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Oxidative stability of sunflower oil as affected by *Carica papaya* leaves extracts during accelerated oxidative storage

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Abstract

Papaya (*Carica papaya* L.) is normally called as paw-paw and it belongs to the family caricaceae. Papaya is normally known for its food and nutritional values throughout the world. This research aims to study the potential of *carica papaya* (CP) leaves extracts for the prevention of sunflower oil oxidation. Extraction was used by soaking method on CP leaves using distilled water, ethanol 70% v/v and methanol 80% v/v at 10% w/v concentration and qualitative test of phytochemical components. Phytochemical screening of ethanol, methanol and aqueous extracts revealed the presence of phenol compounds, flavonoids, tannins , terpinoids, glycosides and steroides in ethanol and methanol extracts whereas Saponinns were present in methanol and aqueous extracts. The bioactivities of the leaves extracts were attributed to their phytochemical constituents. Analysis of heated sunflower oils demonstrated significant increases in peroxide values (PV), acid values (AV) and T.B.A. However, iodine values (IV) of the oils were markedly decreased. Results indicated that CP-Et 600 gave the lowest percentage change in AV, PV and T.B.A and the highest value in IV of sunflower oil, followed by CP-Mt 600 when compared with sunflower oil in the presence of the other antioxidant during the storage period. CP extracts i.e., methanolic, ethanolic, and water protected protective effect against oxidation of sunflower oil and can serve as substitutes for synthetic antioxidants. The highest antioxidant activity occurred in 70% ethanol extract.

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Introduction

Oxidation is one of the major causes of deterioration of fats and oils leading to the development of rancid odours and taste, and causing a reduction in the shelf life of the fat or oil. Oxidation can also decrease the nutritional quality and safety of lipids through the formation of toxic products in foods after cooking and processing (Moure *et al.*, 2001).

Vegetable oils with higher contents of unsaturated fatty acids are more susceptible to the oxidation (Mohdaly *et al.*, 2010), especially when the oil is exposed to oxygen, light, high temperatures or trace Lipids oxidation during food processing or storage led to changes in organoleptic properties, decrease shelf life and nutritional value of the food (Choe and Min, 2007;Iqbal and Bhanger, 2007; Katragadda *et al.*, 2010.).

Sunflower oil contains about 59% of polyunsaturated fatty acids (PUFA), linoleic acid and 30% of monounsaturated fatty acids (MUFA), oleic acid (Normand *et al.*, 2001). Due to its high PUFA content, sunflower oil is highly susceptible to lipid oxidation (Normand *et al.*,2001 and Aladedunye and Przybylski, 2009). The lipid oxidation of sunflower oil not only can produce rancid odors, unpleasant flavor and discolouration but can also decrease nutrition al quality and safety due to degradation products.

Antioxidants are chemical compounds are substance that inhibit oxidation or that retards deterioration by oxidation, especially of fat, oils, and foods. The same examples of antioxidants are vitamins A, C and E, β-carotene, enzymes catalase, superoxide, dismutase and various peroxidases (Subramanian *et al.*,2014).
Both natural and synthetic antioxidants are widely used in protection oils from oxidation (Frutos and Hernandez-Herrero, 2005).

The important antioxidants used in the food industry are butylatedhydroxyanisol (BHA), butyl1-4-hydroxytoluene (BHT), tertbutyl hydroquinone (TBHQ). TBHQ has been found to be the most effective antioxidant (Guzman *et al.*, 2009; Pimpa *et al.*, 2009).

The synthetic antioxidants have been suspected to cause or promote harmful effects to the health (Ozcan and Arslan, 2011; Chen *et al.*, 2014) So that, there are increasing demand for using natural antioxidants from plant sources, which characterized with high Phenolic compounds content can act as either radical scavengers or metal chelators resulting in retardation lipid oxidation (Sikwese and Duodu, 2007).

Carica papaya, belongs to the family of Caricaceae and a few types of Caricaceae have been utilized as cure against a variety of diseases (Mello *et al.*,2008). All parts of the plant can be used by people for food or for medicinal purposes (Dawkins *et al.*, 2003; Fakeye *et al.*, 2007). Its fruits, leaves and flowers are edible. The leaves of papaya have been appeared to contain many active components that can increase the total antioxidant activity and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenic-glucosides and glucosinolates (Otsuki *et al.*, 2010).Papaya leaves are utilized to cook in some tropical countries which contain high calories than papaya fruit (Subramanian *et al.*, 2014).

