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CHEMICAL COMPOSITION, FATTY ACIDS AND OIL STABILITY OF *CHROZOPHORA BROCHIANA* (VIS.) SCHWEINF. SEED GERMINATION

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Abstract

The chemical composition of seeds of *Chrozophora brochiana* (Vis.) Schweinf. as well as stability of the oil extracted from the seeds affected by germination have been investigated. *C. brochiana* seed was found to be a rich source of oil (42.9%) and protein (18.2%). Seed germination increased the moisture, protein, fiber and ash contents and decreased the fat and carbohydrate contents. The fatty acid composition was also influenced by germination where linoleic and oleic acids increased and stearic acid decreased. The concentrations of Na, K, Cu and Ca were higher in germinated seeds. FTIR spectroscopy was utilized to screen – changes in the germinated and ungerminated seeds during the successive heating at 70°C for 72 hrs. It was found that the oil extracted from germinated seeds was oxidized faster than ungerminated seeds when subjected to successive heating.

Introduction

Species of *Chrozophora* A. Juss. (Euphobiaceae) are annual plants. Its leaves, stems and fruits are utilized in food and pharmaceuticals. *Chrozophora brochiana* (Vis.) Schweinf. is a shrubby monoecious herb up to 60 - 150 cm tall. The seeds are ovoid, smooth, yellowish tan, secured by a slender, pale, gleaming aril (Ahmed *et al.* 2014). The plant is found in Sudan (where it is known as Argassi) and other African countries, the seeds are boiled and utilized for food and the seed oil is extracted through oil mills to get clear yellow edible oil.

C. brochiana seeds contain about 26% protein and 37 - 40% oil composed of 11 triacylglycerols with a low melting point (Hussein *et al.* 2006, Mirghani *et al.* 1996). Seeds germinate with absorption of water and activate the reserves within their storage tissues to help seedling development, and the process is completed when the embryonic axis stretches (Bewley *et al.* 2001). Kuo *et al.* (2004) reported that, germination causes vital changes in the biochemical, nutritional and sensory characteristics of legume seeds. The process is accompanied by breakdown of seed-storage lipids and structural proteins causing an increase in the level of dietary fiber.

Fourier transform infrared spectroscopy (FTIR) has been successfully utilized to analyze vegetable oils stability (Russin *et al.* 2004, Rohman *et al.* 2011) and to study the impact of temperature on the stability of oil obtained from roasted and unroasted safflower seeds (Mariod *et al.* 2012a). The aim of this work was to study the impact of the germination process on the constituents of *C. brochiana* seeds and on its oil composition and stability.

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Materials and Methods

Chrozophora. brochiana seeds were obtained from Ghibaish, Western Kordofan State, Sudan. Mature seeds were cleaned with running tap water. Dried for 2 days under shed, brought to the laboratory of Food Science and Technology Department, Sudan University of Science and Technology, Khartoum North, Sudan), and stored at 4°C until further investigations. Chemicals and solvents were of analytical grade, and were supplied by Merck (Darmstadt, Germany).

Seeds were germinated following the technique of Akpapunam *et al*. (1997). Moisture content, crude protein (Kjeldahl technique), crude fat (solvent extraction), crude fiber, and ash were determined in triplicates utilizing standard methods (AOAC 2005) and the available carbohydrate contents were calculated by difference. The oil was extracted from the pulverized seeds with *n*-hexane in a Soxhlet apparatus for 6 hrs following the AOCS method (2009). One hundred mature seeds were chosen randomly and their weight and dimensions were determined using a sensitive balance (Mettler-Toledo, Columbus, OH, USA). Hulls and kernels were weighed and the proportion of weight of the hulls and the kernel was computed. The AOCS official methods (2009) were used to examine the following physicochemical properties of *C. brochiana* seed oil: relative density (Cc 10 a - 25), refractive index (Cc 7 - 25), viscosity (Tq 1a - 64), content of free fatty acids (Ca 5a - 40), peroxide value (Cd 8b - 90), iodine value (Cd 1d - 92), saponification value (Cd 3 - 25), and unsaponifible matter (Ca 6b - 53).

