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ORIGINAL ARTICLE

# Studies on the nutraceuticals composition of wheat derived oils wheat bran oil and wheat germ oil

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**Abstract** Fat-soluble nutraceuticals of cereals are known for number of disease preventive activities. Hence wheat bran oil (WBO) and wheat germ oil (WGO) were extracted from wheat bran and germ which yielded 3.35 % and 7.35 % of oil, containing polyunsaturated fatty acids (PUFA) (64 %, 61.2 %) respectively. Both oils contained tocopherols and carotenoids, which were higher in wheat germ oil (273 mg/100 g, 12.23 mg/100 g) than wheat bran oil (190 mg/100 g, 2.21 mg/100 g). Steryl ferulates were also present in both the oils, but their content was eight-fold higher in WBO than in WGO. Three major steryl ferulates identified by HPLC were campesteryl ferulate and sitostenyl ferulate, campestanyl ferulate and $\beta$-sitosteryl ferulate as in $\gamma$-oryzanol and another ferulate, viz., sitostanyl ferulate. A strong $IC_{50}$ value of 7.5 mg/mL and 21.6 mg/mL DPPH free radicals scavenging for wheat germ oil for wheat bran oil was observed. NMR ($^{13}C$ and $^{1}H$) profile explored the evidence of distribution of antioxidant molecules in the unsaponifiable matter of wheat derived oil. Since oils rich in PUFA and minor components are required for the normal physiological activities, blending such oils with other edible oils of the diet in wheat growing countries like India may be useful to provide health benefits.

**Keywords** Wheat bran oil · Wheat germ oil · Nutraceuticals · Total tocols · Steryl ferulates

## Introduction

Growing population in India has increased the demand for edible oils which is a food component. India is the world's 3rd largest edible oil consumer after China and Europe, consuming over 12–13 million tons every year, of which 50 % is met through imports. Palm oil (mainly imported) and soybean oil account for almost half of total edible oil consumption in India, followed by mustard and groundnut oils. Vegetable oils are consumed for food processing, in houses, industries restaurants and catering institutions. Indian consumer's preference for vegetable oil vary from region to region based on local cultivations Eg; northern and eastern region is accustomed to mustard and rape, west and south to groundnut and coconut. Vegetable oil consumption in India is continuously increasing, according to National council of Applied Economic Research (NCAER) that stands at 16.7 kg/head/year at present (2010). Hence, our aim was to explore new sources of vegetable oil to overcome imports and meet the edible oil demand in India and also to address the importance of its composition.

India is the second largest producer of wheat (79 million tons per annum) in the world which forms the second major staple food. Bran is the outer coat of the grain (15 %) and reproductive part of the plant is called germ (2 %) which constitutes 17 % of the total weight of grain and are produced as byproducts of wheat milling industry. The germ is removed from the endosperm during milling because of its unfavorable baking properties and susceptibility to oxidation. Hence it is used as fodder or for oil production (Appett 1986). These by-products contain valuable fat-soluble substances such as tocopherols, sterols, carotenoids, steryl ferulates and others having biological effects (Wilson et al. 2007; Agudo et al. 1999; Gray et al. 2010). At present these are going as waste and their application in food industry need to be explored.

Both wheat bran (WB) and wheat germ (WG) contain oil to the extent of 3–4 % and 7–9 % respectively. It is reported that removal of lipids and lipid-soluble components from WB increased colon tumorigenesis, while fortification of de-fatted bran diet with bran oil significantly inhibited, suggesting that the bioactive compounds present in the WB oil possess inhibitory properties against colon carcinogenesis (Reddy et al. 2000). An oil rich in unsaturated fatty acids and good amount of minor components is expected to have number of beneficial health effects (Hu et al. 1998). Some of the nutraceuticals of minor components in the oil are unique such as steryl ferulates (known as oryzanol in rice bran oil) which have number of

