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Kinetics and the Effect of Refining Methods on the Physicochemical Properties, Fat Soluble Vitamins and Nutritional Metal Content of *Hura crepitans* Oil

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Abstract. The effect of three refining methods, *viz.* alkali refining, degumming and bleaching was investigated on the physicochemical properties, fat soluble vitamins and nutritional metal content of *Hura crepitans* oil. The processes increased the glyceride content while there was reduction in the nutritional metal content of the oil. The effect of temperature (60-180 °C) and time (upto 90 min) was also considered using the bleaching method with surface active clay and activated charcoal. The adsorption of peroxides was adequately modeled by Arrhenius type of equation and described by the first-order kinetic. The activation energy for bleaching at 120 °C and 45 min was 244.60 cal/mole. Among all the refining methods, bleaching appeared to be the best technique for refining in terms of stability and improvement of physicochemical properties of the *H. crepitans* seed oil.

Keywords: oil refining methods, physicochemical properties, vegetable oil, *Hura crepitans* seed oil, vitamins, minerals

Introduction

Vegetable oils obtained from plant seeds are cheaply processed for fulfilling the demands of food supply. These oils contain some essential minerals accumulated in different parts of plants from the environment and are required by human body. Trace metals have also been detected in such plants. (Liu *et al.,* 2005).

Extraneous substances, present in the crude edible oils of plants, may impart objectionable properties to the oils (Weiss, 1983), which can be removed by refining in terms of increased shelf life and nutritive status of the resultant oils (Haraldsson, 1983).

Among the oil seed-containing plants, *Hura crepitans* is one of the newly known plants; it was underutilised due to lack of information on its composition and utilisation.

The seeds and seed oil of *H. crepitans* (HC) have been characterised in a previous study (Oderinde *et al.,* 2009), wherein the oil yield was found to be 37.78±0.81%; the acid value and iodine were 19.04±0.41 mg KOH/g and 20.81±0.20 mg iodine/g, respectively. The characterisation and lipid profile of the oil suggested its possible application in soap and food industries. In continuation of our search for novel seed oils, the present study aims at improving the physicochemical properties, lipid classes, nutritionally valuable minerals and fat soluble vitamins of *H. crepitans* seed oil by means of effective refining (bleaching, degumming and alkali refining). The kinetic data of the effect of refining on the oil was also studied.

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Materials and Methods

Sample preparation. The *H. crepitans* seed sample was obtained from the surroundings of the University of Ibadan, Oyo State, Nigeria and identified at the herbarium of Botany Department, University of Ibadan. The seeds were subsequently ground in laboratory mill and stored in cellophane bags at 4 °C prior to analysis.

Physicochemical analysis. The *H. crepitans* seed oil was extracted using Soxhlet extractor with petroleum ether (40-60 °C) for 10 h (Ajayi *et al.,* 2004). The extracted oil was immediately analysed for iodine, peroxide saponification and acid values as well as unsaponifiable matter by the method described by the Association of Official Analytical Chemists (AOAC, 1984). Estimation of free fatty acids as oleic acid was performed following the method described by Oderinde *et al.* (1990). The refractive index of the oil (at room temperature) was determined with Abbe refractometer (Oderinde and Ajayi, 2000) and the specific gravity was measured using gravity bottle. The state and colour of the oil were noted at room temperature through visual inspection. Mean molecular mass was estimated by the formula (56/SV)´1000, where SV is the saponification value (Akintayo and Bayer, 2002).

Determination of minerals. The metals determined included lead, cadmium, copper, zinc, iron, magnesium, calcium,

sodium, potassium and manganese. 0.5 g of the oil was digested using 5 ml (2:1) of 69.40% (w/w) nitric acid and 90.00% (w/w) perchloric acid (Oderinde and Ajayi, 1998) and the metals were analysed by atomic absorption spectrophotometry (Perkin-Elmer).

Lipid classes. Lipid classes were separated on 0.75 mm plates (20´20) coated with silica gel (Merck). Plates were developed vertically in a 80/20/1 volume mixture of petroleum ether: diethylether: acetic acid, according to the method described by Oboh and Oderinde (1988).

