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Study of the Effect of Zinc Nanoparticles from Saffron Extract during Thermal Treatment of Soybean Oil

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ABSTRACT

This work aimed to study the effect of nanoparticles of zinc oxide saffron extract and its effect as natural antioxidant. Transmission Electron Microscope (TEM) used as a method showed that a round shape of ZnO nanoparticles of medium size 15 to 50 nm. Results of bioactive compounds elucidated that content of total phenolic being 54.66±1.3 mg GAE\gm in saffron ethanolic extract, while total flavonoids being 70.23 in the same one. Determination of phenolic compounds using HPLC obviously that nine compounds were detected. Safranal acid is the most plentiful one in saffron extract that was 220.11mg\100gm, while hesperitin was the most flavonoid compound in the same extract was 87.51mg\g. Using concentrations of 200 and 300 ppm of ZnO saffron ethanolic extract was added to soybean oil and compared its effect with TBHQ as a synthetic antioxidant. Acquired data for stability of oxidation by rancimat method noticed that addressed sample with Zinc oxide nanoparticles saffron extract using concentration 300ppm have the highest presentation stability time (18months) of storge compared with other simples. Heating process effect of treated soybean oil by ZnO saffron ethanolic extract using concentration 300 ppm was chronicled the lower most ethics of hydrolysis, oxidation and rancidity limitation subsequently twelve hours thermal process. Thus, addition of zinc nanoparticles saffron extract as an alternative to synthetic antioxidants in food oils.

***Keywords*:** Antioxidant Activity, ZnO-Nanoparticles, saffron extract, soybean oil, Thermal stability.

INTRODUCTION

The recognition of natural antioxidants considered as agorgeous extent of study for food and therapeutic submissions. Natural antioxidants can be planned in place of alternates for synthetic antioxidants that consume delimited bids owing to the injurious health glitches like cancerogenic possessions which probably arise payable to their end during ingesting. Antioxidants have a wide-ranging series of compounds which can reduce lipids deprivation during oxidation processes and consequently avoid diseases by their enhanced of free radicals (Najafia et al.,2023)

Saffron (*Crocus sativus L.*) is a triploid antiseptic plant have its place to the Iridaceae family. Which contained bountiful volatile compounds and elements including crocin, picrocrocin and safranal and these compounds are liable for color, taste and odor of saffron respectively. Also, coloring constituent of saffron, so saffron is rummage-sale as a flavoring and coloring manager. Along all of these compounds, it also contains little quantities of other pigments like anthocyanin, α-carotene, β- carotene, and zeaxanthin. Flavonoids and other phenolic compounds were broadly in plants and their varied biological activities such as antioxidant possessions which have been inspected in many studies such as coronary heart diseases, diabetes and cancer (lopez et al.,2019).

Vegetable oils and fats are considered the foremost spring of USFA and their consumption by way of a rare substantial for the construction of nutraceutical and pharmacological products of in height further worth happens in arrears to their low fee. In the greatest convincing studies apropos the modifiable consequence of omega-3, omega-6 and omega-9 on invulnerable cell occupations, these fatty acids have been moreover directed or accompanied in their permitted procedure and at an actual in height gradation of spotlessness. (Ract et al.,2015). Most vegetable fats and oils have limited tenders in their creative forms due to their precise chemical composition. To expand their commercial custom, vegetable oils are modified either actually (by fractionation/blending), chemically by hydrogenation and interesterification (Isailović et al.,2017).

Recently, nanotechnology products have been industrialized in several fields that includes food industries and pharmaceutical manufacturing. Several researches have shown the successful method of the usage of the nanoparticles to enhance stability in the food applications. The nano-dispersions can be simply manufactured on a manufacturing increase. It was prepared Essential oils nano dispersions by the custom of high-pressure homogenization and deliberate their physical and chemical possessions. There are other claims which embrace the encapsulation of lutein, limonene, omega 3 fatty acids and lycopene. The encapsulation of α-tocopherol to diminish lipid oxidation in fish oil and the custom of nanoparticles in the direction of integrate essential oils, oleoresins and oil-based natural flavors obsessed by food products (Nidhi ,2015).

MATERIALS AND METHODS

Materials

Raw materials:

Saffron (*Crocus sativus*)"threads" plucked from crocus flowers were purchased from local Apothecary in El-Dakahlia Governorate, Egypt.

