



P-ISSN: 2349-8528

E-ISSN: 2321-4902

[www.chemijournal.com](http://www.chemijournal.com)

IJCS 2020; 8(3): 1767-1771

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Received: 20-03-2020

Accepted: 22-04-2020

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## Quantification of $\omega$ -fatty acids through GC-MS analysis in flaxseed (*Linum Usitatissimum L.*)

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DOI: <https://doi.org/10.22271/chemi.2020.v8.i3x.9454>

### Abstract

The current research is focused on the evaluation of  $\omega$ -unsaturated fats on dry basis in flaxseed to enhance the implementation of Polyunsaturated fatty acids (PUFA) in the prepared nourishment to get the total eating regimen for customers. These have significant result on infection anticipation like cardiovascular disorder, coronary ailment, sickness possibility, (for instance, prostate lung, and colon, chest, etc.), immune disarray, menopause, and diabetics. The extraction of flaxseed performed with (solvent ratio 1:5v/v) utilizing hexane, methanol, dichloromethane, and acetonitrile. The portrayal and evaluation of  $\omega$ -unsaturated fats was by the GC-MS interpretation with utilizing the popularity of standard fatty acid methyl ester (FAME) solution. The Linolenic acid ( $\omega$ -3), Linoleic acid ( $\omega$ -6), and Oleic acid ( $\omega$ -9) unsaturated fats amount in flaxseed concentrate was found to be 21.36  $\mu$ g/g, 5.6  $\mu$ g/g and 2.9  $\mu$ g/g (dry matter basis) respectively. This would be beneficial to the improvement of practical and nutraceutical nourishment for destitute buyers and investigate the usage of PUFA acids in food products.

**Keywords:** PUFA; GC-MS; extraction; FAME; nutraceutical

### Introduction

Flaxseed (*Linum Usitatissimum L.*) is a little dark brown coloured hard covered oilseed and its local is Mediterranean and western Asia zone <sup>[1]</sup>. It is an individual from Linaceae family and regularly known as the flaxseed or linseed. Flaxseed is one of the most seasoned oilseed crop, which has been developing since ancient times <sup>[2]</sup>. It is utilizing for fiber creation, just as human sustenance and creature nourishes. Practically all piece of the plant utilizes for various reason either legitimately or indirectly <sup>[3]</sup>. Presently individuals aware about the wellbeing advantages of flaxseed in light of the fact that before it was developed as either oil yield or fiber crop with fiber material from the stem of fiber assortments. Beyond this benefits of flaxseed its supplements segment demonstrates the potential capacity of consolidation in nourishment improvement, which are inadequate of some significant bioactive part, which are found in this seed. On the dry weight basis, flaxseed contain 20% protein, 27% total dietary fiber 41% oil, 4% ash and 8% moisture <sup>[4]</sup>. Alfa Linolenic Acid (ALA) and Linoleic acid constitutes 57% and 16.0% and mono saturate fat and saturated fat constitutes 18.0% and 9.0% respectively of total fatty acids respectively in flax making the richest source of essential fatty acids <sup>[5]</sup>. Flaxseed as seeds or seed inferred oil is considered as the useful nourishment for its uncommon dietary benefit because of its high convergence of the fiber based lignans and huge measure of polyunsaturated fatty acids <sup>[6]</sup>.

Canada is the major producer of flaxseed, which contributes about 80% of the worldwide exchange supply. It is all around utilized in the creation of paints and materials where seeds are squashed for oil extraction. It is alluded as flaxseed or linseed relying upon whether is utilizing for human utilization or for industrial purpose separately. Flaxseed is unique crop among oilseeds its oil is source of essential fatty acids <sup>[7, 8]</sup>. It is basic for human to fuse nourishment high in ALA into eating regimen since absence of required desaturation catalysts which essential for the embedding double bonds at 12, 15-carbons <sup>[9-11]</sup>. ALA is metabolically changed over into the more drawn out chain omega-3 unsaturated fat, Eicosapentaenoic acid (EPA) and Docosapentaenoic acid (DHA) <sup>[12]</sup>. It is vital to acquire adequate measures of EPA and DHA from the eating routine, since these two  $\omega$ -3 fats can somewhat diminish the master provocative impacts of the  $\omega$ -6 fats to arachidonic acid. Since flaxseed oil is recognized as one

