

Extraction of Iraqi Okra Seeds Oil and Study of its Properties During Different Periods

Isar A.S. Al-Kanani, Sawsan. A.H. Al-Hilifi* & Ali H. Abd-Al- Kareem

Department of Food Science, College of Agriculture, University of Basrah, Iraq

*Corresponding author e-mail: Sawsanali701@gmail.com

Received 19 September 2019; Accepted 18 November 2019; Available online 22 November 2019

Abstract: This study includes making comparison of Physic-chemical properties between three types of local okra seeds (*Abelmoschus esculentus* L.). Oil which is (Petra, Batera and Husayniyah). The seeds were obtained from the local markets and diagnosed. Then they cleaned, dried and selected for homogeneous seeds in form and colour. The seeds were milled and the chemical composition measured including (moisture, ash, protein, oil and carbohydrate). The extraction of oil was carried out. The antioxidant properties of the extracted oil were studied. The petra seed oil had the highest efficacy of 68%. While Husayniyah seed oil was higher than the other two types with the highest percentage of chelating ferrous ion, scavenging of hydrogen peroxide and reducing power 70.42%, 58.43% and 75.56% respectively. The extracted oil is than stored at 50°C for 60 days follow changes in its antioxidant properties during storage. All antioxidant traits had a significant decrease in the storage period, reaching the lowest values at the end of storage.

Keywords :Extraction , Okra seeds, Oil, Antioxidant Properties.

Introduction

Okra is the only botanical crop of economic importance from the Malvaceae family and is grown in all tropical and semi-tropical regions (Sharma & Prasad, 2015). Okra is the most economically rewarding vegetable (National Research Council, 2006). Okra is an economically vegetable crop (Isail & Ibn Idriss, 2013). Okra has high nutritional value because it contains important essential nutrient as it contains 85% moisture content and varying amounts of protein, fat, carbohydrates, starch and cellulose (Fellows, 2000).

The majority of protein and fat are concentrated in the nucleus of the seed, while the fibre are found in the pulp and crusts.

Also its contain an adequate amount of vitamins such as vitamin A and vitamin C and many other essential elements, such sodium, potassium, magnesium and calcium and a small proportion of trace elements Zinc, iron and nickel (Moyin-Jesu, 2007; Arapitsas, 2008). Okra contains some of vital components that contribute the regulation in the nucleus the action of some enzymes, such as, liver enzymes Alkaline Phosphatase (ALP) and Aspartate transaminase (AST). It works to improve insulin resistance and thus reduces the risk of developing diabetes (Muhammad *et al.*, 2018).

Plants are the most important natural sources of most antioxidants and their derivatives, which include phenols,

flavonoids and carotenoids, phenolic compounds have antioxidant properties because contain hydroxyl groups and phenols ring (Prochazkova *et al.*, 2011). Antioxidant are substances that are able to inhibit oxidation and reduce the proportion of the free radicals and thus prevent or limit the formation of lipids peroxides (Ozsoy *et al.*, 2008). Methew & Abraham (2006) defined Antioxidants as phenolic compound that act as protection against the negative effects of free radicals and effective oxygen classes it provides protection against the oxidation of food in addition to its work as antioxidant within the body *in vivo*, which is a class of chemical compounds that the widely spread in nature with mechanisms the most important role these compounds play is their interaction of stable and ineffective products also, when added to food, they make them less rancid, thus preserving their quality and quality helping to preserve food loner (Fukumoto & Mazza, 2000).

Process of removing free radicals by antioxidant is important for human health and life, however the presence of free radicals in the body is also necessary, as the body uses them to break down germs in addition to the production of energy (Alies, 2003). Okra contributes to being one of the best antioxidants and as a promising chemical protective agent, phenolic compounds have recently gained a lot of interest among other antioxidants by inhibiting the growth of cancer cells by being a rich source of phenolic compounds such as oligomers, catechins, hydroxyl cinnamic derivatives and flavonoids, which are the main antioxidant agents (Devi, 2017). Okra is rich in polyphenol compounds with important biological properties such as quercetin, flavinol derivatives, catechin oligomers and hydroxyl cinnamic derivatives, which play an important role as antioxidants

and their effect on the prevention of many diseases such as cardiovascular disease, diabetes and gastrointestinal diseases and some types of cancers (Gemede *et al.*, 2014).

