the human diet and a major source of biologically active substances such as vitamins, dietary fiber, antioxidants, and cholesterol-lowering compounds. Despite a large amount of information on this topic, the nutritional quality of plants has not been defined [1]. Historically, the value of many plant nutrients and health-promoting compounds was discovered by trial and error. By the turn of the century, the application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites [2]. Approximately 50000 metabolites have been elucidated in plants, and it is predicted that the final number will exceed 200000. Most of them have unknown function. Metabolites such as carbohydrates, organic and amino acids, inorganic elements, vitamins, hormones, flavonoids, phenolics, and glucosinolates are essential for plant growth, development, stress adaptation, and defense [3].

Linseed (*Linum usitatissimum*) is an ancient crop cultivated in temperate zones of Asia and Europe since seven thousand years [4]. Linseed in also known as Flax, Flaxseed, Flax weed, Lint bells, Toad flax, Winterlien etc., Flax is considered a functional food or source of functional ingredients, because it contains alpha-linolenic acid. The seeds also contain many nutraceuticals such as dietary fiber (28%), lignans (15 mg/g) and phenolic components, phytates etc. [5]. The potential health benefits of omega-3 fatty acids have been widely reported for several conditions including cardiovascular disease, hypertension, atherosclerosis, brain development, diabetes, cancer, arthritis, inflammatory, autoimmune and neurological disorders [6].

Within a decade, there was a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC- MS that were powerful tools for separation, identification and structural determination of phytochemicals. The aim of this paper is to determine the organic compounds present in the raw (dried), autoclaving, boiling and roasted *Linum usitatissimum* seed with the aid of Gas Chromatography and Mass spectroscopy (GC-MS) Technique, which may provide an insight in its use in tradition medicine.

MATERIALS AND METHODS

Selection of Linseed

Flax seed, also referred to as linseed, is an oilseed that is flat, oval, pointed at one end, reddish linolenic acid, and phytochemicals such as lignans. Whole flax seed (ground meal, powder or intact seed) contains 28% dietary fiber, (7 – 10% soluble fiber, 11 - 18% insoluble fiber), 40% fat (73% of it being polyunsaturated fatty acids), and 21% protein. Other Linseed nutrients include vitamins E and B, phytosterols, and mineral nutrients such as calcium, iron, and potassium. More than 50% of the fat in linseed is an essential omega-3 fatty acid called Alpha-Linolenic Acid (ALA), which makes linseed the richest plant source of dietary omega-3 fatty acids.-brown in color, smooth, shiny, and slightly larger than a sesame seed. Linseeds were obtained from the local market in Salem, Tamil Nadu, India. The seeds were cleaned by hand to remove foreign materials. The cleaned seeds were processed using autoclave, boiling and roasting methods.

Processing of linseed into powder Food processing is the set of methods and techniques used to transform raw ingredients into food or to transform food into other forms for consumption either in home or by the food processing industry. The processing techniques help to activate the nutrients present into the seeds. Removal of undesirable components is essential to improve the nutritional quality of seeds. It is widely accepted that simple and inexpensive traditional processing techniques are effective methods of achieving desirable changes in the composition of seeds. Food processing is any deliberate change in a food that occurs before it□s available for us to eat. It can be as simple as freezing or drying food to preserve nutrients and freshness or as complex as formulating a frozen meal with the right balance of nutrients and ingredients. Processed foods are more convenient, but longer, safe to eat and improve taste.

Raw (Dried) The cleaned linseeds were washed in water. The hulls were removed then the seeds dried in the sun for 6 to 8 hours. The seeds were then kept warm for 3 to

Volume : 5 | Issue : 8 | August 2015 | ISSN - 2249-555X

4 hours and powdered [7].

Autoclaving The cleaned seeds were autoclaved using vertical autoclave at 15 lb pressure (121 1C) in tap water (1:10, w/v) until 50% of the seeds become soft when felt between the fingers (35 min). afterthat, they were dried and powdered [8].