Refined, Bleached and Deodorized (RBD) soybean oil and Tertiary butylhydroquinone (TBHQ) were attained

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from Arma Company for Oils at 10th of Ramadan City, Cairo, Egypt.

All chemicals and Substances were taken out from El-Gomhouria Pharmaceutical Company, Mansoura city, El-Dakahlia Governorate, Egypt.

Approaches

Accommodation of illustration:

Saffron "threads" was dried in oven dryer model (Officine specialization, TREVISO, Italy) at 45°C for 8 hours. Dried materials were wrapped in plastic air tight polyethylene pending the extraction was accomplished.

Saffron extract apparatus:

100 gm of dried material was crushed into fine powder mill (BRAUN, German). Then was mixed with 1L of ethanol for 10 min under continuous stirring. The assortment was sifted and the obtained liquid was vanished under vaccum consuming a rotary evaporator model (RE-201d RE 201D) then composed and stored in airtight glass containers in a refrigerator at 4°C for use in the experiment (Semnani et al., 2006).

Groundwork of oil illustrations preserved the diverse antioxidants:

Soybean oil (RBD) illustrations were treated with 200 and 300 ppm from prepared nanoparticle saffron extracts related with TBHQ by concentration 200 ppm as synthetic one (Toshiyuki,2019).

Accommodation of zinc oxide nano particle of saffron extracts:

Saffron nanoparticle's extracts consuming zinc oxide nanoparticles was equipped by filtering 20 ml of water extract and ethanolic extract and boiled at 60ºC then 2g. of Zn $(NO3)_2.6H_2O$ was added for each of extract and the assortment was boiled Cynthia et al.2012. Lastly, the extracts were deposited pending analysis.

Transmission Electron Microscope (TEM) Analysis:

Rendering to the method designated by Smith, (2015) by using Transmission Electron Microscopy (TEM) Physical and visual assets of the ZnO nanoparticles were determined.

Size particle measurements of saffron extract:

Size particles of saffron extract were measured using Electron Microscope Malvern (Zeta sizer – nano sizer) conferring to the method pronounced via Khoshnevisan and Barkhi (2015).

Assessments of Antioxidant Activity:

Purpose of total phenolic compounds and total flavonoids:

Folin-Ciocalteu scheme was rummage-sale to guesstimate total phenolic compounds (as gallic acid equivalent) using standardized spectrophotometric as stated by Ivanova et al.,2019 and Flavonoids were take-out and appraised by the technique of AOAC,2000.

Fractionation and Identification of phenolic and flavonoids compounds:

Compounds of Phenolic and flavonoids were resolute consuming HPLC conferring to Goupy et al.,1999.

Antioxidant Activity (DPPH%):

2,2 diphenyl1-picrylhydrazyl (DPPH %) evaluate was passed obtainable conferring to the technique of Brand Williams et al.,1995.

Heating Process of soybean oil samples:

All samples of soybean oil were heated at 0, 6 and 12 hours at 120 ºC in oven (Model WT Binder), then kept at refrigerator and storage at 5±1ºC for examination (Kmiecik et al.,2015).

Chemical Evaluation of Different Soybean Oil:

Acid value (AV), free fatty acids (FFA%) and peroxide value (PV) were resolute by A.O.A.C.2005.

Thiobarbituric acid value (TBA):

Thiobarbituric acid (TBA) value was resolute by spectrophotometer and optical density was restrained at 530nm. TBA value was uttered as mg/malonaldhyde/kg oil, affording the technique pronounced by A.O.A.C. 2005. Consuming the following equivalence:

(TBA = 7.8 x O.D.)

(O.D. = Optical density at 530 nm)

RESULTS AND DISCUSSION

Characterization of Nano particle of saffron extracts:

1-Transmission Electron Microscopy (TEM):

Structure of nano particles of saffron extracts were advised using TEM, which were be used to attain quantifiable dimensions of the structural, automated and chemical assets of materials on length weighing machine dejected to the sub-A level. (Savardekar and Bajaj, 2016).

Figure 1 (a and b) exhibited that saffron extract particles sizes being 178: 200 nm whereas our gained consequences from TEM specified that constituent part size of regular saffron extract particles which administered by nano-particles procedure were smaller than those of the derivation type and vacillated from 15: 50 nm. This is attained results unswerving with results of Anahita,2017 and Fitri et al., 2022.