The mineral content of the *C. brochiana* seeds cake was measured following the method of the Association of Official Analytical Chemists (2005). Oil from germinated and ungerminated *C. brochiana* seeds were derivatized to fatty acid methyl esters (FAME) following the method of Christie (1989). FAME samples (1 µl) were injected into a GC (Shimadzu, GC-2010A series; Shimadzu, Tokyo, Japan) and identified. These determinations were carried out in duplicates. The tocopherols were identified using HPLC a Merck-Hitachi low-pressure gradient system. The mobile phase used was *n*-heptane/tertiary butyl methyl ether (99 + 1, v/v) (Balz *et al.* 1992). These analyses were carried out in duplicates. The oil stability was measured as peroxide value following the AOCS official methods (AOCS 2009), and Fourier Transform Infrared Spectroscopy (FTIR) (Van de Voort *et al.* 1994). All analyses concerning the oxidative stability of oils were carried out in duplicates.

Unless otherwise stated, all experiments were carried out in triplicate and the results were expressed as means ± Sd. Statistical analysis was carried out using a one-way ANOVA with a significance level of $p \leq 0.05$. The software used for the statistical analysis was the SPSS for Windows statistical package (v.10.0.6; SPSS, Chicago, IL, USA).

Results and Discussion

Seeds of *Chrozophora brochiana* resemble those of sorghum in shape and size, but they are somewhat darker in color. The average weight of 100 seeds was 3.27 g, which was similar to that reported by Hussein *et al.* (2006). The weight of 100 kernels was 2.07 g and the weight of 100 hulls was 1.2 g. The ratio of the hull to the kernel was 43 : 57 and the percentage of kernel in the whole seed was 63.3 while it was 36.7 of the hull.

Table 1 demonstrates the proximate composition and physicochemical properties of ungerminated and germinated *C. brochiana* seed oil. The results revealed the following percentages for moisture (4.9 ± 0.05), protein (18.20 ± 0.42), crude fiber (21.7 ± 0.28), and ash (1.05 ± 0.08) in ungerminated seeds. These values were found to be significantly affected by the germination process ($p \leq 0.05$) and were found to increase to 6.79 ± 0.06, 18.64 ± 0 .15, 27.4 ± 0.28, and 1.9 ± 0.42%, respectively. On the other hand, the percentages of fat and carbohydrates before germination were 42.9 ± 0.14, and 11.28 ± 0.70, respectively, and were decreased to 37.7 ± 0.17

and 8.7 ± 0.14 after germination. The results showed that *C. brochiana* seed is a good source of oil (42.9%) and protein (18.2%), the values of protein and oil were compared favorably with those reported by Hussein *et al.* 2006, Mirghani *et al.* 1996. The protein and fat content of seeds from other plants of the same family seem to differ e.g. the undecorticated castor seeds contain 20.78% protein and 51.20% fat (Dastagir *et al.* 2013), while *Chrozophora tinctoria* seed contain 6.8% protein and 7.6% fat (Annongu *et al.* 2008).

The increase of the protein content as a result of germination, increases the nutritional value of the germinated seeds. This might be due to the net enzymatic synthesis of protein leading to the production of some amino acids during protein synthesis (Kim *et al.* 2012). The slight increment in protein is due to the degradation of the high molecules of protein to the simpler peptides during germination which is in agreement with the findings of Tian *et al.* (2010), who reported that the protein concentration of oat seeds gradually increased because of germination.

The reduction in fat and carbohydrate contents (Table 1) could be ascribed to their utilization as energy sources during the germination process. These results are in agreement with the results reported by Akpapunam *et al.* (1997), who reported that the process of germination reduced fat and starch contents and increased the protein and fiber content of jack beans. However, these results are in contrast with Mariod *et al.* (2012b), who reported that germination of black cumin seeds increased both oil and protein contents while other constituents decreased. Colmenares and Bressani (1990) reported a marked increase in moisture content during germination of amaranth species and they also reported that, protein, oil, crude fiber, and ash contents did not change significantly.

Table 1. Proximate analysis of seed and physicochemical properties of oil extracted from germinated and ungerminated *Chrozophora brocchiana* seeds*.

