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Role of Microwave assisted treatments in the extraction of Flax seed oil from Linum usitatissimum

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Introduction:

Flax (Linum usitatissimum), commonly names as flax or linseed, belonging to the family Linaceae. This is a nutritional food, composed of 41% fat, 28% total dietary fiber, 20% protein, 7.7% moisture, 3.5% ash, and 1% simple sugars. Flaxseed is rich in saturated and unsaturated fatty acids like α-linolenic acid, monounsaturated fatty acids and poly unsaturated fatty acids playing a major pharmacological role reducing plasma triglycerides with a potent arrhythmic activity and anti-inflammatory activity1,2. This is principal source of Omega 3fatty acids for vegetarians3.

Conventional extraction methods of this oil are pressing and solvent extraction. Pressing is observed to be less effective when compared to solvent extraction which is not economical and having a hazard of environment pollution. So the extraction can be improved by assisted techniques like ultrasound and microwave assisted with appropriate control measures4. The poly unsaturated fatty acids present in the linseed oil are studied to improve hemorheology and coagulation of atherosclerotic rats, reduce oxidative stress, improve endothelial function and inhibit atherosclerotic plaque formation.

The aim of the present study is to emphasize that although concentration of poly unsaturated fatty acids has improved when assisted by microwave relative to the conventional method, but application of microwave treatment over longer duration of time resulted in absence of unsaturated acid and accumulation of alkanes, carboxylic acids and some ketones.

The anticoagulant activity of linseed oil was also studied.

Objectives:

Materials and Methods

The linseed samples were procured from the local vender, dried and stored in refrigerator. Seeds were dried, crushed and sieved through 16 and 32 mesh sizes. The material which is retained in 32 mesh size is used for extraction of linseed oil.

Extraction methods

Organic solvent Extraction

Extraction was carried by using n-hexane, 95% purity in a Soxhlet extractor at 60–80 °C for 24h taking 30gm of ground powder. The extraction was followed by concentrating in rotary evaporator under vacuum at 40°C. All extractions were performed in triplicate.

Enhanced extraction method using microwave

30g of linseed was arranged in petri dishes and kept on the centre of turntable of microwave. Samples were subjected to a frequency of 2450 MHz for a period of 5min and 10min. Samples were cooled to room temperature, crushed in the mixer.

Estimation of linseed oil by Gas chromatographic analysis

Fatty acid analysis of the oils extracted using conventional and microwave assisted methods were performed by GC after converting to fatty acid methyl esters. The sample injected was 1.0 L. Nitrogen, having a flow of 1.0 mL/min, was used as the carrier gas and a spilt/spilt less injector was used with a split ratio of 20:1. The injector temperature was 250 0 C and the detector temperature was 2700 C. The column temperature was programmed from 100 to 1800 C at 200 C /min, and then to 2300 C at 100 C /min and held at 2300 C for 5 min. Fatty acid methyl esters were quantified based on the peak areas6

Determination of Coagulation time

Unsaturated fatty acids are found to have considerable influence on thromboxane-prostaglandin balance, thus delaying the platelet activation7. In order to understand the influence of these fatty acids, blood coagulation time was determined by Capillary tube method. The time taken for appearance of fine thread of fibrin when capillary tube is placed in a slanting direction is noted.

Results: From our statistical analysis was concluded that 95.19% 3.1 GC Analysis for the determination of mono unsaturated and poly unsaturated fatty acids

Fatty acid analysis of the oil extracted by conventional solvent extraction technique and microwave assisted technique was investigated (Table 3.1). Gas chromatographic results indicate that unsaturated fatty acids dominated over saturated fatty acids in conventionally extracted linseed oil.

It was noted that samples of linseed oil composed majority of unsaturated fatty acids while saturated fatty acids were at a lesser rate. From mass spectral analysis it was found that linoleic acid, 9,12,15-octadecatrienoic acid methyl ester are observed . Along with these some saturated fatty acids and their reduced products in the form of alcohols like 2-decy-1-ol, 13-Tetradece11-yn-1-ol was also observed. Table 3.2 reveal the composition of fatty acid extracted after microwave treatment for 5min. The results explains that the concentration of unsaturated fatty acids were found to be increased when subjected to microwave for 5min and further increase in time of 10min resulted in the accumulation of oxidative products like carboxylic acids and ketones (Table 3.3). The experimental interpretation correlates with the literature8,9,10.Thus microwave assisted extraction techniques allows the solvents to penetrate easily and increase the surface contact between hexane used here and seed components 11.