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of the largest sources of ALA, it has also been titled as a health promoting, functional food. There are Many investigations centered around flaxseed utilization, which have indicated the expanded medical advantages to patients experiencing manifestations brought about via cardiovascular malady [13, 14], diabetes [15], and malignant growth [16]. In spite of the fact that fish oil is extraordinary source of these  $\omega$  unsaturated fat (EPA and DHA) [17], yet flaxseed can be the elective vegetarian source for the individuals who has concern with animal source. Lipids are viewed as the most considered natural supplements for people. Lipid atom produces numerous bioactive lipid particles, which are principal element between various signalling pathways and they are indispensable mixes of cell membranes. The human body cant synthesize the PUFAs with the double bonds from methyl end because lack of the appropriate enzymes [9]. Hence, these unsaturated fats are basic and must be gotten from an eating regimen. Consequently, our goal is to measurement of these unsaturated fats the  $\omega$ -3,  $\omega$ -6 and  $\omega$ -9 fully expecting getting the careful convergence of these three fundamental unsaturated fats in flaxseed extricate.

### Materials and methods

Organic Flaxseed was procured from Shreshta Natural bio product Pvt Ltd (Hyderabad, Telangana). Methanol, Hexane, Dichloromethane (DCM), Acetonitrile (ACN) for Mass Spectroscopy grade were handled from Sigma-Aldrich Chemicals, Germany. Ethanol and hypochlorite was procured from Hayman Limited, Eastways park, Witham, Essex, CMB 3YE, England.

### Sample preparation

The flaxseed was ground into powder with the assistance of mortar and pastel and extricate were removed utilizing following natural solvents methanol, hexane, DCM, ACN individually with 1:5 w/v for 6 hours at 40-45°C under magnetic stirrer (Remi-2, Remi Elektrotechnik Limited, Thane, India) condition. After which the supernatant was taken into centrifuge tube and centrifuged (R-8C, Remi Elektrotechnik) at 8000g to acquired clear supernatant bit. A clear part moved to another vial and dried by centrifugal vacuum concentrator.

### Derivatization

The extraction procedure was the equivalent up to the flaxseed oil acquired. From that point forward, we had played out the derivatization procedure to make unpredictable of the flaxseed oil segment for the best possible recognizable proof in the GC-MS instrument. Derivatization is the procedure of artificially changing a part to deliver another compound, which has properties that are a reasonable investigation with GC-MS instruments. The synthetic structure of the compound continues as before and simply alters the particular practical gathering of responding compound to the subsidiary of going amiss of concoction and physical properties to make them discernible and analysable. We have utilized derivatization reagent of Trimethylsilyl (TMS), pyridine and O-Bistrifluoroacetamide (BSTFA) reagent for derivatization of flaxseed remove (FSE) for GC-MS examination [18, 19]. The

improvement of derivatization with 20mg/ml of TMS in pyridine and we have taken 10  $\mu$ l FSE from various dissolvable extraction taken in the blend of derivatization reagents. It was heating for 90 minutes at 37°C in glass vials. After this, included 100ul of BSTFA in the vial and again fore-heating at 70°C for an hour under magnetic stirrer [20, 21]. Than after cooling the derivatized sample was promptly infused into GC-MS with the assistance of auto sampler.