The aim of this work is to carry out a phytochemical analysis of some extracts of *Carica papaya* leaves and evaluate the antioxidant effectiveness of ethanol, methanol and aqueous extracts of CP in preventing sunflower oil rancidity under 20 days of accelerated storage conditions. Oxidative changes were monitored by the peroxide value, iodine value, 2-thiobarbituric acid reactive substances and acid value, as well as the calculated total oxidation value.

Materials and methods

Preparation of Carica Papaya leaf extracts

Fresh leaves of CP used for this research were collected from Minia University farm. The leaves of CP were washed with distilled water, air-dried for two weeks and ground to fine powder with a mill. Ground powdered leaves were soaked in distilled water , ethanol 70% v/v and methanol 80% v/v at 10% w/v concentration at room temperature under stirring for three hours and filtered Plant: solvent ratio was 1:10. The soaking and filtration were repeated twice.

The solvents were removed by rotary evaporation at 45°C. Concentrated extracts were weighed to find the extraction efficiency on dry weight basis. The extracts were stored in the refrigerator prior to analysis. Extraction efficiency was calculated as follows;

Extraction efficiency % = (Final dry weight of extract ÷ Initial weight of dried plant Material) × 100 (Shilpakar *et al.*, 2011).

Phytochemical screening

Presence or absence of selected phytochemicals: terpenoids, phenols, glycosides, flavanoids, steroids tannins and glycosides in each extract was determined by performing qualitative chemical tests as described by Harborne, 1987.

Sample preparation

CP extracts 300 and 600 ppm, gallic acid , citric acid and synthetic antioxidants TBHQ at 200 ppm level, for each were added to virgin sunflower oil before being subjected to oven test to evaluate their capability in retarding the oxidation processes. Control samples bearing no antioxidants were also placed under the same storage conditions.

Oven test

Samples of oil 10 g were placed in a separate 50 mL open beakers and held in an oven at 65°C for up to 0, 5, 10, 15, 20 days. After each storage period, oil samples were immediately analyzed. The temperature of 65°C was used as a rapid method to simulate the storage in real conditions (Besbes*et al.*, 2004) and each day under such oven storage test at 65°C is equivalent to one month of the storage at ambient temperature (Chong *et al.*, 2015; Yim *et al.*, 2013).

Chemical analysis

Determination of acid value

Each oil sample 1.0 g was weighed and dissolved with 50 ml of ethanol in a conical flask. Two drops of phenolphthalein indicator were added and titrated to pink end point which persisted for 15 minutes with 0.1 N potassium hydroxide solution KOH. Acid value was calculated according to the method described by Okpuzor*et al.*, 2009:

Acid value = 56.1 × V× C / m

Where 56.1 is equivalent weight of KOH, V is the volume in ml of standard volumetric KOH solution used, C is the exact concentration in KOH solution used 0.1 N; m is the mass in grams of the test portion 1 g.

Determination of iodine value

The iodine value (IV) of an oil is a measure of its level of unsaturation. It is defined as the number of grams of iodine that is added to 100 gram of oil Allen, 1955. The iodine value was calculated from the equation,

1cm^3 of 0.1N $Na_2S_2O_3$≡ 0.01269 g of iodine

IV = 1.26 (a-b)/w

Where, a = volume of 0.1N $Na_2S_2O_3$for the blank. , b = volume of 0.1N $Na_2S_2O_3$for the sample and w = weight of the sample

Determination of Thiobarbituric acid (T.B.A.)

Thiobarbituric acid value TBA, the method of Sidwell *et al.* 1954 was conducted to determine the TBA value as follows. A known weight of oil 3g was dissolved in a carbon tetrachloride 10ml followed by the addition of TBA reagent 10ml, 0.67% TBA in 50% acetic acid.

The mixture was transferred to a separatory funnel and the aqueous layer was drawn into a test tube and immersed in a boiling water bath for 30 min. The absorbance of the developed pink colour was then recorded at 532nm against a blank reagent.