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biological activities viz., reducing serum cholesterol levels (Wilson et al. 2007), inhibiting tumoroigenesis (Yasukawa et al. 1998), anti-diabetic properties (Myoung et al. 2011) and others. Some of the nutrients like carotenoids (β-carotene, pro vitamin A), tocopherols (vitamin E), cannot be synthesized de novo and hence have to be supplemented exogenously through foods. Studies have shown that carotenoid rich fruits and vegetables reduce the risk of cardiovascular diseases (Agudo et al. 1999). Lutein and zeaxanthin are the carotenoids, especially abundant in retina (macula) which can prevent development of macular degeneration at the older age (Olmedilla et al. 2001). Tocopherols exhibit vitamin E activity and also other biological activities such as, anti-diabetic property by improving insulin sensitivity (Gray et al. 2010) and anti-cancerous activity (Yang et al. 2011). Extensive studies have been carried out on tocopherols in wheat germ oil but not much work has been done on carotenoids and oryzanol in wheat germ oil (WGO) and wheat bran oil (WBO).

With this background the present investigation was carried out to evaluate the nutraceutical composition of minor components in oil extracted from wheat bran and wheat germ and the results are presented in this paper.

## Materials and methods

Fresh wheat bran was procured from the ISMT Mill situated at our institute, Dept. of FMBCT, CFTRI and M/S Shakthi Agro Industries, Mysore, supplied wheat germ. Folin-Ciocalteau reagent from Sisco Research Laboratory Pvt. Ltd, Mumbai. India. Gallic acid (97.5–102.5 %), ferulic acid (99 %), α-tocopherols (>96 %), β-carotene (>93 %) and lutein (99 %) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA) and Oryzanol (98.5 %) was procured from Tsuno, Japan. Other chemicals and solvents used were of analytical grade.

*Moisture and fat content* Wheat bran and wheat germ were powdered and material passing through 100 mesh sieve were collected and used for determination of moisture content by the method of Firestone (1998) Method No Ca 2c-25 and oil content by using soxhlet extraction apparatus for 8 h using hexane as the solvent. Oil quality was assessed by determination of free fatty acid value (FFA) (Ca 5a-40), peroxide value (PV) (Cd 8–53) and unsaponifiable matter (Ca 6a-40), using the standard methods of Firestone (1998).

*Fatty acid composition* Methyl esters of the fatty acids were prepared using boron trifluoride/methanol according to Firestone (1998) Method No. Ce 1–62, and were analyzed using a gas liquid chromatograph (GC-15A, Shimadzu Corp. Koyto, Japan) equipped with a data processor (model CR-4A, Shimadzu Corp., Japan) a flame ionization detector (FID) and stainless steel column, 3 m length×3.3 mm i.d. packed with 15 % diethylene glycol succinate coated on chromosorb WAW 60–80 mesh. The GC was operated under the following conditions: nitrogen flow 40 mL/min; hydrogen flow 40 mL/min; air flow 300 mL/min; column temperature 180 °C; injector temperature 200 °C and FID temperature 220 °C. The fatty acids were identified by comparing the retention time of sample with those of reference fatty acid methyl esters.

### Analysis of minor components in wheat bran oil and wheat germ oil

*Carotenoids analysis* β-Carotene and lutein were determined through RP-HPLC analysis by injecting known amount of oil in acetone using LC-6A, Shimadzu instrument equipped with a Photodiode array detector (PDA) and fitted with Bondapak, $C_{18}$ column (25 cm×4.6 mm, 5 μm i.d.), detector set at 450 nm; mobile phase acetonitrile/methanol/dichloromethane (6:2:2 v/v) with 0.1 % ammonium acetate; flow rate 1 mL/min β-Carotene with Limit of Detection (LOD) 2–10 μg and lutein with 0.2–0.5 μg were used as reference standards for identification and quantification.

