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Liposome nanocapsules of aqueous extract from defatted wheat germ as by-products to enhance stability of biodiesel

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Abstract

Biodiesel is an alternative to fossil fuels and it's known by its low stability. Natural antioxidants attract researcher interest as safe replacement of the toxic synthetic ones to improve the oxidative stability. The aim of this work is to evaluate the antioxidant activity of natural extracts from wheat industry by products (wheat germ and defatted wheat germ) using low cost, safe and eco-friendly solvents. Loading the more active aqueous extract in liposome nanocapsules to overcome on its solubility problem in lipid then using it to enhance the stability of biodiesel is the novelty of this work. The antioxidant activity of different extracts was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and β-carotene-linoleic acid oxidation method (Coupled autoxidation). Total phenolic content and total flavonoid content were also determined for the different extracts. The oxidation and storage stability of biodiesel treated with defatted wheat germ aqueous extract loaded in liposome, in comparison with control sample and biodiesel treated with synthetic antioxidant were evaluated using accelerated oven test. Water extracts of wheat germ and defatted wheat germ gave higher bioactive content and higher antioxidant activity than other solvents extracts. Moreover, biodiesel treated with liposome nanocapsules of aqueous extract gave higher stability than both control and samples treated with synthetic antioxidants. These results concluded that natural antioxidants from wheat industry by-products are a promising alternative to synthetic antioxidants and attract attention to more research about using liposome nanocapsulation in improving biodiesel stability.

***Key words*:** Defatted wheat germ aqueous extracts, Natural antioxidant, Phenolic content, Antioxidant activity, Liposome nanocapsules, Biodiesel stability

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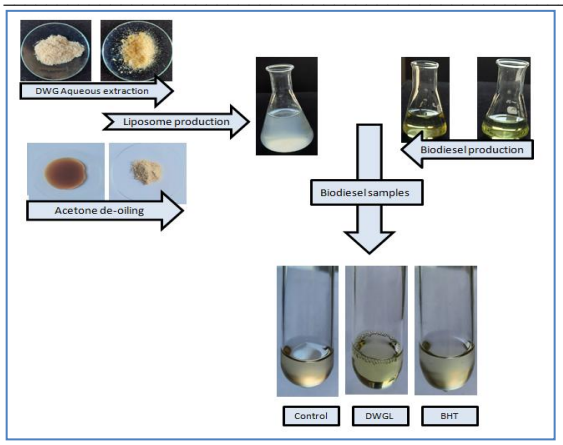


Graphical abstract

1. Novelty

Usage of food industry by-products (especially wheat industry) as an economically cheap and environmentally safe natural antioxidant for improving the oxidation, thermal and storage stability of biodiesel. Liposome nanoparticles loaded with bioactive compounds are widely used in food, medical and pharmaceutical industries. As we didn't find any research talk about using liposome in biodiesel industry to improve its oxidative stability we think that, this work is the first one to done in this field.

