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Comparative analysis of various processing on total phenolic content and antioxidant activity of flaxseed

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Abstract
Flaxseed, a minor oilseed possesses various biologically important components which makes it a potential food source. Along with its high nutritional quality, it has high antioxidant properties which protect human from various diseases. This study focused on analyzing and comparing the effect of different processes viz. soaking, roasting, mating, two-phase solvent extraction method, dehulling on the nutritional composition and biochemical properties (total phenolic content and antioxidant activity). Positive Pearson correlation was observed between crude protein & crude fiber (0.472) and negative correlation of crude fat with crude protein (0.56) & crude fiber (0.91). The TPC ranged from 354.82 to 748.3 GAE mg/100g while antioxidant activity ranged from 19.61% to 49.51%. Salt soaking methods slightly affected the TPC and AA while T4 significantly increased the both TPC and AA due to germination step. However, dehulling process exposed the flaxseed to damages from light and heat, thus significant reduction was observed.

Keywords: Flaxseed, malting, dehulling, nutritional composition, total phenolic content, antioxidant activity

Introduction
Flaxseed has emerged as a potential functional food recently, containing high amount of proteins, PUFA (alpha linolenic acid), lignans and fibers. It is a member of family Linaceae of genus *Linum* belonging to *usitatissimum* species. Its plant bears blue colored flowers and seed color varies from dark brown to yellow because of the differences in amount of pigment present in seed coat in different species [1]. Canada is leading producer and exporter of flaxseed in the world while India ranks third in the production. Proximate composition of brown Canadian flaxseed showed moisture 7.7%, fat 41%, protein 21%, ash 3.4% and dietary fiber 28% [2]. Variation in nutritional composition may be due to environmental, genetical, processing of seed and analytical procedures [3]. Plants phenolic compounds and dietary fibers also contribute to the nutritional properties of flaxseed [4]. Polyphenols, lignans, tocopherols, phenolic acids, flavanoids possesses anti-cancerous activity and also reducing risks of various health related diseases like diabetes, heart attack, artherosclerosis and cancer [5]. Polyphenols have direct correlation with the antioxidant capacity of flaxseeds [6]. Raw flaxseed should not be consumed because it has toxic components which in high doses can be dangerous. However, these can be reduced with the application of different processing pre-treatments. Various processing has been employed to decreased toxic components especially cyanogenic components while maintaining its nutritional properties becomes equally important. Germination is considered a very good option to enrich the flaxseed with nutrients and also it decreases the amount of non-nutrients [7]. Roasting of flaxseeds not only reduce the cyanogenic compounds but also enhances the taste of flaxseeds. The presence of seed coat containing mucilage affects the availability of proteins and oils present in the embryo [8]. Therefore, the process of malting which is a combination of soaking, germination and kilning (roasting) can be beneficial for enhancing its overall composition.
As seed coat of flaxseeds is hard it is not digested by the human gut and its mucilage leads to less absorption of nutrients. Polysaccharides present in the seed coat swells in an aqueous medium causing obstruction in protein separation. So, there should be a process which can separate seed coat.

Dehulling is a process in which the outer mucilaginous layer of seed coat is removed so that the availability of nutrients is increased. Dehulled flaxseed is rich in fat and protein content and less of carbohydrates which can be a potential ingredient in food. Removal of hull concentrated the oil and protein present in flaxseed [9]. Mucilage present in flaxseed may also hinder in the functional properties of dough for making bakery products and may decrease its acceptability. Hence, with demand of high-quality protein nutrition in the present era, use of dehulled flaxseeds can be considered beneficial.
The present study focused on the effect of different processing treatments and its comparison on the nutrient composition, total phenolic content and the antioxidant activity of flaxseed.

