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Evaluating the Nutritional Composition, Anti-oxidative, and Prebiotic Properties of de-oiled Sesame and Linseed Meals

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A B S T R A C T

Sesame and linseed are two abundantly grown oil crops around the world. Following extraction of sesame and linseed oil, the respective de-oiled meals are acquired. In this paper, we have analyzed the nutrient composition of de-oiled sesame and linseed meals. The de-oiled meals were found to contain substantial amounts of various nutrients, especially protein and crude fibre. This was followed by evaluation of their anti-oxidant and anti-nutrient. Lastly, this paper includes assessment of their prebiotic property with respect to probiotic bacterial strain— *Lactobacillus acidophilus*. Both sesame and linseed de-oiled meals were also found to be rich in both polyphenol and flavonoids. The assessment of prebiotic activity scores revealed that de-oiled sesame meal could selectively promote growth of probiotic bacteria, as it possessed a prebiotic activity score comparable to that of commercial inulin, a known prebiotic. These de-oiled meals are generally dispensed as garbage, out of which a small quantity is used to feed animals. The requirement for animal feed is rising and therefore, unconventional sources are replacing the market to fulfil this demand gap. Utilization of de-fatted sesame and linseed meals, owing to their nutritional, anti-oxidative as well as prebiotic properties could be a valuable alternative for feeding animals and fishes.

***Key words*:** Edible oil industry waste, Waste valorisation, Proximate composition, Anti-oxidative property, Prebiotic property, Anti-nutritional factors

Oil crops contribute to enormous economic benefits not only as a source of oil but also by serving as a raw material for several industries, manure, and food ingredient for animals [1]. India produces a variety of oil crops and is accounted as a major producer of oil seeds in the world. The oilseeds are predominantly used for oil extraction, which leads to the generation of enormous amounts of by-products in the form of de-oiled meals/cakes [2]. These de-oiled meals derived as a result of oil extraction are extremely valuable sources of nutrients like carbohydrates, protein, dietary fibre, and phytochemicals [3]. These de-fatted meals are generally used to feed animals and fishes while the rest is treated as waste. Due to dearth of knowledge, the utilization of de-oiled meals is undermined [4]. With the notable hike in the cost of animal feed over the past few years, simultaneous with the increasing rate of demand for animal-feed stuff, these nutrient-packed de-oiled meals are a perfect alternative for feeding animals and fish [5-6]. Utilization of by-products of the edible oil industry for feeding animals will serve as an operative technique of waste utilization and waste valorisation.[3] In this paper, we have characterized the de-oiled meals— sesame and linseed, that has been procured after extraction of sesame and linseed oil. The de-oiled sesame and linseed have been then evaluated for their anti-oxidative and prebiotic properties.

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MATERIALS AND METHODS

The de-oiled cakes— sesame and linseed were collected from Vinayak Oil and Fats Private Limited, Howrah, West Bengal India, after oil production. The chemicals and reagents used for this study were of analytical grade.

De-hulling and de-oiling of seed meals

The de-oiled meals were dehulled, ground, and the powders were collected after sieving in a Soxhlet apparatus to remove any remanent amount of oil, using food-grade hexane.

The powders obtained from dehulled and de-oiled meals were then stored for further analysis.

Proximate composition Analysis

The protein content of the de-fatted meals was estimated by the Kjeldahl method. A conversion factor of 6.25 was used to calculate the amount of protein present in the de-oiled meals. The total amount of carbohydrates in the de-oiled meals was evaluated by the method of Dubois *et al*. [7]. The content of moisture, fibre, and ash was determined by the standardized AOCS Official Method 1991. a) method no. Ba 2a-38; b) method no. Ba 6-84; and c) method no. Ba 5a-49, respectively [8].

Estimation of anti-nutritional factors

The anti-nutritional factors of the de-oiled meals, viz., phytate and tannate were estimated by Wheeler and Ferrel [9] and using Folin-Denis reagent, respectively.