2. Introduction

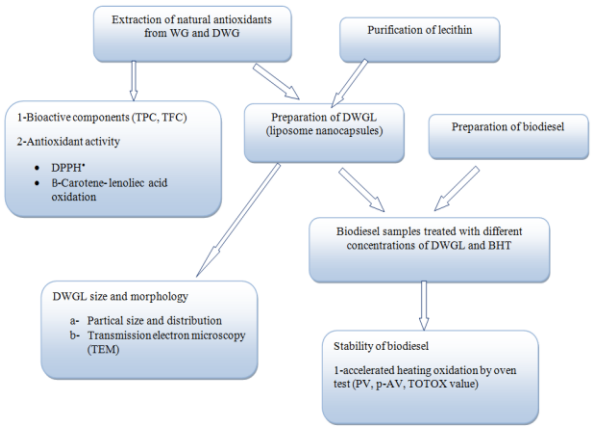
Biodiesel is a promising alternative to limited crude fossil fuel [1]. However, poor oxidation stability is a significant problem that faces commercialization of biodiesel [2]. The parent feedstock fatty acid profile impacts greatly the biodiesel stability. The higher the concentration of the unsaturated fatty acids (linoleic acid, linoleinc acid ...etc) the more acceptable of biodiesel for oxidation [1,3]. The naturally present antioxidants in the parent oils are deactivated during the process of biodiesel production (estrification) or removed during purification and separation of the products which cause the decrease of the biodiesel stability than that of the parent oil [3]. Light, air, minerals, moisture and storage container material are also affect on the biodiesel stability [1]. The products of oxidation process such as sediments, acids, gum and polymers compromise the fuel quality, fuel properties and the performance of the engine that can reduce the service life of the system of the fuel engine [4]. The higher molecular weight products will increase the viscosity of the biodiesel leading to many mechanical problems such as block fuel pumps and lines, injector and fuel filter as well as the formation of deposits in the combustion chamber [5,6]. To decelerate the rate of oxidation some of chemical compounds are used as antioxidant additives in the fuel. These antioxidants can donate hydrogen atom to delay the propagation step in the oxidation mechanism [2]. Although being toxic and non-biodegradable, synthetic antioxidants are widely used to increase the stability of biodiesel. Recently, natural antioxidants have been investigated as biodegradable and safe fuel additives [7]. Franca *et. al.* (2017) studied the potential effect of extract from *Moringa Oleifera* Lam leaf on the oxidative stability of biodiesel [8]. In 2019, Freitas *et. al*. study assessed the activity of curcumin , catechin and quercetin as antioxidants on methylic biodiesel from cotton seed oil [9]. The comparative study that done by Rodrigues *et.al*. (2020) showed that, the natural antioxidants exhibit higher retarding to the oxidation process of biodiesel than the synthetic antioxidants [10].

Wheat is one of the most used food ingredients and cereals in the world as it can be grounded into flour. The by-products of wheat milling industry including wheat bran, wheat germ, and endosperm parts were found to be about 23% - 27% of the output milling. Wheat germ (WG) is widely used for wheat-germ oil production as it containing about 8% - 14% oil which used in food, cosmetic and medical industries. However, huge amounts of wheat germ produced annually as wheat milling industry by-product in Egypt [11]. Inspite of being the main by-product of the wheat germ oil production, few systematic studies have been done on defatted wheat germ (DWG) antioxidants, while most of the papers were interested only about the oil from WG [12,13]. Moreover, DWG phenolics content and its contribution to the DWG antioxidant activities have been less studied [14]. Wheat antioxidants include tocopherols, carotenoids, phenolic acids

and flavonoids. Tocopherols, lipophilic antioxidants, located mainly in germ and bran. The phenolic compounds are present in both free and bound forms that linked to the cell wall of the caryopses outer layers. The main phenolics in wheat are hydroxybenzoic derivatives such as vanillic acids, hydroxycinnamic acids, and ferulic acids [15,16]. DWG extracts exhibits antioxidant activity in radical scavenging assay DPPH and ABTS. It is also notable that they have antimicrobial activity mainly on Gram-positive and Gram-negative bacteria. Thus, WG and DWG can be used as a vital source of antioxidant and antibacterial additives [11,17].

The hydrophilic compounds in the aqueous extracts suffer poor solubility in lipids that limits their bioavailability. Therefore, the improvement of the aqueous extracts solubility may enhance its bioavailability and its overall antioxidant efficiency [18]. Lecithin is a residue produced from the degumming process of vegetable oils. Alemán *et al.* (2021) reported that the liposomal encapsulation can improve chemical and physical stability of the bioactive compounds and prevent their activity possible loss and improve their bioavailability [19]. Soybean lecithin (contains 65-75% phospholipids) widely used in liposomes encapsulation due to its wide availability in low cost and its softy in use. The crude soybean phospholipids costs only 5% of that of the pure one, so it's good alternative and attractive choice [18]. The liposames are spherical vesicles of phospholipid bilayer with particle size range from 30 nm to several micrometers [20]. Liposome encapsulation emerging technology for either hydrophilic or lipophilic bioactive agents encapsulation, due to the presence of both aqueous and lipid phases[21]. The aqueous extracts were loaded on liposome to overcome the problem of its solubility. Liposome encapsulation widely used in food, medical and pharmaceutical industries [22].