Materials and Methods

Sample preparation and chemicals

Flaxseeds (Linum usitatissimum) were purchased from local market of Hisar. The seeds were cleaned and stored in airtight containers at room temperature. Chemicals used were Hexane (Thermofisher), anhydrous Na_2CO_3 (HPLC Grade), methanol, ethanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma), Standard Tannic acid (Sigma), Standard Gallic Acid (Sigma), Folin- Ciocaltaeu Reagent, conc. H_2SO_4 (Sigma-Aldrich). The study was carried out in the Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar India.

Treatments on Flaxseeds

Samples were prepared with whole cleaned flaxseeds. After performing each process, the seeds were ground in mixer and stored in separate plastic sealable bags for further use. Unprocessed ground seeds were taken as control.

Soaking in 0.10 N Salt Solution: Soaking was done according to the previous method described with some changes [8]. Seeds were soaked in 0.10 N NaCl solution at a ratio of 1:10 (w/v) of seed-to-solvent ratio for 6 hours in a container at room temperature (RT), followed by removal of excess water using a mesh and then washed 5 times with water (1:10, w/v). Then the seeds were dried at 45°C in oven for 6-12 hours or till complete drying.

Soaking In 0.15 N Salt Solution: It was done according to the previous method described [8] with some modifications as mentioned above using 0.15 N NaCl.

Roasting: Roasting was done according to the earlier method [10]. Fifty (50 g) of flaxseeds were taken and placed on petri dishes in a single layer and roasted in conventional oven at 180°C for 10 mins. Then the seeds were allowed to cool at room temperature for 30 min.

Malting: Malting was done in three steps as steeping/soaking, germination and kilning/ roasting as described in literature [11] with some modifications. Soaking was done in water for 6 hours followed by germination in germination chamber at temperature 25˚C and relative humidity (RH) of 85˚C for a period of 3 days or till the acrospire (shoot of germinated seed) length was approximately 6.5-8cm. After that roasting of germinated seeds was done at a temperature of 60-62˚C for 2-3 hrs or until seeds dried completely. Then the sprouts were rubbed between hands to separate seeds from acrospire, followed by sieving to obtain seeds without acrospire.

Two Phase Solvent Extraction: The process was done according to the previous method described [12] with some modifications. Sixty (60 g) of flaxseeds were first crushed in a grinder, then it was first blended with 400ml of 95% (v/v) Methanol with addition of 10% dissolved ammonia at 10,000 rpm for 2 min which was then followed by 15 min of rest period. After that 400ml of Hexane was added to the mixture and blended for 2 min at 10,000 rpm. The treated meal was vacuum filtrated on Whatmann No. 41 filter paper, and washed three times with methanol 125 ml total volume and dried overnight at 40°C in an oven.

Dehulling: Desired amount of flaxseeds were taken from previously cleaned flaxseed batch. They were first pretreated with ethanol according to the method described in literature [13]. These pre-treated seeds were then dehulled using tangential abrasive dehulling device (TADD) to remove hull of the flaxseed at maximum level as previously described [14]. The mixture was then cleaned for kernels or embryo by hand shortening/ manually.

Proximate analysis

Chemical analysis of the flaxseed done according to the official AOAC (2005) [15] methods for ash content, protein content using micro-kjeldahl method and using conversion factor of 6.25, crude fat, and crude fibers [16].

Color analysis

Color of control and different processed ground flaxseed were analyzed by using Konica Minolta Chroma Meter CR-400 in the Department of Food Technology, GJUS&T, Hisar. The readings were taken in L*, a*, b* color space where L* shows brightness, a* is redness and b* is yellowness. The color measurements were taken in triplicates and Chroma and hue angle were calculated using standard formula.

Solvent Extraction of Ground Flaxseed

Control and processed ground flaxseed were analyzed for TPC and antioxidant activity using different methods after extraction with methanol. According to Anwar and Przybylski [5] pure methanol was found to be most efficient solvent for extracting phytochemical compounds like polyphenols, flavonoids and lignans. Twenty grams (20g) of sample was extracted for 1 hr at room temperature with 300 ml of methanol. Centrifuged the solvent-meal mixture at 1500 rpm for 15 min and collected the supernatant for further use.

