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Research Article

Studies on Screening of Phyto Chemicals of Flax Seed Oil and its Defatted Flaxseed Meal and Oxidative Stability of the Oil Extracted by Polar and Non Polar Solvent System

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Abstract

The present study evaluate the screening of phyto chemicals and oxidative stability of Flax seed (*Linum usitatisimum* L.) Oil after extraction by polar (iso propanol) and non polar (hexane) solvent yielding 35% and 40% flax seed oil respectively. Phyto chemical screening of defatted flax seed meal extracted by polar and non polar solvent was also conducted. Phyto chemicals indicated the occurance of Alkaloids, Glycosides, Saponin, Flavonoids, Phenolic compounds in the oil and defatted flax seed meal which also contained carbohydrate and protein. Result also showed that the oil and defatted flax seed meal from both polar and non polar solvent extraction did not contain Tannin. Oxidative stability test of the two kinds of solvent extracted oil was performed after one month of storage at the temperature of 7 degree celcius. Results of oxidative stability test of polar solvent extracted flax seed oil indicated the acid value 3.06 ± 0.095 mg KOH/g, peroxide value 5. 02 ± 0.07 mEq/g oil, anisidine value 4.02 ± 0.22 and TBA value 3.93 ± 0.27. While non polar solvent extracted flax seed oil showed acid value 3. 62 ± 0.14 mg KOH/g, peroxide value 4.82 ± 0.48, anisidine value 4. 25 ± 0.28 and TBA value 4. 65 ± 0.42. From the results of the present report it can be concluded that flax seed oil samples from the two solvent systems are highly oxidative stable.

Keywords: Flaxseed; Phytochemicals; Oxidative Stability

Introduction

Flaxseed are used in food industry, mainly they are used in bakery products and in feed due to the presence of nutraceutical components including α-linolenic acid (ALA), lignans and fiber [1]. Flax can be classified into two groups-fibered flax (rich in fiber) and oily flax (rich in oil). The stem of flax is used to produce the fiber of linen and the flaxseed are used for oil and feed production [2]. Flax seed contain 35-45% oil, which contains 9-10% saturated fatty acids (pamitic and stearic acid), about 20% mono unsaturated fatty acid (mainly oleic acid) and 70% poly unsaturated fatty acid (linoleic acid and linolenic acid). Protein content of flaxseed varies from 20-30%. Protein of flaxseed is limited by lysine content but their biological value (77.4%) and digestibility (89.6%) is high [3]. It also contain 30% dietary fiber, 4% ash and 6% moisture [4,5]. There has been a growing interest for the proboitic properties of flaxseed and its beneficial effects in coronary heart disease, some kinds of cancer, neurological and hormonal disorders [6]. Mainly flaxseed are produced in Canada, Argentina, U. S. A, Chaina, India and Europe [7]. Flaxseed oil is rich α-linolenic acid (55%), and omega 3 fatty acids, which is higher than any other vegetable oils [8]. α-linolenic acid can be metabolized to form eicosa pentanoic acid and docosa hexanoic acid in human intestine, which helps in the reduction of lifestyle diseases [9]. Previous studies also have shown that flaxseed oil helps in the reduction of many diseases like hyper lipidaemia, atherosclerosis [10], mammary cancer [11] and cardiovascular disease [12]. Flaxseed oil is also used in the production of linoleum, paints, ink, cosmetics, coating, vernishes [13,14]. The medicinal importance of a plant is due to the presence of some special substances like alkaloids, glycosides, resins, volatile oil, gum and tannins etc. The active principles usually remain concentrated in the storage organs of the plants [15]. Considering all these facts the present study is designed to investigate the presence of various phytochemicals in the flaxseed, a plant which evokes various therapeutic effects.

The aim of the present study is to extract the flaxseed oil by polar solvent (iso propanol) and non polar solvent (hexane) and phytochemical screening of both polar and non polar solvent extracted flaxseed oil and defatted flaxseed meal after oil extraction and to determine the oxidative stability of both polar and non polar solvent extracted flaxseed oil after storage of one month in refrigerator at 7 degree Celsius temperature.

Experimental

Materials

The flaxseed was bought from local market of Sealdah. Kolkata (West Bengal, India). All chemicals were purchased from MERK, INDIA.