Antioxidants are widely used as ingredients in dietary supplements to maintain health and prevent disease (Bjelakovic *et al.*, 2007). Since the industrial antioxidants BHT and BHA are unhealthy and unsafe for humans because of the health problems, the research continues natural sources such as antioxidants and their substitution for industrial antioxidants to get rid of oxidation reactions, as the most important tocopherols (Malecka, 2002), of the most common natural antioxidants tocopherols (α , β and γ) and important in plant and animal tissues where vegetable oils have higher concentrations compared to animal fats, as tocopherols as antioxidants with high effectiveness against the oxidation of animal fats than in vegetable oils (Alanbarry, 2006). Okra seed genotypes have substantial amounts of total phenols and antioxidant activity (Shui & Peng, 2004; Tian *et al.*, 2015).

The aim of this study is to investigate the effect of storage periods on the antioxidant properties of okra seeds oil .Okra is properties by its antioxidants activity in all its parts as well as seed oil rich antioxidants.

Materials & Methods

Okra seeds were obtained for the three varieties (Petra, Batera and Husayniyah) from the local markets of Maysan city in 2015/12 province for the purpose of extracting oil and conducting the study.

Methods

Dry seeds crushed and broken by ordinary mortar for each variety separately, taking into account the homogeneity of the seeds in size and shape exclude what is damaged, if any,

and grind that powder in an electric mill to get the seeds, which was dried in the heater at a 50°C for two hours and save powder seeds in airtight polyethylene bags at 4-5°C in the refrigerator until use.

Chemical Analysis

The percentage of moisture was estimated according to the method described in A.O.A.C. (2000), by taking 5g of ground seed varieties using standard drying oven on the temperature of 105 for three hours. protein was estimated in the total percentage in seeds depending on the total nitrogen and as mentioned in A.O.A.C. (2000). And using the method of semi-microkjldahl, and the total protein was calculated by multiplying the nitrogen by the coefficient of 5.7 and the percentage of ash by burning the specimens at the incinerator muffle furnace was calculated at a temperature 550 C for 16 hours according to A.O.A.C. (2000). The fat content of soxhlet It was estimated as in A.O.A.C. (2000), and hexane was used in the extraction process under 40-60°C boiling point. The carbohydrates ratio was calculated by the difference between the aforementioned compounds as Pearson (1976).

Oil Extraction

The oil was extracted from the samples of the ground okra seeds according to the method adopted by Bligh & Dyer (1959) with some modification, by taking 100 g of grounded seeds and adding 200 ml of chloroform and homogenized with an electric mixer at 2000 rpm. then the same amount chloroform was added and blended at the same speed for 30

seconds, after which the mixture was filtered by the Whitman No. 1 filter papers and collected extracted oil and the solvent rotary evaporator at a temperature 40°C and saved oil extracted in opaque glass containers in the refrigerator. The oil produced is known as cud oil.

Oil storage

Oil varieties (Petra, Batera and Husayniyah) are stored in degree 50± 5°C to 60 days and study his retirement during some Physic-chemical properties and antioxidant l adjectives.

Determination of antioxidant activity for oils

Antioxidant activity of the okra seeds was determined by the method Osawa & Namiki (1981) by preparing a mixture of 4.1 ml linoleic acid (2.5% dissolved in ethanol), 4 ml of oil and 8 ml of phosphate soluble solution 0.05M and PH 7 and 3.9 ml of distilled water, incubate the mix in sealed opaque containers at 40 °C for 24 hours. Thiocyanate oxidation was estimated by taking 0.1 ml of this mixture and adding 9.7 ml of 75% ethanol and 0.1 ml ammonium thiocyanate (30% concentration). Three minutes later, 0.1 ml of ferrous chloride was added to its concentration of 0.02 M(recorded in 3.5% hydrochloric acid). Absorbance was measured at 500 nm. The control sample was present in the same way except for the addition of chloroform instead of the sample. The percentage of inhibition of linoleic acid peroxides was calculated as% of antioxidant activity according to the following equation:

$$\text{Antioxidant activity \%} = \left[1 - \frac{\text{Absorbance of sample}}{\text{absorbance of the control}} \right] \times 100$$

Measurement of Reducing Power

Reducing power was determined by Oyaziu (1986) method of mixing 2.5 ml of oil and