Boiling The rinsed and soaked seeds were cooked in tap water (100°C) in the ratio of 1:10 (w/v) on a hot plate until they became soft when felt between the fingers (90 min). They were dried and powdered for product development [9].

Roasted Roasting involves the application of dry heat to linseeds using a hot pan or at a temperature of 150 to 200°C for a short time. They were powdered at the end of roasting [10].

Fatty acids profile analysis by Gas Chromatography-Mass spectrometry (GC-MS)

GC-MS analysis of the fatty acid was carried out after methylation. GC-MS analysis was performed with GC Clarus 500 Perkin Elmer equipment. Compounds were separated on Elite-5MS capillary column (5% diphenyl /95% Dimethyl poly siloxane), 30× 0.25mm×0.25µm df). Oven temperature was programmed as follows: isothermal temperature at 110°C for 2 min., later increased to 200°C at the rate of 10°C/minutes and then increased up to 280°C at the rate of 5°C/min and held for 9 min. Ionization of the sample components was performed in the EI mode (70eV). The carrier gas flow rate was 1ml/min, and 3µl of sample was injected. The detector was Mass detector turbo mass gold-Perkin Elmer. The total running time for GC was 36 min. and software Turbomass 5.2 was used in this GC-MS study. The determination of the components was done by comparing their retention time with those of authentic specimens on the capillary column as well as peak enrichment.

RESULTS AND DISCUSSION

Effect of Processing on Fatty Acid Composition of Linseed Powder

The fatty acid composition of the various processed linseed powders is analyzed using GC-MS method and the results are enumerated with molecular formula, retention time, molecular weight and peak area. The identification of fatty acid compounds were based on peak area and molecular formula.

Fatty Acid Composition of Raw Linseed Powder

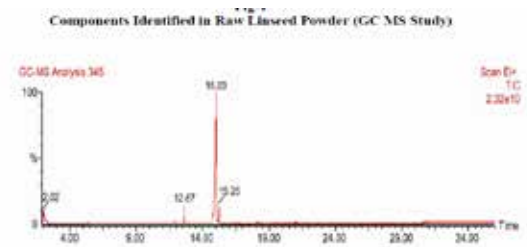
Fatty acid composition of raw linseed powder analyzed using GC-MS is shown in table-1 and fig-1.

Table 1 components identified in Raw linseed powder by G C MS

No	RT	Name of the compound	Molecular formula	MW	Peak Area%
1.	12.67	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	5.26
2.	15.03	9,12,15-Octadecatrienoic acid Methyl ester (Z,Z,Z).	$C_{19}H_{32}O_2$	292	88.38
3.	15.25	Octadecanoic acid Methyl ester	$C_{19}H_{38}O_2$	298	5.96
4.	17.53	9-Octadecatrienoic acid 12-hydrox-, Methyl ester (Z).	$C_{19}H_{36}O_3$	312	0.25
5.	17.99	Eicosanoic acid methyl ester	$C_{21}H_{42}O_2$	326	0.15

The maximum peak area represented in the raw linseeds was 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (88.38%) ($C_{19}H_{32}O_2$) with retention time of 15.03. This compound is called as Alpha-Linolenic acid. Alpha-linolenic acid is a kind of omega-3 fatty acid found in plants. Linolenic acid, an *n*-3 fatty acid is a member of the group of Essential Fatty Acids (EFAs), because they cannot be produced within the body and must be acquired through diet. Linolenic acid is the most abundant unsaturated component of several seeds and oils particularly flaxseeds and its oil. In recent years there has been considerable interest in the beneficial physiological effects of the omega-3 fatty acids [11]. Dietary α-linolenic acid has been assessed for its role in cardiovascular health. Clinical benefits have been concluded that modest dietary consumption of α-linolenic acid (2 to 3 g per day) will help in the primary and secondary prevention of coronary heart disease [12].

Fig 1 Chromatogram obtained from the GC/MS with Raw linseed powder



Fatty Acid Composition of Autoclaved Linseed Powder

Fatty acid composition of autoclaved linseed powder analyzed using GC-MS is shown in table-2 and fig-2.

