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EXTRACTION OF TOTAL POLYPHENOLS FROM OATS AND FLAX SEEDS BY SOXHLET EXTRACTION AND FIXED BED EXTRACTION COLUMN

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ABSTRACT: This paper presents the Total Polyphenol Content (TPC) extraction from the oats and flax seeds by Soxhlet extraction and fixed bed extraction column. The extraction effects of various individual and combined solvents were studied. The combined solvent ratio of (100:0,75:25,50:50,25:75,0:100) methanol: water were studied. The effects of the different parameters such as temperature, time were carried out. The total polyphenol content was evaluated by the Folin-Ciocalteu method. The combined solvents methanol and water (50:50) showed better extraction efficiency. The results conclude that the increase in temperature, there is a decrease in concentrations were observed. The content of total polyphenol present in the flaxseed was higher than oats. The extracted amount also higher in Soxhlet when compared to the fixed bed extraction method.

KEYWORDS: Total Polyphenol Content, solvent selection, Soxhlet, Fixed bed column

I. INTRODUCTION

Polyphenols naturally occur in the plant kingdom, it is the group of phytochemicals and also phytonutrients as an important antioxidant for humans[1][2][3]. These polyphenols have several classifications in it but the two broad classes are flavonoids and non-flavonoids. Many food materials contain polyphenols like cereals, oilseeds, fruits, vegetables[4], and beverages, etc. The TPC (total polyphenol) from cereals has been the interest of study and studied by several authors [5][6][7][8][9][10]. Several methods have been used for the extraction of total polyphenols (TPC), solid-liquid extraction (SLE), pressurized liquid extraction (PLE), ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE)[11], and supercritical fluid extraction (SFE)[2][12] of these methods, Soxhlet is one of the standard methods when compared to the other methods [13]. The application, advantages, and disadvantages of Soxhlet methods were already well documented [14]. Use of suitable solvent for the extraction also a crucible rule in the extraction of total polyphenol from plant materials. Various solvents were used for the extraction of polyphenols, and the results also concluded that the solvent with different polarity significantly affects the polyphenol content in plant material [15]. In many cases the polar solvents methanol, water, ethanol, ethyl acetate [16] were used, and also the use of dual solvent was performed, from which the methanol-water mixture showed better perfomance. Polyphenols were extracted from many kinds of cereals like rice [17], oats, wheat, corn, millet, maize, ragi, flaxseed, etc.

In the present study, experiments were carried out to extract TPC from oats and flaxseed with a single solvent and dual solvent, the results are presented in two parts. Firstly, the TPC from two food materials oats and flaxseeds were extracted using the Soxhlet apparatus with four different single solvents namely methanol, ethanol, isopropanol, and water. Secondly, the two combined solvent methanol and water were selected and used at different ratios (25:75, 50:50, 75:25 methanol: water) at different temperatures 30ºC, 40ºC, 50ºC in Fixed bed extraction column (it is known that above 60 ^{0}C temperature leads to the degradation of TPC). The extract phase was then concentrated in a rotary vacuum evaporator and further analyzed by the Folin-Ciocalteu method.

II. Materials and Methods

2.1 Materials

Oats, Flaxseed, are purchased from the market. The solvents methanol is purchased from Avantor Performance Materials India Ltd, ethanol (99.9%), and isopropanol (995%) were received from Sisco Research Laboratory Pvt Ltd, the distilled water was collected from Millipore unit available in our lab. All the chemicals were used as it in the received form without any purified form.

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2.2 Methods

The experiments are carried out using two types of extraction i) Soxhlet apparatus and ii) Fixed bed column extraction.

2.3 Soxhlet extraction

Both single (methanol, ethanol, isopropanol, &water) and dual solvent(methanol and water at different ratio 25:75, 50:50, 75:25) were studied by taking 15g of powdered material (oats/flaxseed) in a thimble holder and 300mL of solvents were taken for extraction in a Soxhlet extractor. Initially, the number stages were optimized after stage five the presence of (total polyphenol) TPC content was not changed, so the apparatus was run up to five stages. The extract was collected from the apparatus and then concentrated using a rotary vacuum evaporator (Heidolph). The samples were then analyzed by the Folin-Ciocalteu UV analysis method [3][18][6][19]. The results were then analyzed and compared and the presence of total polyphenol was presented in terms of gallic acid equivalent per mL of extract.