The aim of this work is to evaluate the antioxidant activity of natural extracts from wheat industry by-products (wheat germ and defatted wheat germ) as cheap and widely available source using safe and eco-friendly hidrophlic and lipophilic solvents mixtures. The hydrophilic aqueous extract of defatted wheat germ loading in liposome nanocapsules to overcome on its lipid solubility problem and its effect on the enhancement of the oxidation stability of biodiesel is a novel work in biodiesel stability research area



Flow chart of methodology

3. Materials and methods

All solvents are analytical grade and were purchased from Elnasr Pharmaceutical Chemicals Co. (ADWIC), Egypt. Folin-Ciocalteu reagent was purchased from Sisco Research Laboratories Chemicals, India. DPPH was purchased from Sigma-Aldrich (St Louis, MO, USA). Cholesterol was purchased from S d Fine Chem. limited Co, India. Wheat germ and lecithin were purchased from local market in Egypt.

3.1. Preparation of WG and DWG extracts

WG was divided into two parts and oil was extracted from one of them to give DWG. 25g of each part was extracted twice in 100 ml of different solvents (water, water : isopropanol (1:1) and water : ethyl lactate (1:1)) for 1 hour under sonication in ultrasonic water bath (NEY Ultrasonik 28 H, 220 V 50/60 Hz 9A 200 W).

3.2. Determination of bioactive component in different extracts

3.2.1. Total phenolic content

Total phenolic content in the extracts was determined according to Folin-Ciocalteu colorimetric method using an UV-visible spectrophotometer (Shimadzu, UV-spectrophotometer UV-240) [23].

3.2.2. Total Flavonoid (TFC) content

Total Flavonoid (TFC) contents were determined by colorimetric method [24]. TFC content was expressed as mg equivalent quirecetin / 10 g dry extract.

3.3. Measurement of the extracts antioxidant activity:

3.3.1. DPPH• radical scavenging assay (R.S.A %)

DPPH• radical scavenging assay depends on measuring the change in the DPPH• solution color results from the scavenging the DPPH• radical by protons from the natural antioxidants. 3.9 mL of DPPH• solution (0.0024mg/100mL) was mixed with different concentrations of antioxidant extracts in test tubes. They left to stand for 30 min prior to being detected spectrophotometrically at 515 nm. The radical scavenging activity (R.S.A %) were calculated as following

$$R.S.A\% = [(A_{DPPH^{\cdot}} - A_S)/ A_{DPPH\bullet}] \times 100$$

Where, $A_{DPPH^{\bullet}}$ is the absorbance of the blank DPPH• solution and A_S is the absorbance of the solution contain the extract. EC_{50}, the extract concentration providing 50% inhibition was calculated from plotting of different concentration of each extract under optimum conditions against R.S.A% via relation equation. Also antiradical power (ARP) is used to define antioxidant action of antioxidant and it's defined as: $ARP= 1/EC_{50}$ [25]

3.3.2. β-Carotene-linoleic acid oxidation method (coupled autoxidation)

β-Carotene-linoleic acid bleaching method (coupled autoxidation) was also used to determined the antioxidant activity of the extracts. The Miller (1971) spectrophotometric method based on measuring the ability of different extracts to decrease oxidation of β-carotene in the used emulsion of β-carotene/linoleic acid [26].