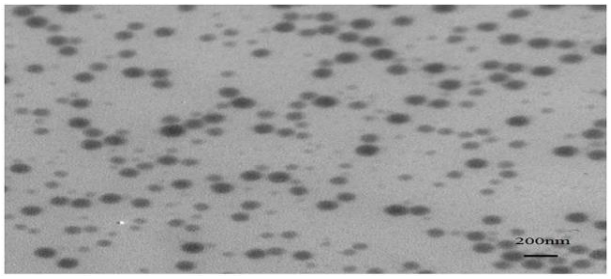

Fig. 1. (A) Normal Saffron Extract Particles

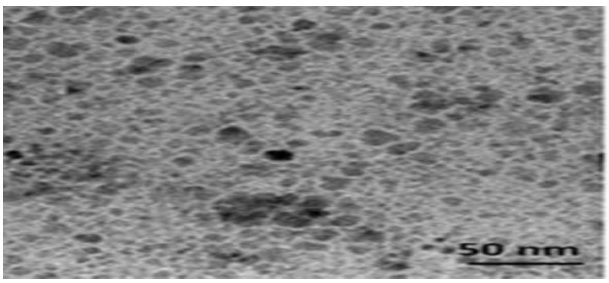

Fig. 1. (B) Nano Particles Saffron Extract

Figure 1. (A and B) TEM micrographs depiction exemplifying the size and geomorphology of saffron extract at 200nm and which by nanotechnology at 50nm

2- The size particle measurements:

Zeta potential is a significant structure that illustrate the external assets of nanoparticles and is associated to electrostatic exchanges among constituent part in colloid schemes. It is also case off to govern physical constancy. The particles in a colloidal different or emulsion frequently convey

an electrical responsibility. This is more often negative than positive and it may arise in a number of customs. Sometimes the superficial of the particles encompasses chemical groups that can ionize to produce a thrilling surface. Occasionally the surface the issue favorably adsorbs ions of one sign of charge in penchant to custodies of the opposite sign. zeta-potential mensuration methods (electrophoresis, electroosmosis, streaming potential and sedimentation) are designated besides the measuring principles of the conforming maneuvers (Pongsumpun, et al.,2020).

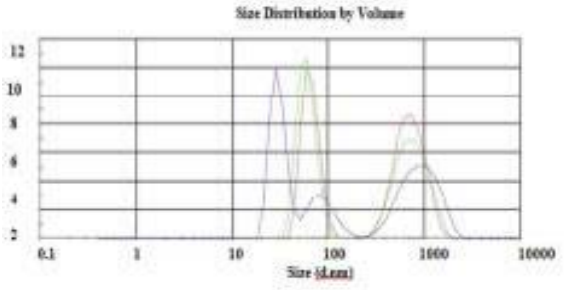

Fig. 2. (A) Normal saffron extract particles

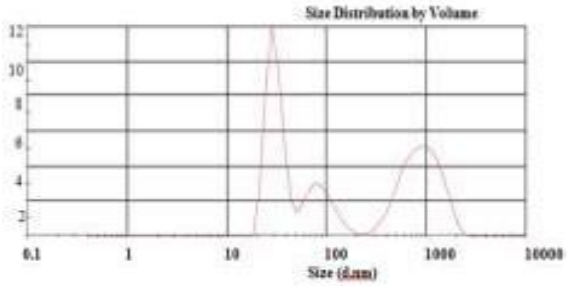

Fig. 2. (B) Saffron extract with nano particles

Figure 2. (A and B) Zeta potential dimensions of regular saffron extract and saffron extract with nano particles

From accessible statistics in Figure 2 (A) it possibly will be potted that the supply the typical size, density and the construction of masses of constituent part were more constant in saffron extract with nano particles than those other regular saffron extract as accessible in Fig 2 (B). Consequently, it could be abridged from obtained outcomes in Figure 1 (A and B) and Figure 2 (A and B) that saffron extract with nano particles intensification constancy of particles size and this belonging is enormously imperative for antioxidant effect.

Radical scavenging activity (DDPH)of saffron extract:

The phenolic hydroxyl assemblies involved to flavonoids and are branded to remain antioxidants by scavenging free radicals, inhibiting lipid oxidation, or chelating brass ions (Liu et al.,2018.