Total Phenolic Content (TPC)

Total Phenolic Content estimation was done using the folin-ciocalteu reagent [17]. Briefly, in 1 ml of sample extract 0.5 ml of FC reagent was added then incubation was done at room temperature for 5 min and 5 ml of 20% anhydrous sodium carbonate was added. Solution was kept undisturbed for 30 min and absorbance was recorded at 750 nm in UV-VIS Spectrophotometer (Thermo Scientific). The results were expressed in gallic acid equivalent (GAE)/g of fruit on the dry weight basis.

Antioxidant activity by DPPH free radical scavenging activity

The antioxidant activity as Free Radical Scavenging activity using DPPH in flaxseed samples were analyzed according to the method described by Barthet *et al.*, [18] with minor modifications. One ml of sample extract was mixed with 4 ml of 0.1 mM DPPH (2, 2 diphenyl-1-picrylhydrazyl) solution.

The mixture was kept in dark for 45 min at room temperature for reaction. The absorbance of mixture was recorded at 517 nm and scavenging activity against DPPH radical.

Statistical Analysis

The data generated from experiments were represented as mean±SD when appropriate. Analysis of data was performed by one-way ANOVA using IBM SPSS software. Significant differences among the mean values were determined by duncan's multiple range comparison at 5% level of significance.

Results and Discussion

Effect on Crude fat

As per observation unprocessed flax seed contained 35.73 percent of crude fat content and a significant difference ($p<0.05$) decrease in fat content was observed in salt concentration treatment. It was found that treatment T1 has lower concentration of salt resulted in higher fat content than the treatment T2 salt concentration and similar pattern was noticed in Indian *Brassica* species salt treatment [19]. T3 has shown non-significant effect on fat content w.r.t control, i.e. fat content was unaffected by roasting at 180 ºC temperature and remained same during this process. T4 treatment include germination process and as per study shown by Ogbonna *et al.*, [20], fat content in Sorghum grist during the germination fat content was increased. Therefore, in flaxseeds combination of treatments such as steeping and germination enhance the release of fat content by lossening the outer hard cover of seed which increases availability of fat from seeds. So it observed that after T4 treatment fat content in flaxseed was increased as 39.46 percent which was significant differ ($p<0.05$) from control [21]. T5 treatment possess solvents which reduced the fat content due to solubility of fat in organic solvents. Solvent extracted most of fat from seeds thus resulted in seed flour with low fat content. Therefore, T5 treatment decreased (9.57%) fat content in flax seed and observed the lowest fat percentage in flaxseed flour during T5 treatment while using organic solvent hexane [12]. T6 treatment flour contained maximum fat content because it was a dehulled process. During the dehulling outer hard cover of seed was removed so that availablity of fat was tremendously increased. As per observations, the study showed that dehulled flour contained upto 49.84 percent of fat that was significantly higher than control flour. A study of Figueiredo *et al.* [22], showed that dehulled sunflower seed has higher fat content as compared to un-dehulled sunflower seeds.

Effect on Protein

Flaxseeds are rich source of proteins (majorly globulins and albumins) and amino acids such as aspartic acid, glutamic acid and arginine are abandoned whereas the limiting amino acids are cystine, lysine and methionine [1]. During salt soaking methods i.e. T1 and T2, a significant increase in protein content was observed, this happened due to the synthesis of nitrogen components and sugars that protect the plants structure and maintain its osmotic balance under salt stress conditions [23] or might be due to decrease in viscosity after mucilage removal that increased protein availabiltiy [8]. Similarly, according to Qados [24], increase in protein concentration was observed during the salt soaking of *Vicia fava (L.).* T3 treatment showed non-significant difference w.r.t. control, it might be because protein content was un-affected due to less roasting duration. Treatment 4 showed great reduction in protein content of flaxseed flour because of hydrolysis of proteins during germination process. A study of Ogbonna *et al.*, [20] on sorghum grist reported that developing embryo during germination period has utilised amino acids hydrolized from total protein content and also the kilning process might have cause degradation of some proteins. Therefore, malting treatment caused decrease in crude protein content of flaxseed. In T5 treatment flaxseed flour has highest protein content possibly due to the extraction of polar matter in alkanol medium and removal of fat. The flaxseed flour in T6 showed an increase in level of total protein due to removal of hard coat of seed which enhanced the availability of protein in flaxseed flour [9]. Similar observation was reported in red lentils by Wang [25].

