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Antioxidative Activity of Nanoparticles of Rosemary

Rania E. El-Gammal

Food Industries Dept., Faculty of Agric. Mansoura University, Egypt

Abstract : This work aimed to study the antioxidative activity of nanoparticles ZnONps Zinc oxide using rosemary water and ethanolic extracts .Structural and optical properties of nanoparticles using transmission electron microscope showeda spherical shape of ZnO nanoparticles of average particle size 2.8 to 3.8 nm . Zinc oxide nanoparticles of rosemary water and ethanolic extract was added to sunflower oil (200 and 300 ppm) as natural antioxidant compare with Tertiary Butylhydroxy Quinone (TBHQ) as synthetic one. Oxidative stability and thermal process for 12 hours at 120^{o}C for treated sunflower oil with different antioxidants were estimated. Obtained results indicated that the total phenolic contents being 45.44 and 58.36 mg of GAE/g. in water and ethanolic rosemary extract respectively, while total flavonoids being 22.58 and 88.26 in the same extracts respectively .Rosemary ethanolic extract exhibited the highest DPPH activity in compare with the water extract .Identification and fractionation of phenolic compounds using HPLC cleared that ten phenolic compounds(162.6 and 88.32 mg/100g) were separated. Cinnamic acid the most abundant phenolic compound in rosemary extracts was, while rosmarinic was the most abundant flavonoids compound in the same extracts 78.83 and 67.91 mg /100 g. respectively. Obtained data for oxidative stability using rancim at showed that treated sunflower oil with ZnONps rosemary ethanolic extract at the concentration of 200 and 300 ppm the highest showed stability time (20 months of storage) in compare with other treated sunflower oil samples**.** Results for thermal process of treated sunflower oil ZnONPs using RME at the concentration of 300 ppm were recorded the lowest values of hydrolysis, oxidation and rancidity parameter after 12 hours heating. So, addition of ZnONps rosemary extract to sunflower oil showed a positive effect on the oxidative and thermal stabilities of such raw material and could be recommended as an alternative antioxidant in oil.

Keywords: Antioxidative Activity, Nanoparticles, Rosemary.

Introduction

Nanoparticles produced by plants extracts are more stable, and the rate of synthesis is faster than that in the case of other organisms. Nowadays, Green synthesis of metal nanoparticles is an interesting issue of nano science and nanotechnology[1].

Zinc oxide, with its special physical and chemical properties, such as high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photo-stability, were synthesized by different methods. It is confirmed that the various applications of ZnO nanoparticles depend upon the control of both physical and chemical properties such as size, size dispersity, shape, surface state, crystal structure[2].

Lipid oxidation is one of the major forms of deterioration in fatty foods, because it leads to the formation of off-flavors and potentially toxic compounds, essential bioactive compounds in food namely flavonoids, phenolic diterpenes, tannins and, Thermo-oxidation process subjects oils or fats to high temperature, similar to the frying process, but without the presence of food. Therefore, the temperature and the oxygen content are the variable that determines thermo-oxidation rates[3].

At high temperatures the formation of new compounds is very rapid, the oxygen pressure is reduced and the hydro-peroxides decompose rapidly and are practically absent above 150 ºC indicating that the decomposition of hydro-peroxides becomes faster than their formation[4].

Natural antioxidants are more ideal as food additives, not only for their free radical scavenging properties, but also on the belief that natural products are healthier and safer than synthetic ones; thus they are more readily acceptable to the modern consumers[5].

Phenolic acids were acting as natural antioxidants factor for preventing rancid odors and flavors, have attracted increasing attention for their antioxidant behavior and beneficial health-promoting effects and they account for about one-third of the phenolic compounds in plant foods. It is assumed that many antioxidative phenolic compounds in plants are usually presented in a covalently-bound form. Therefore, reliable and practical methods for liberation of natural antioxidants from plant materials are of considerable interest, Rosemary (*Rosmarinus officinalis* L.) was a special widely natural antioxidants around the world., has been considered as one of the spices with have the highest antioxidant activity, It effects on the quality of the oil due to prevent the formation of free radicals and losses of a desirable effective compounds[6].

