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Research Article

Study on Proximate Composition, Phytochemical Content, Oxidative Stability, Antioxidant Activity and Fatty Acid Composition of Enzyme Aided Aqueous Extraction of Flax Seed Oil

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Abstract

The study reports on the production of flax seed oil by water, a bio renewable solvent aided by enzyme. The amount of oil extracted by this process is 35%.Total protein (20%), carbohydrate (28%) and fiber (25%) content were also measured. Analytical values such as iodine number (170 gm iodine/kg oil) and saponification value (182 mg KOH/gm oil), total phenolic content (1822 mg GAE/100 gm) and total flavonoids content (434 µg CE/mg sample) were determined. Oxidative stability test of flax seed oil was done based on acid value (.86mgKOH/gm oil), peroxide value (.96 mili euivalent/kg oil), anisidine value (3.56) and TBA Value (2.96). The antioxidant activity of water extracted flaxseed oil on the basis of determination of DPPH Free radical scavenging activity (80%), FRAP assay (10µ mol/ml), ABTS assay (40%) was evaluated. Fatty acids composition of the oil was also analysed.

Keywords: Flax Seed; Antioxidant Activity; Oxidative Stability; Fatty Acid Composition

Introduction

Both agri –food and pharmaceutical industry have shown large interest in flax seed due to their nutritional composition such as lignin, α linolenic acid and soluble flax seed gum (SFG). SFG is also called mucilage. It is mainly present in outer most layer of flax seed hull [1]. Flax seed is mainly rich in lignans example - secoisolariciresinol diglucoside (SDG) [2]. Lignans helps to protect against some types of cancer and heart disesse [3]. Flax seed contains almost 40% oil, 30% dietary fiber, 20% protein, 4% ash and 6% moisture [4]. It also contain 73% polyunsaturated fatty acid. Around 50% of total fatty acids consist of α linolenic acid (ALA), precursor of many essential fatty acids in human diet [5]. Flax seed also helps to prevent hyperglycaemia, mammary cancer, atherosclerosis and cardiovascular disease [6]. In comparison with other vegetable oils, linseed oil is distinguished by the highest content of a-linolenic acid, which recently has been found as especially important for human organism (Rudnik., *et al*. 2001). Flaxseed (*Linum usitatissimum* L.), a plant widely cultivated in Europe for fiber or oil for industrial use [7]. The most important linseed or flaxseed producing countries are Canada, Argentina, USA, China, India and Europe [8].

Objectives of the study is to extract the flax seed oil by water as a solvent along with the use of enzyme and to determine the extent of oxidative stability, antioxidant properties and fatty acids composition of the enzyme aided aqueous extracted flax seed oil.

Materials and Methods

Samples and chemicals

The flax seed was brought from local market of Kolkata (West Bengal, India). All chemicals were purchased from MERCK, INDIA.

Oil extraction and proximate composition of flaxseed

This process was carried out according to R. Sengupta., et al. [9] method. Flax seed and water were fixed in a ratio of 1:6 and the Ph of the solution was maintained at 4.5 with 1:1HCl. The mixture was then treated by cellulase enzyme (2% of flaxseed by weight).Then the solution was placed in a magnetic stirrer for 3 hours at 50 degree Celsius temperature at 300 – 600 r. p. m. The pH of the solution was kept constant throughout the process. After enzymatic reaction for a definite period, the temperature of the solution was raised to 80 degree Celsius for 5 minutes to deactivate the cellulase enzyme. After that 1.5 volumes of n-hexane based on the weight of flaxseed and then the solution was placed in a magnetic stirrer for 15 minutes to de-emulsify the oil water

emulsion. Then centrifugation of the solution was done at 7000 r. p. m. for 20 minutes. After centrifugation the liquid layer consisted of two layers. The upper layer consisted of oil in hexane and the lower layer contained dissolved carbohydrate in the syrup. The oil from the upper layer was separated by separating funnel (24 hrs) and the solvent was evaporated by rotary evaporator, then the crude oil was obtained. The solid phase consisted of an upper layer of sedimented protein and the lower level contained fiber.

Oil	35% ± 0.87
Protein	20% ± 0.56
Carbohydrate	28% ± 0.11
Fiber	25% ± 0.92

Table 1: Oil Extraction and Proximate Composition of Flaxseed.

Analytical characteristics

Saponification value, iodine value of enzyme aided aqueous extracted flax seed oil were estimated according to the AOAC official method [10].

Phytochemical content

Total phenolic content

Total Phenolic content was determined spectrophotometrically using Folin–Ciocalteu reagent by the method of McDonald., *et al.* [11]. A calibration curve of gallic acid was prepared and the results were expressed as gallic acid equivalents (mg GAE/gm). An aliquot of the extract (100 µl) was mixed with 250 µl of Folin Ciocalteu's reagent and incubated in room temperature for 5 minutes. 1.5 ml of 20% sodium bi carbonate was added to the mixture and absorbance was measured at 765 nm. against a blank which were composed of the same reagents except test extract. A calibration curve of gallic acid was prepared and the results were expressed as gallic acid equivalents (mg GAE/100 ml) and were calculated by the formula - T = (C × V)/M Where, T=Total content of phenolic compounds, milligram per gram dry weight of plant extract, in GAE; C = the concentration of Gallic Acid established from the calibration curve, milligram per milliliter; V = the volume of extract, milliliter; M = the weight of plant extract, gram.