Determination of total polyphenol and total flavonoid contents

The methanolic extracts of de-oiled meals were obtained by the modified method of China *et al*. [10] using 8:2 methanol: water. The extracts were collected, evaporated, lyophilized and stored to be analyzed later.

Polyphenols are secondary metabolites produced by plants with diverse structures [11]. The Total Phenolic content (TPC) of the de-oiled meals— sesame and linseed were determined spectrophotometrically [12]. The results were expressed as Gallic acid equivalents (GAE) (mg GAE/gm dried extract). The total flavonoid content (TFC) of the de-oiled meals was determined by the colorimetric method [13] with some modifications. Results were expressed as quercetin equivalents (mg QE/gm dried extract).

α-amylase inhibitory activity assay

The lyophilized methanolic extracts of the de-oiled meals— sesame and linseed, reconstituted with DMSO were used to analyze the α-amylase inhibitory activity [14. DMSO was used as the control. The following formula was used to calculate the α-amylase inhibitory activity:

$$\% \text{ Inhibition} = \frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \times 100$$

Where, $Abs_{Control}$ was the absorbance without sample, and Abs_{sample} was the absorbance of sample extract.

Prebiotic activity assay

The prebiotic activity score of the de-oiled meals was estimated by the method designed by Huebner, Wehling, and Hutkins [15]. The growth of probiotic strains, *Lactobacillus acidophilus* and *Lactobacillus plantarum*, in media containing glucose/de-oiled meals, was compared with the growth of enteric bacteria— *Escherichia coli*. MRS nutrient agar and Tryptic Soy agar were used as the selective media for the growth of probiotic microorganisms and enteric microorganisms, respectively. The following equation was used to assess the prebiotic activity score:

$$\text{Prebiotic activity score} = \frac{\text{probiotic log cfu ml}^{-1} \text{ on the prebiotic at 24 hr} - \text{probiotic log ml}^{-1} \text{ on the prebiotic at 0 hr}}{\text{probiotic log CFU ml}^{-1} \text{ on glucose at 24 hr} - \text{probiotic log CFU ml}^{-1} \text{ on the glucose at 0 hr}} - \frac{\text{enteric log CFU ml}^{-1} \text{ on the prebiotic at 24 hr} - \text{enteric log CFU ml}^{-1} \text{ on the prebiotic at 0 hr}}{\text{enteric log CFU ml}^{-1} \text{ on glucose at 24 hr} - \text{enteric log CFU ml}^{-1} \text{ on the glucose at 0 hr}}$$

The standard prebiotic, Commercial Inulin (C-Inulin), has been used as the standard.

Statistics

The statistical analysis were carried out using GraphPad Prism version 9.0.

RESULTS AND DISCUSSION

Proximate composition analysis

The protein, carbohydrate, crude fibre, ash, and moisture content (%w/w) of deoiled sesame meal has been determined as 48.03 ± 2.56, 20.99 ± 1.56, 11.25 ± 0.896, 7.5 ± 0.347, and 8.73 ± 0.332. The proximate composition of sesame meal was reported as follows— crude protein (46.14%,) crude fibre (3.5%), total ash (10.66%) [16]. The protein, carbohydrate, crude fibre, ash, and moisture content (%w/w) of deoiled linseed meal has been determined as 22.56 ± 1.61, 19.03 ± 0.58, 38.049 ± 1.05, and 6.50 ± 0.96. The proximate composition of the de-oiled meals—sesame and linseed have been presented in (Fig 1). The proximate composition of linseed meal as reported by various studies are as follows— crude protein (37.10%), total ash (9.50%), crude fibre (5.50%) [17]; crude protein (22.25%), total ash (4.70%), crude fibre (29.82%), moisture (6.88) [18]. The proximate composition of the de-oiled meals was found to be in agreement to the existing literature. The protein, carbohydrate, crude fibre, ash, and moisture content of de-oiled sesame and linseed meals are represented in (Fig 2). The protein content of sesame was significantly higher than the protein of de-oiled linseed ($p<0.05$). On the other hand, the crude dietary fibre content of de-oiled linseed the was significantly greater than that of de-oiled sesame meal.