Accordingly, this study was conducted to investigate the antioxidant activity of nanoparticles of rosemary extract using zinc oxide nanoparticles as natural antioxidant compared with TBHQ. Also, oxidation stability and thermal stability of sunflower oil treated with nanoparticles of rosemary extracts were studied.

Materials and Methods

Materials

Rosemary leaves (*Rosmarinus officinalis L.*) were obtained from local spice market in Cairo, Egypt.

Refined, bleached, and deodorized (RBD) sunflower oil and Tertiary Butylhydroxy Quinone (TBHQ) were obtained from Arma Company For Oils at 10 th of Ramadan City, Cairo, Egypt.

All chemicals and reagents were purchased from El-Gomhouria Pharmaceutical Company, El-Mansoura City, El-Dakhaleia Governorate, Egypt.

Methods :

Sample preparations:

Preparation of Rosemary leaves powder :

Rosemary leaves were washed using distilled water ,the leaves were dried in oven dryer at 45 ºC for 8 hours with air circulation dryer model (Officine specializzate, GARBUIO, Essiccatioi, TREVISO, ITALY) .Dried materials were ground in a domestic mill (Braun, German). Then sieved using mesh particle size less than 80 and packaged in plastic air tight polyethylene until the extraction were performed .

Preparation of rosemary extract.

Ethanolic extract was prepared according to the method described by (Semnani et al.,[7] and water extract was prepared according to the method reported by Mishra and Sharma[8].

Nano particle of rosemary extracts using zinc oxide preparation :

Nanoparticle of rosemary extracts using zinc oxide nanoparticles was prepared according to the method described Cynthia et al., [1] at Nano Technology Center , Faculty of Engineering , Mansoura University.

20 ml of water extract and ethanolic extract were filtrated using whatmam No.1. boiled at 60ºC then 2g. of zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) was added for each of the extracts . the mixture was boiled and the color turned to deep yellow . finally , the extracts were stored until further analysis were carried out.

Transmission Electron Microscope (TEM) Analysis:

Structural and optical properties of the ZnO nanoparticles were determined by using Transmission Electron Microscopy (TEM). Electron Microscope Unit . Faculty Of Agriculture Mansoura University.

The size particle measurements : were performed using Electron Microscope Malvern (Zetasizer – nanoseizer)

Preparation of oil samples treated with different antioxidants :

Sunflower oil samples were treated with 200 and 300 ppm from synthesized nanoparticle rosemary extracts (water and ethanolic) compared with TBHQ (200 ppm) as synthetic one (maximum legally permitted level) [9,10].

Antioxidant activity assessments:

Determination of total phenolic compounds and total flavonoids :.

Folin-Ciocalteu method was used to estimate total phenolic compounds (as gallic acid equivalent) using standardized spectrophotometric according to*Ivanova et* al., [11] and Flavonoids were extracted and estimated by the method of AOAC, [12] at Food Tech. Res. Institute ,Agricultural Research Center, El-Giza, Egypt.).

Fractionation and identification of phenolic and flavonoids compounds .

Phenolic and flavonoids compounds were determined using HPLC according to Goupyet al., [13].

Determination of antioxidant activity:.

2,2 diphenyl1-picrylhydrazyl(DPPH %)assay was carried out according to the method of Brand-Williams etal., [14].

Thermal process:

All sunflower oil samples were heated at 120 ºC for 0, 6 and 12 hours according to Iqbal etal., [15] in oven Model WT Binder Then oil samples were refrigerated and storage at 5±1ºC till further analysis were carried out.

Chemical properties of oil samples:

Acid value (AV), free fatty acids (FFA%) and peroxide value (PV) were determined according to the methods described by A.O.A.C. [16].

Thiobarbituric acid value(TBA):

Thiobarbituric acid (TBA) value was determined using spectrophotometer, model : SPECTROUV-VISAUTO,UV-2602 and absorbance was measured at 530nm. TBA value was expressed as mg/malonaldhyde/kg oil, according the method described by A.O.A.C. [16]. Using the following equation :TBA = 7.8 x O.D.