Parameters	Ungerminated seeds (%)	Germinated seeds (%)
Moisture	4.87 ± 0.05^{a}	6.79 ± 0.06^{b}
Oil	42.90 ± 0.14^{a}	37.73 ± 0.17^{b}
Protein	18.20 ± 0.42^{a}	18.64 ± 1.15^{a}
Fiber	21.70 ± 0.28^{a}	27.40 ± 0.28^{b}
Ash	1.05 ± 0.08^{a}	1.92 ± 0.42^{b}
Carbohydrate	11.28 ± 0.70^{a}	7.65 ± 0.14^{b}
Refractive index, (25°C)	1.4720 ± 0.00^{a}	1.4718 ± 0.00^{a}
Free fatty acid	2.66 ± 0.01^{a}	6.30 ± 0.14^{b}
Unsaponifiable matter	0.22 ± 0.00^{a}	0.40 ± 0.00^{b}

*All determinations (except RI, FFA%, unsaponifiable and color) were carried out in triplicate and mean value ± standard deviation (Sd) are reported. Significant differences in a same row are shown by different letters ($p < 0.05$).

Table 1 also shows that the oil extracted from the ungerminated seeds had 2.66% of free fatty acids and that this value increased to 6.3 ± 0.14% by the germination process. The content of free fatty acids was higher compared to results of Hussein *et al*. (2006) who reported that freshly extracted oil from *C. brochiana* initially had a low free fatty acid value but it increased upon long storage. The unsaponifiable matter of the oil extracted from ungerminated seeds was 0.22% and

the refractive index was 1.4720 at 25°C, which agreed with results of Hussein *et al*. (2006) and is in the range found for sunflower, groundnut and soybean oils (Al-Kahtani 1983). The level of the unsaponifiable matter was different from that reported by Mirghani *et al.* (1996). It is also found in this experiment that the germination process caused an increase in the free fatty acids and unsaponifiable matters (to 6.3 and 0.40%, respectively), while the refractive index decreased to 1.4718.

Mineral content of ungerminated and germinated *C. brochiana* seeds is presented in Table 2. Potassium was the predominant element in the seeds followed by magnesium and sodium then iron, calcium, and manganese. Ungerminated seeds had a higher content (in mg/100 g) of potassium (32.01 ± 0.014), magnesium (9.77 ± 0.014) and sodium (1.589 ± 0.001), of which the levels of potassium and sodium were significantly ($p \leq 0.05$) increased by germination to 58.70 ± 0.14 and 5.80 ± 0.014 while magnesium showed a slight decrease to 9.52 ± 0.14. The content of calcium, manganese, and copper increased slightly in geminated seeds, while the content of iron decreased. The zinc and cobalt content also increased during germination process. The increments in mineral concentrations are likely to be due to removal of phytate as a result of germination (Sokrab *et al*. 2012). Germination activates endogenous grain phytase which can degrade phytate. During germination, phytins are broken down by endogenous phytase enzymes, releasing their P, inositol and mineral contents for use by the growing seedling (Afify *et al*. 2011). Phytate and polyphenols, are considered as antinutritional factors because of their interactions with food constituents such as minerals that render them unavailable for absorption by the human body. Reductions of such antinutritional factors by processing methods such as soaking, sprouting, cooking, malting, fermentation, and germination have been shown to increase the levels of ingested minerals (Steve 2012). Many authors reported increases in major and trace mineral contents, such as zinc, manganese, copper and cobalt, by germination (Sokrab *et al.* 2012), likely to be due to removal of phytate (Colmenares and Bressani 1990). These results are sensibly great in light of the fact that potassium assumes an essential part in human physiology, and sufficient measures of it will lessen the danger of heart stroke, while calcium assumes a vital part in building stronger, denser bones early in life and keeping bones solid and healthy later in life (Dawson-Hughes *et al.* 1997).