### GC-MS analysis

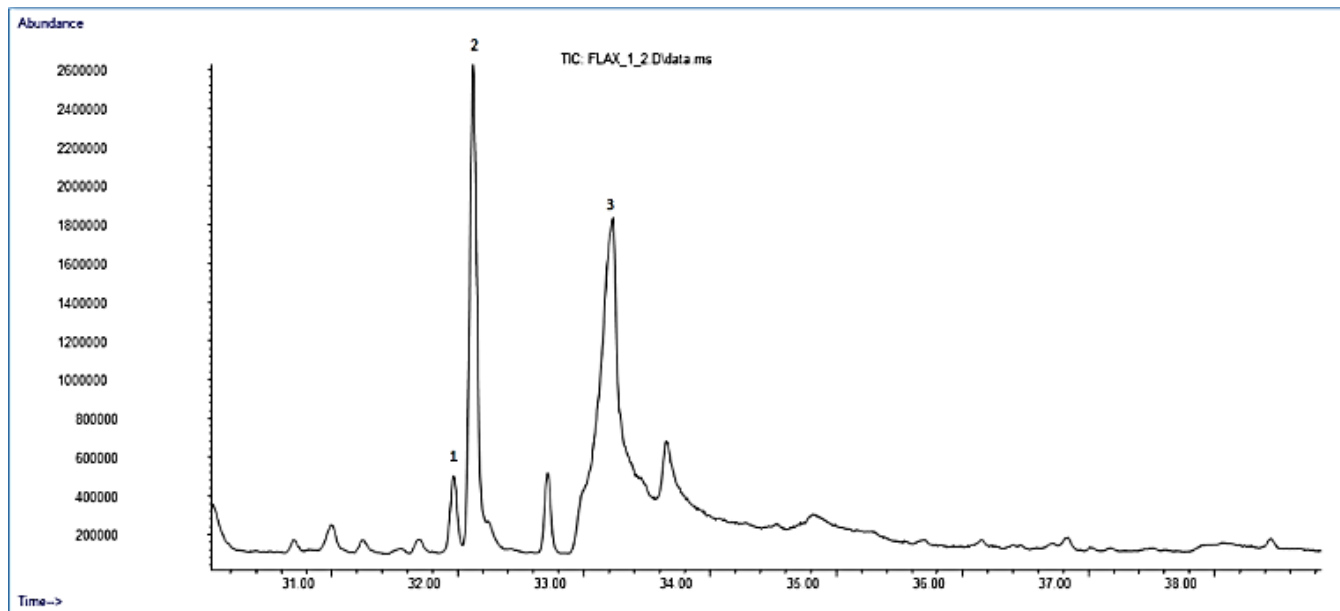
Flaxseed extracts diluted at ratio of 1:1 with their extraction solvent and injected into the GC-MS. GC-MS analysis of this extract was performed using Agilent technology 7890B gas chromatography system interfaced to a 5977A mass spectrometer (GC-MS) equipped with a HP-5ms ultra-inert column (30m x 250 $\mu$ m x 0.25 $\mu$ m) back stain less steel inlet. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.999%) was used as carrier gas at constant flow rate of 1 ml/min and an injection volume of 1 $\mu$ l was employed split less. Injector temperature 250°C. The oven temperature was programmed from 50°C (isothermal for 2 min) with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 6 min isothermal at 320°C. Mass spectra were taken at 70eV; a scan interval of 2.0 seconds and fragments from 50 to 550 Da. Total GC run time was 70 min.