O.D. = Optical density at 530 nm

Results and Discussions

Structural and Synthesized zinc oxide nanoparticles (ZnONPs):

Transmission Electron Microscopy (TEM):

Data in (Figure 1) showed that TEM images of ZnO nanoparticles indicated clearly the size and the shape which have a spherical , smooth and granular shape from ZnO nanoparticles were observed and particle size ranged between 2.8 to 3.8 nm, this is considered as nano-particle size were presented this resulted were in accordance with [17]

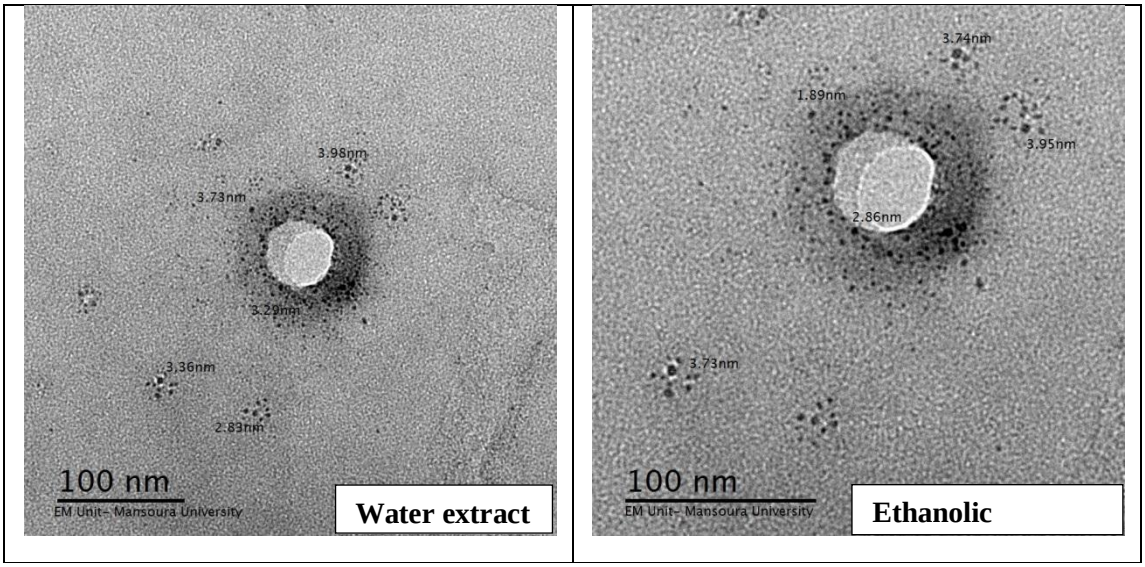

Figure (1):Transmission Electron Microscopy(TEM) images of ZnO nanoparticles of water and ethanolic extracts.