The fatty acid composition of oil from ungerminated and germinated *C. brochiana* seeds is shown in Table 3. The percentage of myristic (C14 : 0), palmitic (C16 : 0), palmitoleic (C16 : 1), stearic (C18 : 0), oleic (C18 : 1), and linoleic (C18 : 2) acids in the ungermenated seeds were 0.1, 8.2, 0.2, 16.6, 24.9 and 49.3%, respectively, which is in good agreement with the results reported by Hussein *et al.* (2006) and Mirgani *et al.* (1996). Other plants of the same family contain higher amount of unsaturated fatty acids e.g. *Caryodendron orinocense* contains 75.13% linoleic acid (Mde and de Padilla 1994), while *Chrozophora plicata* contains 60 - 75% linolenic and linoleic acid. During germination the content of oleic acid, and linoleic acid showed a significant increase ($p \leq 0.05$), while stearic acid showed a significant decrease.

The levels of tocopherols (vitamin E) and their changes as a result of the germination process are presented in Table 4. The amounts of these tocopherols were compared with three commercial available oils mainly used in the Sudanese diet, *viz.* sunflower, sesame, and groundnut oils (CODEX 1999). The total vitamin E contents were 87.1 and 64.3 mg/100 g in oil from ungerminated and germinated seeds, respectively, amounts that were within the ranges which were reported by Codex (1999) for sunflower oil (44.0 -152 mg/100 g), sesame (33.0-101 mg/100 g), and groundnut (17.0 - 130 mg/100 g) oils.

The main tocopherol of ungerminated seeds was gamma-tocopherol with 73.6 mg/100 g, representing 84.6% of the total tocopherols, followed by beta-tocopherol with 13.5 mg/100 g, representing 15.4% of total tocopherols. These amounts decreased during germination to 60.0

mg/100g (93.3%) and 4.3 mg/100 g (6.7%), respectively. Tocopherols induce a protective effect against oxidative stress linked to metabolic syndrome and they are also essential for normal neurological function (Dias 2012).

Table 2. Minerals composition of germinated and ungerminated *Chrozophora brocchiana* seeds*.

Minerals	Ungerminated seeds (mg/100 g)	Germinated seeds (mg/100 g)
Sodium (Na)	1.589 ± 0.001^a	5.80 ± 0.014^b
Potassium (K)	32.01 ± 0.014^a	58.70 ± 0.14^b
Calcium (Ca)	0.340 ± 0.001^a	1.204 ± 0.001^b
Magnesium (Mg)	9.77 ± 0.014^a	9.52 ± 0.014^a
Copper (Cu)	0.145 ± 0.001^a	0.525 ± 0.001^b
Iron (Fe)	0.548 ± 0.001^a	0.502 ± 0.001^a
Manganese (Mn)	0.304 ± 0.001^a	0.312 ± 0.001^a
Zinc (Zn)	0.828 ± 0.001^a	1.570 ± 0.014^b
Cobalt (Co)	0.018 ± 0.001^a	0.119 ± 0.01^a
(Pb)	Tr.	Tr.
(Cr)	Tr.	Tr.
(Cd)	Tr.	Tr.

*All determinations were carried out in duplicate and mean value ± Sd were reported. Significant differences in a same row are shown by different letters ($p < 0.05$).

Table 3. Effect of germination process on fatty acid composition of *Chrozophora brocchiana* oil (%).*

Fatty acids (%)	Ungerminated seeds	Germinated seeds
Myristic 14 : 0	0.1 ± 0.11^a	0.1 ± 0.10^a
Palmitic 16 : 0	8.2 ± 0.21^a	8.2 ± 0.20^a
Palmitoleic 16 : 1	0.2 ± 0.10^a	0.1 ± 0.10^a
Stearic 18 : 0	16.6 ± 0.21^a	15.8 ± 0.22^b
Oleic 18 : 1	24.9 ± 0.31^a	25.1 ± 0.31^b
Linoleic 18 : 2	49.3 ± 015^a	50.1 ± 0.13^b
Linolenic 18 : 3	0.5 ± 0.2^a	0.5 ± 0.2^a
Gadoleic 20 : 1	0.1 ± 0.1^a	0.1 ± 0.1^a
Saturated	24.9	24.1
Mono-unsaturated	25.2	25.2
Di-unsaturated	49.3	50.1
Tri-unsaturated	00.5	00.5

*All determinations were carried out in duplicate and mean value ± Sd were reported. Significant differences in a same row are shown by different letters ($p < 0.05$).