BHT with 2.5 ml of sodium phosphate buffer (0.2 M and pH 6.6) and 2.5 ml of potassium ferricyanide 1% ,were added and mixed well

the mixture was incubated for 20 min at 50°C. The 2.5 ml of 1% Trichloro acetic acid was added, and the mixture was centrifuged 2000 rpm for 10 min. The top layer of solvent was separated as 5 ml distilled water and 1 ml ferric chloride 0.1% solution was added and

mixed. Absorbance was measured at 700 nm. The control sample was prepared by adding all the previous materials except the addition of 2.5 ml chloroform instead of oil models. According to the following equation;

$$\text{Reducing Power \%} = \frac{(\text{Absorbance of sample} - \text{absorbance of the control})}{\text{absorbance of the control}} \times 100$$

chelating of ferrous Iron

The connectivity of oil to chelating ferrous iron was measured according to the method created by Decker & Welch (1990) modified by Al-Hilifi (2009) involving the mixing of 2

ml of oil with 0.4 ml FeCl₂ 2 mM with 0.4 ml of 5 mM 8- hydroxy quinolone (prepared in ethanol 98%), after 10 minutes the absorbance was measured at 562 nm. The ability of the oil to chelating was calculated according to the following equation:

$$\text{Chelating of ferrous Iron} = 1 - \left[\frac{\text{Absorbance of sample}}{\text{absorbance of the control}} \right] \times 100$$

Scavenging hydrogen Peroxide activity

The hydrogen peroxide scavenging method was measured by Ruch *et al.* (1989). To determine the oil to hydrogen peroxide scavenging by mixing 1 ml of oil model with 0.6ml hydrogen peroxide was prepared in

phosphate buffer (0.1M, pH 7.4) after 10 min the absorbance was measured as 230 nm. The control sample was prepared from 1 ml phosphate buffer solution and 0.6ml hydrogen peroxide with adding oil. percentage of hydrogen peroxide scavenging of oil and standard compounds were calculated:

$$\% \text{ Scavenger} = \frac{\text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

Oil storage

Oil varieties (Petra, Batera and Husayniyah) are stored in degree 50± 5 °C to 60 days and study his retirement during some Physic-chemical properties and antioxidant 1 adjectives.

Statistical analysis

A factorial experiment with complete randomized design was used to analyse data. RLSD at 0.05 level has been utilized to compare among means (Al-Rawi & Khalafallah, 2002).

Results & Discussion

Chemical content of okra seed powder

The results in Table (1) show the content of moisture in Okra seeds (Petra, Batera and Husayniyah) as the percentage of moisture (8.16, 8.13 and 7.26%) respectively. the chemical content of the okra seeds is due to variety of seed the varieties. The results were no significant differences (P <0.05), for the seeds of (Petra, Batera & Husayniyah). The highest percentage of moisture was found in the

Petra and Batera varieties and to the lowest was in the Husayniyah, and the percentage was lower than that found in Adalakun *et al.* (2017) for Nigerian seeds types (11.08-13.35)%, but the comparable to the findings of Badgujar *et al.* (2018) for one of the Pakistani okra seeds 8.51%, while Acikgoz *et al.* (2016) reported that the percentage of moisture in one Turkish okra seeds 9.95% and were slightly higher than the recorded moisture values. Ndangui *et al.* (2010) found that the percentage of moisture in the varieties of okra seeds was 9.45%. The percentage of protein for okra types are, 24.5, 29.23 and 30.94% respectively. The results were higher than those found in Adalakun *et al.* (2017) during their study for types of Nigerian seeds, which ranged between 18.37 - 19.36 %, while the percentage of protein in this study exceeds what found Acikgoz *et al.* (2016) in the estimation of the proportion of protein for the Turkish okra seeds, which amounted to 28.21% while Hassan & Ali (2015) found that the proportion of protein for three varieties of okra seeds powder ranged 22.52-26.81, while Soares *et al.* (2012) found that the percentage of protein in Brazilian seeds 22.14%, Ndangni *et al.* (2010) has concluded that the percentage of protein in the powder seeds were 24.85%, which is an

approach to the percentage of protein for the petra seeds category and less than the two varieties. The results showed that no significantly ($P < 0.05$), differences in the percentage of ash for Okra seeds powder for the types of Husayniyah, Petra and Batera the content ash were 5.16, 5.13 and 5.1% respectively. These results were similar to those of Ndangui *et al.* (2010), Anwar *et al.* (2011) and Acikgoz *et al.* (2016) (5.68%, 5.18% and 5.21%) respectively, but higher than those found by Adalakun *et al.* (2017). The percentage of ash in the Nigerian okra seeds ranged from 2.13 to 4.40%, while Hassan & Ali (2015) found that the ash ratio of three classes of powder was between 5.76 - 10.54%.