Determination of peroxide value (PV)

The peroxide value was determined according to AOAC method 2000 A known weight of the oil sample 2.5 g was dissolved in a mixture consisted of glacial acetic acid: chloroform 30 ml, 3 : 2, v/v then 1 ml solution of freshly prepared saturated potassium iodide was added. Distilled water 30 ml was added then titrated slowly with sodium thiosulphate solution 0.1N.

The antioxidant activity calculated while using the following equation :

100-(PV sample ÷ PV control ×100).

Statistical analysis

Statistical analysis were performed in triplicate, Values are the mean of six determinations ± SD Kenney and Keeping, 1962 and the ANOVA analysis using the MASTATC program vergion 3 and means

were compared using L.S.D.-rang according to Gomez and Gomez,1984.

Results and discussion

Extraction yield

The percentage yield extract of the different used solvents was shown in Table 1.

Tabulated data showed that the yield of aqueous extract 5.1% had the lowest value compared to the two other extracts. Based on statistical analysis, it appears that the yield of ethanol extract 70% and 80% methanol extract were not significantly different, but the yield of both extracts were significantly different with Aqueous extract Table1.

Table 1. Percentage yield extract of the different *Carica papaya* leaves extracts.

Samples	Solvent	Yield corrected %
Carica papaya leaves	Ethanol 70%	9.9^{A}
	Methanol 80%	9.6^{A}
	Aqueous	5.1^{B}

A, B show different correlation or not significantly different between data.

Phytochemical screening

Phytochemicals occur naturally in plants and they were responsible for colour and organoleptic properties. Saidu and Nweri,2013 indicated that phytoconstituents in fruits and vegetables may reduce the risk of cancer possibly due to dietary fibers, polyphenols, antioxidants and anti-inflammatory effects .Phytochemical screening of different extracts of CP showed the presence of different group of active constituents in ethanol, methanol and aqueous extracts(Table 2).

Table 2. Qualitative phytochemical analyses of *Carica papaya* leaves extracts.

S. No	Tests	Ethanol	Methanol	*Aqueous*
1	Phenol	+++	++	+
2	Flavonoid	+++	++	+
3	Saponinns	-	+	++
4	Tannins	++	+++	+
5	Terpinoids	+++	++	+
6	Glycosides	++	+	-
7	Steroides	+	++	-

+Slightly present, + + Moderately present, + + + Highly present, - Absent.

The results obtained were tabulated as follows. As per result ethanol extract contained phenol, flavonoids, tannins, terpinoids, glycosides and steroids.

The methanol extract contained phenol, flavonoids, saponins,tannins, terpinoids glycosides and steroids. And aqueous extract contained phenol, flavonoids, saponins,tannins and terpinoids .

These findings were in agreement of similar nature of study conducted by Okoye, 2011; Sumathi and Gowthami, 2014; Itani *et al.*, 2017. This observation indicates that the difference in activity could be due to the differences in the phytochemical composition of the extracts (Arunkumar and Muthuselvan, 2009;Yebpella *et al.*, 2011).

These phytocompounds are known to be biologically active and are believed to be responsible for the observed antibacterial effects.

Effect of Carica papaya leaves extracts on acid values of sunflower oil under accelerated storage at 65 ^{o}C for 20 days as meq O_2/ Kg oil

Formation of free fatty acids might be an important measure of rancidity of fats and oils. An increase in the amount of free fatty acids in an oil or fat sample indicates hydrolysis of the triglycerides (Frega *et al.*, 1999).

Acid values (AV) were used as a measure of the formation of acidic compounds and secondary products dissent formed during oxidation (Chavan *et al.*,1992).

Results showed that the acid value increased gradually in all sunflower oil samples during storage Table 3.

Table 3. Effect of *Carica papaya* leaves extracts on acid values of sunflower oil under accelerated storage at 65 °C for 20 days as meq O_2/ Kg oil.