Total flavonoid content

The total flavonoids were measured using the Aluminium chloride colorimetric method of Chang C., *et al*. [12]. The sample extract (250 µl) was added to 4.5 ml distilled water followed by 5% $NaNO_2$ (.03 ml).After incubation of 5 minutes $AlCl_3$ (.03 ml, 10%) was added at 250°C. The reaction mixture was treated with 2 ml of 1 M NaOH. The reaction mixture was then diluted to 10 ml distilled water and absorbance was measured at 510 nm against a blank which were composed of the same reagents except test extract. A calibration curve of catechin was prepared and the results were expressed as catechin equivalents (µg CE/100 ml) and were calculated by the formula: T = (C × VV)/M Where; T = total content of flavonoid compounds, mg per gram dry weight of plant extract, in Catechin equivalent, C = concentration of Quercetin established from the calibration curve in mg/ml, V = volume of extract in ml and M = weight of plant extract in gram.

Oxidative stability test

- **Acid Value (A.V.):** This analysis has been conducted according to AOCS Ca 5a-40 official method. 1 gm of oil was mixed with hot ethyl alcohol and 2-3 drops phenopthalein indicator was added to this mixture. The mixture was titrated with standard aqueous solution of alkali, shaking the solution vigorously during titration. Titration was continued till the solution turns pink [13].

- **Peroxide Value (P.V.):** This analysis has been conducted according to AOCS Cd 8-53 [14].

- **Anisidine Value:** This analysis was done according to AOCS Cd 18-90. 1 ml of .25% of p-anisidine in glacial acetic acid made up to 100 ml with iso-octane, 1 gm of oil was dissolved in the mixture and allowed to react for 10 minutes at room temperature and absorbance was measured at 350 nm [15].

- **TBA Value:** This analysis has done according to Hekmat and Mc Hamon [16]. TBA reagent is prepared by dissolving 200 mg TBA in 100 ml 1-butanol and leave it for one night and filter or centrifuge the suspension to remove the undisolved residue and makeup the filtrate to 100 ml with 1-butanol. 50-200 mg sample was taken in a volumetric flask (25 ml), dissolved in small amount of 1- butanol and makeup to volume with the same solvent. 5 ml sample solution was mixed with 5 ml reagent solution and placed the solution into a thermostat bath at 95 degree Celsius temperature. After 120 minutes remove the solution from thermostat and cooled at running water and absorbance was measured at 530 nm.

Antioxidant activity

DPPH Free radical scavenging activity assay

The oil was assessed using 1, 1-diphenyl 2-picryl hydrazyl (DPPH) radical scavenging assay according to Gorinstein., *et al.* [17] method. 01Mm solution of DPPH in methanol was prepared.

An aliquot of .2 ml of sample was added to 2.8 ml of this solution and kept in the dark place for 30 minutes. The absorbance was measured at 517 nm. The ability to scavenge the DPPH radical was calculated with the following equation.

Inhibition percentage (I%)=(A0 – A1)/A0.

(A0 =Absorbance of the control, A1 = Absorbance of the sample.)

Ferric reducing antioxidant power (FRAP)

The assay was based upon the methodology of Benzie and Strain [18]. The FRAP reagent consist of 10 mM TPTZ in 40 mM HCL, 250 mM sodium acetate buffer (pH - 3.6) and 20mM $FeCl_3$.The reagent was freshly prepared by mixing TPTZ solution, $FeCl_3$ solution and acetate buffer in a ratio of 1:1:10. An extract solution (100 µl) was mixed with 900 µl of FRAP reagent. The mixture was incubated at 37 degree Celsius for 4 minutes and the absorbance was measured at 593 nm.

ABTS Free radical scavenging activity

ABTS assay of flax seed oil was measured using the method described by Fatma Bouaziz. A solution of ABTS (7 µM) was prepared in distil water and mixed with a solution of potassium per sulphate (2.45 µM). The mixture was kept in the dark place for 16 hours at room temperature. The resulting intense colour matches the ABTS radical cations. The obtained solution was subsequently diluted with distil water and absorbance was measured at 734 nm. 1 ml of ABTS diluted solution was mixed with 10 µl of sample at different concentration and the reaction mixture was kept for 6 minutes before measuring the absorbance. ABTS scavenging activity was calculated by the following equation.

Inhibition Percentage (I %) = (1-A/AO) × 100.

(A=Absorbance of the sample. A0 = Absorbance of the ABTS solution.)

Fatty acids profiles

The fatty acids composition of enzyme aided aqueous extracted flaxseed oil was analyzed by Gas Chromatography (Agilent technologies, Model NO-7890B) after converting the fatty acids of the oil into their methyl esters according to AOCS official method Ce 2-66. Here DB-Wax capillary column (30 mL, 0.25 mm I.D., 0.25 µm F.T.) and FID (Flame Ionization detector) were used. The carrier gas was nitrogen, at a flow rate of 1 ml/minute. Injector and detector temperature was 2000C and 2400C respectively. Column temperature was maintained from 1500C to 2400C. Samples of 1 µl were injected by manually, in the split mode.