Isolation of unsaponifiable matter. 10 g oil was dissolved in 200 ml of 2 M ethanolic potassium hydroxide and refluxed for one hour. The reaction mixture was later diluted to 400 ml with distilled water and transferred to a one litre separating funnel. The unsaponifiable matter was then extracted thrice with 100 ml diethylether. The ether extract was first washed with 100 ml aqueous solution of 0.5 M potassium hydroxide in order to remove any residual fatty acids; it was further washed and cleaned with 5´100 ml distilled water and dried over anhydrous sodium sulphate. The solution was filtered and dried.

Separation of unsaponifiable matter. Chloroform solution (50%) of the unsaponifiable matter (30 mg/plate) was applied uniformly along the line from the edge of the 20´20 cm plate coated with 0.55 mm layer of silica gel and developed thrice with hexane/ethyl acetate (6:1 v/v) as mobile phase. The developed plates were dried and irradiated at 254 nm with ultraviolet radiations. Three zones corresponding to *n*-alkanes, triterpene alcohols and sterols were marked, carefully scrapped and extracted with petroleum ether (Adebowale *et al.,* 2001).

Alkali refining. 30 g of the oil was heated at 50 °C for 10 min then 2 ml 18 M potassium hydroxide was added with continuous agitation for 30 min. The resultant mixture was, thereafter, heated to 80 °C in order to break the soap stock formed. Neutral oil was separated from the soap stock by centrifugation at 3000 rpm. The separated oil was washed with boiling water for 10 min and finally separated from the soap solution by centrifugation.

Degumming. 45 g of the oil sample was taken in a conical flask and phosphoric acid (85%) 2% w/w was added. The flask was corked tightly and the content was agitated for 30 min. The oil was separated from the contents by centrifugation at 3000 rpm for 5 min.

Bleaching. Activated carbon and clay (bentonite) (1:1 mixture) was used for bleaching. 35 g of the oil was warmed to 50 ° C and the bleaching mixture was gradually added. The flask was corked, the temperature was increased to 120 °C and maintained at it for 45 min. The oil was filtered while hot through a sintered glass funnel using suction pump.

Statistical analysis. Data were analysed by one way analysis of variance (ANOVA). Means were compared by the Duncan's multiple range tests. Significance was accepted at 5% level (P£ 0.05) (Duncan, 1955).

Results and Discussion

Effect of refining on physicochemical properties of *H.crepitans* oil. Refining processes have significant effect on the physicochemical properties of *H. crepitans* oil (Table 1). Acid and free fatty acid values were reduced in all the processes used. The value obtained after treatment was the highest in the degummed fraction. Free fatty acid value was the lowest (2.80 ± 0.30%) in the bleached oil while the acid value was the lowest (5.00 ± 0.20 mg KOH/g) in the alkali refined sample. This reduction is an indication of the removal

Table 1. Effect of refining on the physicochemical properties of HCoil

Parameter	Crude oil	Alkali refined oil	Degummed oil	Bleached oil
Free fatty acid (%)	7.65 ± 0.11^{a}	3.11 ± 0.10^{b}	6.81 ± 0.10^{c}	2.80 ± 0.30^{d}
Acid value (mg KOH/g oil)	19.04 ± 0.41^{a}	5.00 ± 0.20^{b}	14.71 ± 0.10^{c}	6.30 ± 0.10^{d}
Saponification value (mg KOH/g oil)	294.04 ± 0.11^{a}	268.00 ± 3.70^{b}	204.00 ± 2.20^{c}	288.00 ± 7.10^{d}
Iodine value (mg iodine/g oil)	20.81 ± 0.20^{a}	18.61 ± 3.80^{b}	30.17 ± 2.10^{c}	31.70 ± 6.80^{d}
Peroxide value (mg O_2/g oil)	1.20 ± 0.12^{a}	8.30 ± 0.30^{b}	0.90 ± 0.10^{c}	0.40 ± 0.10^{d}
Specific gravity	0.8711 ± 0.04^{a}	0.8011 ± 0.08^{b}	0.8615 ± 0.01^{c}	0.8598 ± 0.01^{d}
Refractive index	1.5480 ± 0.40^{a}	1.5210 ± 0.1^{b}	1.6780 ± 0.10^{c}	1.6870 ± 0.20^{d}
Unsaponifiable matter (%)	1.02 ± 0.01^{a}	4.51 ± 0.11^{b}	1.62 ± 0.56^{c}	0.82 ± 0.72^{d}
Colour	Light yellow	Light yellow	Creamy	Light cream