Antioxidant properties of oil

Antioxidant activity effect of storage

The results in fig. (1) indicated that the effect of storage at 50 °C on the antioxidant activity of okra seeds oil which was the highest percentage of antioxidant activity in Petra seed oil 68.11% followed by Batera 66.28% and then Husayniyah 64.40% at zero period and all were less than the antioxidant activity of BHT 80%, and these results were similar to what Herchi *et al.* (2014).

Table (1): Chemical content of Okra seeds for Petra, Batera and Husayniyah.

The chemical content %	Seed varieties			
	Petra	Batera	Husayniyah	R.L.S.D _{0.05}
Moisture	8.16	8.13	7.45	0.866
Protein	24.5	29.23	30.94	1.706
Ash	5.13	5.10	5.16	
Fat	10.23	11.32	11.6	1.00
Carbohydrate	51.98	46.22	44.85	1.480
Caloric value Kcal/100 g	397.99	403.68	407.56	

The antioxidant activity of palm oil was 64.23%,Graham & Agbenorhevi (2017) found in a study of five varieties of okra seeds in Ghana that the highest antioxidant activity was in the range of 34.39 -53%,. A decrease in antioxidant activity was observed with the advancement of storage periods and for all types of oil. However, these decreasing values varied between the oil classes. With the continuation of the storage period, the antioxidant activity decreased to a minimum of 50.36%, 51.64% and 53.88% in seed oil Patera, Husayniyah and Petra after 60 days of storage, okra oil features a lot of antioxidant , including procyanidin compound, which is actively abducted to take advantage of the free radical root of the fatty acid of acidic acids as linoleic and oleic acid (Jarret *et al.*, 2011). This is consistent with the decrease in the antioxidant activity of the studied seed oil during different storage periods.

The reason for the low activity of antioxidant it may attributed to the extension of the oil stage is due to the low declaration of phenolic compounds in the storage, which is important to Liao *et al.* (2012) a relevant relationship between the total content of the phenolic compounds and flavonoids and antioxidant activity in okra seed. There is not necessarily a direct correlation between the total phenols and antioxidant activity,this was confirmed by Harich & Anandappa (2016) that the antioxidant activity may be due to other compounds such as vitamin E, A and others.

Reducing Power

The results in fig. (2) illustrated the effect of storage at 50 °C on the reducing power of okra seed oil of Petra, Batera, Husayniyah.