3.4. Purification of lecithin

Lecithin was purified by acetone de-oiling process to remove the remainder oil and fatty acids. Few grams of the crude lecithin were washed several times by acetone with vigorous stirring. The precipitate was filtered of and dried to give the acetone insoluble (AI) [21].

3.5. Preparation of DWG liposome.

DWG liposome (DWGL) was prepared by film dispersion method. Blank liposome film was prepared by dissolving acetone insoluble lecithin and cholesterol in chloroform in l:l molar ratio followed by evaporation in round bottom flask in Heidolph rotary evaporator at 45°C under vacuum. DWG aqueous extract dissolved in distilled water was added to the blank liposom film in ratio 1:3 and stirred until dissolving all the blank liposome. The formed DWGL was homogenized by high speed homogenizer to reduce its particle size followed by sonication to reach to the nano size [27,28].

3.6. Characterization of DWGL size and morphology

3.6.1. Particle size and distribution

Particle size (PS), and polydispersity index (PDI) were determined using a Zetasizer Nano ZS (Malvem Insterament Ltd., Worcestershire, UK).

3.6.2. Transmission electron microscopy (TEM)

The sample was diluted with distilled water and sonicated for 10 min. one drop of DWGL was deposited on carbon coated grid, dried and stained with 1% phosphotungestic acid. The sample was dried and studied using Joel-JEM 2100 HRTEM (Japan).

3.7. Biodiesel preparation

Biodiesel has been prepared using alkali catalized transestrefication. 4:1 molar ratio of methanol/oil was used with 1% of KOH (w/w, oil/catalyst) as the alkali catalyst. Initially, potassium methoxide was prepared by dissolving KOH in methanol. Thereafter, it was added to the oil in a round bottom flask coupled with reflux under stirring at 900 rpm for 2 h at 60°C. Methanol was evaporated under vacuum. The reaction mixture was then transferred to a

separating funnel until the two phases were separated with the upper phase contains biodiesel and lower one contain glycerin. Glycerin phase was removed and the biodiesel was washed with distilled water many times to remove the catalyst. After washing, anhydrous sodium sulphate was added to the separated biodiesel to get rid of the residual moisture followed by filteration under layer anhydrous sodium sulphate [9].

3.8. Biodiesel sample preparation

Different biodiesel samples were prepared by adding different concentrations of DWGL and BHT to biodiesel samples (500, 1000, 1500 ppm) in addition to a control sample without any additives.

3.9. Storage stability (oven test)

The control and treated samples were heated at about 175 °C in an oven for 6 hours. The oil samples were removed from the oven at certain time intervals (0.5, 1, 2, 3, 4, 6 h). Oxidative stability was studied by measuring peroxide value (PV) for primary oxidation and *p*- anisidin value (*p*-AV) for secondary oxidation [29]. PV was determined according to AOCS official method Cd 8b-90 (2005) [30]. *p*-AV was determined as described in El-Malah *et al*., (2018). Total oxidation value **(**TOTOX value) is the sum of both PV and *p*-AV which used to estimate the total oxidative deterioration of oil [31]. It was calculated according to the equation:

$$\text{TOTOX value} = p\text{-AV} + 2\ \text{PV}$$

3.10. Statistical analysis

Results are presented as the mean ± standard deviation from three replicates of each experiment. A P-value < 0.05 was used to denote significant differences between mean values determined by the Microsoft Excel 2010. One-way analysis of variance (ANOVA) was used [32].

4. Results and discussion

Different extracts of WG and DWG extracted by three safe and eco-friendly different hydrophilic (water) and lipophilic solvents mixtures (isopropanol and ethyl lactate mixture with water) using USE method. The active component content and antioxidant stability of the different extracts were determined.