Values are mean ± standard deviation of triplicate determinations; data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$).

of the free fatty acids which could have been produced due to hydrolysis of the glyceride bonds. Reduction in the free fatty acid and the acid values show some level of acceptability in the food industry. It also reflects the affinity of the adsorbent used for the acid functional groups. The level of unsaturation increased after treatment except in the case of alkali refining which might be due to the reaction of potassium hydroxide with the double bonds of unsaturated fatty acids in the triacylglycerol chain leading to production of peroxides and hyperoxides; it may eventually lead to oxidative rancidity and deterioration of the oils. This increase was also in accordance with the trend of refractive index which increased after treatment and so was the iodine value. Since double bonds (unsaturation) increase the refractive index of organic compounds, the higher the unsaturation, the greater is the effect on refractive index (Oderinde *et al*., 2008a). Peroxide value was reduced after treatment, with the exception of the alkali treatment. The value increased from 1.20 ± 0.12 mg /g oil in the crude (untreated) oil to 8.30 ± 0.30 mg /g oil in the alkali refined fraction. This is an indication of some degree of deterioration as expressed by the iodine value. There was also an improvement in the colour of the oil after treatment which changed from light yellow to cream and light cream which must have been the result of the removal of some secondary metabolites responsible for colouration.

Effect of refining on lipid classes, unsaponifiable matter and vitamin composition of the oil. Refining of the seed oil of *H. crepitans* using alkali refining, degumming and bleaching methods lead to significant differences between the lipid profile of the crude seed oil and the treated oil (Table 2). Triacylglycerol is the major component of the oil. The tri, di and mono acylglycerol content of the oil increased after treatment applying all the methods. Removal of impurities and other extraneous substances from the oil might account for this increase (Haraldsson, 1983). Alkali refining had been reported to remove fatty acids, phospholipids, pigments, trace metals, sulphur, water soluble and insoluble impurities from oil, whereas degumming removes phospholipids, trace metals, pigments and carbohydrates; bleaching selectively removes pigments, oxidative products, trace metals and sulphur from the oil (Jung *et al*., 1989). Removal of these materials led to increase in the triacylglycerol content of the oil. The monoacylglycerol and the diacylglycerol content of the oil also increased by all the applied methods. Polar lipids, free fatty acid and sterol content reduced after the treatment. The highest reduction, both for the polar lipids and the sterols, was noticed in degumming. For the polar lipids, the values reduced from 4.10 ± 0.50% to 1.50 ± 0.50% and for the sterol, from 1.60 ± 1.10% to 0.20 ± 0.80%. This reduction in the polar lipids and increase in the triacylglycerol content of the oil is of great importance, being an indication of the possibility of use of this oil in the food industry and its safety in consumption.

The unsaponifiable content of the oil was reduced through application of all the methods except *n*-alkanes (Table 3)

Table 2. Effect of refining on the lipid classes (%) of HC oil

Parameter	Crude	Alkali refined	Degummed	Bleached
Polar lipid	4.10 ± 0.50^{a}	2.80 ± 0.20^{b}	1.50 ± 0.50^{c}	1.80 ± 0.70^{d}
Sterol	1.60 ± 1.10^{a}	0.40 ± 1.50^{b}	0.20 ± 0.80^{c}	0.50 ± 0.50^{d}
Diacylglycerol	2.40 ± 1.20^{a}	3.00 ± 0.80^{b}	2.20 ± 1.00^{c}	2.70 ± 1.10^{bd}
Monoacylglycerol	1.10 ± 0.50^{a}	1.20 ± 1.00^{b}	1.40 ± 0.50^{c}	1.80 ± 0.50^{d}
Triacylglycerol	85.20 ± 0.80^{a}	88.80 ± 0.50^{b}	91.60 ± 1.20^{c}	91.00 ± 0.70^{d}
Hydrocarbon	3.00 ± 0.60^{a}	3.80 ± 0.50^{b}	1.90 ± 0.30^{c}	1.30 ± 0.50^{d}
Free fatty acid	2.60 ± 0.40^{a}	ND	1.20 ± 0.50^{b}	0.90 ± 0.20^{c}

Values are mean ± standard deviation of triplicate tests; data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$); ND = not detected.