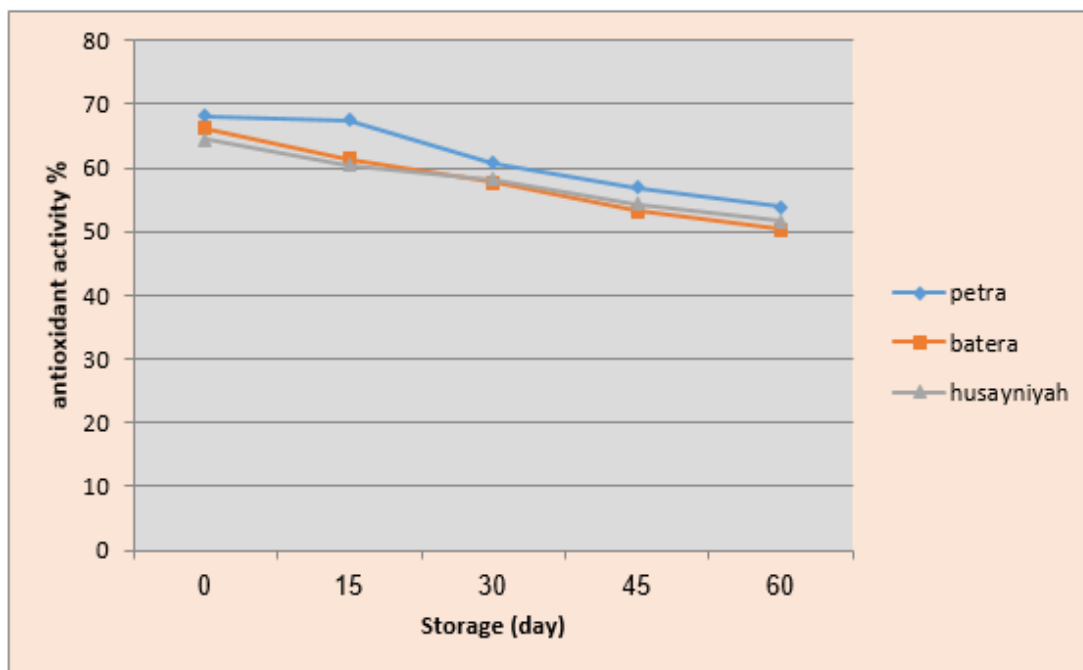


Fig. (1): Effect of storage on the antioxidant activity of the Okra seed oil.

R.L.S.D Effectiveness of oil varieties 0.5033 R.L.S.D for storage periods 0.5627
 R.L.S.D for the effect of interaction between classes and storage periods 1.1253

The results showed that were significantly ($P < 0.05$) in the reducing power values of the stored oil. Reducing power decreased during storage to its lowest of 59.21%, 60.20%, 62.43% in the seed oil of Patera, Petra and Husayniyah after 60 days of storage, the results were similar with Adetuyi *et al.* (2008) they observed decreased of reducing power during storage of six types of okra in polypropylene bags for 10 days with the highest reduction in reductive strength in Auchi okra cultivar 73.27%. The decrease in reducing power during storage may be due to

the progression of the oil storage period may be due to decrease in the active compounds such as Carotenoids, phenols, vitamins E and A, with the antioxidant activity thus, the reduction of ferric ions to ferrous. this is what Oboh *et al.* (2008) found that reductive power is directly proportional to the total content of phenols. while the Tachakittirungrod *et al.* (2007), however it has found that reductive power indicates that the antioxidant compound is an electron that can reduce intermediate substances contributing to hydrogen peroxide for motion processes.

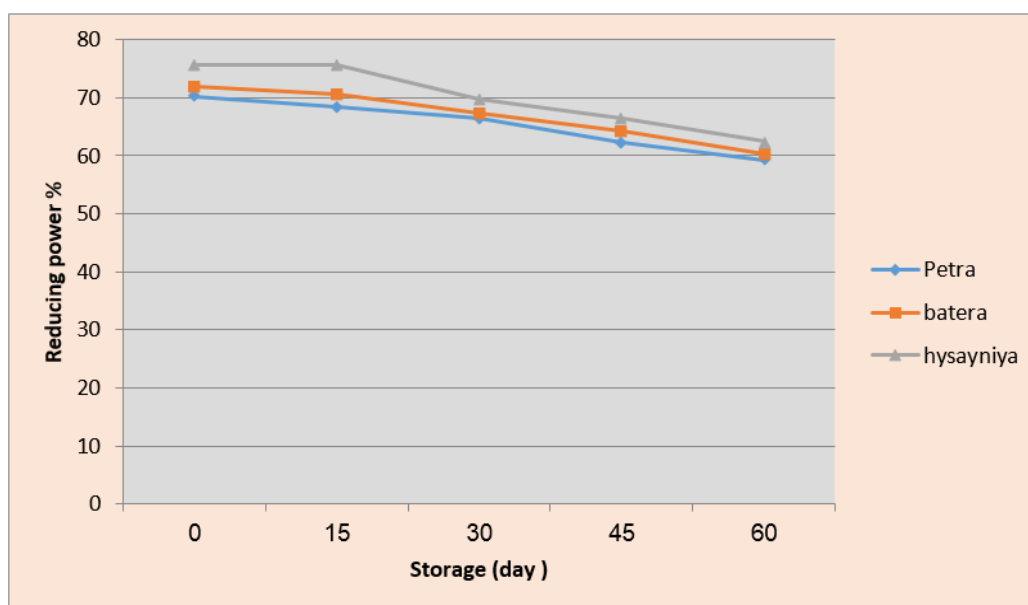


Fig. (2): Effect of storage time on reducing power of Okra seed oil.

R.L.S.D for the reduction power of oil varieties 0.5005 R.L.S.D for storage periods 0.5595.

R.L.S.D for the effect of interaction between classes and storage periods 1.1190.