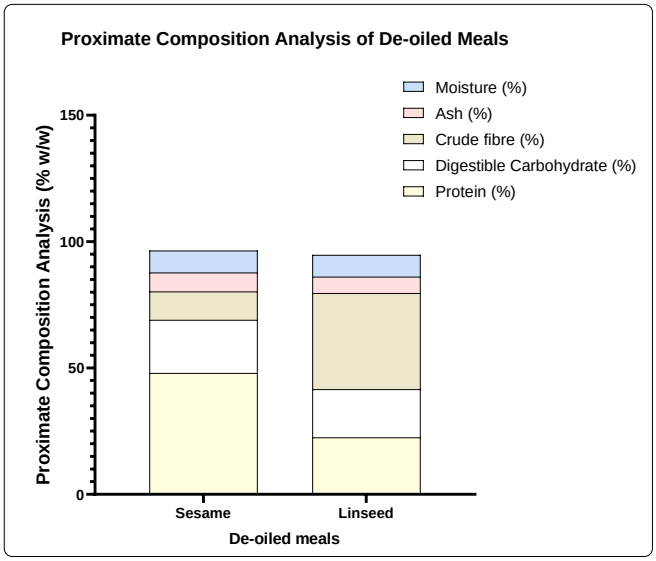

Fig 1 Proximate composition analysis of de-oiled meals

Anti-nutritional Factors

Phosphorus is predominantly stored in plants as phytates. They are considered to be anti-nutritional factors as they bind with essential minerals, reducing their absorbability [19]. Tannins are secondary metabolites that interfere with and

hinder the digestion and absorption of nutrients like amino acids, and Vit B_{12} by forming insoluble complexes [19-20].

The content of phytate and tannate of the de-oiled meals— sesame and linseed have been presented in figure 3. The means of the total phytate contents of sesame were significantly greater than linseed. The tannate content of sesame although numerically greater than that of linseed, had no significant difference ($p>0.05$). The levels of phytate in sesame seeds and linseed respectively as follows— 285 mg/100 gm and 104 mg/100 gm [21]. They also reported the levels of tannins in sesame and linseed varieties ranging between 85 to 660 mg/100 gm and 96 to 695 mg/100 gm, respectively. In one study, tannic acid was not detected in sesame and linseed meals and a very small amount of phytic acid, i.e., 23 µg/gm and 27 µg/gm of meals respectively [22]. 15 gm/kg and 7.2 gm/kg of tannins and phytic acid, respectively was reported in raw sesame meal [23].

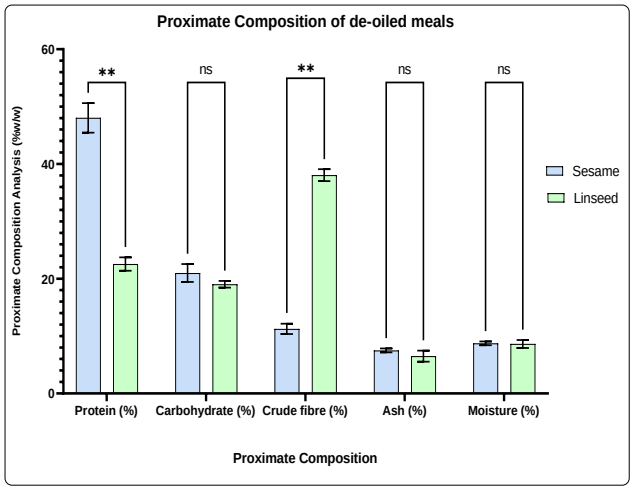

Fig 2 Protein, carbohydrate, crude fibre, ash, and moisture content of sesame and linseed, respectively. All values are expressed as mean ± standard deviation for three parallel measurements. All the * here are indicative of statistical differences between the nutrient contents of different de-oiled meals. The statistical significance was calculated using one-way ANOVA. One * indicate significant difference at significance level of 0.05, whereas two or more ** indicate significant difference at significance level of 0.01. The superscript ns denotes not significant