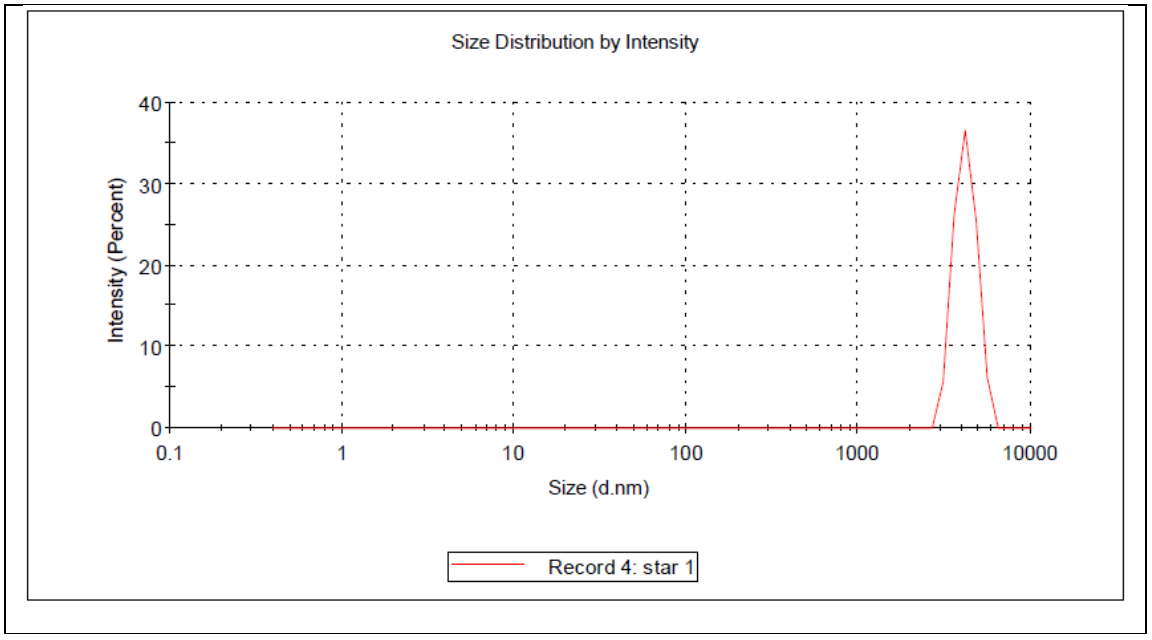

Figure (2): Size distribution by intensity of ZnO nanoparticles of water extracts.

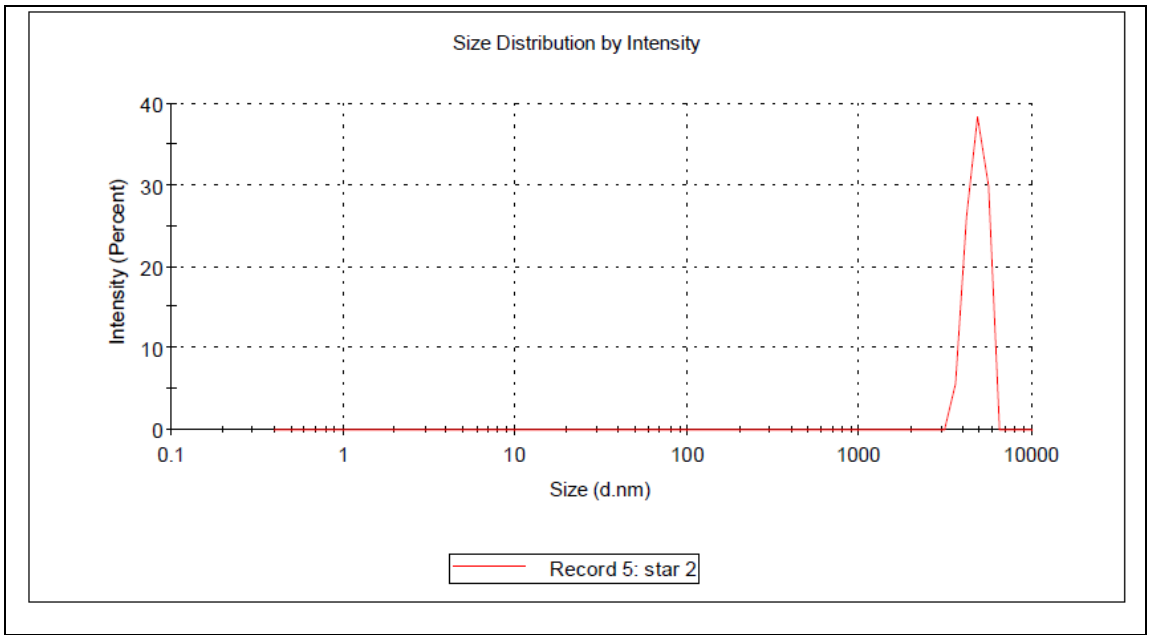

Figure (3): Size distribution by intensity of ZnO nanoparticles of ethanolic extracts.

The zeta potential is a key indicator of the stability in solutions results in Fig. (2and 3) for The zeta - average size for both of ZnONps for ethanolic and Methanolic extract showed that the density and the aggregates of particle were more stables as stated by [18,19].

Radical scavenging activity (DDPH)of rosemary extracts:

Radical scavenging activity were evaluated by DPPH technique which depend on donate hydrogen to free radical and inhibiting the propagation stage in lipids oxidation pathway [20].