A.V	Zero	5 days	10 days	15 days	20 days
Control	0.086^{W}±0.04	1.498^{OPQ} ±0.3	3.972^{C} ±0.2	4.68^{B} ±0.3	7.013^{A} ±0.2
TBHQ	0.086^{W}±0.04	0.496^{UV}±0.2	1.342^{PQR}±0.1	2.541^{JK}±0.1	3.281^{EF}±0.1
Citric acid	0.086^{W}±0.04	0.531^{UV}±0.2	1.122^{RS} ±0.2	2.712^{HIJ}±0.2	3.012^{FGH}±0.2
Gallic acid	0.086^{W}±0.04	0.422^{VW}±0.1	1.240^{QR} ±0.3	2.089^{LM} ±0.3	3.189^{FG} ±0.1
CP -Et300	0.086^{W}±0.04	0.862^{ST}±0.1	1.744^{MNO}±0.1	2.572^{JK} ±0.2	3.582^{DE} ±0.2
CP-Et600	0.086^{W}±0.04	0.727^{TUV} ±0.2	1.524^{OPQ} 0.1	2.015^{LMN}±0.1	3.124^{FG} ±0.1
CP-Mt300	0.086^{W}±0.04	0.874^{STU} ±0.3	1.805^{MNO}±0.2	2.924^{GHI}±0.2	3.792^{CD} ±0.2
CP-Mt600	0.086^{W}±0.04	0.761^{TUV}±0.40	1.683^{NOP}±0.2	2.012^{LMN}±0.3	3.280^{EF} ±0.2
CP-W 300	0.086^{W}±0.04	0.987^{TUV} 0.2	1.963^{LMN}±0.3	2.251^{KL} ±0.4	3.875^{CD} ±0.1
CP-W600	0.086^{W}±0.04	0.881^{ST}±0.4	1.748^{MNO}±0.1	2.642^{IJ}±0.1	3.773^{CD} ±0.3
LSD0.05	0.3479				

All Values are means± SD of 3sample .Means in a row with superscripts without a common letter differ, P<0.05.

The increasing of AV may be due to occurrence hydrolysis in oils result to heating (Rabie *et al.*, 2014).However, the rate of increase was dependent on antioxidant used.

The acid value of the control was 0.086, after 20 days it reached 7.013 oil. Whereas, the values of the AV of sunflower oil containing CP extracts were less.

This indicated that CP extracts slowing the hydrolysis of the triglycerides during heating. Based on statistical test, addition of CP extracts, citric acid and gallic acid as well as TBHQ, resulted in significant decrease in AV p<0.05 relative to the control during heating process. Accordingly, it could be concluded that CP extracts had obvious antioxidant activity following the order: CP-Et 600>CP-Mt 600> CP-Et 300> CP-Mt 300> CP-W 600> CP-W 300.

Effect of Carica papaya leaves extracts on iodine values of sunflower oil under accelerated storage at 65 °C for 20 days as meq O_2/ Kg oil

Sunflower oil contains more than 70% polyunsaturated fatty acids (PUFAs). These PUFAs are highly prone to lipid oxidation (Zhang *et al.*, 2010).

During storage, the double bonds of these PUFAs are attacked by free radicals, which results in the formation of conjugated bonds (Kanner *et al.*, 2012). Hence, measuring the amount of unsaturated fatty acids present in sunflower oil can be used as a reference to determine the freshness of the oil (Winne Sia *et al.*, 2014).

The freshness of sunflower oil can be determined quantitatively by adding iodine monochloride to the oil samples. The unsaturated fatty acids react with iodine monochloride to release free iodine. The free iodine can then react with sodium thiosulphate for the determination of iodine value (IV)(O'Keefe and Pike, 2010).Abdulkarim *et al.* 2007 reported that the IV indicates the degree of unsaturation of oils. Anwar *et al.* 2007;Nor *et al.* 2008 demonstrated that the IV values greatly reduced with the increase of storage time.

The IV values of the stabilized *Carica papaya* leave extracts, TBHQ, citric acid, gallic acid and control sunflower oil, over an accelerated storage period of 20 days at 65°C, was shown in table 4.

Table4. Effect of Carica papaya leaves extracts on iodine value of sunflower oil under accelerated storage at 65 °C for 20 days as meq O_2/ Kg oil.