Metal chelating activities

The results in fig. (3) showed the effect of storage at 50 °C on the chelating ratio of the studied Okra seed oil. The chelating at zero period was 69.37, 68.24 and 70.42% (Petra, Batera and Husayniyah) respectively. The results of the statistical analysis have indicated that their differences to the significant ($P < 0.05$) where there was a decrease in the chelating ability with the increased of storage periods and this decrease appeared in all the types of oil and if there is

a difference in the value of the decline between these c types, the lowest level was 53.38, 55.45 and 57.03% was found in the seeds of the Batera, Petra, Husayniyah after 60 days of storage. This may be due to heat and different storage periods negatively affecting the bonding potential due to the oxidation of double bonds resulting in fewer hydroxyl aggregates and consequently reduced bonding, Jarret *et al.* (2011) said the okra oil has a procyanidin compound with

multiple hydroxyl bodies and donor for hydrogen and thus connect metal ions. Nagulendran *et al.* (2009) found that the multiple hydroxyl aggregates of phenolic compounds On the grant of hydrogen and therefore the binding of metal ions such as iron and copper depends on the content of

those multiple hydroxyl stored for 10 days in polypropylene bags. The amount of Fe can be monitored and quantified by measuring the blue at 700 nm where high absorption refers to a higher activity of that susceptibility (Zarena & Sankar, 2009).

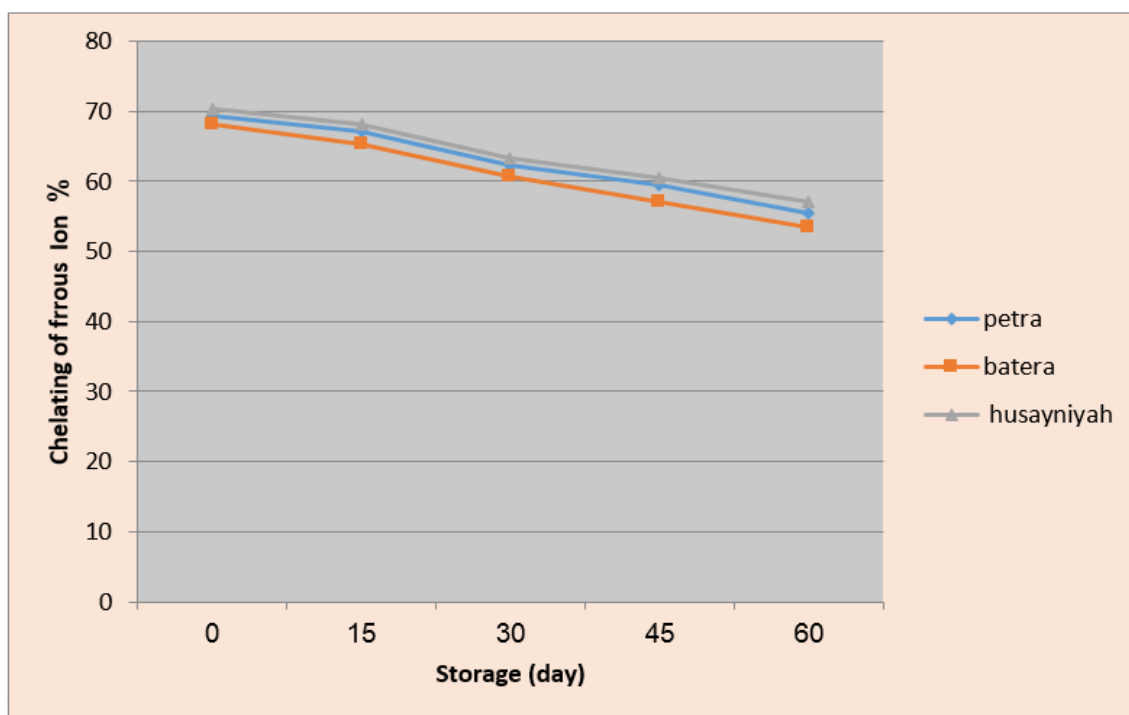


Fig. (3): Effect of the storage period on the iron bonding of the studied Okra seed oil.