Table (1) exhibited the ratio of total phenolic gratified in saffron extract which was 54.66±1.3 mg/ g as gallic acid, whereas the antioxidant activity which was appraised consuming the DPPH method grasped to 68.80±3.6 %. Cerdá-Bernad et al., (2022) settled that saffron had a significantly higher concentration of total phenolic compounds.

Table 1. Total phenolic compounds as Gallic acid, radical scavenging activity (DPPH) %Total flavonoids of nano saffron extract:

Antioxidants Parameters	TBHQ	Saffron Extract
Antioxidant activity %	80.75	68.80±3.6
Total phenolic Compounds (mg of GAE/g)	-	54.66±1.3
Total flavonoids (mg RE/g)	-	70.23

Compounds of Total phenolic of saffron ethanolic extract were detached and acknowledged by HPLC and the results were revealed in Fig (3), it could be observed that nine phenolic compounds were detached.

Safranal was the furthermost plentiful phenolic compounds in saffron ethanolic extract (220.11 mg/100g) tracked by crocin (185.43 mg/100g) and picrocrocin was (95.32 mg/100g). Furthermore, ellagic and alfa carotene were also detected in medium amounts. Conversely, the vanillic acid and catechein also detected in small amounts.

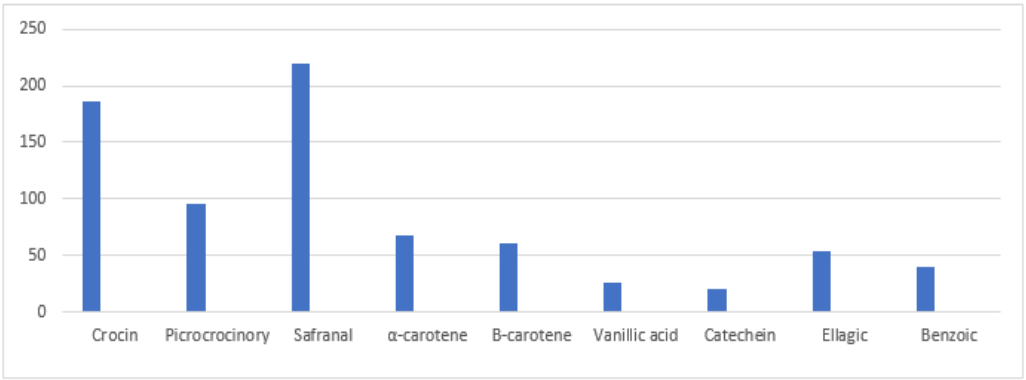

Figure 3. Phenolic compounds in nano saffron ethanolic extract

Flavonoid compounds are vital payable to their aptitude to serve as antioxidants. Flavonoids, which are found frequently in the leaves, flowering tissues and pollens are a vital part of the regime because of their possessions on human nutrition. The furthermost imperative function of flavonoids is their antioxidant activity, as they have been exposed to be exceedingly active scavengers of furthermost types of oxidizing molecules, as well as vest oxygen and several free radicals (Bensebia et al., 2021).

As recorded results in fig (4), seven flavonoid compounds were fractionated and identified. Hesperitin was the furthermost flavonoid compounds in the extract being (87.51 mg/g), followed by apigenin and kaempferol were (59.14 and 48.15 mg/g) respectively.

Effect of addition saffron nanoparticles on the oxidative stability of soybean oil:

Oxidative decline, frequently identified as lipid oxidation or rancidity, off-flavors, staining, disagreeable aromas, and reductions comestible oil nutritional worth. Lipid

oxidation is a problematic of monetary apprehension to edible oils, as it decreases the positive quality characteristics (e.g., flavor, smell, and color) and curtails their shelf life (Lucía and Irwin(2021).