*Tocopherol and tocotrienol composition* Total tocopherols and tocotrienols (Total tocols) content and composition were determined by HPLC (model LC-10 AVP Shimadzu Corp., Tokyo, Japan) coupled with silica column ( 250 mm×4.6 mm, 5 μm i.d.) and flourescence detector (FLD). Following conditions were used: $\lambda_{Ex}$290 nm, $\lambda_{Em}$330 nm; mobile phase hexane: isopropanol (99.5 : 0.5 v/v); flow rate 1 mL/min and α-tocopherol with LOD 2–10 ng was used as standard for identification and quantification.

Total tocols content was spectrophotometrically determined in the unsaponifiable matter of the oil by the method of Anon (1959) and the values were expressed as mg/100 g oil.

*Oryzanol analysis* Total γ–oryzanol like compounds were determined in oil samples either spectrophotometrically by absorbance measurement of hexane solution of oil at 314 nm or by using HPLC Shimadzu LC 10A system coupled with Shimadzu $C_{18}$ column (150 mm×4.6 mm, 5 μm i.d.) and Shimadzu SPD-M10 AVP UV detector. Following conditions were used: Wavelength (λ) 325 nm; mobile phase acetonitrile: methanol: isopropyl alcohol (10:9:1 v/v/v); flow rate 1 mL/min and using γ-oryzanol as standard with LOD 20–100 μg for steryl ferulate quantification (Gopala Krishna et al. 2001).

### Antioxidant assay

*Free radical scavenging effect* The stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effect of oil was carried out as described by Ramadan et al. (2006). The oil samples (5–80 mg) were mixed with 900 μM DPPH in

toluene and the final concentration was made to 750 μM. The mixture was incubated for 20 min. at room temperature and the absorbance was read at 515 nm along with sample blank. Gallic acid, ferulic acid, and α-tocopherol were used for the comparative study, the inhibitory percentage of DPPH was calculated according to the following equation:

$$I\% = \left(A_{blank} - A_{sample} \Big/ A_{blank}\right) \times 100$$

*Nuclear magnetic resonance (NMR) spectroscopy* Samples for NMR spectroscopy were prepared by dissolving 20 mg of oil in $CDCl_3$ for the measurement of $^1H$ and $^{13}C$ spectra respectively. All NMR experiments were performed on a Bruker 500 MHz spectrometer at 25 °C using a TXI probe. Proton chemical shifts are given relative to residual $CDCl_3$ signal (7.26 ppm), whereas, carbon chemical shifts are given relative to the residual $CDCl_3$ (77.16 ppm).

*Statistical analysis* All experiments were carried out in triplicates ($n$=3) and the results were expressed as mean ± standard deviation (S.D.). The significance of difference was calculated by using Student's $t$-test, and values<0.05 were considered to be significant.

## Results and discussion

*Physico-chemical properties of wheat bran oil and wheat germ oil* Fresh wheat bran and wheat germ were analyzed for the moisture and fat content (Table 1). Moisture content (8.45 %) was found to be more in wheat germ (1.7 fold) than in wheat bran (4.8 %) and also the oil content (7.45 %) was 2.2 fold higher in wheat germ than in wheat bran (3.35 %). It is known that the cereal germs contain more oil than bran as reported by Yongzhi and Wang 2005. Furthermore, oil quality was assessed by estimating peroxide value (PV) and free fatty acid value (FFA) followed by unsaponifiable matters which are given in Table 1. FFA in wheat germ oil (WGO) was high at 11.0 %, whereas wheat bran oil (WBO) had a lower FFA content of 7.9 %. PV was also found to be slightly higher in WGO (12.3 meq$O_2$/kg) than in wheat bran oil (9.9 meq$O_2$/kg) while unsaponifiable matter was almost similar in both WBO (2.8 %) and WGO (3.0 %).