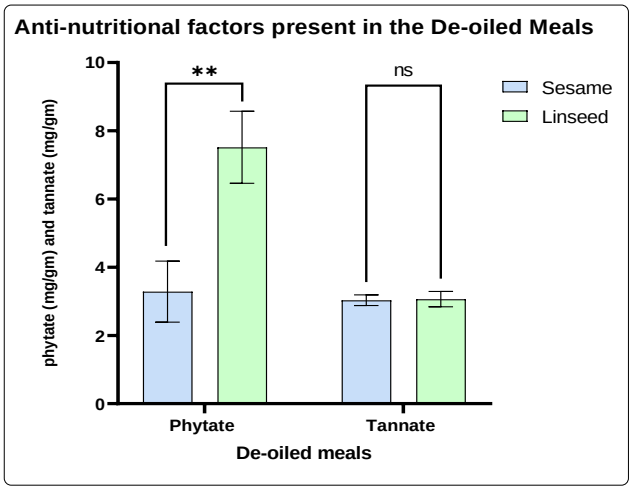

Fig 3 Phytate and tannate content of de-oiled meals of de-oiled sesame and linseed meals. All values are expressed as mean ± standard deviation for three parallel measurements. The statistical significance was calculated using one-way ANOVA. All the * here are indicative of statistical differences between the respective variables of different de-oiled meals, respectively. One * indicate significant difference at significance level of 0.05, whereas two ** indicate significant difference at significance level of 0.01. The superscript ns denotes not significant

Total polyphenol and flavonoid content

The means of the total polyphenolic contents of the de-oiled meals— sesame and linseed had no significant difference ($p=0.171$). The means of the total flavonoid contents of sesame was significantly greater than that of linseed ($p<0.05$). The TPC and TFC of the de-oiled meals were graphically represented in the (Fig 4). The polyphenol and flavonoid content of the de-oiled meals displayed a strong positive co-relationship as indicated by the Pearson's Correlation Coefficient that was recorded as r= 0.991 (p= 0.008). Our findings were similar to the values reported previously. The TPC of de-oiled flaxseed meal was reported as 162-362 mg GAE /100 gm [24]; 1.20 mg GAE/gm [25]; 844.30 mg GAE/ 100 gm [26]. The TFC of flaxseed obtained from previous literature are 9.78 mg Lutein equivalents /100 gm [26]; and 12.24-19.98 Rutin equivalents /100 gm [24].

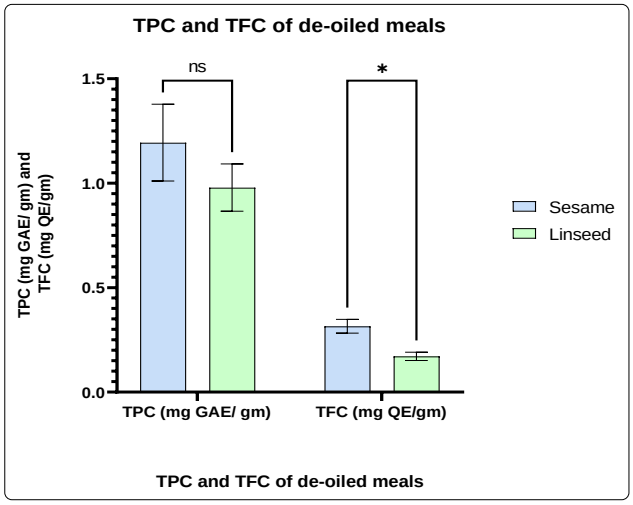

Fig 4 Total polyphenolic and flavonoid content of de-oiled meals. All values are expressed as mean ± standard deviation for three parallel measurements. The statistical significance was calculated using one-way ANOVA. All the * here are indicative of statistical differences between the respective variables of different de-oiled meals, respectively. One * indicate significant difference at significance level of 0.05, whereas two ** indicate significant difference at significance level of 0.01. The superscript ns denotes not significant