### Characterization of compounds

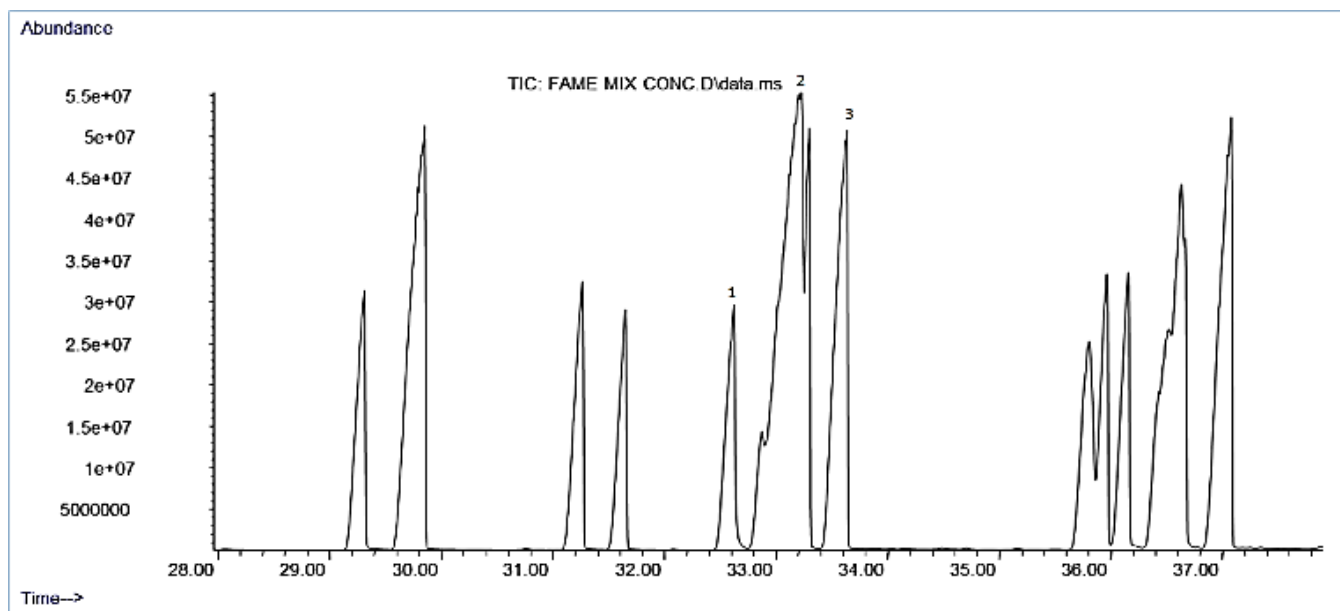
Elucidation on mass range GC-MS was directed utilizing Chemstation programming. Metabolic distinguishing proof was performed utilizing the database of national organization standard and innovation (NIST) having in excess of 62000 mixes. The range of the obscure segment was contrasted and the range realized parts put away in NIST library. The name sub-atomic weight and structure of the part of the test material were discovered. Correlation of Metabolic profiling of FSE test the two strategies (derivatized and underivatized test) [22].

### Quantification of $\omega$ -fatty acids with FAME standard solution

Flaxseed methanol concentrate test infused into various dilutions 1:2 v/v, 1:5 of v/v, 1:10 v/v (test in dissolvable proportion) and likewise FAME test infused in same dilutions ratio (FAME to dissolvable proportion). Dilution 1:2 v/v of both example (fig.1 and fig.2) flaxseed concentrate and FAME blend has the comparative maintenance time and zone standardization. The concentration and area of FAME's  $\omega$ -fatty acids recorded and compared with the extricates particulars (table.1 and table.2). The regression curve plotted for the FAME standard with targeted component in extract and calculated the concentration of each component (fig.3). These three PUFA were distinguished in flaxseed extricate by examination of maintenance times with FAMEs standard blend (SUPELCO FAMEs Mix 37 parts shifted fixation in dichloromethane, Sigma Aldrich St. Louis MO USA) and evaluated utilizing territory standardization. Pinnacle region of the concentrate for every unsaturated fat worth put into every relapse bend condition and focus was estimated distinctive unsaturated fats.