The samples were subjected to successive heating at 70°C for three days and the oxidative stability was monitored using the peroxide value (Table 5) and FTIR analysis. Peroxide values (PV) of ungerminated *C. brochiana* oil increased gradually from 1.1 ± 0.14 meq O_2/kg oil at the starting time to 23.2 ± 0.14 after 3 days of storage at 70°C. These results are comparable to the change from 2.2 ± 0.14 meq O_2/kg oil at the beginning to 24.2 ± 0.14 meq O_2/kg oil after 72 hrs of storage at 70°C in the oil sample from germinated seeds. The PVs in the two oils increased significantly ($p < 0.05$) after 72 hrs of storage to 23.2 ± 0.14 and 24.2 ± 0.14 meq O_2/kg oil, respectively, as incubation at 70°C accelerates the oxidation in the oils (Mariod *et al.* 2012b). Oxidative stability decreased as a result of germination; this can be explained by the decrease in some bioactive components e.g. tocopherols. This natural antioxidant plays a very important role in the oil stability. The loss of such antioxidants will decrease oil stability. Also Herchi *et al.* (2015) reported that germination causes a decrease in germinated flax seed oil stability.

Table 4. Tocopherol composition (mg/100 g) of germinated and ungerminated *Chrozophora brocchiana* seed oils compared with commercial oils*.

Tocopherols	Ungerminated seed	Germinated seed	Sunflower oil	Sesame oil	Groundnut oil
Alpha	ND	ND	40.3 - 93.5	ND - 0.33	4.9 - 37.3
Beta	13. 5 ± 0.1[a]	4.3 ± 0.2[b]	ND - 3.4	52.1 - 98.3	ND - 2.2
Gamma	73.6 ± 0.4[a]	60.0 ± 0.3[b]	ND - 0.70	0.4 - 2.1	8.8 - 38.9
Total	87.1	64.3	40.3 - 97.6	52.5 - 100.73	13.7 - 78.4

*Values for sunflower, sesame, and groundnut oils were from the Codex Standard 210 - 1999. All determinations were carried out in duplicate and mean values were reported. Significant differences in a same row are shown by different letters ($p < 0.05$). ND = Non-detectable, defined as $p \leq 0.05$ mg/100 g.

Table 5. Peroxide value of oil extracted from germinated and ungerminated *Chrozophora brocchiana* seeds and stored for 0 - 72 hrs under oxidative conditions*.

Samples	0 hr	6 hrs	12 hrs	24 hrs	28 hrs	72 hrs
Ungerminated	1.1 ± 0.14[a]	2.2 ± 0.14[a]	3.6 ± 0.14[a]	10.1 ± 0.21[a]	17.7 ± 0.14[a]	23.0 ± 0.14[a]
Germinated	2.2 ± 0.14[b]	2.9 ± 0.07[a]	3.9 ± 0.14[a]	12.2 ± 0.14[b]	22.9 ± 0.14[b]	24.2 ± 0.14[b]

*All determinations were carried out in triplicate and mean values were reported. Significant differences in a same column are shown by different letters ($p < 0.05$).

Fig. 1 shows the FTIR spectra of the fresh oils extracted from germinated (A) and ungerminated (B) seeds of *C. brochiana*. The prevailing groups in these oils are the same as in other edible oils. Fourteen visible peaks were observed in ungerminated *C. brochiana* seed oil at frequencies of 3473, 3008, 2923, 2854, 2677, 1745, 1649, 1463, 1377, 1236, 1163, 1099, 914, and 723/cm. Henna and Tan (2009) reported that absorption peaks at 3600 - 2800 and 1800 - 700/cm are the dominant bands in vegetable oils. Comparison of the results of germinated seed oils with those of oils from ungerminated seeds reveals several changes in the peak intensities (absorbances). For example, (i) the band at frequency 723/cm, associated to bending of –(CH2) n-, HC = CH- (cis) (Henna and Tan 2009), was changed to 721/cm and its intensity was increased to 56, (ii) the band near 1163/cm experienced an increased in wave number and intensity and gave

a broad band as affected by germination, while (iii) the band near 1236/cm disappeared as a result of germination (Fig. 1).