2.4. Fixed bed extraction

The arrangement of the fixed bed extraction was shown in Figure 1. The powdered oats/flaxseed were taken in a teabag filter (made up of cotton material) and packed in the entire column, the mixed solvent methanol and water at the ratio of 50:50 were taken into the solvent tank (at this ratio only we achieved the better extraction). The solvent mixture was circulated from the bottom to the top of the column at a constant flow rate. The solvent was circulated from the tank to the column again sent back to the solvent tank. The experiments were performed for different temperatures 30°C, 40°C, and 50°C. The temperature of the solvent in the solvent tank is maintained and controlled by the PID controller. At every 30 min, the sample was collected in the sample collection port at the end of the column and then analyzed using the Folin-Ciocalteu UV analysis method.

2.5. Folin- Ciocalteu method

The 10 % Folin-Ciocalteu Standard solutions were prepared using dissolving 10 mL of the reagent in 100 mL of distilled water. The stock solution of Galic acid (0.1 mg/mL) was prepared by using methanol as the rest of the solution, then it is diluted into five different concentrations.

The standard graph was shown in Figure 2. A 25 ml sample was prepared from 1 ml of the extract using a 5ml Folin-Ciocalteu solution and kept in dark room for 5 min. Then 4 mL of Na_2CO_3 (7.5 %) solution was added and the rest of the solution was methanol and then analyzed in a UV spectrophotometer. The TPC presence was given in terms of equivalent to GA gm/mL of extract.

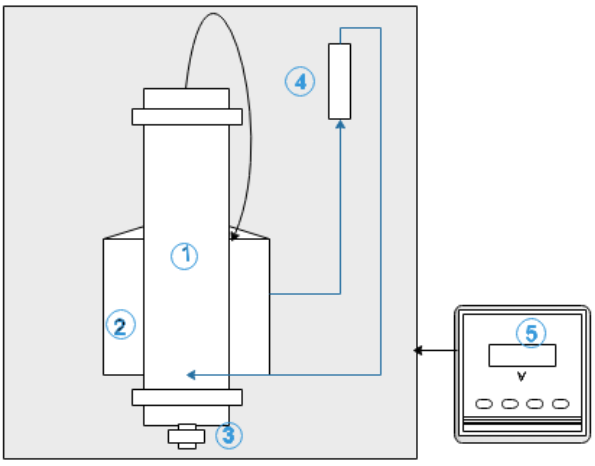

Figure 1. Schematic representation of fixed bed extraction column (1.Fixed bed column, 2.Solvent tank, 3.Sample collection port, 4.Rotameter, 5.PID controller)

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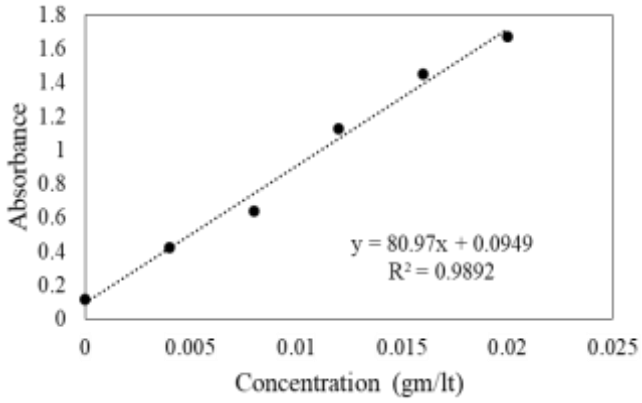

Figure2. Standard graph

III. Results and discussion

3.1. Extraction of TPC using Soxhlet for oats and flaxseed using single a solvent

The total polyphenol content of oats and flaxseed using acetone, ethanol, water, and methanol as solvents are shown in Figure 3. The highest concentration of TPC was obtained with methanol (0.025 equivalent to GA gm/mL of extract) when compared with the other solvents. While comparing the different polar solvents, methanol showed better results and this is well agreed with reported literature [20][21][22]. The flaxseed contains 0.025 equivalent to GA gm/mL of extract which is higher than the oats contained TPC amount of 0.009 equivalent to GA/mL of extract.