The crude fiber of control flaxseed was in range (4.43%) with the findings of Amin and Thakur [26], however slight variation might be due to regional, genetical, environmental differences in flaxseeds. As per this study, an increment in crude fibre in case of soaking in 0.1 N and 0.15 N salt concentrations was observed but it was contradictory with the observations reported in mustard seeds by Singh *et al.*, [19]. The crude fibers of T3 flour was increased, that closed to the pattern as observed in chia seeds [27] and similar study on sorghum grist was reported with increment in fiber [20]. A study of Martín-Cabrejas *et al.*, [28], observed increase in total dietary fiber (TDF) after the germination in pea because of increase in both the soluble and insoluble dietary fiber fractions. Therefore, crude fiber was increased in T4 (8.21%) with significant difference of $p \leq 0.05$. However, it was reduced considerably in dehulled (T6) flour because of removal of seed coat which contains most of the fibrous compounds.

Table 1: Nutritional Analysis of Ground Flaxseed Flour after Various Treatments*

Samples	Crude Fat (%)	Protein (%)	Crude fibers (%)
Control	35.73±0.30^{d}	19.18±1.55^{b}	4.43±0.60^{b}
Treatment (T1)	29.6 ±1.70^{b}	21.16±0.66^{c}	17.5±2.29^{e}
Treatment (T2)	26.93±0.40^{c}	21.55±0.62^{c}	12.7±2.75^{d}
Treatment (T 3)	35.86±2.95^{d}	20.77±0.65^{bc}	9.5±1^{cd}
Treatment (T 4)	39.46±0.35^{a}	15.76±1.75^{a}	8.21±2.29^{c}
Treatment (T5)	9.57±1.07^{e}	31.28±0.22^{e}	22.17±3.25^{f}
Treatment (T6)	49.84±1.23^{f}	25.15±0.22^{d}	0.73±0.25^{a}

*Values are mean ±SD of three triplicates. Different superscript letters (a, b, c, d, e, f) within the same column indicates significant differences ($p \leq 0.05$) among the treatments.

(Whereas, T1 – soaking in 0.10N salt concentration, T2 – soaking in 0.15 N salt concentration, T3 – Roasting, T4 – Malting, T5 – Two-phase solvent extraction, T6 – Dehulling)

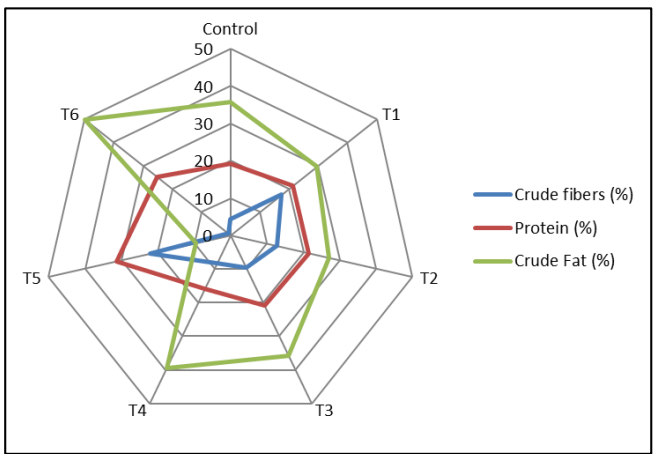

Fig 1: Effect of various processing on protein, crude fat and crude fibers (values are mean of three triplicates)

Color Analysis

Since flaxseed can be used for fortification or enhancement of various bakery products, therefore its color is an important parameter for product acceptance by consumers. The L*, a* and b* values were recorded and Chroma and Hue angles were calculated (Table 2). The color of ground flaxseeds with control and other treated samples were mainly in range of yellow-red and red color on The Munshell Hue Circle.