Oil extraction

To obtain oil by solvent extraction method flaxseed powder was extracted with polar solvent (iso propanol) and non polar solvent (hexane) using Soxhlet apparatus (Borocil) for 5 hours at 70 degree Celsius temperature and the remaining solvent was removed by rotary evaporator (BUCHI).

Phytochemical screening

Flaxseed extract was tested for the presence of active phytochemicals such as Triterpenoids, Streroids, Glycisodes, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free amino acids, Carbohydrates, Phenolic compounds and Vitamin C [16,17]. Following standard procedures were used.

Test for steroids and triterpenoids

Liebermann Burchard test – The sample extract was mixed with few drops of acetic anhydride, after that the mixture was boiled and cooled. Then concentrated sulphuric acid was added to the test tube and the formation of a brown ring at the junction of two layers was observed. Green coloration of the upper layer and the formation of deep red color in the lower layer confirm the presence of steroids and triterpenoids respectively.

Test for glycosides

Keller Killiani Test – The sample was treated with few drops of glacial acetic acid and Ferric chloride solution. Then concentrated sulphuric acid was added to the mixture, and two layers formation was observed. Lower reddish brown layer and upper acetic acid layer which turns bluish green indicated the presence of glycosides.

Bromine water test – The sample was dissolved in bromine water and the formation of yellow precipitate confirmed presence of glycosides.

Test for saponins

Foam Test – Sample was mixed with water and shaken and the formation of froth, which is stable for 15 minutes, confirmed the presence of saponins.

Test for alkaloids

Hager's Test – Few drops of Hager's reagent (saturated picric acid solution) was added to the sample. Formation of yellow precipitate indicated the presence of alkaloids.

Test for flavonoids

The flaxseed was bought from local market of Sealdah. Kolkata (West Bengal, India). All chemicals were purchased from MERK, INDIA.

- **Ferric chloride test:** Few drops of Ferric chloride solution was added to the sample and the formation of blackish red color confirmed the presence of flavonoids.
- **Alkaline reagent Test:** Sample was treated with sodium hydroxide solution, atfrist yellow colour was appeared. Then few drops of dilute Hydrochloric acid was added and the test solution turned colourless, that indicated the presence of flavonoids.
- **Lead acetate solution Test:** Test solution when treated with few drops of lead acetate (10%) solution, formation of yellow precipitate showed the positive result.

Test for tannins

Gelatin Test – Sample solution was treated with gelatin solution, appearance of white precipitate indicated the presence of tannins.

Test for proteins

Biuret Test – The sample was treated with 10% sodium hydroxide solution and two drops of 0. 1% copper sulphate solution was added to the mixture and formation of violet or pink color confirmed the presence of protein.

Test for free amino acids

Ninhydrin Test – Sample was boiled with 0. 2% solution of Ninhydrin, the formation of purple color suggested the presence of free amino acids.

Test for carbohydrate

Benedict's test – Few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) was added to the sample and the mixture was boiled in water bath, the formation of reddish brown precipitate confirmed the presence of carbohydrate.

Test for carbohydrate

- DNPH Test – Sample was treated with Dinitrophenyl hydra zine dissolved in concentrated sulphuric acid. The formation of yellow precipitate indicated the presence of vitamin C.
- Test for Phenolic compounds
- Ferric chloride test
- The sample was dissolved in water or a mixture of water and ethanol and a few drops of dilute ferric chloride solution was added. The formation of green colour showed the presence of phenolic compounds.

Oxidative Stability test

Acid Value (A.V.)- This analysis was done according to AOCS Ca 5a-40 official method. Hot ethyl alcohol was added to 1 g of sample and then 2-3 drops of phenopthalein indicator was added to this mixture. The mixture was titrated against standard aqueous solution of alkali, shaking the solution vigorously during titration. Titration was continued till the solution turns pink from its yellowish colour.

Peroxide value (P.V.)

This analysis was done according to AOCS Cd 8-53 [18-20].

Anisidine value

This analysis has been conducted according to AOCS Cd 18-90.1 ml of. 25% of p-anisidine was prepared in glacial acetic acid and its volume made up to 100 ml with iso-octane, 1 g of oil sample was dissolved in the mixture and allowed the test reagent to react for 10 minutes at room temperature and then absorbance was measured at 350 nm using spectra photometry.