**Fig 1:** Ion chromatogram of flaxseed extract 1-Linoleic acid, 2-Linolenic Acid, 3-Oleic Acid



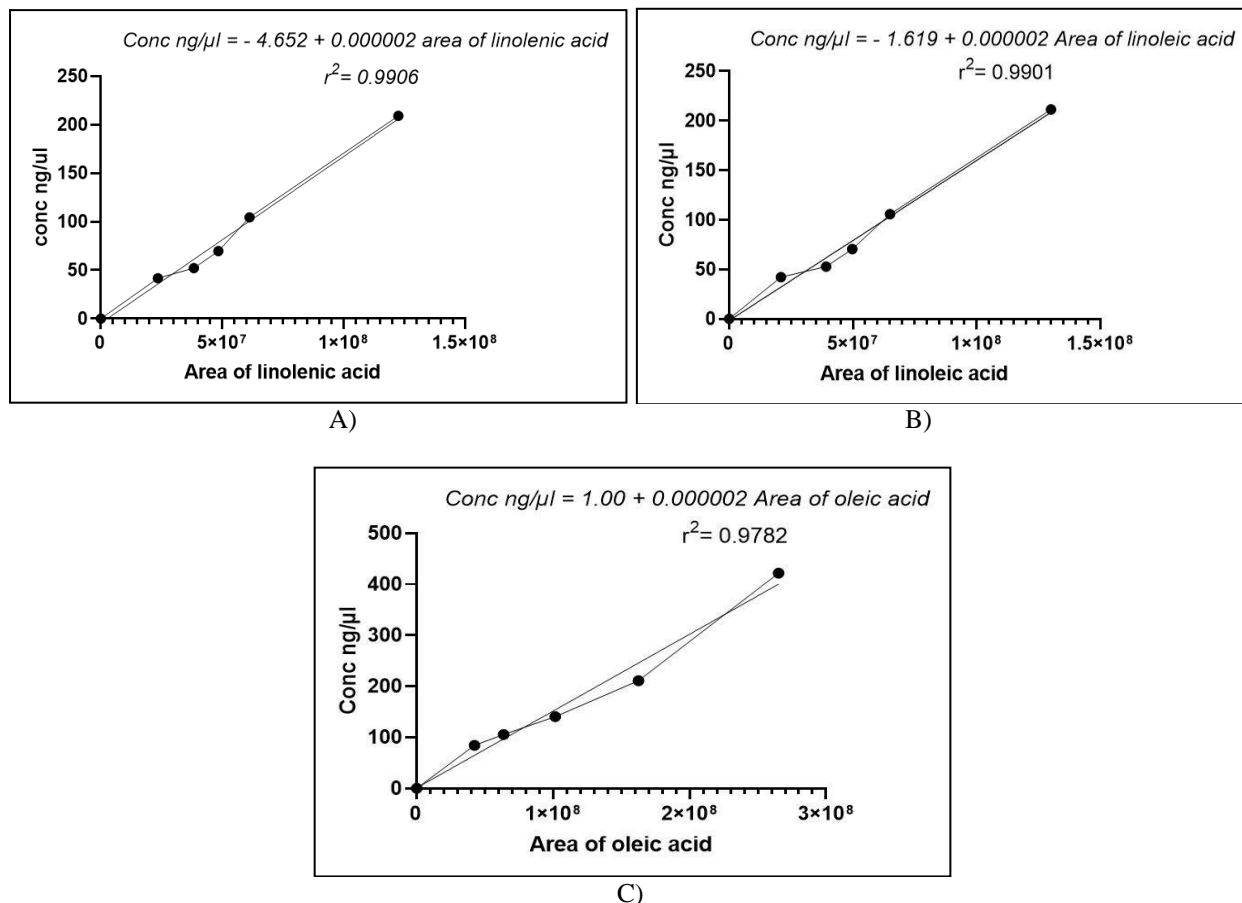
**Fig 2:** Ion chromatogram of FAME solution; 1-Linoleic acid, 2-Linolenic Acid, 3-Oleic Acid

**Table 1:** concentration and area of Linolenic acid, Linoleic acid and oleic acid in FAME standard mixture chromatogram analysis.

Dilution	Linolenic acid		Linoleic acid		Oleic acid	
	Con. ng/ul	Area	Con. ng/ul	Area	Con. ng/ul	Area
FAME 0	0	0	0	0	0	0
FAME_1_5.D	41.8	23518258.84	42.28	20978341.53	84.36	42426506.16
FAME_1_4.D	52.25	38387483.33	52.85	39181217	105.45	63817070.63
FAME_1_3.D	69.67	48458538.84	70.47	49781779.98	140.6	101543325.8
FAME_1_2.D	104.5	61308910.51	105.7	65075767.9	210.9	162647796.7
FAME concentrated	209	122617821	211.4	130151535.8	421.8	265295593.4

**Table 2:** Concentration of major PUFA Linoleic acid, Linolenic acid, and Oleic acid. The result based on the chromatogram area analysis with the standard FAME mix

FUFA	Flax Area
Linolenic acid	73533895
Linoleic acid	19372694
Oleic acid	9453316



**Fig 3:** concentration of omega fatty acid irrespective of the area of omega fatty acid in FAME mix. A) Linolenic acid B) Linoleic acid C) Oleic acid.