Table 3.1 GC analysis data showing Area% of fatty acid extracted by conventional solvent extraction method

Sl.no	R.Time	Area%	Name
1	17.939	0.01	10-Undecynol
2	20.689	1.74	10-Undecyn-1-ol
3	20.819	0.23	3,7-Octadien-2-one, (E)-
4	22.030	0.02	Linoleic acid methyl ester
5	21.025	0.02	alfa-Methyl linolenate
6	21.045	0.03	2-Nonynoic aicd
7	24.110	0.03	13-Tetradece11-yn-1-ol

Table 3.2 GC analysis data showing Area% of fatty acid extracted by microwave treatment of 5min

Sl.No	R.Time	Area%	Name
1.		20.689	1.77 10-Undecyn-1-ol
2.		20.819	0.33 3,7-Octadien-2-one, (E)-
3.		21.045	0.13 Linoleic acid methyl ester
4.		22.435	0.14 alfa-Methyl linolenate
5.		24.110	0.03 2-Nonynoic aicd
6.		24.496	0.13 13-Tetradece11-yn-1-ol
7.		24.731	0.05 3-Hexanone
8.		25.511	0.11 1-Iodoundecane
9.		25.795	0.02 Nitrous Oxide
10.		26.267	0.18 Decane, 6-ethyl-2-methyl-
11.		26.994	0.29 1-Iodoundecane
12.		27.703	0.30 1-Iodoundecane
13.		28.250	0.02 Propanedioic acid, oxo-, bis(1-m
14.		28.466	0.33 Decane, 6-ethyl-2-methyl-
15.		29.328	0.28 1-Iodoundecane
16.		29.921	0.09 (CH3)2CHCH2ONO
17.		30.250	0.01 2-Propenoic acid, ethenyl ester
18.		30.308	0.28 Decane, 6-ethyl-2-methyl-
19.		30.869	0.12 (CH3)2CHCH2ONO
20.		31.452	0.08 Decane, 1-iodo-

Conclusions: This work is envisaged to the study the effect of microwave treatments on the extraction of flax seed oil from flaxseed. Results revealed that there is marginally increase in the concentration of unsaturated fattyacids upon microwave treatment than conventional solvent extraction method for a treatment time of 5min.The results proclaim that microwave assisted treatments when used controlledly improve oil extraction kinetics and thus their efficiency. There was improvement of clotting time when linseed oil was added to collected blood thus demonstrating an synergistic therapy in cardiotonic treatments.

Table 3.3 GC analysis data showing Area% of fatty acid extracted by microwave treatment of 10min

Sl.no	R.time	Area%	Name
1	0.110	0.73	2-Butynoic acid
2	0.140	1.18	Argon
3	0.214	1.09	Argon
4	0.296	1.56	Nitrous Oxide
5	0.735	0.90	Argon
6	1.115	19.83	Ethane, 1-chloro-1-fluoro-
7	1.174	10.98	2-Propanol, 1-bromo-
8	1.269	3.83	Hexane
9	1.293	3.12	Ethyl Acetate
10	1.329	9.11	1-Propene, 2-methoxy-
11	1.795	0.72	Benzeneethanamine, 3,4-benzyl
12	2.859	1.00	Nitrous Oxide
13	4.225	0.95	2-Butynoic acid
14	7.650	0.85	Argon
15	28.616	1.17	anti-2-Acetoxyacetaldoxime
16	29.100	0.85	Nitrous Oxide

3.2 Determination of coagulation time

Coagulation time was determined by capillary tube method. Linseed oil extracted using microwave assisted treatment (for period of 5min) mixed to the blood sample was observed to prolong the clotting time for a period of 12min whereas conventional extracted oil produced a clotting of 10min(Table 3.4). This shows that polyunsaturated fatty acids present in the linseed oil plays a pivotal role in the clotting mechanism thus thrusting out to be one of therapeutic molecules in the treatment of atherosceloris

Table 3.4 Comparison of clotting behavior of Linseed oil extracted by the two methods

Sl.no	Coagulation time (minutes)
1	Control 8min
2	Blood sample with conventional linseed oil 11min 46sec
3	Blood sample with microwave assisted linseed oil 16min13sec.