The fatty acid composition of WBO and WGO is given in Table 1. Both the oils were found to be rich in polyunsaturated fatty acids and low in saturated fatty acids that are comparable with earlier reports (Kwon et al. 2010; Go-Woon et al. 2010). Presence of high amount of linoleic acid (18:2) was observed in both WBO (54.0 %) and WGO (58.6 %) and hence are good sources of ω-6 fatty acids along with small amount of ω-3 fatty acids. ω-6 and ω-3 fatty acids are required for the

**Table 1** Characteristics of wheat bran and wheat germ and the oils extracted from them

| Parameters studied | Wheat bran | Wheat germ |
|---|---|---|
| Moisture (%) | 4.81±0.02 | 8.41±0.11 |
| Fat (% wet basis) | 3.35±0.14 | 7.45±0.22 |
| | Wheat bran oil | Wheat germ oil |
| PV(meq$O_2$kg$^{-1}$) | 9.91±0.55 | 12.33±0.20 |
| FFA (% as oleic acid) | 7.90±0.47 | 11.01±0.75 |
| Unsap (%) | 2.80±0.47 | 03.00±0.04 |
| Fatty acid compositions (%) | | |
| 16:0 | 18.9 | 20.3 |
| 18:0 | 0.4 | 0.6 |
| 18:1 | 16.4 | 17.6 |
| 18:2 | 58.6 | 54.0 |
| 18:3 | 5.5 | 7.2 |

Values are mean ± Standard Error Mean of three experimental results ($n$=3)

*PV* peroxide value

*FFA* Free fatty acids value

*Unsap* Unsaponifible matter

**Table 2** Nutraceuticals composition of wheat bran oil and wheat germ oil

| Nutraceuticals | Wheat bran oil | Wheat germ oil |
|---|---|---|
| Tocopherols | | |
| T.Tocols (mg/100 g wrt α-T.) by Spec. | 190±16 | 273±90 |
| T.Tocols (mg/100 g wrt α-T.)by HPLC | 202±25 | 288±40 |
| α T (%) | 18.63 | 162.24 |
| α T3 (%) | 8.17 | – |
| γ T (%) | 12.92 | 91.26 |
| γ T3 (%) | 161.24 | 34.32 |
| Carotenoids | | |
| Total carotenoids (mg/100 g) | 2.22 | 12.23 |
| Lutein (mg/100 g wrt lutein) | 0.39 | 2.95 |
| β - carotene (mg/100 g wrt β-carotene) | 0.22 | 2.42 |
| Other caroteonids (mg/100 g wrt β-carotene) | 1.60 | 6.89 |
| Steryl ferulates | | |
| T. Steryl ferulates (mg/100 g wrt γ-oryzanol) | 311.4 | 48.80 |
| Campesteryl ferulate & sitostenyl ferulate (%) | 37.83 | 6.03 |
| Campestanyl ferulate & β-sitosteryl ferulate (%) | 172.48 | 27.59 |
| Sitostanyl ferulate (%) | 101.08 | 15.09 |

Values are mean ± Standard Error Mean of three experimental results ($n$=3)

*WBO* Wheat bran oil, *T* Tocopherol

*WGO* Wheat germ oil, *T3* Tocotrienol

*wrt* with respect to, *T.Tocols* Total tocopherols and tocotrienols

normal growth, health and development of body (Cunnane and Anderson 1997).