Radical scavenging activity of rosemary extracts depends greatly on the concentration of active compounds as showed in Table (1). Rosemary ethanolic extract exhibited the highest DPPH activity in compare with water extract. The highest scavenging activity being 89.91 % for TBHQ, as synthetic antioxidants.

Table (1):Total flavonoids , Total phenolic compounds as Gallic acid and radical scavenging activity(DPPH) % of rosemary extracts.

Antioxidants / Parameters	TBHQ	Rosemary water extract	Rosemary ethanolic extract
Antioxidant activity %	89.36	77.56	82.67
Total phenolic Compounds (mg of GAE/g)	-	45.44	58.36
Total flavonoids (mg RE/g)	-	22.58	38.26

Phenolic compounds are considered as a major group of antioxidant molecules that contribute to the antioxidant activities of plants because of their ability to scavenge free radicals by virtue of the presence of hydroxyl groups [21].

Total phenolic compounds content was expressed as Gallic acid equivalent and the values were expressed as mg of Gallic acid (GAE)/g of extract.

Obtained results (Table 1) showed that the total phenolic contents in two examined extracts being 45.44 and 58.36 mg of GAE/g respectively. The highest concentration of phenols was measured in ethanolic extract (RME) followed by the water extract.

These obtained results were in full agreement with those reported by[22] who stated that the percent of phenolic compounds from the plants depends on the nature of the solvent used in the extraction process of the plant. Also, there were a positive relationship between phenolic content of the examined extracts and their abilities as antioxidant factors.

Also, results in the same table showed that total flavonoids content of the extracts were expressed as rutin equivalents (RE) in milligram per gram dry extract. Results in Table 1 showed that rosemary ethanolic extract has the highest flavonoid content (88.26) followed by the water extract (22.58).

Results indicated that there were a negative relation between the total antioxidant activity (DPPH) of the examined extracts of rosemary and the two studied fractions,which were derived from this extract and their phenolic and flavonoid contents.

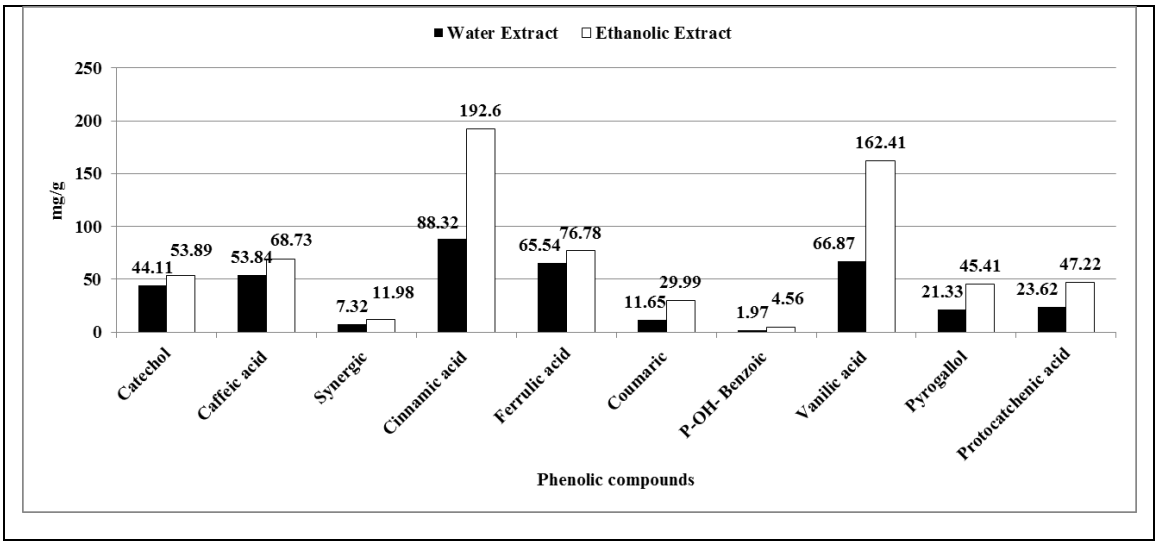

Figure (4):Fractionation and identification of some phenolic compounds in rosemary extracts as mg/g.