I.V	Zero	5 days	10 days	15 days	20 days
Control	$110.5^{A}\pm2$	$089.7^{GH}\pm1.2$	$77.9^{KL}\pm2.0$	$65.6^{ST}\pm3.2$	$53.5^{V}\pm2.2$
TBHQ	$110.5^{A}\pm2$	$098.6^{EF}\pm1.2$	$84.3^{IJ}\pm3.0$	$74.2^{MNO}\pm1.4$	$66.5^{ST}\pm2.0$
Citric acid	$110.5^{A}\pm2$	$102.9^{BCD}\pm2.5$	$89.6^{GH}\pm1.1$	$78.4^{K}\pm2.5$	$67.2^{RST}\pm3.5$
Gallic acid	$110.5^{A}\pm2$	$101.9^{BCDE}\pm4.3$	$85.8^{IJ}\pm2.1$	$75.6^{KLMN}\pm3.2$	$67.0^{RST}\pm3.1$
CP -Et300	$110.5^{A}\pm2$	$099.7^{DEF}\pm2.2$	$84.5^{IJ}\pm3.1$	$74.4^{LMNO}\pm2.6$	$65.7^{ST}\pm1.2$
CP-Et600	$110.5^{A}\pm2$	$105.4^{B}\pm3.4$	$87.5^{HI}\pm2.2$	$77.6^{KLM}\pm1.6$	$69.1^{QRS}\pm1.1$
CP-Mt300	$110.5^{A}\pm2$	$092.4^{G}\pm2.0$	$82.6^{J}\pm1.2$	$73.7^{NOP}\pm2.1$	$64.4^{T}\pm1.3$
CP-Mt600	$110.5^{A}\pm2$	$103.3^{BC}\pm1.3$	$85.4^{IJ}\pm2.0$	$75.7^{KLMN}\pm1.2$	$68.6^{QRS}\pm2.4$
CP-W 300	$110.5^{A}\pm2$	$097.8^{F}\pm2.1$	$83.2^{J}\pm3.1$	$72.9^{NOP}\pm1.0$	$63.8^{TU}\pm1.0$
CP-W600	$110.5^{A}\pm2$	$100.4^{CDEF}\pm1.0$	$82.9^{J}\pm1.5$	$71.3^{OPQ}\pm3.0$	$60.4^{U}\pm2.1$
LSD0.05	3.570				

All Values are means± SD of 3sample .Means in a row with superscripts without a common letter differ, P<0.05.

It was observed that the IV value decreases significantly p <0.05 with the increase time of storage for all samples. The rate of reduction in control was higher than that in sunflower oil containing both synthetic and natural antioxidants. A decreasing IV value of oils is generally attributed to the destruction of the fatty acid double bonds caused by oxidation process.

Throughout the 20 days storage period, the total reduction in IV among the samples were in the following order: Control > CP-W 300 >CP-W 600>CP-Mt300 >CP-Et 300 > TBHQ>Citric acid> Gallic acid > CP-Mt 600 >CP-Et 600(Table 4).

From this, it can be deduced that *carica papaya* extracts antioxidant is higher effective as the synthetic antioxidants in preventing lipid oxidation.

Table 5. Effect of *Carica papaya* leaves extracts on peroxide values of sunflower oil under accelerated storage at 65 °C for 20 days as meq O_2/ Kg oil.

	Zero	5 days	10 days	15 days	20 days
Control	$8.5^{O}\pm1.0$	$19.5^{M}\pm1.7$	$31.1^{JK}\pm4.7$	$43.9^{EF}\pm1.4$	$65.2^{A}\pm6.8$
TBHQ	$8.5^{O}\pm1.0$	$14.5^{N}\pm2.1$	$24.8^{L}\pm4.4$	$34.8^{HIJ}\pm1.6$	$47.9^{CDE}\pm3.2$
Citric acid	$8.5^{O}\pm1.0$	$14.9^{N}\pm1.3$	$25.5^{L}\pm6.3$	$36.5^{GHI}\pm2.7$	$47.2^{CDE}\pm2.4$
Gallic acid	$8.5^{O}\pm1.0$	$14.5^{N}\pm0.9$	$26.0^{L}\pm2.8$	$36.9^{GH}\pm1.0$	$47.5^{CDE}\pm3.7$
CP -Et300	$8.5^{O}\pm1.0$	$15.3^{MN}\pm1.7$	$25.7^{L}\pm5.2$	$35.5^{GHIJ}\pm4.7$	$49.6^{CD}\pm4.5$
CP-Et600	$8.5^{O}\pm1.0$	$12.8^{NO}\pm1.2$	$24.5^{L}\pm3.7$	$32.2^{IJ}\pm3.4$	$44.5^{E}\pm5.8$
CP-Mt300	$8.5^{O}\pm1.0$	$15.6^{MN}\pm3.0$	$26.6^{KL}\pm2.0$	$38.7^{GH}\pm3.9$	$50.5^{C}\pm4.9$
CP-Mt600	$8.5^{O}\pm1.0$	$15.9^{MN}\pm0.6$	$25.8^{L}\pm2.5$	$35.6^{GHIJ}\pm2.8$	$45.6^{DE}\pm2.0$
CP-W 300	$8.5^{O}\pm1.0$	$16.1^{MN}\pm1.0$	$26.2^{L}\pm5.7$	$39.7^{FG}\pm1.6$	$56.5^{B}\pm3.1$
CP-W600	$8.5^{O}\pm1.0$	$15.7^{MN}\pm2.0$	$25.8^{L}\pm3.8$	$36.7^{GHI}\pm1.2$	$50.8^{C}\pm2.4$
LSD0.05	4.554				