Table 3. Effect of refining methods on the unsaponifiable composition (%) of HC oil

Treatment	Triterpene alcohol	Sterol	*n*-Alkane	Unidentified
Crude oil	22.40 ± 0.30^{a}	36.50 ± 0.10^{a}	25.90 ± 0.10^{a}	15.20 ± 0.50^{a}
Alkali refined oil	30.20 ± 0.50^{b}	30.00 ± 0.30^{b}	39.60 ± 0.80^{b}	0.20 ± 0.50^{b}
Degummed oil	36.10 ± 0.40^{c}	33.20 ± 0.10^{c}	24.80 ± 0.50^{c}	5.90 ± 0.10^{c}
Bleached oil	39.20 ± 0.20^{d}	30.20 ± 0.50^{bd}	21.50 ± 0.50^{d}	9.10 ± 0.70^{d}

Values are mean ± standard deviation of triplicate tests; data in a column with different superscript letters are statistically different ($P \leq 0.05$).

whose content increased from 25.90 ± 0.10% to 39.60 ± 0.80% through alkali refining. There is significant difference between the composition of crude oil and the refined fractions applying all the methods and also among the values obtained using different methods. This may be the result of the removal of some of the extraneous materials belonging to the same functional groups as those of the identified compounds. The unidentified fractions got also reduced through treatment. The fat soluble vitamins in the oil included vitamins A, D and E. HPLC result of the vitamin content is presented in Fig. 1 while that of the bleached oil is shown in Fig. 2. These vitamins vary in amount in the oil, with vitamin A being the highest in concentration as shown in Table 4; other peaks on the graph represent compounds other than the vitamins present in the oil. On treatment, the concentration of the vitamins was reduced. Vitamin A reduced from 243.51 ppm to 196.08 ppm while vitamin D and E were not detected after treatment. Some unidentified peaks also disappeared after treatment, some of which are likely to be glycerides, hydrocarbons, peroxides or even high molecular weight fatty acids. This shows the affinity of the adsorbents used for these organic molecules (Oderinde *et al*., 2008b), though their concentrations were found to be low in the oil.

Effect of refining on the nutritional metal composition. Of all the nutritionally valuable metallic nutrients, sodium had the highest concentration (206.50 ± 0.20 ppm) which reduced (145.50 ± 0.30 ppm) after bleaching (Table 5). There were significant differences in the concentration of Na, Mg and Mn between the raw and the bleached oils. There was total removal of most of the heavy metals in the oil after treatment. Concentrations of Cu, Pb and Cd were 1.50 ± 0.30 ppm, 1.50 ± 0.40 ppm and 0.90 ± 0.20 ppm, respectively, in the crude oil but they were not detected after treatment. Removal of these toxic metals from oil by the adsorbent provides some level of safety in the use of oil in food industries. The concentration of Fe, an important part of haemoglobin, was reduced from 90.70 ± 0.10 ppm to 50.20 ± 0.10 ppm, while that of Mn, also an essential component of co-enzymes and important in growth and photosynthesis, reduced from 110.10 ± 0.50 ppm to 70.20 ± 0.10 ppm. Despite removal of some of such nutritional elements, the oil can still serve as a good source of other nutritional elements, their concentrations through bleaching still being within the tolerable limit (Abou-Arab *et al.*, 1999).

Kinetic data for bleaching of oil. As is evident from Table 6, at all the temperatures of treatment, adsorption of peroxides from the oil increased with the increase in temperature, whereas the acid value increased at different levels of treatment. This may be the result of the hydrolysis of the ester bond at these temperatures leading to liberation of fatty acids. The iodine value as well as the refractive index increased at different levels of treatment.