Statistical analysis

Results were expressed as mean value± standard deviation of three replications. Statistical differences were analyzed using one way ANOVA followed by post-hoc Tukey HSD (Honestly Significant Difference) at level $p \leq 0.01$.

Results and Discussion

Oil extraction and proximate composition of flaxseed

35% oil was extracted from enzyme aided aqueous extraction of flax seed. Usually flax seed contains 40% oil. Many polar and non polar solvent are used to extract oil from flax seed. Hexane provides highest amount of oil from flax seed.

Above study shown that flax seed contain 20% protein, 28% carbohydrate and 25% fiber.

Analytical characteristics

To determine the analytical charatetrisyics of the oil iodine value and saponification value were performed. table 2 show the results of analytical values.

Iodine value	170 ± 1.78 gm iodine/kg oil
Saponification value	182 ± 1.52mg KOH/gm of oil

Table 2: Analytical characteristics.

Correlation is significant at the p≤0.05 level.

Phytochemicals content

The phytochemical analysis revealed the presence of phenolics and flavonoids content shown in table 3. Phenolics, main secondary metabolite of plant origin. They have multiple biological effects including antioxidant properties. They are not only essential for plant growth but also produced as a response against injury of plant from pathogens.

Total phenolic content	1822 ± 3.08 mg GAE/100 gm)
Total flavonoid content	434 ± 2.12µg CE/mg sample

Table 3: Phytochemicals Content.

Correlation is significant at the p≤0.05 level.

Flavonoids are important in the plant kingdom for normal growth, development and give protection from infection and injury. It also contain anti cancer, anti allergic, anti inflammatory and anti microbial properties.

Oxidative stability test

Among oxidative stability test acid value, peroxide value, anisidine value and TBA test were performed.

A.V. measures the content of free fatty acids formed upon the hydrolytic degradation of lipid molecules, thus contributing the reduction of shelf life of oil. According to Codex Alimentarius Commission standard acid value up to 5 mg KOH/gm of oil is safe for consumption. Here the acid value (table 4) is bellow 5 mg KOH/gm of oil so it is safe for human consumption.

Acid value	.86 ± 0.54 mg KOH/gm of oil[a]
Peroxide value	.96 ± 0.26 mequ/kg oil[b]
Anisidine value	3.56 ± 1.12[c]
TBA value	2.96 ± 0.97[c]

Table 4: Oxidative stability test.

The data are presented as mean value ± standard deviation of triplet analyses. Different letters in the same column indicate statistically significant values (p ≤ .05).

P.V. defines the content of lipid hydroxides in oil formed under conditions of auto and photo –oxidation. Here the oil shows the very low P.V. (table 4) which does not exceed the recommended limit of P.V. of oil (10 mequ O_2/Kg oil.)

Anisidine value indicates the content of secondary products of lipid oxidation resulting from the decomposition of hydroxides. Anisidine value along with P.V. indicates the rancidity of oil. Flax seed oil show low anisidine value (table 4).

TBA value

TBA value indicates the degradation of oil. Flax seed oil shows the low TBA value (table 4) which is good for health.

Antioxidant activity

The antioxidant activity of flax seed oil samples were evaluated using DPPH, FRAP, ABTS assay and the results are shown in table 5.

DPPH	80 ± 0 .77%
FRAP	10 ± 0.06µ mol/ml
ABTS	40 ± 1.02 %

Table 5: Antioxidant activity.

Correlation is significant at the p ≤ .05 level.

Fatty acid profile

The fatty acid profile of enzyme aided aqueous extracted flax seed oil was presented in table 6. There were five fatty acids found in the profile including pamitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:0), linoleic acid (C18:2), linolenic acid (C18:3). The major saturated fatty acids found in the oil were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) with the concentration of 6.51%, 4.32% and 18.23% respectively. Unsaturated fatty acids found in the flax seed oil were linoleic acid (C18:2) and linolenic acid (C18:3) with the concentration of 19.01% and 55.23% respectively.

Palmitic acid (C16: 0)	6.51%
Stearic acid (C18:0)	4.32%
Oleic acid (C18:1)	18.23%
Linoleic acid (C18:2)	19.05%
Linolenic acid (C18 :3)	55.23%

Table 6: Fatty acids Composition of Flaxseed Oil.

Correlation is significant at the p≤.05 level.

Conclusion

Above study shows that if water is used as solvent in case oil extraction rather than other chemical solvent the oil extraction is slightly low but other nutritional components such as protein, fiber and carbohydrate contents are same. Enzyme aided aqueous extracted flax seed oil shows high antioxidant activity. This oil is also oxidatively stable at room temperature and it is safe for consumption. It is not only used in oil industry but also used in the production of medicine, paints, petroleum etc.

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Volume 3 Issue 4 April 2019