Lower L* values in treatment T3 and T4 showed less brightness or dark color of flaxseed flour which might be due to the roasting process. Roasting causes degradation and oxidation of carotenoids which caused the reduction of a* and b* values. Whereas, T6 flaxseed flour was more saturated among all flour due to the removal of dark brown colored hull of flaxseed, thus observed brightness in flour [29]. The increase in the brightness of dehulled flaxseed flour was in line with the findings of Wang [25] in red lentils.

Table 2: Color measurement of Control and Treated Flaxseeds*

Samples	L* (brightness)	a* (red/green)	b* (yellow/blue)	Chroma (C*)	Hue angle (h) (˚)
Control	45.25	9.30	9.24	13.10	44.71
T1	45.29	8.69	7.97	11.79	42.52
T2	44.72	8.22	7.73	11.28	43.22
T3	35.62	9.59	6.87	11.79	35.60
T4	31.24	8.95	5.30	10.40	30.62
T5	48.15	5.78	6.30	8.54	47.43
T6	47.68	7.16	16.31	18.81	66.29

* Values are mean of three determinants. T1 – soaking in 0.10N salt concentration, T2 – soaking in 0.15 N salt concentration, T3 – Roasting, T4 – Malting, T5 – Two-phase solvent extraction, T6 – Dehulling

Fig 2: Color gradation among different processed ground flaxseed (Left to Right: Control, T1 T2, T3, T4, T5, and T6); T1 – soaking in 0.10N salt concentration, T2 – soaking in 0.15 N salt concentration, T3 – Roasting, T4 – Malting, T5 – Two-phase solvent extraction, T6 – Dehulling

Effect on Total Phenolic Content

As per the observations in the present study, soaking in salt solutions (T1 and T2) caused the increase in the phenolic content of flax seed i.e. 499.18 and 520.23 mg GAE/100g respectively. As reported by Falcinelli *et al.*, [30] the sprouts and wheatgrass observed increment in phenolic content after salt process due to salinity stress that help in triggering the formation of phenolic compounds. Whereas, the reduction in TPC was observed in T3 due to the thermal degradation that causes changes in its molecular structure [31]. According to Carciochi *et al.*, [11] observations malting treatment caused the reduction of the TPC in quinoa seeds due the degradation of phenolic compounds during kilning process at high temperatures. The significant increase in TPC was observed in treatment 4 (748.3 GAE mg/100g). The changes of phenols content during the malting are shown in table 3. Malting process contain germination and roasting steps however, more than 2-fold increases of phenolic content were found in germination step, this was caused by elevation in metabolic activities and formation of phenolic compounds. While in roasting step degradation in TPC was observed because of thermal effect on phenolic compounds which slightly brings down the phenolic content. Similar pattern was noticed in *Chenopodium album* during malting process [32]. Treatment 5 caused the reduction in TPC of flaxseed, which might be due to the use of aqueous methanol and hexane in combination with ammonia that extracted most of the phenolic components due to the polar nature of solvent as reported in Wanasundara and Shahidi [12] in flaxseed and Felhi *et al.*, [33] in seeds of *Ecballium elaterium*. There was significant decrease of phenolic content of flaxseed flour during treatment 6 and correlate negatively with untreated flaxseed flour as shown in table 3. The major reason might be that phenolic compounds mainly Secoisolariciresinol Diglucoside (SDG) found in hull of flaxseed and during dehulling process the hull is removed, therefore reduction of phenolic content in flaxseed flour were found [6].