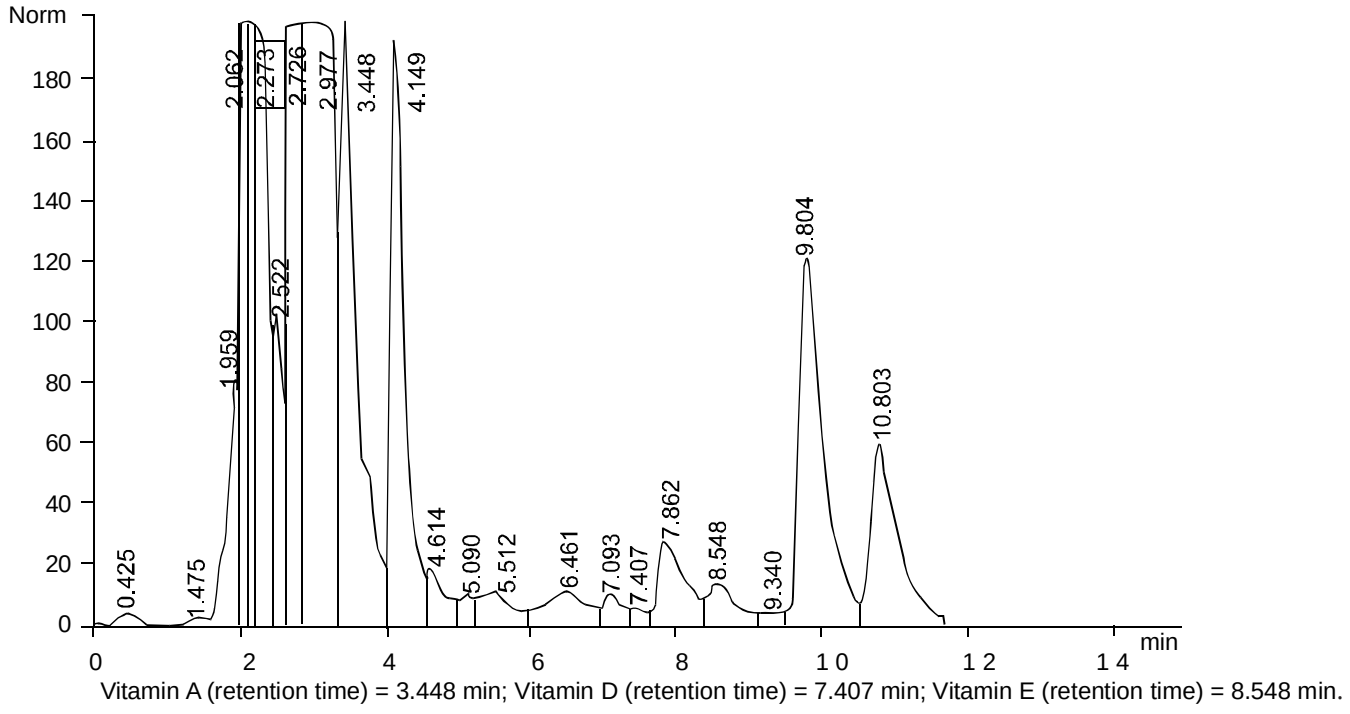

Fig. 1. HPLC of the fat soluble vitamins of HC crude oil.