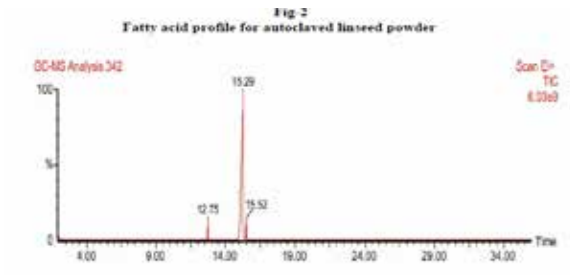
Table 2 components identified in autoclaved linseed powder by G C MS

No	RT	Name of the compound	Molecular formula	MW	Peak Area%
1.	12.75	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	2.88
2.	15.29	9,12,15-Octadecatrienoic acid Methyl ester (Z,Z,Z).	$C_{19}H_{32}O_2$	292	93.19
3.	15.52	Octadecatrienoic acid Methyl ester	$C_{19}H_{38}O_2$	298	3.85
4.	17.80	9,12,15-Octadecatrienoic acid 2,3 dihydroxy prophyl ester (Z,Z,Z)	$C_{21}H_{36}O_2$	352	0.08

The maximum peak area represented in the autoclave flaxseeds was 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (93.19%) ($C_{19}H_{32}O_2$) with retention time of 15.29. This compound is called as Alpha-Linolenic acid. Alpha-linolenic acid is a kind of omega-3 fatty acid found in plants. Linolenic acid, an *n*-3 fatty acid is a member of the group of Essential Fatty Acids (EFAs), because they cannot be produced within the body and must be acquired through diet. Linolenic acid is the most abundant unsaturated component of several seeds and oils particularly flaxseeds and its oil. In recent years there has been considerable interest in the beneficial physiological effects of the omega-3 fatty acids [11]. Dietary α-linolenic acid has been assessed for its role in cardiovascular health. Clinical benefits have been concluded that modest dietary consumption of α-linolenic acid (2 to 3 g per day) will help in the primary and secondary prevention of coronary heart disease [12].

Volume : 5 | Issue : 8 | August 2015 | ISSN - 2249-555X

Fig 2 Chromatogram obtained from the GC/MS with autoclaved linseed powder



Fatty Acid Composition of Boiled Linseed Powder

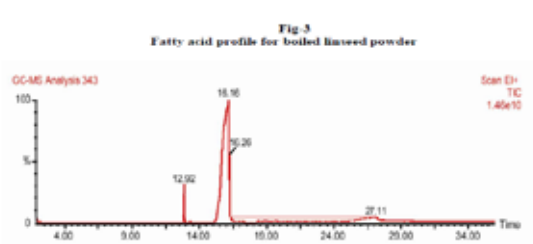
Fatty acid composition of boiled linseed powder analyzed using GC-MS is shown in table-3 and fig-3.

Table 3 components identified in boiled linseed powder by G C MS

No	RT	Name of the compound	Molecular formula	MW	Peak Area%
1.	10.46	Tetradecanoic acid methyl ester	$C_{15}H_{30}O_2$	242	3.85
2.	12.92	Hexadeconic acid methyl ester	$C_{17}H_{34}O_2$	270	88.33
3.	16.16	Linolenoic acid methyl ester	$C_{19}H_{38}O_2$	292	7.22
4.	16.26	Octadecanoic acid methyl ester	$C_{21}H_{34}O_2$	298	0.15
5.	18.42	5.8.11.14-Eicosatetraenoic acid methyl ester.(all-Z)	$C_{21}H_{40}O_2$	318	0.23
6.	18.97	11-Eicosenoic acid methyl ester	$C_{21}H_{40}O_2$	324	0.21
7.	19.70	Eicosanoic acid methyl ester	$C_{21}H_{42}O_2$	326	3.85

The maximum peak area represented in the boiled linseeds was Hexadecanoic acid, methyl ester (88.33%) ($C_{17}H_{34}O_2$) with retention time of 12.92. This compound is called as Palmitic acid. Palmitic acid is also called hexadecanoic acid and is one of the most common saturated fatty acids found in animals and plants. As its name tells us, it is found in palm oil but also in butter, cheese, milk and meat. Palmitic acid is used as an indicator of adulteration of flaxseed oil by palmolein, since flaxseed oil has a palmitic acid content of between 8 and 10%, whereas palm olein contains around 40% palmitic acid. Information regarding linoleic acids is used for the detection of flaxseed adulteration with soybean oil. Soybean oil contains about 50% linoleic acid, whereas flaxseed oil contains 15% [13].