Fig. 2 shows FTIR spectra of the oil extracted from germinated and ungerminated *C. brochiana* seeds stored at 70ºC for 24 hrs. The peak intensities of oil extracted from ungerminated seeds changed in comparison with oil extracted from germinated seeds. An increase in the band absorbing at 3473, corresponding to an increase in the concentration of hydroperoxides as a consequence of oxidation of the oil extracted from germinated seeds is quite clear. Upon progressive heating, increments in absorbances of bands corresponding to carbonylic compounds such as aldehydes, esters, ketones, and lactones should be evident (Rohman *et al*. 2011).

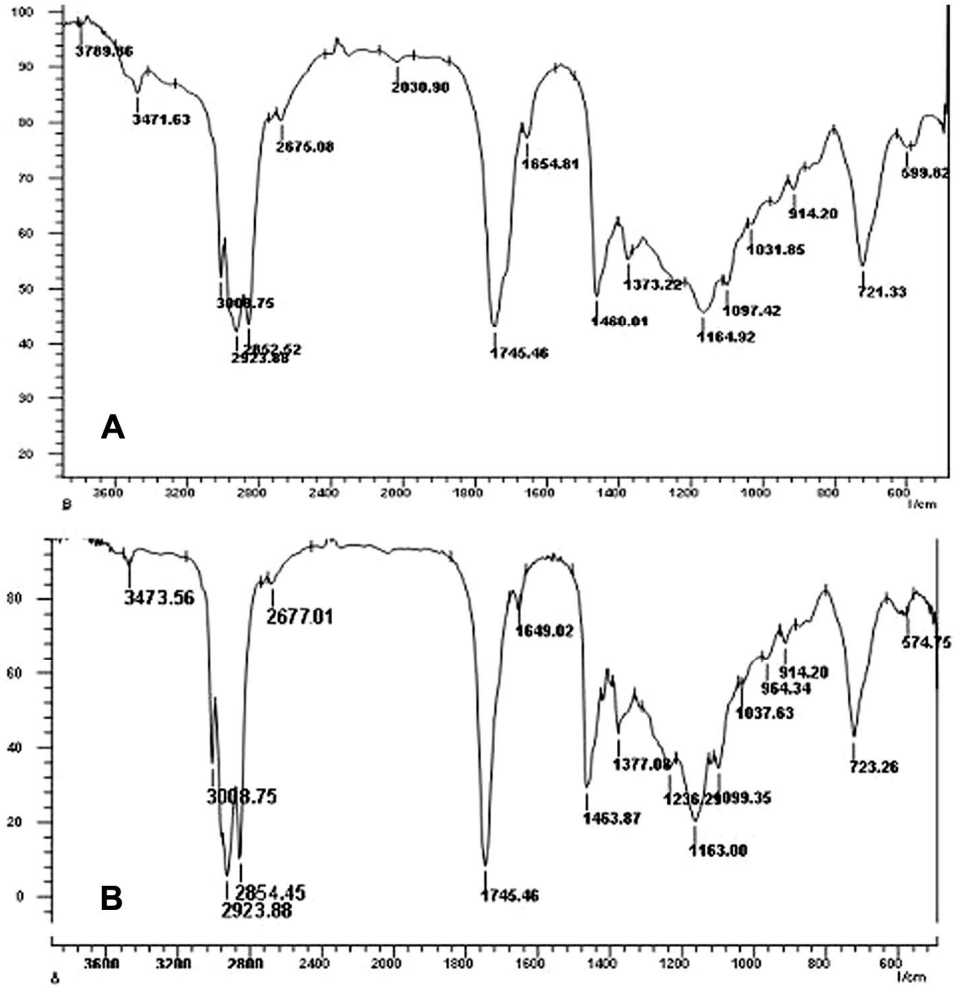

Fig. 1. FTIR spectra of oil extracted from germinated (A) and ungerminated (B) *C. brocchiana* seeds storaged at 70ºC for zero hr.