4.1. Bioactive Components

4.1.1. Total phenolic content (TPC)

Table1, indicates the TPC of different WG and DWG extracts expressed as mg equivalent gallic acid /10 gm dried extract. TPCs were ranged from 68. 69 to 166.88 mg equivalent gallic acid /10 gm dried extract for all extracts. The lower values were given by water: ethyl lactate extracts for both WG and DWG. The higher values were found to be in water extracts for both WG and DWG this may be refers to the water polar nature which dissolves higher amounts of polar phenolics. Water extract give very slightly higher TPC in WG than in DWG but vice versa was found for water: isopropanol and water: ethyl lactate extracts. The range of water and water: isopropanol extracts TPC was similar to that range reported previously by K.X. Zhu et. al., (2011) [14].

4.1.2. Total flavonoid Content

TFC represented in Table 1, showed that lower TFC were given by water: ethyl lactate extracts (42.78 and 44 mg equivalent quirecetin/10 g dried extract for WG and DWG respectively). Meanwhile, the higher values were given by water: isopropanol extracts which equal about 54 mg equivalent quirecetin / 10 g dried extract for both WG and DWG extracts.

Table 1: Bioactive components and antioxidant activity of WG and DWG extracts by different solvents.

Extract	TPC mg/10g	TFC mg/10g	EC_{50}	$1/EC_{50}$ *1000	A.O.A %
WGE (water)	166.88 ± 5.89	52 ± 0.66	42.39	23.59	83.54 ± 4.5
WGE (water:isopropanol)	139.69 ± 4.56	54.67 ± 0.47	89.95	11.11	92.3 ± 0.7
WGE (water:ethyl lactate)	67.299 ± 0.58	42.78 ± 0.77	217.54	4.59	53.84 ± 4.6
DWGE (water)	165.73 ± 9.87	50 ± 0.001	46.57	21.47	92.06 ± 2.6
DWGE (water:isopropanol)	144.06 ± 3.68	54.33 ± 0.88	48.87	20.46	93.62 ± 1.7
DWGE (water:ethyl lactate)	68.65 ± 0.44	44 ± 0.67	270.35	3.69	36.6 ± 5.4
BHT	---	---	38.05	26.28	75.21 ± 1.1

WG= wheat germ, DWG= defatted wheat germ, BHT=butylatedhydroxytoluene. TPC= Total phenolic content (mg gallic acid/ 10g dry extract), TFC: Total flavonoid content (mg quirecetin equivalents / 10 g dry extract), EC50: Concentration of extract that causes a 50% decrease in DPPH absorbance, 1/EC50 antiradical power, A.O.A%= Antioxidant activity (value represent the percent inhibition of oxidation of the linoleic acid/β-carotene emulation).

4.2. Antioxidant activity:

The antioxidant activity of different extracts was measured by DPPH$^{\bullet}$ free radical scavenging assay and β- carotene- linoleic acid oxidation method.

4.2.1. DPPH$^{\bullet}$ free radical scavenging assay:

The antioxidant activity was indicated by the ability of the extract to scavenging The DPPH$^{\bullet}$ free radicals. R.S.A% of the different extracts in Figure1 shows the higher values by water and water: isopropanol extracts for both WG and DWG. Data in Table 1, showed the different extracts EC_{50} which is the extract concentration that can scavenge 50% of The DPPH$^{\bullet}$ free radicals. The antiradical power $1/EC_{50}$ was also included in Table 1. The decrease in the EC_{50} value is an induction in the increase in the natural extract antioxidant activity. On the contrary, as the antiradical power increased the antioxidant activity increased. Higher EC_{50} and lower $1/EC_{50}$ were given by water: ethyl lactate extracts that means they have lower antioxidant activity. Water extracts gave lower EC_{50} and higher $1/EC_{50}$ as they have higher antioxidant activity, while water: isopropanol extracts gave moderate values. Water extracts higher antioxidant activity was may be attributed to their higher TPC as the increase in TPC leads to the increase in R.S.A% [33]. It's noteworthy to mention that the antioxidant activity of water extracts was approaching of that of the synthetic one (BHT) which make it a promising natural alternative antioxidant with higher safety and very low Cost.