*Nutraceuticals composition of minor components in wheat bran and wheat germ oil* Both wheat bran and wheat germ oils have tocopherols and tocotrienols (total tocols), and the total tocols were high in wheat germ oil 288 mg/100 g. Comprising alpha tocopherol (~57 %), γ tocopherol (~30 %) and 11 % tocotrienols in wheat germ oil (Table 2). The results are comparable with the earlier report of Hassanein and Abedel-Razek (2009) for wheat germ oil made in Egypt. In the present study we found that wheat bran oil had a total tocols content of 202 mg/100 g. Recently, Kwon et al. (2010) have shown that wheat bran contains 63 to 210 mg/100 g of total tocols varying with different temperature and pressure by using supercritical carbon dioxide extraction. Wheat bran oil when subjected to HPLC showed the presence of high content of total tocotrienols (83.91 %) and lower amount of tocopherols (16 %) which is presented in Table 2. The results obtained through spectrophotometry for total tocols of wheat germ (273 mg/100 g) and wheat bran (190 mg/ 100 g) oils are also presented in Table 2 and values were slightly less than the HPLC result. However, there is no significant difference between them. Tocotrienols have been shown to possess cardioprotective effects besides their lipid-lowering action via modulating the proliferator-activated receptor gamma (PPARγ) (Theriault et al. 1999; Li et al. 2010). Both wheat bran oil and wheat germ oil possess carotenoids, but total carotenoids were 5.51 fold higher in wheat germ oil (12.23 mg/100 g) when compared to wheat bran oil (2.21 mg/100 g) by HPLC (Table 2, Fig. 1). Lutein and β-carotene content in wheat bran was 0.39 mg/100 g and 2.26 mg/100 g respectively and remaining carotenoid constituted 1.6 mg/100 g. Palm oil (sources of all type of carotenoids) was used to confirm separation of wheat bran/germ oil carotenoids wherein we

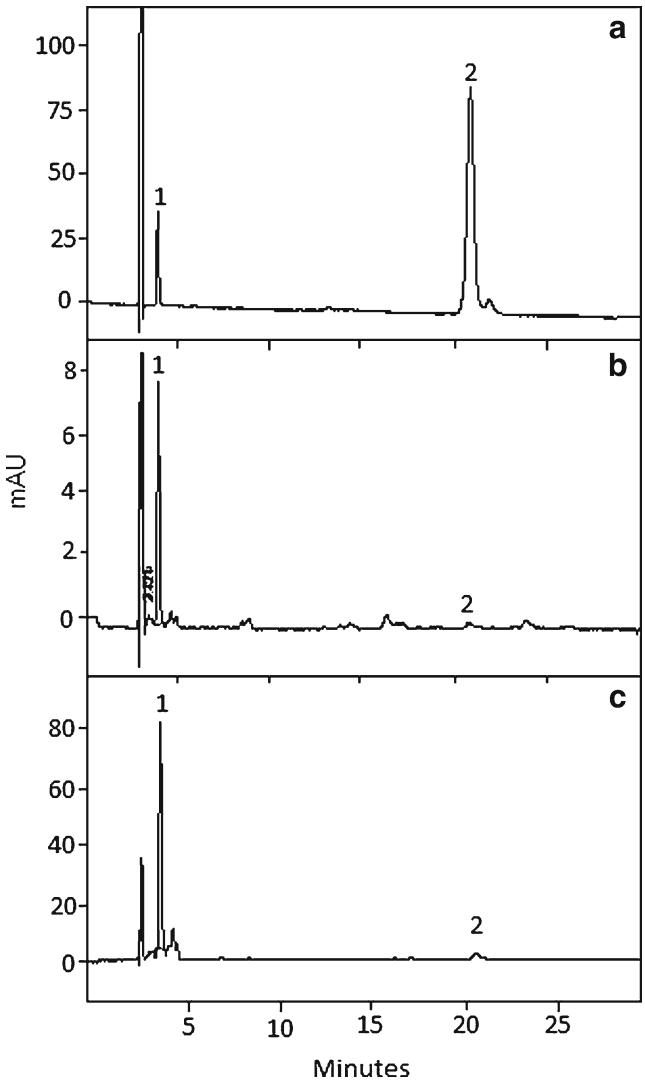


**Fig. 1** Total carotenoids of oil (taken in acetone) were determined through RP- HPLC fitted with Bondapak, $C_{18}$ column and PDA detector. Mobile phase used was acetonitrile/methanol/dichloromethane (6:2:2 v/v) with 0.1 % ammonium acetate having 1 mL/min flow rate. β-Carotene with LOD of 2–15 μg and lutein with 0.2–0.5 μg as reference standards for identification and quantification. Chromatogram of carotenoids at 450 nm: **a** Standards Peak 1. Lutein and Peak 2. β-carotene, **b** Wheat bran oil, **c** Wheat germ oil