Total phenolic compounds of rosemary water and ethanolic extract were separated and identified by HPLC and the results were shown in figure (4), it could be noticed that ten phenolic compounds were separated. Cinnamic acid was the most abundant phenolic compound in rosemary leaves (192.6 and 88.32 mg/100g) followed by Vanaillic (162.41 and 66.87 mg/100g). Moreover, Ferulic acid, Caffeic, Catechol, Protocatchuic acid and Pyrogallol were also detected in medium amounts. On the other hand, the Coumarine, Synergic acid and P.OH Benzoic acid were also detected in small amounts.

These phenolic compounds were more active as antioxidants because they have a double bond in their structure, providing stability for the free radical formation [23].

Flavonoids were one of the most widely studiedclass of polyphenols with respect to their antioxidant and biologicalactivities. They have powerful antioxidant activities in vitro,being able to scavenge a wide range of reactive oxygen species[24].

As recorded in figure (5), eight flavonoid compounds were fractionated and identified. In ethanolic and water extracts, Rosmarinic was the most abundant flavonoid compounds in the extracts being (78.83 and 67.91 mg/g), followed by quercitrin and rutin were (36.29 and 33.65) and (35.41 and 33.42) respectively.

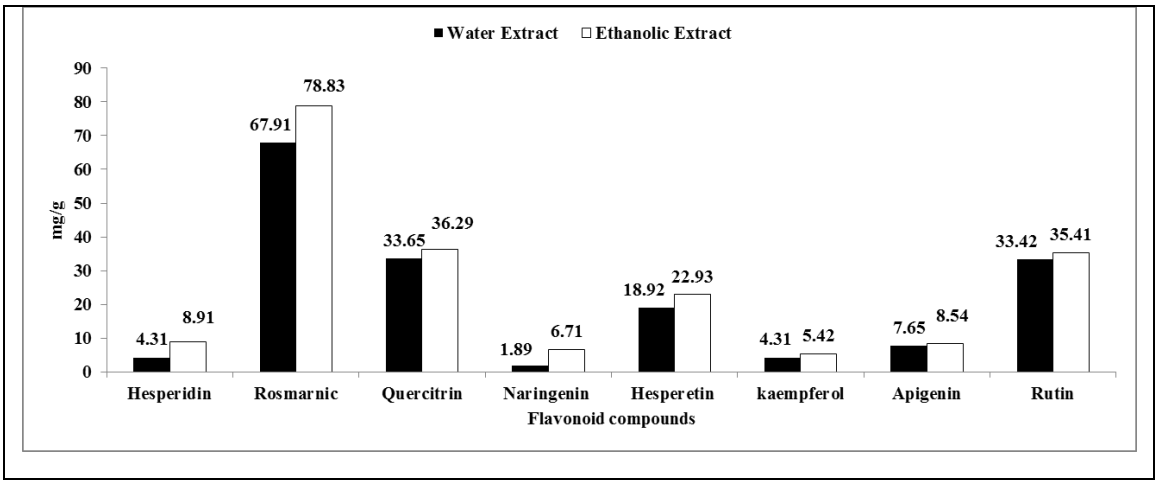

Figure (5):Fractionation and identification of some flavonoids in rosemary extracts as mg/g.

Rancimat index is a fast oxidation measurement of the degree of its resistance to oxidation, examine the oxidative stability of edible oils and predict their shelf life of oil which expressed as induction period. The induction period represents the time needed for decomposition of hydroperoxides produced by oil oxidation [25].

The rancimat time at 110 ^{o}C indicated that induction period of oil was varied from 8.18 to 20.83 hours in oil samples treated with different type of antioxidants.It was clear that addition of rosemary extracts with different concentration due increasing in induction period, which related to its active phenolic and flavonoid compounds composition [25].

Treated sunflower oil with ZnO NPs rosemary ethanolic extract at the concentration of 200 and 300 ppm showed the highest stability time (20 months of storage) in compare with other treated sunflower oil samples. these results could be due that ZnO NPs could bound the essential compounds and functional groups namely phenolic compounds, flavonoid and terpenoids were bound to the surface of ZnO NPs, also ethanolic extract could effectively dissolve the essential compounds this function could enhance the bio-reduction reaction in the biosynthesis of nanoparticles .