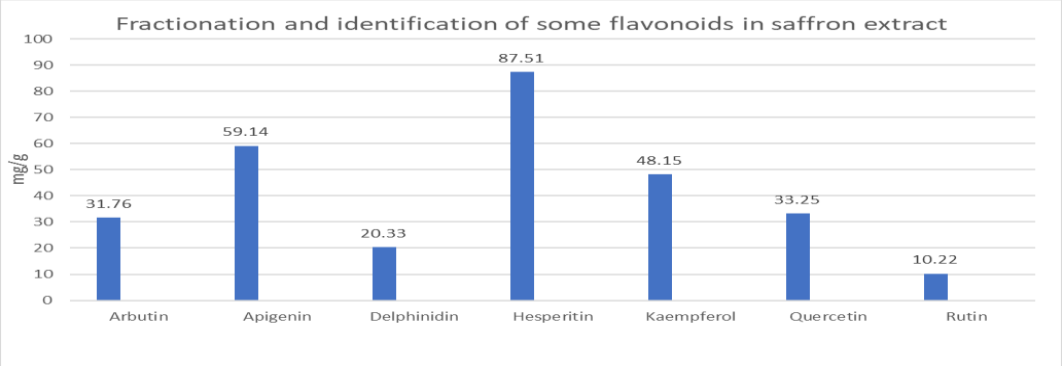

Figure 4. Flavonoid compounds in nano saffron extracts as mg/g.

Induction period or oxidative stability index is one of the methods used to scrutinize the degree of edible oils to struggle oxidation at raised up temperatures. The results obtained by the Rancimat test would provide the indicative measure stability of oils with regards to induction period (Azmil et al., 2016).

Rancimat time at 120ºC signalized that stability time of oil was diverse from 7.45 to 25.35 hours in illustrations preserved by diverse category of antioxidants. Adding saffron extracts with dissimilar concentration increases time of stability, which related to its bioactive components.

Treated soybean oil with ZnO NPs saffron ethanolic extract at the concentration of 200 and 300 ppm showed the highest constancy time (18 months of stowage) in equivalence with other preserved soybean oil illustrations. These results could be due to the effect of ethanolic extract dissolving the essential compounds and this occupation could augment the reaction of bio-lessening in the biosynthesis of nanoparticles, also ZnO NPs could certain the essential compounds and purposeful assemblies specifically flavonoids, phenolic compounds and terpenoids were destined to the superficial of ZnO NPs (Lambrianidou et al., 2021).

Our obtained results were in accordance with Salama et al., 2017 who showed that ZnO NPs has a probable authority on the way to rheostat the oxidation progression throughout heating procedure of comestible oils and could be cast-off as synthetic antioxidant manager. Supplementary studies regarding the solubility of ZnO NPs and its nutritional effects.

Changes of some chemical properties of soybean oil treated with saffron ethanolic extract and zinc oxide nanoparticles during thermal process throughout 12 hours at 120º C:

1-Changes of Acid value (A.V):

It is a qualified quantity of rancidity as free fatty acids are generally molded that breakdown of oil glycerides. The rate is similarly articulated as percent of free fatty acids premeditated as oleic acid. (Negash et al.,2019).

Consequences in Table (3) exhibited control soybean oil illustration revealed the uppermost quantity of acid value existence 0.723 and 1.430 mg KOH/gm oil after 6 and 12 hours of heating process, whereas the oil illustrations preserved with artificial antioxidant (TBHQ) exhibited the slowest one. ZnONps saffron antioxidant ethanolic extract in attentiveness 300ppm had the uppermost antioxidant activity after 6 and 12 h of heating process at 120 °C. It was detected that the saffron extract had the antioxidant activity which was knowingly nearly TBHQ in soybean oil.

Table 3. Oxidative stability of soybean oil preserved with saffron ethanolic extract and zinc oxide nanoparticles.

Stability Time	Soybean Oil samples			
	control	Saffron extract with ZnoNps		TBHQ
"Induction		200PPm	300PPm	
period"	7.45	22.60	22.75	25.41
(Hours)	7.45	23.08	23.15	25.35

2- Changes of Free fatty acids % (FFA)

Free fatty acid % (FFA) is a vital oil superiority pointer throughout apiece period of oils dispensation, a high acidity side by side means an ailing refined oil or fat interruption after stowage or usage (Jacobsen,2019).

Consequences in the similar table exposed also the fluctuations in FFA% were in equivalent with the vicissitudes in acid values. All preserved oil illustrations with different antioxidants reduced progressively in comparability with control one. Preserved oil samples with ZnONps saffron antioxidant ethanolic extract at the concentration of 200 and 300 ppm unveiled the lowest expanse of FFA% in compare with all preserved oil samples with TBHQ and control up to 12 hours of thermal treatment at 120 °C.