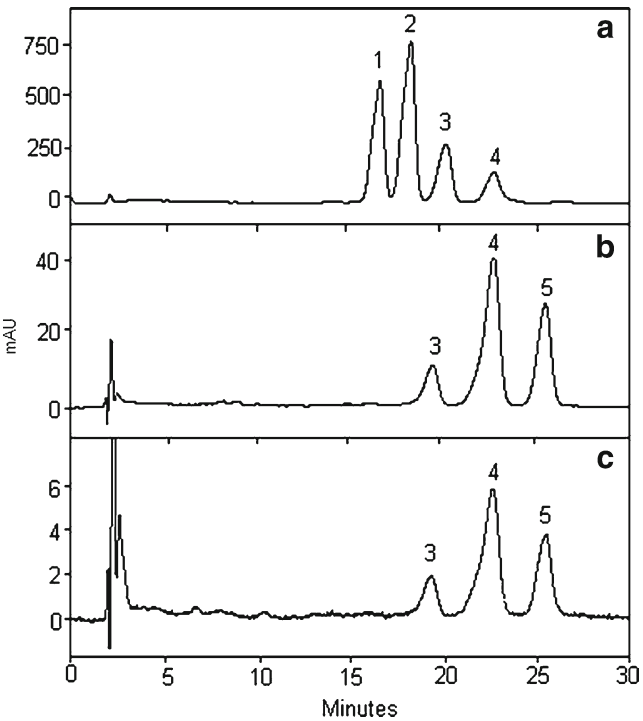


**Fig. 2** Total γ–oryzanol like compounds were determined by injecting oil (taken in chloroform) to HPLC coupled with $C_{18}$ column and PDA detector. Mobile phase used was acetonitrile: methanol: isopropyl alcohol (10:9:1 v/v/v) with 1 mL/min flow rate with γ-oryzanol as standard for steryl ferulate quantification. Chromatogram of steryl ferulate at 325 nm. **a** Oryzanol standard Peak 1. cycloartenyl ferulate; Peak 2. 24-methylene cycloartanyl ferulate; Peak 3. campesteryl ferulate; Peak 4. campestanyl ferulate & β-sitosteryl ferulate and, **b** Wheat bran oil and Peak 5. sitostanyl ferulate, **c** Wheat germ oil

**Table 3** Comparative $IC_{50}$ (concentration of sample or standard required to scavenge 50 % of the DPPH free radicals) values of wheat bran oil and wheat germ oil compared with standard antioxidants (listed in ascending order)

| Samp. | Sample/standard | $IC_{50}$ |
|---|---|---|
| 1 | Gallic acid | 04.3±0.1 μg/mL[a] |
| 2 | α-Tocopherol | 22.8±0.5 μg/mL[b] |
| 3 | Ferulic acid | 54.8±1.2 μg/mL [b] |
| 4 | Wheat germ oil | 07.9±0.5 mg/mL[c] |
| 5 | Wheat bran oil | 21.6±0.6 mg/mL[d] |

Values are mean ± Standard Error Mean of three experimental results ($n$=3)

[a] Values in the same column with different superscripts are significantly($p \leq 0.05$)different

were able to separate α and β carotenes (data not shown). Commercial edible oils, having highest lutein content in unrefined mustard oil (772 μg/100 g) and refined palm oil (11.5 μg/100 g) and absence of lutein in refined sunflower, olive, almond, rice bran and corn oils have been reported (Aruna et al. 2009). Very few reports have shown the presence of carotenoids in wheat germ oil (Panfili et al. 2003), but according to our findings, presence of lutein in unrefined wheat germ oil was the highest reported so far (2.9 mg/100 g). Beta-carotene (2.42 mg/100 g) and other carotenoids (6.89 mg/100 g) were also present and this may be the first report on the presence of lutein in wheat bran oil. A report was available on the total carotenoids of wheat bran oil wherein, carotenoid content ranged from 2.7 to 3.9 mg/100 g at varied temperature and pressure in the supercritical carbon dioxide (CO2) extraction (Go-Woon et al. 2010).