All Values are means± SD of 3sample .Means in a row with superscripts without a common letter differ, P<0.05.

Effect of Carica papaya leaves extracts on Peroxide values of sunflower oil under accelerated storage at 65 °C for 20 days as meq O_2/ Kg oil

Peroxide value (PV) measures hydroperoxide products formed in the initial stages of lipid oxidation. Peroxide value is one of the most widely-used tests for the measurement of oxidative rancidity in oils and fats. In this work, oxidation degree on sunflower oil samples was determined by measuring PV in the absence and presence of antioxidants at 65 °C for 20 days. The influence of antioxidants during storage on PV in the sunflower oil samples are shown in shown in Table 5.

Results showed that peroxide value increased with storage time and increased in acceleration after the fifth day.

The PV increases only when the rate of peroxides formation exceeds that of its destruction Poiana, 2012 .Sunflower oil samples without the antioxidant control reached a maximum PV of 65.2 ± 6.8 meq/kg after 20 days of storage. A significant difference $p < 0.05$ in PV was observed between the control and sunflower oil samples containing natural and synthetic antioxidants, all of which slowed the rate of peroxide formation.

Table 6. Antioxidant activity for different samples during oven test. Calculated as 100-(PV sample /PV control ×100). (According to Rafiee *et al.*, 2012).

	5 days	10 days	15 days	20 days
TBHQ	25.9^{CD} ±4.3	20.5^{IJKL} ±2.2	20.7^{HIJKL} ±1.1	26.3^{C} ±2.8
Citric acid	23.6^{EFG} ±0.0	18.8^{LMNO} ±8.1	16.9^{OPQ}±3.5	27.3^{C} ±3.9
Gallic acid	25.5^{CDE} ±1.9	16.0^{Q} ±3.7	15.9^{Q} ±0.4	27.0^{C}±1.9
CP -Et300	21.7^{GHIJ} ±1.9	17.8^{MNOPQ} ±4.4	19.3^{KLMN} ±8.1	23.9^{DEF} ±1.1
CP-Et600	34.4^{A} ±0.4	21.2^{HIJK} ±0.0	26.8^{C} ±5.4	33.9^{AB} ±2.1
CP-Mt300	20.5^{IJKL} ±8.5	13.8^{R} ±6.7	11.9^{R}±6.1	22.6^{FGH} ±0.5
CP-Mt600	18.2^{MNOP} ±4.1	16.6^{PQ} ±4.6	18.9^{LMNO} ±3.8	31.9^{B}±1.8
CP-W 300	17.3^{NOPQ} ±2.1	16.3^{PQ}±5.8	9.6^{S}±0.76	13.1^{R}±4.3
CP-W600	19.7^{JKLM} ±3.3	17.0^{OPQ} ±0.3	17.2^{OPQ}±1.4	21.8^{GHI}±4.5
LSD0.05	2.062			

All Values are means± SD of 3sample .Means in a row with superscripts without a common letter differ, P<0.05.

This indicates that without addition of antioxidants to vegetable oil, there may be rapid deterioration during storage period. The presence of CP-Et 600 gave the lowest percentage change in peroxide value of sunflower oil, followed by CP-Mt600 when compared with sunflower oil in the absence of the antioxidant during the storage period. Results reported by other authors indicate that only extracts from plants with high antioxidant activity cause a slowing down of peroxide formation in oils with the comparable level of unsaturated fatty acids (Anwar *et al.*, 2006).