Table(2):Oxidative stability of sunflower treated with rosemary water extract and zinc oxide nanoparticles.

oil samples	Control	RMW		ZnoNps		TBHQ
		200ppm	300 ppm	200ppm	300 ppm	
Stability time in hours(i.p)	8.18	13.68	15.38	17.65	17.05	20.83
		RME		ZnoNps		TBHQ
		200 ppm	300 ppm	200ppm	300 ppm	
	8.18	14.08	16.61	20.63	20.82	20.81

RMW (Rosemary Water Extract), RME (Rosemary Ethanolic Extract) and I.P. means induction period.

Also, from the same table, it could be detected that ZnO NPs with rosemary water extract can involve with the bio-reduction reaction with the water soluble phenolic acids and can form the intermediate complexes with phenolic OH groups presented hydro-stable state with zinc oxide nanoparticles which prevent oxidation and rancidity as reported by [26,27].

Our obtained results were in accordance with [28] who stated the mechanism of ZnONps stabilization related to the interaction between phenolic acids such as Caffeic acid, Cinnamic acid and Ferrulic acid and zinc ions.

Table (3): Some chemical indices of sunflower treated with rosemary water extract and zinc oxide nanoparticles during thermal process throughout 12 hours at 120^{o} C.

Chemical indices	Time in/ hr	control	TBHQ	RMW		ZnONps		RME		ZnONps	
				200 ppm	300 ppm	200 ppm	300 ppm	200 ppm	300 ppm	200 ppm	300 ppm
Acid value	zero	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
	6	6.68	2.56	4.42	3.54	3.41	2.98	2.51	2.13	2.45	1.89
	12	13.58	7.32	11.02	10.64	8.97	8.65	7.30	7.12	6.92	6.87
F.F.A. %	zero	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035
	6	2.84	1.28	2.21	1.77	1.71	1.49	1.23	1.07	1.23	0.95
	12	6.79	3.66	5.51	5.32	4.48	4.28	3.65	3.56	3.46	3.44
Peroxide value Ml.eqv/kg oil	zero	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
	6	8.34	1.89	3.89	3.72	3.43	3.61	2.35	2.01	1.89	1.72
	12	6.79	8.20	11.64	11.48	10.53	10.07	9.82	9.61	8.72	8.33
Thiobarbituric acid value (mg mal/ Kgoil	zero	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042
	6	10	3.41	8.65	8.12	6.95	6.69	4.88	4.65	3.71	3.71
	12	14.25	6.01	11.11	11.02	9.71	9.11	7.94	7.81	5.90	5.87

Effect on acid value(AV) and free fatty acid %:

Acid value is one of the most measurements of oil decomposition analysis [29]. Results in Table (3) showed control sunflower oil sample exhibited the highest amount of acid value being 6.68 and 13.58 after 6and 12 hours of thermal process, while the oil samples treated with synthetic antioxidant (TBHQ) showed the slowest. ZnONps Rosemary antioxidant ethanolic extract had the highest antioxidant activity after 6 and 12 h of thermal process at 120 °C. It was found that the rosemary extract had the antioxidant activity which was significantly higher than TBHQ in sunflower oil.

Free fatty acid % (FFA) is an important oil quality indicator during each stage of oils processing, a high acidity level means a poorly refined oil or fat breakdown after storage or use.

Results in the same table showed also, the changes in FFA% were in parallel with the changes in acid values. All treated oil samples with different antioxidants decreased gradually in compare with control one. Treated oil samples with ZnONPs at the concentration of 200 and 300 ppm exhibited the lowest amount of FFA% in compare with all treated oil samples and control up to 12 hours of thermal treatment at 120 °C.

The changing rates in FFA% in compare with control oil sample after 12 hours of thermal process indicated addition of antioxidant at the concentration of 300 from ZnONps could lowering the change rate in FFA% to 49.85 % followed by treated oil samples with RME 49.04% and the other treated with RMW was 21..64% (as mentioned in Fig. 6).