Table 3: Free Radical Scavenging activity as DPPH and total phenolic content

Samples	Total phenolic content (as GAE mg/100g)	Antioxidant activity (%)
Control	480.15	33.72
T1	499.18	34.14
T2	520.23	35.24
T3	432.23	31.95
T4	748.31	49.51
T5	442.55	27.99
T6	354.82	19.61

T1 – soaking in 0.10N salt concentration, T2 – soaking in 0.15 N salt concentration, T3 – Roasting, T4 – Malting, T5 – Two-phase solvent extraction, T6 – Dehulling

Effect on Antioxidant Activity

The antioxidant activity of flaxseed is positively correlated with total phenolic content. As discussed above, there was an increase in the accumulation of TPC in T1 and T2 due to salt stress, which was responsible for the increase in its antioxidant activity because of their free radical scavenging activity. Similar pattern was reported in *Schizonepeta tenuifolia* [34]. There was significant reduction in antioxidant activity of flaxseed flour during treatment 3 because of high temperature of roasting that degrade endogenous antioxidants [35]. The antioxidant activity in T4 and T5 were in line with the trend of TPC as antioxidant activity was attributed due to

the release of phenolic compounds responsible for scavenging activity. Germination step caused the activation and release of bound phenolic components leading to high antioxidant activity in T4 while the solvent extraction removed majority of those components thus leading to its reduction.

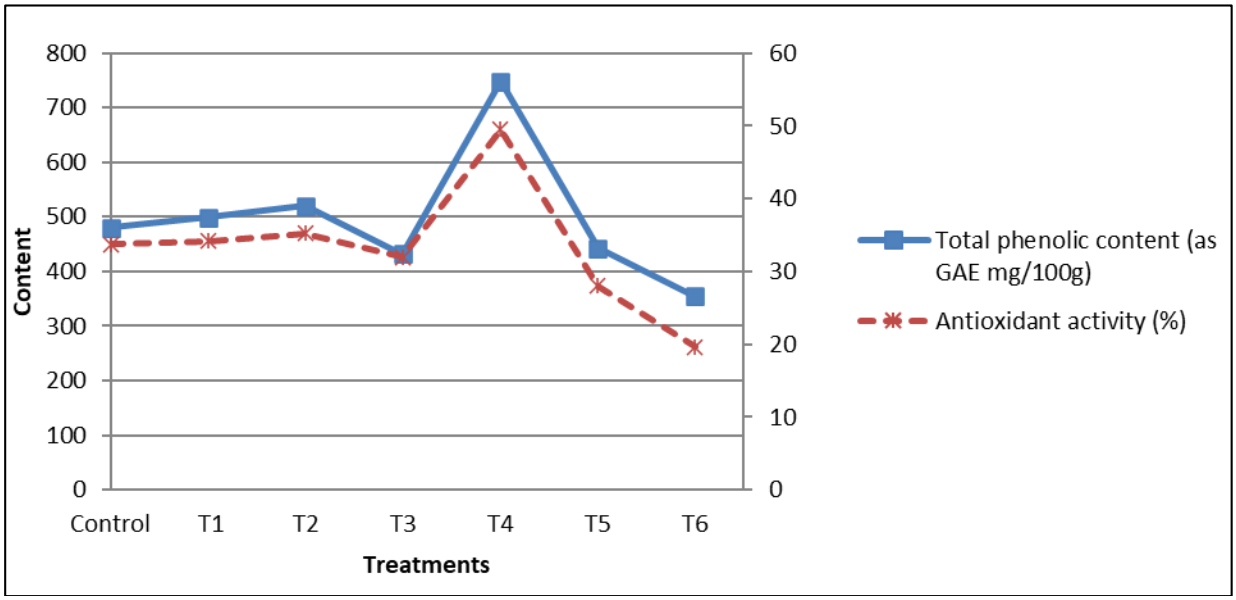

Fig 3: Variation in TPC and Antioxidant Activity of ground flaxseed extracts after various processing indicating direct correlation between them. T1 – soaking in 0.10N salt concentration, T2 – soaking in 0.15 N salt concentration, T3 – Roasting, T4 – Malting, T5 – Two-phase solvent extraction, T6 – Dehulling