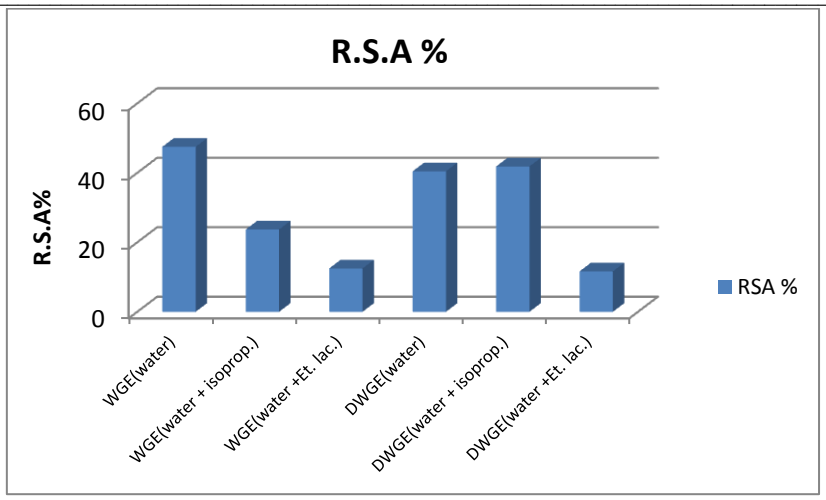


Figure 1: Radical scavenging activity (R.S.A%) of different wheat germ extracts (WGE) and defatted wheat germ extracts (DWGE) at concentration of 40 mg/ml.

4.2.2. β- Carotene- linoleic acid oxidation (Coupled autoxidation)

This method depends on the ability of the extract to reduce β- carotene oxidation in presence of lenoleic acid. The antioxidant activity (A.O.A%) was determined by measuring the change in the intensity of β-carotene color. Water: ethyl lactate extracts which have lower TPC was found to have lower A.O.A% while, water and water: isopropanol extracts for WG and DWG were almost closer to each other (ranged from 83.67 to 93.62 %). WG water extract as well as DWG extracted by water and water: isopropanol gave higher A.O.A% than that of synthetic antioxidant (BHT) (Table 1). This considered as another evidence on the suitability of WG and DWG natural antioxidant as good alternative. However, the best results were given by water extracts and the difference between WG and DWG extracts activity is very slight. For this reason we prefer to apply DWG water extract in the improvement of the oxidative potency of biodiesel, while WG can be used in the production of the healthy and important wheat germ oil.

4.3. Liposomal DWGL

The main problem which facing us is the aqueous extracts (hydrophilic) solubility in biodiesel (lipid medium). To overcome this problem the aqueous extract was loaded on liposome using low cost crude lecithin which has hydrophilic and lipophilic phases. To get lecithin with higher content of phosphorlipids the ordinary strategy of acetone de-oiling is used [34,35].The purified lecithin produced from acetone de-oiling was found to be about 50 % of the crude one so it was used in the preparation of DWGL.

Size and morphology of DWGL:

After preparation of DWGL its particle size was reduced to nano size by homognation and sonication. The particle size (PS), and polydispersity index (PDI) of DWGL were studied by particle size analyzer. The average particle size of DWGL was 42.33dynamic nanometer and give good PDI (0.671) (Fig 2). PDI indicates the uniformity of liposome size which related to the aggregation, homogeneity and the quality of the colloidal system. PDI of DWGL was found to be 0.671. TEM graph of DWGL showed the nanospherical vesicles of liposome of <100 nm (Fig 2). Particle size is an important property, as it can affect of on the stability and bioavailability of the liposome [27]. Therefore, the particle size of DWGL (<100 nm) were expected to improve lipid solubility, stability and activity of DWG aqueous extract in biodiesel. These data provide us an evidence about feasibility of crude lecithin phospholipids for preparation of nano-liposomes loaded with

aqueous extract of DWG. Therefore, it can be used for basic formulation of DWGL that provide a prolonged protection effect against biodiesel oxidative stress.