Steryl ferulates were present in both wheat bran and wheat germ, the content in wheat bran oil was 6.4 fold higher than in wheat germ oil (Table 2) but both the chromatogram profiles were showing similar pattern (Fig. 2). Out of three peaks in both the oils, peak 3 and peak 4 were identified using standard oryzanol components namely campesteryl ferulate & sitostenyl ferulate, campestanyl ferulate & β-sitosteryl ferulate in wheat bran oil and wheat germ oil (Fig. 2). Similar HPLC pattern for γ-oryzanol analogues were reported by Gopala Krishna et al. (2001) Peak 5 was identified as sitostanyl ferulate by Hakala et al. (2002), wherein, they have shown the presence of steryl phenolic acid esters in cereals including wheat bran. Among the available vegetable oils very few have steryl ferulates namely rice bran oil (γ-oryzanol), rye, corn oil along with wheat bran oil and have been shown to be inhibitory to Epstein-barr virus activation in the tumor formation (Iwatsuki et al. 2003). Presence of minor components of steryl ferulates in wheat germ oil (48 mg/100 g) observed in the study was also reported by Yongzhi and Wang (2005). Presence of ferulic acid moiety in steryl ferulates, a powerful antioxidant, whose esterified form (ferulates) is observed to be more active than the free form (ferulic acid) in liposomes (Nystrom et al. 2005).

*Antioxidant activity of wheat bran oil and wheat germ oil* Antioxidant activity study was performed on wheat bran and wheat germ oils using free radical DPPH method and $IC_{50}$ values were calculated with respect to concentration of sample