The altering tariffs in FFA% in comparability with control oil sample after 12 hours of current procedure specified addition of antioxidant at the concentration of 300 from ZnONps saffron antioxidant ethanolic extract could letting down the revolution frequency in FFA% to 0.986 % followed by the other treated with Saffron extract was 1.301%.

3-Changes of peroxide value:

Peroxide value is usually articulated in terms of milliequivalents of oxygen per kilogram of fats. Peroxides are the main preliminary products of Autoxidation. It also resolves bounces a degree of the level to which an oil sample has suffered primary oxidation (Abdullahi et al.,2022).

Consequences in Table (4) exposed that, throughout the first 6 hours of heating at 120 ^{0} C, peroxide value of the oil illustration with further antioxidant were amplified to 4.058 ml. eqv. / kg oil in comparability to 6.210 ml.eqv/ kg oil in control oil sample, the intensification in PVs befell more increasingly thru heating up to 12 hours. All the values of PV treated with ZnONps with different concentration 200 and 300 ppm which were 4.058 and 4.039 ml. eqv. / kg oil respectively not exceeded 10 ml. eqv / kg oil, which is the superior border for vegetable oils.

4-Changes of Thiobarbituric acid value (TBA):

TBA values for indicating hydroperoxide and aldehyde formations, respectively, can describe the extension of oxidation. The TBA reactive substance is commonly used as an indicator of lipid oxidation (Feri, 2020).

Gained information in Table (4) bare the consequence of heating conduct and accumulation of dissimilar antioxidants extracts on the TBA value. The consequences designated that, all preserved soybean oil had a bolted TBA values be disposed to increase during the thermal process up to 12 hours at 120ºC. Control oil sample recorded the higher TBA values in compare with other oil preserved illustrations being (1.021 mg malonaldhyde /kg oi), whereas the oil samples with supplementary Saffron extract with ZnoNps at the concentration of 200, 300 ppm were recorded the lowest TBA values after 12 hours at 120º C being (0.809 and 0.798 mg malonaldhyde /kg oil) mg malonaldhyde / kg oil, respectively.

The intensification in TBA that heating process of control soybean oil samples might be ascribed to the foundation of malonaldhyde products explicitly aldehydes and ketones and the free radicals from unsaturated fatty acid putrefaction (Thamer, 2021).

Table 4. Some chemical properties of soybean oil treated with saffron ethanolic extract and zinc oxide nanoparticles throughout heating process all over 12 hours at 120º C.

Chemical properties	Heating time (hours)	Oil samples					
		control	TBHQ	Saffron extract		Saffron extract with ZnoNps	
				200ppm	300ppm	200ppm	300ppm
Acid value (*mg KoH/gm oil*)	0	0.644	0.511	0.531	0.528	0.511	0.505
	6	0.723	0.699	0.801	0.790	0.690	0.685
	12	1.430	0.851	0.980	0.969	0.720	0.710
FFA %	0	0.566	0.355	0.361	0.357	0.331	0.325
	6	1.033	0.967	0.986	0.980	0.889	0.850
	12	1.889	1.280	1.313	1.301	1.011	0.986
Peroxide value (*ml.eqv/Kg oil*)	0	3.555	2.440	2.500	2.476	2.425	2.420
	6	4.954	2.688	3.554	3.322	2.693	2.640
	12	6.210	4.058	5.232	5.181	4.058	4.039
Thiobarbituric acid value (*mg mal/ Kg oil*)	0	0.765	0.636	0.646	0.640	0.602	0.600
	6	0.902	0.772	0.660	0.651	0.610	0.605
	12	1.021	0.801	0.827	0.822	0.809	0.798

In supposition, the accumulation of ZnO NPs saffron extract to soybean oil exhibited a positive upshot on chemical indicators and could be commercially exploited and unveiled respectable source of antioxidants possessions having a constant method in the product thru thermal process of edible oils.