Fig 3 Chromatogram obtained from the GC/MS with boiled linseed powder



Fatty Acid Composition of Roasted Linseed Powder

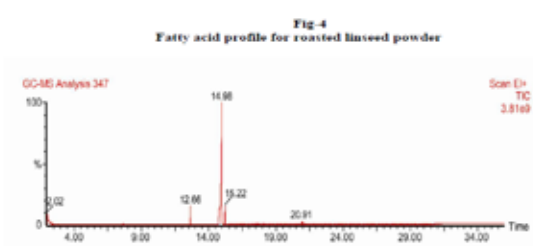
Fatty acid composition of roasted linseed powder analyzed using GC-MS is shown in table-4 and fig-4.

Table 4 components identified in roasted linseed powder by G C MS

No	RT	Name of the compound	Molecular formula	MW	Peak Area%
1.	12.66	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	5.97
2.	14.98	9,12,15-Octadecatrienoic acid Methyl ester (Z,Z,Z).	$C_{19}H_{32}O_2$	292	93.74
3.	15.22	Octadecatrienoic acid Methyl ester	$C_{19}H_{38}O_2$	298	0.07
4.	17.29	9,12,15-Octadecatrienoic acid 2,3 dihydroxy prophyl ester (Z,Z,Z)	$C_{20}H_{34}O_2$	306	0.06
5.	17.52	9-Octadecenonic acid 12-hydroxy methyl ester (Z).	$C_{19}H_{36}O_3$	312	0.16

The maximum peak area represented in the roasted flaxseeds was 9, 12, 15-Octadecatrienoic acid, methyl ester (93.74%) ($C_{19}H_{32}O_2$) with retention time of 14.98. This compound is called as Alpha-Linolenic acid. Alpha-linolenic acid is a kind of omega-3 fatty acid found in plants. Linolenic acid, an *n*-3 fatty acid is a member of the group of Essential Fatty Acids (EFAs), because they cannot be produced within the body and must be acquired through diet. Linolenic acid is the most abundant unsaturated component of several seeds and oils particularly flaxseeds and its oil. In recent years there has been considerable interest in the beneficial physiological effects of the omega-3 fatty acids [11]. Dietary α-linolenic acid has been assessed for its role in cardiovascular health. Clinical benefits have been concluded that modest dietary consumption of α-linolenic acid (2 to 3 g per day) will help in the primary and secondary prevention of coronary heart disease [12]. Among the four processed linseeds, roasted linseed showed a maximum retention time with maximum peak range ie. 93.74%. The compound found in this linseed is Alpha-Linolenic acid. α-Linolenic acid, an *n*−3 fatty acid, is a member of the group of essential fatty acids (EFAs), so called because they cannot be produced within the body and must be acquired through diet. Dietary α-linolenic acid has been assessed for its role in cardiovascular health. Based on the above results roasted linseed is chosen for incorporation in designer foods

Fig 4 Chromatogram obtained from the GC/MS with roasted linseed powder



Results of the present study concluded that the maximum peak area represented in the raw, roasted and autoclaved linseeds were 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z) - (88.38%) ($C_{19}H_{32}O_2$) with retention time of 15.03. This compound is called as Alpha-Linolenic acid. The maximum peak area represented in the boiled linseeds was Hexadecanoic acid, methyl ester (88.33%)

Volume : 5 | Issue : 8 | August 2015 | ISSN - 2249-555X

($C_{17}H_{34}O_2$) with retention time of 12.92. This compound is called as Palmitic acid.

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