Fig. 3 shows the FTIR spectra of oils extracted from germinated and ungerminated *C. brochiana* seeds incubated at 70°C for 72 hrs. Fourteen peaks were visible at the above mentioned

frequencies (3473, 3008, 2923, 2854, 2678, 2028, 1745, 1654, 1460, 1373, 1164, 1099, 914, and 723/cm). After 72 hrs of incubation, there were sharp changes in the intensities (absorbances) of other peaks such as the peak at 1164/cm corresponding to the –C–O, –CH2– of extending vibration and the band at 1745/cm related to C = O of extending vibration, for which the intensity is influenced by germination and by progressive storage at 70ºC. The absorbance in the region 2800 - 3200/cm was expanded showing that the bands 2854, 2923 and 3008/cm accomplished a sharp increase in intensity. The band at 3008/cm was related to the extending vibration of the CH groups of *cis* double-bonds while the other two bands indicated stretching vibration of carbon-carbon double bonds. Hence, the progression of oxidation was accompanied by reduction in the number of cis double-bonds and a decline in the degree of unsaturation. Germination caused a more adverse influence on the oxidative stability of the oil extracted from germinated *Chrozohpora brochiana* seeds as indicated by changes in the intensities and absorbance of most bands (Figs 1-3).

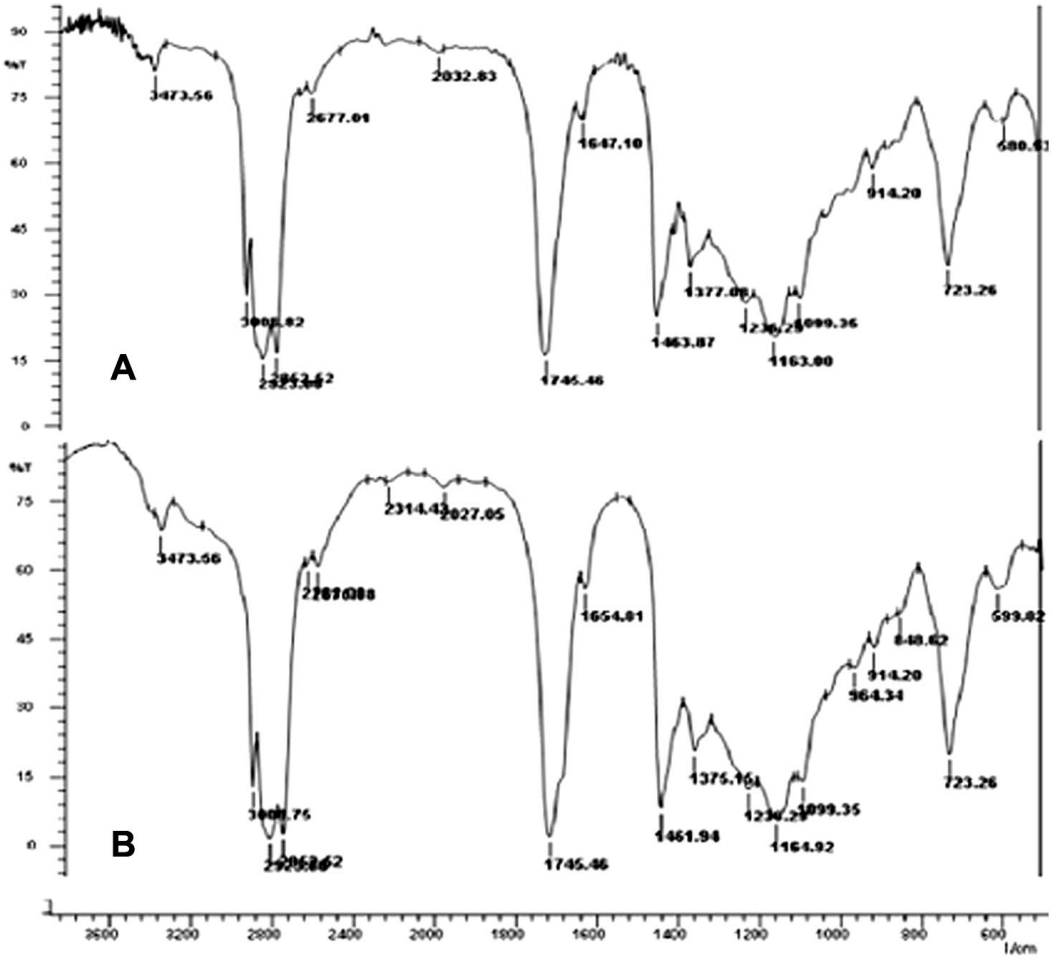

Fig. 2. Fourier transform infrared spectra of oil extracted from germinated (A) and ungerminated (B) *C. brocchiana* seeds storaged at 70ºC for 24 hrs.