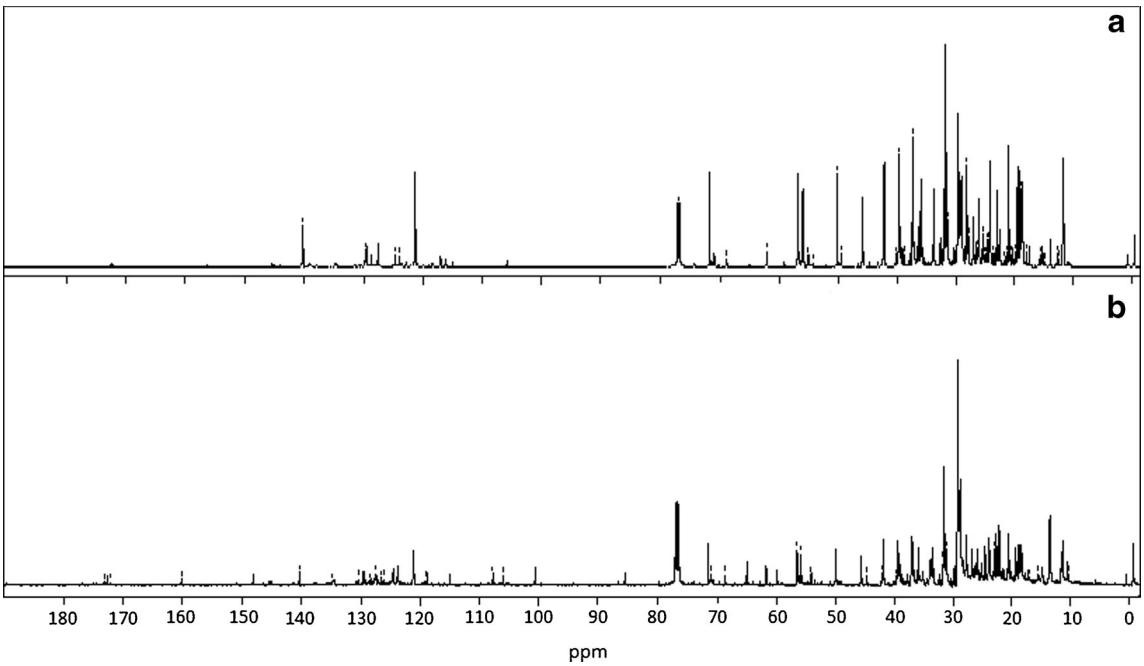


**Fig. 3** $^{13}C$ NMR in $CDCl_3$ spectra **a** unsaponifiable matter of wheat germ oil and **b** unsaponifiable matter of wheat bran oil depicting the presence of steryl ferulates and other components

or standard required to scavenge 50 % of the DPPH radicals. $IC_{50}$ values observed were in the following decreasing order gallic acid (GA) (4.3 μg) > α-tocopherols (22.8 μg) > ferulic acid (54.8 μg) > wheat germ oil (7.9 mg) > wheat bran oil (21.6 mg) (Table 3). In the present study, wheat germ oil showed strong $IC_{50}$ than wheat bran oil and lesser than other standard phenolics (GA, α-tocopherols and ferulic acid). Next to GA, α-tocopherol showed strong $IC_{50}$ which is the major component of the wheat germ oil minor components.

To understand the differences in antioxidant activities of wheat bran oil and wheat germ oil, the unsaponifiable fractions of the respective oils were subjected to $^{1}H$ NMR and $^{13}C$ NMR analyses instead of oils, primarily to avoid extreme interference of abundant fatty acids. Another reason comprises the fact that the unsaponifiable matter (unsap) due to the presence of various phytochemicals correctly represents the antioxidant potential of the wheat germ / wheat bran oil.

The $^{1}H$ NMR spectra of unsaponifiable fraction of wheat bran oil and wheat germ oil are quite complex with many peaks between 0.8 and 2.6 ppm where peaks due to α-tocopherol appear (spectra not shown). However, $^{13}C$ NMR spectrum is more resolved and the wheat germ unsap fraction (Fig. 3a) indicates higher fraction of α-tocopherols compared to wheat bran unsap fraction in the region 10.5 to 40.5 ppm. On the other hand, the $^{13}C$ NMR of wheat bran unsap fraction (Fig. 3b) clearly shows several peaks between 100 and 160 ppm corroborating with higher concentration of steryl ferulates. Antioxidant lutein was detected in HPLC, however it was not observed due to low concentration and also due to interference of α-tocopherol, steryl ferulates and fatty acids in the proton NMR spectra between the regions consisting of 1.0–3.8 ppm and 6.05–7.1 ppm, whereas in the $^{13}C$ NMR spectra, across the region comprising 12.5–65.5 ppm and 126–139 ppm.

From these studies, it can be concluded that, due to higher concentration and greater antioxidant activity of the principal antioxidant α-tocopherol ($IC_{50}$=22.8 μg), relatively higher antioxidant activity for wheat germ oil was observed in comparison to wheat bran oil. Nilufer et al. (2009) and Kwon et al. (2010) have also observed the presence of phenolics, along with much of tocopherols in wheat germ oil which might be responsible for its higher scavenging activity. It has been reported that the synergistic action of tocopherols with phenolics in olive oil had higher scavenging activity than the hydrophilic fraction of olive oil (Espin et al. 2000).

## Conclusions

This study indicated that minor components in wheat derived oils such as wheat germ oil formed the richest source of total tocols, lutein and smaller amount of other carotenoids among the vegetable oils and also contains oryzanol like compounds along with high amount of polyunsaturated fatty acids (PUFA). The presence of ideal amount of oryzanol like compounds (steryl ferulates), total tocols and lutein in WBO might be of sufficient quantity to prevent diseases like, cardiovascular disease, diabetes and cancer. Therefore, the oils from wheat bran and wheat germ may be used as a source of minor components in our daily food menu to promote good health.

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