**Table 3:** Concentration of major PUFA Linoleic acid, Linolenic acid, and Oleic acid. The result based on the chromatogram area analysis with the standard FAME mix

PUFA	Concentration ( $\mu\text{g/g}$ of flaxseed dry weight)
Linolenic acid	21.36
Linoleic acid	5.6
Oleic acid	2.9

### Results and discussion

Flaxseed extract contains 21.36 $\mu\text{g}$ , 5.6 $\mu\text{g}$  and 2.9 $\mu\text{g}$  (dry matter basis)  $\omega$ -3,  $\omega$ -6 and  $\omega$ -9 respectively per gram of flaxseed (table.3). We have evaluated the  $\omega$ -fatty acids by contrasting the pinnacle regions of these unsaturated fats in flaxseed separate with that of standard from FAME standard blend.

The quantification of the micro compounds on dry matter basis will encourage the incorporation in the food. The extraction of the flaxseed extract with the solvent extraction for the quantification by GC-MS performed solvent extraction method along with the centrifugal dryer. Three targets in this examination concentrated on the metabolic profiling of flaxseed extricate, distinguishing proof of key unsaturated fats and the evaluation of some key unsaturated fats, which are basic fats require for the best possible eating regimen nourishment. In the present examination, the flaxseed extraction was upgraded utilizing four unique solvents viz. methanol, hexane, dichloromethane (DCM) and Acetonitrile (ACN). In which, flaxseed extricate has the simple great number of unsaturated fats significantly the unsaturated fats. Unsaturated fats parts for the most part are the  $\omega$ -3 fat known as Linolenic acid,  $\omega$ -6 unsaturated fat as Linoleic acid and  $\omega$ -9 as oleic acid, which are the fundamental poly unsaturated fat. The quantification of selected PUFA in the extract

21.36 $\mu\text{g}$ , 5.6 $\mu\text{g}$  and 2.9 $\mu\text{g}$  per gram of flaxseed  $\omega$ -3,  $\omega$ -6 and  $\omega$ -9 respectively, which supports the ALA and linoleic acid, constitutes 57% and 16.0% and mono saturate fat and saturated fat constitutes 18.0% and 9.0% respectively of total fatty acids respectively in flax [5]. The quantification of our examination uncovered the measure of this unsaturated fat (Linolenic acid, Linoleic acid, oleic acid) present in per gram of the flaxseeds. It also contains several bioactive, fibre, lignan and FUFA responsible for the potential modulate gut health. Earlier studies show that the flaxseed exhibit the beneficial outcome on colon health primarily upon anti-cancer effect. It illustrates the result of reducing effect on growth and development of colon cancer *in vitro* and *in vivo* animal models [23-25]. It is well established that fibre particularly fermentable fibre (inulin, pectin, oligofructose, and resistant starch) can reduce the colorectal cancer (CRC) risk [26, 27] soluble fibre and their fermentable effect by promoting two main contents of gut health (micro biota and mucosal layer) [28, 29]. Modulating the immune response and inflammation [30-32], even though exact compound responsible for these result is unknown, studies either purified mammalian lignans [33, 34] or FS oil [24]. Although the further studies are yet to be done in *in-vitro* and *in-vivo* beneficial effect of flaxseed nutrients on human trial viz. hyper-cholesterolemic, several prostanoids, reduces blood pressure in hypertensive and lowers triglycerides and cholesterol, anti-carcinogenic activity which some research on the animal examination results exhibit.

### Conclusion

The aim of the work is to provide information regarding the approximate concentration of the targeted  $\omega$ -fatty acids on dry matter basis, which possibly encourage the incorporation flaxseed to formulate with flaxseed in food to meet the

requirement of consumers. Since the diet has grossly imbalanced omega fatty acid ratio, it is important to incorporate foods that constituents higher in  $\omega$ -fatty acid composition. Flaxseed is the one of highest vegetarian source of  $\omega$ -fatty acids. The calculation of the incorporation of the flaxseed in the food would become easy to meet the dose of the  $\omega$ -fatty acid per day allowance. The extraction and analysis method could be varying according to the different condition. The degree of the sample homogeneity and separation of different parts of the flaxseed analysed could positively influence the accuracy of the results. Future works included to optimize the concentration of these essential fatty acids, perform and present the potential evidence report of *in vivo* analysis of flaxseed based doses and signify the beneficial effect against diseases.

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