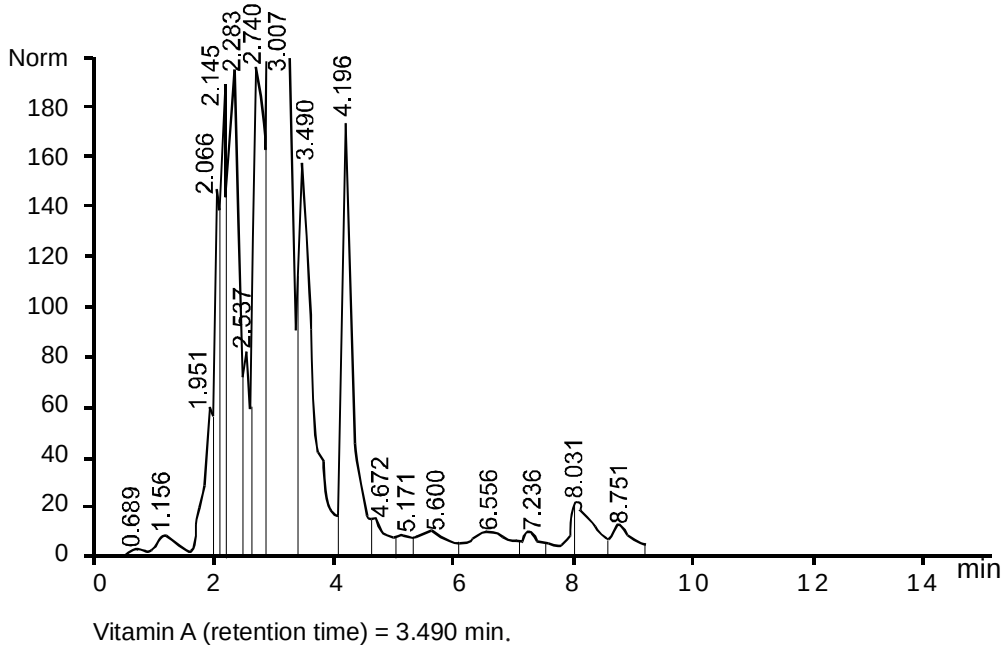

Fig. 2. HPLC of the fat soluble vitamins of HC refined oil.

Table 4. Effect of bleaching on fat soluble vitamins (ppm) of HC oil at 120 °C and 45 min

Vitamin	Crude oil	Bleached oil
A	243.51	196.08
D	2.36	ND
E	6.16	ND

ND; Not detected.

Table 5. Nutritionally valuable and trace metals (ppm) of HC oil at 120 °C and 45 min

Metal	Crude oil	Bleached oil
Na	206.50 ± 0.20[a]	145.50 ± 0.30[b]
K	154.00 ± 0.50[a]	110.20 ± 1.00[a]
Ca	145.50 ± 0.50[a]	110.10 ± 0.10[a]
Mg	152.50 ± 0.10[a]	105.50 ± 0.50[b]
Fe	90.70 ± 0.10[a]	50.20 ± 0.10[a]
Cu	1.50 ± 0.30[a]	ND
Zn	41.10 ± 0.10[a]	27.00 ± 0.10[a]
Mn	110.10 ± 0.50[a]	70.20 ± 0.10[b]
Pb	1.50 ± 0.40[a]	ND
Cd	0.90 ± 0.20[a]	ND

Average concentration ± standard deviation of triplicate determinations (ppm) (mg/kg); data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$); ND = not detected.

A first order bleaching was presumed to obtain the kinetic data (Fig. 3). 'In C_t/C_o' was plotted *vs.*'t', from which rate constant 'K' was calculated as the slope (Fig. 3.) (Van Boekel, 1996). $T_{1/2,}$ which is the time taken for half peroxide to be removed from the oil by 50% of its original value was calculated from the rate constant as '0.693/k'. The rate constant and $T_{1/2}$ are shown in Table 7. The activation energy, E_a (cal/M) was calculated as a product of gas constant, R (1.987 cal M/K) and the slope of the graph obtained by plotting 'In K' *vs.* '1/T'. The linear nature of the plot obtained in Fig. 4 gave the activation energy of the adsorption of peroxide in HC as 244.60 cal/mole.

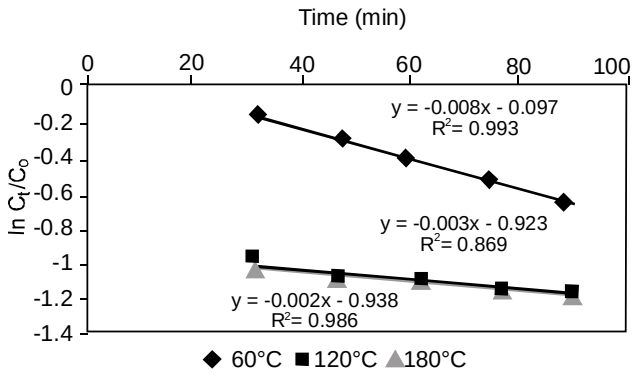

Fig. 3. First order plot of the adsorption of peroxide in the oil.