TBA value

This analysis has been conducted according to Hekmat and Mc Hamon [21]. TBA reagent was prepared by dissolving 200 mg TBA in 100 ml 1-butanol and leave it for one night and then the solution was filtered or centrifuged to remove the undisolved residue and makeup the volume of the filtrate to 100 ml with 1-butanol. 50-200 mg sample was taken in a volumetric flask, dissolved in small amount of 1- butanol and makeup to volume with the same solvent. 5 ml sample solution was added with 5 ml reagent solution and placed the solution into a thermostat bath at 950 Celsius temperature. After 120 minutes solution was cooled at running water and absorbance was measured at 530 nm using spectra photometer [22].

Results and Discussion

Oil extraction

Many polar and non polar solvents are used to extract oil from flaxseed. Here iso propanol was used as polar solvent and hexane was used as non polar solvent. 35% flaxseed oil was extracted by polar solvent (iso propanol) and 40% flaxseed oil was extracted by non polar solvent (hexane). Table 1 shows the oil yield percentage by polar and non polar solvent extraction method.

Phytochemical Screening of polar and non polar solvent extracted flaxseed oil and defatted flaxseed meal

Phytochemical screening for Flaxseed oil showed the presence of glycerides, saponins, alkaloids and flavonoids. Test for sterols, terpinoids and tannins were found to be negative.

Solvent	Oil
Polar solvent (iso propanol)	35%
Non polar solvent (hexane)	40%

Table 1: Oil content in flaxseed as extracted by polar and non polar solvents.

Phytochemicals	Polar solvent	Non polar solvent
Glycerides	Positive	Positive
Saponins	Positive	Positive
Alkaloids	Positive	Positive
Flavonoids	Positive	Positive
Sterols	Negative	Negative
Terpinoids	Negative	Negative
Phenolic Compounds	Positive	Positive
Tannins	Negative	Negative

Table 2: Phytochemical screening of polar and non polar solvent extracted flaxseed oil.

Phytochemical screening of defatted flaxseed meal contains alkaloids, phenolic compounds, carbohydrate, protein, amino acid, flavonoids and saponin as phytochemical components.

Phytochemicals	Polar solvent	Non polar solvent
Alkaloids	Positive	Positive
Phenolic Compounds	Positive	Positive
Carbohydrate	Positive	Positive
Protein	Positive	Positive
Amino acid	Positive	Positive
Flavonoids	Positive	Positive
Saponin	Positive	Positive

Table 3: Phytochemical screening of defatted flaxseed meal.

Oxidative Stability test of flaxseed oil (polar and non polar solvent extracted)

Oxidative stability test based on acid value (A.V.), peroxide value (P.V.), anisidine value (A.N.V.), TBA test was examined.

Acid value measures the amount of free fatty acids formed due to the hydrolytic degradation of lipid molecules, that is the main contributing factor for the reduction of shelf life of oil. According to Codex Alimentarius Commission standard acid value below 5 mg KOH/g of oil is safe for our consumption. Here the acid value of the samples are below 5 mg KOH/g of oil so they are safe for human consumption.

Peroxide Value helps to measure the amount of lipid hydroxides formed in oil under conditions of auto and photo –oxidation. The flaxseed oil shows very low peroxide value and which does not exceed the recommended limit of peroxide value of oil (10 milliequivalents/Kg oil).

Anisidine value measures the amount of secondary products of lipid oxidation resulting from the decomposition of hydro peroxides. Anisidine value along with peroxide value indicate the rancidity of oil.

TBA Value indicates the degradation of oil. The low TBA value of flaxseed oil is beneficial for our health.

Test	Polar solvent extracted flaxseed oil	Non polar solvent extracted flaxseed oil
Acid Value (A.V.)	3.06 ± 0.95 mg KOH/g oil	3.62 ± 0.14 mg KOH/g oil
Peroxide Value (P.V.)	5.02 ± 0.07 mEq/g oil	4.82 ± 0.48 mEq/g oil
Anisidine Value (A.N.V)	4.02 ± 0.22	4.25 ± 0. 28
T.B.A. Test	3.93 ± 0.27	4.65 ± 0. 42

Table 4: Oxidative stability test values.

Conclusion

From the present above study, it can be concluded that the oil yield percentage is more with the non polar solvent extraction system compared to the polar solvent extraction system. Phytochemical composition reveals that both polar and non polar solvent extracted flaxseed oils are same. Both polar and non polar solvent extracted flaxseed oils are oxidatively stable after storage of one month at 7 degree Celsius temperature in a refrigerator.

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