Antioxidant activity

The antioxidant efficacy of all tested antioxidants during storage are shown in Table 6.Results showed that the antioxidant activity of CP-Et 600 on the fifth day was 34.4 as meq O_2/ Kg which reached 33.9 as meq O_2/ kg of sunflower oil on the 20 th day. On the other hand, in other treated samples i.e, CP-Mt 600 and CP-W 600, this value increased from the fifth day to the 20 th day, this can be attributed to the oxidation phenomenon of antioxidants during their storage period.

Effect of carica papaya leaves extracts on T.B.A values of sunflower oil under accelerated storage at 65 ^{o}C for 20 days as mg malonaldhyde /kg oil.

The most common method for measuring oxidative changes in the biological samples and food products is the TBA test (Che and Tan 1999; Rania, 2001). In fact, TBA test is more reliable than P.V in determining oil deterioration as it measures the secondary stages of oxidation or accumulation of secondary products. It was assumed that these products are accumulated during heating (Ramadan, 1998; Rania, 2001).

Results in Table 7 mentioned that TBA values of samples were increased during heating process. For control sample, TBA value was increased sharply from 1.144±0.5mg malonaldhyde/Kg oil to 12.65±1.0 mg malonaldhyde/Kg oil after 20 days at 65°C, but TBA values for treated samples were increased gradually. Oil samples which treated with CP at concentration of 600PPM was more effective than other treated oil samples with antioxidants. On the other hand, the results of CP -Et at concentration of 600 PPM were nearly close to the other treated oil with TBHQ.

Table 7. Effect of carica papaya leaves extracts on T.B.A values of sunflower oil under accelerated storage at 65 °C for 20 days as mg malonaldhyde/kg oil.

T.B.A	Zero	5 days	10 days	15 days	20 days
Control	1.144^{W}±0.5	3.276^{T} ±1.1	5.487^{MNO}±1.2	8.50^{BCD} ±0.8	12.65^{A} ±1.0
TBHQ	1.144^{W}±0.5	1.850^{UV} ±0.2	4.024^{RS}±0.5	5.89^{KLM} ±0. 3	6.89^{IJ} ±0.4
Citric acid	1.144^{W}±0.5	1.721^{UVW} ±0.1	3.939^{RS}±0.2	5.68^{LMN} ±0.1	7.64^{FGH} ±0.1
Gallic acid	1.144^{W}±0.5	1.587^{VW} ±0.1	4.114^{R}±0.3	6.01^{KLM} ±0.1	7.84^{EFG} ±0.3
CP -Et300	1.144^{W}±0.5	2.047^{UV} ±0.3	4.758^{PQ}±0.2	7.09^{HI} ±0.2	8.98^{B} ±0.3
CP-Et600	1.144^{W}±0.5	1.529^{VW} ±0.2	3.471^{ST}±0.5	4.89^{OPQ} ±0.3	6.28^{JKL} ±0.2
CP-Mt300	1.144^{W}±0.5	2.102^{UV} ±0.5	4.407^{QR} ±0.6	7.65^{FGH} ±0.1	8.34^{CDE} ±0.2
CP-Mt600	1.144^{W}±0.5	1.616^{VW}±0.3	3.276^{T}±0.3	5.26^{NOP} ±0.2	7.37^{GHI} ±0.2
CP-W 300	1.144^{W}±0.5	2.250^{U} ±0.4	4.096^{R}±0.4	7.82^{EFG} ±0.2	8.97^{BC} ±0.3
CP-W600	1.144^{W}±0.5	2.127^{UV} ±0.2	4.536^{QR}±0.5	6.35^{JK} ±0.3	8.00^{DEF} ±0.4
LSD0.05	0.6134				

All Values are means± SD of 3sample .Means in a row with superscripts without a common letter differ, P<0.05.

The results of the present study are in agreement with that of Semwal and Arya, 2001 who reported that thiobarbituric acid values on storage for oils increase the storage period of oils.

In conclusion, this work reveals that ethanol extract of Carica papaya CP leaves exhibited the highest antiradical activity among other solvents and showed the highest antioxidant capacity compared to synthetic and natural food antioxidants such as TBHQ, Citric acid and Gallic acid.

In general, it could be concluded that the CP-Et600 had the most potent antioxidant activity and secured protective effect against oxidation of oils and can serve as substitutes for synthetic antioxidants.

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