Table 6. Effect of bleaching on PV, IV, RI and AV of HC oil

Temp. (°C)	Time (min)	PV(mg/g)	IV (mg/iodine)	RI(25 °C)	AV(mg KOH/g)
60	30	1.00 ± 0.20^{a}	24.00 ± 1.20^{a}	1.5890 ±0.20^{a}	13.10 ± 0.10^{a}
	45	0.91 ± 0.10^{b}	25.80 ± 0.60^{b}	1.5890± 0.10^{b}	13.00 ± 0.50^{b}
	60	0.80 ± 0.10^{c}	28.00 ± 1.50^{b}	1.6000± 0.10^{c}	12.90 ± 0.20^{c}
	75	0.69 ± 0.20^{d}	29.70 ± 1.20^{c}	1.6020± 0.04^{d}	12.70 ± 0.20^{d}
	90	0.60 ± 0.50^{d}	30.80 ± 1.00^{d}	1.6040± 0.02^{e}	12.60 ± 0.10^{e}
120	30	0.45 ± 0.20^{a}	31.50 ± 3.10^{a}	1.6850± 0.06^{a}	6.10 ± 0.20^{a}
	45	0.40 ± 0.10^{b}	31.70 ± 6.80^{a}	1.6870 ± 0.20^{a}	6.30 ± 0.10^{b}
	60	0.40 ± 0.10^{b}	31.70 ± 2.50^{b}	1.6870 ± 0.10^{a}	6.50 ± 0.10^{c}
	75	0.38 ± 0.20^{c}	31. 91 ± 1.00^{c}	1.6890 ± 0.10^{b}	6.80 ± 0.10^{d}
	90	0.37 ± 0.20^{d}	32.10 ± 1.20^{c}	1.6900 ± 0.05^{c}	6.90 ± 0.30^{d}
180	30	0.43 ± 0.10^{a}	31.60 ± 1.70^{a}	1.6880± 0.05^{a}	6.80 ± 0.10^{a}
	45	0.41 ± 0.10^{b}	31.80 ± 2.10^{b}	1.6900± 0.07^{b}	7.10 ± 0.10^{b}
	60	0.40 ± 0.50^{c}	31.90 ± 1.50^{c}	1.3910± 0.20^{c}	7.40 ± 0.20^{c}
	75	0.38 ± 0.30^{d}	32.20 ± 0.60^{d}	1.6940± 0.10^{d}	7.60 ± 0.30^{d}
	90	0.36 ± 0.10^{e}	32.10 ± 1.00^{c}	1.6950± 0.10^{e}	7.60 ± 0.10^{d}

Values are mean ± standard deviation of triplicate determinations; data in a column with different superscript letters are statistically different ($P \leq 0.05$); PV = peroxide value; IV = iodine value; RI = refractive index; AV = acid value.

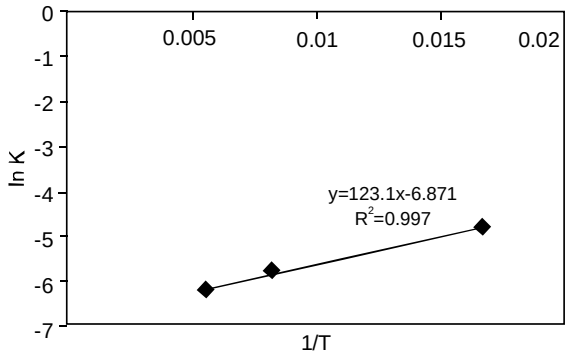

Fig. 4. Arrhenius plot for the adsorption of peroxide in the oil.

Table 7. Rate constant and half-life of adsorption of peroxide in HC oil

Temperature (°C)	Regression equation for adsorption (R^2)	$T_{1/2}$(min)
60	Y = –0.008´ + 0.097 (0.99)	86.63
120	Y = –0.003´ – 0.923 (0.87)	231.00
180	Y = –0.002´ – 0.986 (0.99)	346.50

Conclusion

It may be concluded that among different refining procedures, including degumming, alkali refining and bleaching, studied for their effects on the properties of *H. crepitans* oil, bleaching process showed a first-order kinetic with the Arrhenius plot, yielding a straight line with a slope equivalent to activation energy of 244.60 cal/mole at 120 °C and 45 min. Thus bleaching process seems to be the best technique for refining of this oil in terms of stability and improvement of the physicochemical properties of *H. crepitans* oil.

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