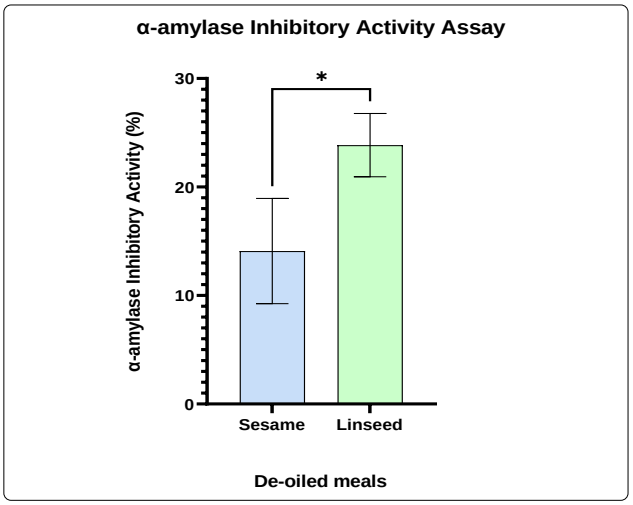

Fig 5 α-amylase inhibitory activity of de-oiled meals. All values are expressed as mean ± standard deviation for three parallel measurements. The statistical significance was calculated using one-way ANOVA. All the * here are indicative of statistical differences between the α-amylase Inhibitory Activity of different de-oiled meals, respectively. One * indicate significant difference at significance level of 0.05, whereas two ** indicate significant difference at significance level of 0.01. The superscript ns denotes not significant

α-amylase inhibitory activity assay

The mean percentage α-amylase Inhibitory Activity of the de-oiled sesame and linseed meals were 1.09% and 23.85%, respectively, and is represented in figure. The difference between them is significant ($p<0.05$). We found strong correlation between the TPC and amylase inhibitory activities of the de-oiled meals ($r= 0.965$, $p= 0.034$). The mean percentage α-amylase Inhibitory Activity of the de-oiled sesame and linseed meals are represented in (Fig 5).

Prebiotic activity score

Prebiotic activity score helps in calculating the capability of a substance to selectively promote the growth of a probiotic organism [15]. The growth of *L. acidophilus* and *E. coli* at 0 and 24 hours in sesame, linseed, and C-inulin, have been represented in Figure 6 and 7. The means of the prebiotic activity scores of sesame, linseed and C-Inulin were recorded as— 0.91, 0.28, and 0.77. The prebiotic activity score sesame de-oiled meals showed no significant difference from the values of commercial inulin. The prebiotic activity scores have been represented in (Fig 8).

CONCLUSION

The de-oiled meals sesame and linseed were abundant in nutrients like protein, carbohydrates, and crude fibre. Consumption of these de-oiled meals will help to suffice the nutritional requirements of animals and fishes. They also have ample amount of anti-oxidants like polyphenols and flavonoids. Sesame had a very good prebiotic activity score which was comparable to commercial inulin. Intake of sesame will therefore promote desirable changes in the gut microbiota. The anti-nutritional constituents present in the de-oiled meals might pose hazard but their ill-effects can be overcome by application of various techniques.