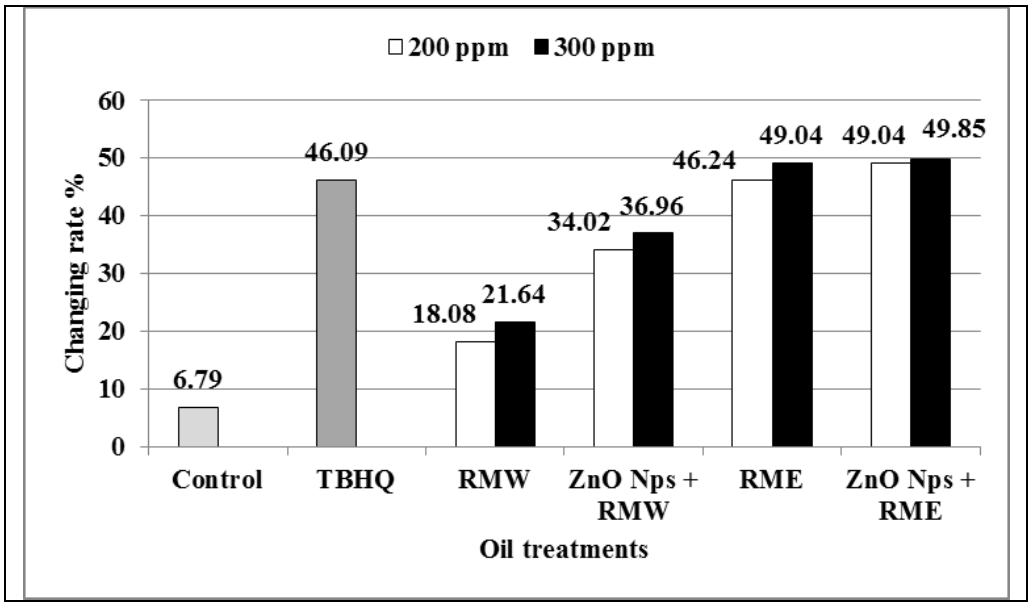

Figure (6):Changing rate% in FFA% after12 hours of thermal process.

Effect on peroxide value:

Peroxide value (PV) is a measure of first stage primary oxidation products namely peroxides and hydro peroxides formed during the initial stages of oil oxidation, which is transitory phase and pass by time into other compounds which contain no reactive oxygen [30].

Results in Table (3) showed that, during the first 6 hours of thermal process at 120 0 C, PVs of the oil with added antioxidant were increased to 3.89 ml .eqv./ kg oil in compare to 8.43 ml .eqv/ kg oil in control oil sample, the increase in PVs occurred more progressively throughout thermal process up to 12 hours. The values of peroxides reached 15.55 in control oil sample, while in treated oil samples reached 11.64 ml .eqv./ kg oil.

All the values of PV treated with ZnONps with different concentration 200 and 300 ppm not exceeded 10 ml. eqv / kg oil, which is the upper limit for vegetable oils according to [31].

Effect of Thiobarbituric acid value (TBA):

Thiobarbituric acid (TBA) considered as widely used test for estimating extent of lipid oxidation oils and fats.It can be expressed as malonaldhyde (Daker et al., [31]).

Obtained data in Table (3) revealed the effect of thermal treatment and addition of different antioxidants extracts on the TBA value. The results indicated that, all treated sunflower oil had a closed TBA values tended to increase prolonged the thermal process up to 12 hours at 120oC. Control oil sample recorded the higher TBA values in compare with other oil treated samples being (14.25 mg malonaldhyde /kgoil, whereas the oil samples with added ZnONPs using RME at the concentration of 200, 300 ppm were recorded the lowest TBA values after 12 hours at 120^{o} C being (5.60 and 5.87) mg malonaldhyde / kg oil, respectively.

The increase in TBA throughout thermal process of control sunflower samples might be attributed to the formation of malonaldhyde products namely aldehydes and ketones and the free radicals from unsaturated fatty acid decomposition. These results are in agreement with those of [32].

In conclusion, the addition of ZnO NPs rosemary extract to sunflower oil showed a positive effect on the oxidative and thermal stabilities of such raw material and could be recommended as an alternative antioxidant in oil conservation.

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