R.L.S.D Likability of oil items 1.356, R.L.S.D for storage periods 1.516

R.L.S.D for the effect of interaction between classes and storage periods 3.032

Hydrogen peroxide Scavenging

The results shown in fig. (4) explained the effect of storage at 50 °C on the scavenging hydrogen peroxide from okra seed oil for the Petra ,Batera and Husayniyah types. The highest rate of scavenging was initially storage in Husayniyah seed oil 58.43%, followed by Petra seed oil 57.46% and 54% Batera. The results of the statistical analysis showed that there were significant differences at (P <0.05) in the percentage of scavenging at storage as this inability gradually decreased with the progress of storage and for all oil

types under study and the decline rates varied to reach the lowest rate 42.26%, 44.37% and 46.53% in the seeds oil (Petra, Batera & Husayniyah) after 60 days of the storage. The seeds of okra containing good amounts of phenolic compounds of the procyanidin, pronin and epicatechin, which increases their antioxidant adapters and their capabilities of free roots as the oil is a source of vehicles (Khomsug *et al.*, 2010). Yildirim (2001) has shown that there is a positive correlation between hydrogen peroxide scavenging capture

potential and phenolic compounds content and with extended storage periods and higher temperatures, this negatively affects the work of these components, Ashokkumar *et al.* (2018) in the study of the physical and chemical properties of olive oil at different storage temperatures, the total phenolic content of oil 168.53 mg.kg⁻¹ decreased to

137.22 mg.kg⁻¹ when stored at room temperature for 30 days, while the level of decline in storage at the refrigerator temperature is 165.78 mg.kg⁻¹ where the lower content of the phenolic components, means the lower the susceptibility, because phenolic components are natural antioxidants.

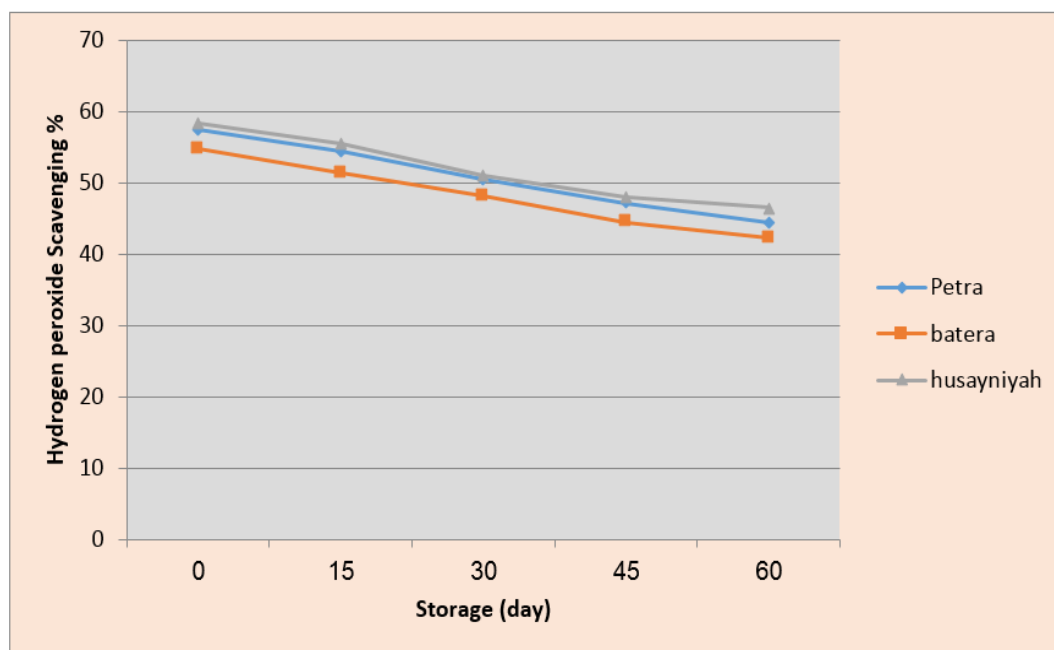


Fig. (4): Effect of storage time on the hydrogen peroxide capture potential of the studied Okra seeds oil.

.L.S.D for the ability to acquire shares Oil 0.2764, R.L.S.D for storage periods 0.3090
R.L.S.D for the effect of interaction between types and storage periods 0.6180

Conclusions

The results found that the antioxidant and free radical scavenging activity of okra seeds oil were decrease during storage 60 days, and they oils high phenolic, carotenoids and vitamins E, A and C contents showed good antioxidant properties such as antioxidant activity, chelating and scavenging hydrogen peroxide. reducing power, therefore can be the oil of okra seeds considered for prevention and treatment of human diseases and its complication as potent antioxidant.

Acknowledgement

The authors are grateful to staff of Department of Food Sciences, College of Agriculture, University of Basrah, for using all laboratory facilities.

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