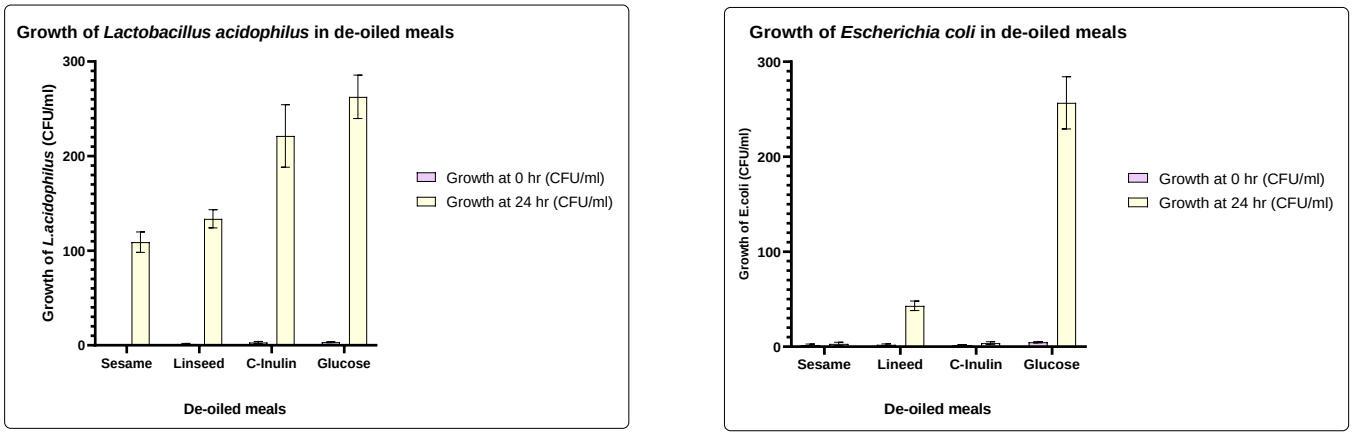

Fig 6-7 Growth of *Lactobacillus acidophilus* and *E. coli* at 0 hr and 24 hr, respectively in de-oiled sesame meal, de-oiled linseed meals, control (glucose), and commercial inulin. All values are expressed as mean ± standard deviation for three parallel measurements. The statistical significance was calculated using one-way ANOVA. All the * here are indicative of statistical differences between the growth of *L. acidophilus* at 0 hr and 24 hr, respectively. One * indicate significant difference at significance level of 0.05, whereas two ** indicate significant difference at significance level of 0.01. The superscript ns denotes not significant

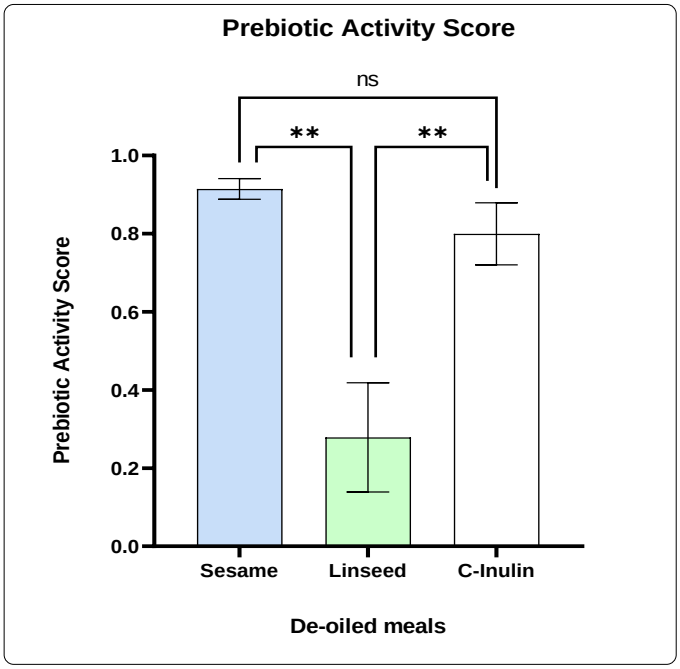

Fig 8 Prebiotic activity score of de-oiled sesame meal, de-oiled linseed meal, and commercial inulin with *L. acidophilus*. All values are expressed as mean ± standard deviation for three parallel measurements. The statistical significance was calculated using one-way ANOVA. All the * here are indicative of statistical differences between the prebiotic activity score of various de-oiled meals. One * indicate significant difference at significance level of 0.05, whereas two ** indicate significant difference at significance level of 0.01. The superscript ns denotes not significant

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