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دراسة تأثيرجزيئات الزنك النانونية من مستخلص الزعفران أثناء المعاملة الحرارية لزيت فول الصويا

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الملخص

يهدف هذا العمل إلى دراسة تأثير الجسيمات النانوية لمستخلص أكسيد الزنك والزعفران وتأثيرها كمضاد أكسدة طبيعي. أظهرت الخصائص الهيكلية والبصرية للجسيمات النانوية باستخدام الميكروسكوب الإلكتروني TEM تشكلاً كرويًا لجسيمات ZnO النانوية بمتوسط حجم الجسيمات من 15 إلى 50 نانومتر. تمت إضافة جزيئات أكسيد الزنك النانوية من مستخلص الزعفران الإيثانولي إلى زيت فول الصويا بتركيزات 200 و 300 جزء في المليون تمت مقارنتها مع TBHQ كمضاد أكسدة صناعي. تم تقدير الثبات التأكسدي والمعاملة الحرارية لمدة 12 ساعة في علي درجة حرارة 120° مئوية لزيت فول الصويا المعامل بمضادات الأكسدة المختلفة. اظهرت النتائج التي تم الحصول عليها إلى أن إجمالي المحتويات الفينولية هو 54.66 ± 1.3 ملجم من GAE/g. في مستخلص الزعفران الإيثانولي، بينما يبلغ محتوي الفينولات الكلية 70.23 في نفس المستخلص. أظهر نتائج الفصل باستخدامHPLC وجود تسعة مركبات فينولية. كان حمض Saffon هو المركب الفينولي الأكثر وفرة في مستخلص الزعفران 220.11 مجم/100 جم، بينما كان الهيسبريتين من أكثر مركبات الفلافونويد في نفس المستخلص وكان 87.51 مجم/جم. أظهرت البيانات التي تم الحصول عليها لاستقرار الأكسدة باستخدام الرانسيمات أن زيت فول الصويا المعالج باستخدام مستخلص الزعفران الإيثانولي ZnONps عند التركيز 300 جزء في المليون، وهو أعلى مستوى أظهر ثباتًا لمدة 18 شهرًا من التخزين بالمقارنة مع العينات الأخرى. سجلت نتائج المعاملة الحرارية لزيت فول الصويا المعالج بتركيز 300 جزء في المليون أدنى قيم التحلل المائي والأكسدة ومعامل الحساسية بعد 12 ساعة من التسخين. لذلك، أظهرت إضافة مستخلص الزعفران ZnONps إلى زيت فول الصويا تأثيرًا إيجابيًا على الثبات التأكسدي والحراري ويمكن التوصية به كبديل لمضادات الأكسدة الصناعية في الزيوت الغذائية. أظهر المجهر الإلكتروني النافذ (TEM) المستخدم كطريقة أن شكل دائري من جسيمات أكسيد الزنك النانوية متوسطة الحجم يتراوح من 15 إلى 50 نانومتر. أوضحت نتائج المركبات النشطة بيولوجيا أن محتوى الفينول الكلي هو 54.66±1.3 ملغم\GAE غم في مستخلص الزعفران الإيثانولي، في حين بلغ مجموع الفلافونويدات 70.23 في نفس المستخلص. تحديد المركبات الفينولية باستخدام HPLC من الواضح أنه تم اكتشاف تسعة مركبات. ويعتبر حمض سافرانال هو الأكثر وفرة في مستخلص الزعفران بنسبة 220.11 ملغم/100غم، بينما كان الهسبيريتين أكثر مركبات الفلافونويد في المستخلص نفسه بنسبة 87.51 ملغم/غم. باستخدام تراكيز 200 و 300 جزء في المليون من مستخلص الزعفران الإيثانولي ZnO تم إضافته إلى زيت فول الصويا ومقارنة تأثيره مع TBHQ كمضاد أكسدة صناعي. لوحظت البيانات المستحصلة عن ثباتية الأكسدة بطريقة رانسيمات أن العينة المعالجة بمستخلص الزعفران بجزيئات أكسيد الزنك النانوية بتركيز 300 جزء في المليون تمتلك أعلى زمن ثبات للعرض (18 شهراً) من التخزين مقارنة مع البسيطات الأخرى. تم تسجيل تأثير عملية تسخين زيت فول الصويا المعالج بمستخلص أكسيد الزنك الإيثانولي للزعفران باستخدام تركيز 300 جزء في المليون على أنها أقل أخلاقيات التحلل المائي والأكسدة والحد من النتانة بعد اثنتي عشرة ساعة من العملية الحرارية. وبالتالي، فإن إضافة جزيئات الزنك النانوية ومستخلص الزعفران كمضاد طبيعي للأكسدة إلى فول الصويا يمكن أن يقلل من التغيرات التأكسدية ويؤدي إلى تحسين جودة زيت فول الصويا.