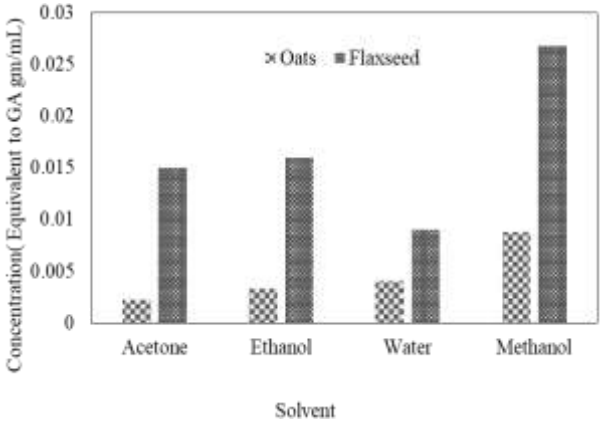

Figure 3. The total polyphenol content in oats and flaxseed with different solvents

3.2. Extraction of TPC by soxhlet for oats and flaxseed using a combined solvent

Based on the above discussion to bring better synergism, the combined solvent methanol and water at different ratios (100:0, 75:25, 50:50,25:75,0:100) were taken for further study and the results obtained from the experiments are presented in the Figure 4. The solvent ratio of 50:50 (water: methanol) showed a higher concentration extract of 0.028 equivalent to GA gm/mL of extract for flaxseed and 0.012 equivalent to GA gm/mL of extract for oats was obtained. When compared to the pure solvent (methanol and water) the TPC content in both extracts has improved in the case of combined solvent. The amount of TPC was observed to be higher in flaxseed when compared to the oats in the dual solvent. With the pure solvent (water), the TPC content in both flaxseed and oats were found to be very lower when compared to the combined solvent. The methanol to water ratio as 50:50 was fixed as an optimum ratio for combined solvent extraction, and further, the ratio was used in a fixed bed extraction column to determine the effect of temperature with respect to time.

3.3. Extraction of total polyphenol content using fixed bed extraction column

The continuous extraction of total polyphenol using flaxseed and oats were studied using a fixed bed extraction column and presented in Figure 5. The figure shows that the TPC content of flaxseed was increased with an increase in extraction time and the steady-state value attained at after 250 min, even though the

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temperature is varied. For the case of oats, the steady-state was attained at 150 min itself. For both the cases (flaxseed and oats) it is observed that the temperature had a reverse effect. For an increase in temperature, there was a decrease in the concentration of TPC, a better concentration of TPC was obtained at 30^0C. In the case of oats at 40^0C and 50^0C, the TPC concentration was decreased after 150 min. When compared to the Soxhlet extraction the amount of TPC in the fixed bed extraction column was found to be lower for both oats and flaxseed.

IV. Conclusions

The extraction of total polyphenol content in flaxseed and oats were studied using the Soxhlet apparatus and fixed bed extraction column. The better results of TPC content were obtained at the combined solvent ratio of 50:50 (vol/vol) (methanol: water). With an increase in temperature, the TPC content present in the extract was decreased. When compared to the oats the TPC content in flaxseed was higher. The combined solvent showed better results when compared to the single solvent.

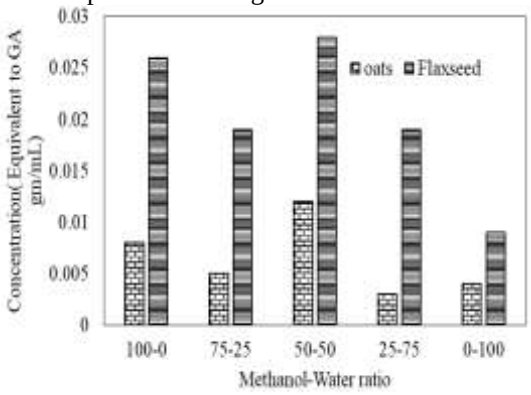

Figure 4. Effect of dual solvent (methanol and water) on total polyphenol content of oats and flaxseed

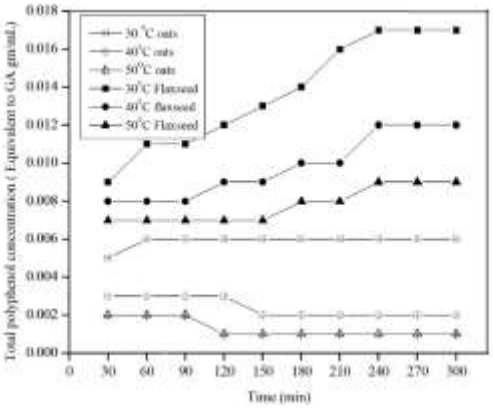

Figure 5. Effect of temperature and solvent flow rate on TPC in a fixed bed extraction column

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