The dehulling of flaxseed caused the exposure of endosperm to light, air and moisture therefore causing its oxidative rancidity and production of free radicals. Removal of hull fraction containing SDG, main phenolic acid of flaxseed, was responsible for the reduction in its DPPH free radical scavenging activity [6]. In a study conducted on flaxseed hull oil, reported that it exhibits higher antioxidant activity [36].

Table 4: Correlation Analysis between Nutritional Properties of Treated Flaxseed Flour

	CFT	CP	CF	AA	TPC	L*	a*	b*	Hue
CFT	1								
CP	-0.5691	1							
CF	-0.9172	0.47215	1						
AA	0.01344	-0.6606	0.07525	1					
TPC	-0.0286	-0.7332	0.10994	0.96721	1				
L*	-0.3138	0.71096	0.19293	-0.7105	-0.7602	1			
a*	0.48961	-0.8979	-0.4096	0.36239	0.52789	-0.6224	1		
b*	0.60736	0.23019	-0.6349	-0.6359	-0.7433	0.52028	-0.2349	1	
Hue	0.23419	0.58716	-0.3116	-0.7446	-0.8746	0.77309	-0.5856	0.91077	1

CRT- crude fat, CP- Crude Protein, CF- Crude Fibers, AA- Antioxidant activity, TPC- Total Pheniolic Content, L*- brightness, a*- redness, b*- yellowness

***** Pearson correlation coefficient

Conclusion

The study showed that processing such as salt soaking, roasting, malting, two-phase solvent extraction method and dehulling affect the nutritional composition of flaxseed. Dehulling caused a significant increase in both crude fat and crude protein, however great reduction in crude fiber, TPC and antioxidant activity was observed due to removal of hull. The crude fat content significantly correlated positively with crude protein during T6 but crude fiber content, phenolic content correlate negatively with them.

The crude protein correlates positively with the crude fibers while TPC and antioxidant activity correlates negatively with crude fat during two-phase solvent extraction process (T5).

Malting process (T4) increased the crude fat as well as crude fibers while reduction observed in crude protein that signifies the positive correlation between crude fiber and crude fat however, in phenolic content and antioxidant activity correlated negatively with the protein content due the growth of acrospire. Roasting caused non-significantly affects both crude fat and protein but crude fiber correlates negatively with TPC and anti-oxidant activity during T3 process. Soaking in salt solution has increased the phenolic content thus the DPPH scavenging activity as they are positively correlated.

Color of a product is used as an important parameter for its acceptance or appealing and therefore it can be useful for bakery industries. Further, an interesting phenomenon was observed that the brightness of flours showed negative correlation with the crude fat however yellowness of the flour showed strong positive correlation with it as represent in Table 4. Crude protein was positively correlated with brightness of the flour whereas it was strongly negative correlated with redness of the flour. These observations suggest that crude fat and protein can be considered as indicators of color of the flaxseed after various treatments.

The color analysis showed that dehulled flaxseed flour has bright color with most saturation due to better protein and fat content whereas solvent extracted flour was dull colored as large amount of fat was removed during processing. This can be considered for making bakery products with high nutritional content and desirable their color of the product.

The processing method can be employed in food industry for producing product of interest. Malting treatment showed beneficial effect on the flaxseed enhancing its nutritional aspects along with its anti-oxidative property. This process can be exploited in production of malted beverages or bakery products with high nutritional value. However, dehulled treatment showed poor antioxidant activity but quality proteins and fats are easily available to the body. Thus, keeping the results in mind, future researches can be done to enhance or prevent degradation of antioxidant activity of dehulled flaxseed which can form the basis for future researches.

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