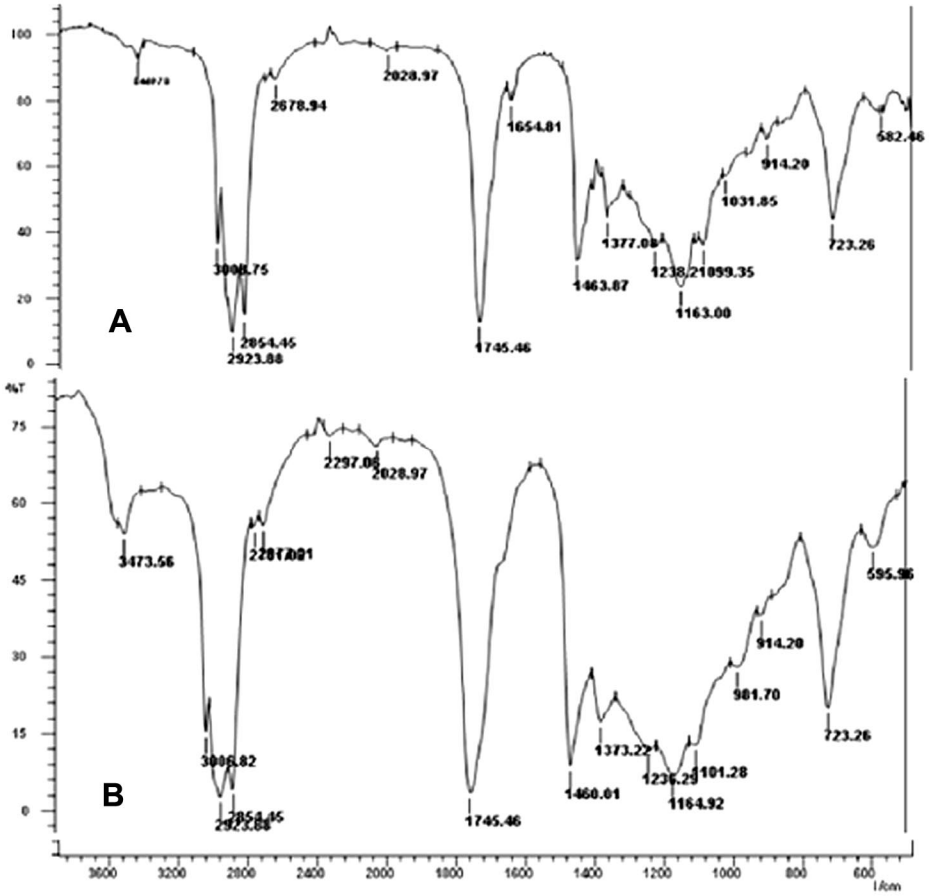

Fig. 3. Fourier transform infrared spectra of oil extracted from germinated (A) and ungerminated (B) *C. brocchiana* seeds storaged at 70ºC for 72 hrs.

The results demonstrated that *Chrozophora brochiana* seeds contain high amounts of protein, oil, and carbohydrates. The results also show a potential for utilization as new source for vegetable oil high of unsaturated fatty acids, especially linoleic acid. It was observed that germination prompted increments in moisture and fiber and a decrease in fat and carbohydrates. Germination of the seeds increased the availability of minerals such as sodium, potassium or zinc, but the overall quality of the seed oil decreased. The content of tocopherols was remarkable lower in oil from germinated seeds and successive heating at 70ºC resulted in a lower stability of oil from germinated *C. brocchiana* seed oils. Through FTIR, which appears suitable for studying the oxidative stability of this oil, the degree of oil oxidation was found to be lower in ungerminated than in germinated *C. brocchiana* seed oils.

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