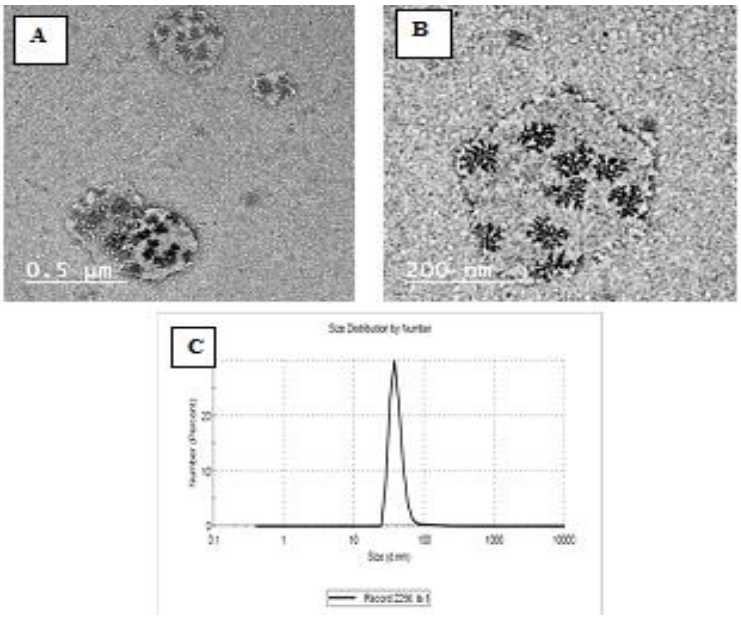


Figure 2: Morphology and particle size analysis of DWGL, [A, B] Transmission Electron Microscopy (TEM) graph of DWG liposome, [C] particle size distribution.

4.4. Storage stability of biodiesel:

Sunflower biodiesel was prepared by alkali catalized transestrefication. The prepared biodiesel was found to have peroxide value (PV) of 7.67 and *p*- anisidine value (p-AV) of 3.15. The accelerated oxidation of biodiesel samples was carried out at about 175°C for 6h to estimate the decline of biodiesel oxidation progress rate after addition of DWGL. Both PV and *p*- AV were used to determine the ability of the added DWGL to delay the primary and the secondary oxidation of the biodiesel during accelerated heating.

4.4.1. Change in PV:

The primary oxidation was followed by PV. PV increased when the formation rate of peroxides is higher than its rate of composition. Data in Table 2a expressed the effect of the addition of DWGL to biodiesel on the PV change rate during heating sunflower biodiesel at 175°C for 6 h. PV was increased by increasing heating time. The rate of PV increasing was lower in samples containing the nanocapsulated natural antioxidant DGWL than that of the control and the samples containing BHT as seen from Table 2a.

4.4.2. Power of inhibition of lipid oxidation (IO%) :

IO% is a term used to monitor the improvement of oxidation stability during heating. IO% of different biodiesel samples were recorded in Table 3. The demonstrated data showed that the higher IO% values were given by biodiesel samples contain different concentration of DWGL than the IO% of control and samples with BHT, the IO% increased by increasing concentration of DWGL.

4.4.3. Change in p-AV:

The hydroperoxides decomposition to secondary oxidation products (aldhydes, ketons, acids....) were detected by *p*-AV. The *p*-AV results were observed to be similar to PV results. Biodiesel with DWGL gave lower rate of *p*-AV increasing followed by biodiesel with BHT and control which has higher increasing rate (Table 2b).

4.4.4. Total oxidation value (TOTOX value)

TOTOX value is a mathematical prediction of total oxidation stability in the sample calculating from both PV and *p*-AV. From data in Table 4, it was found that higher TOTOX value was given by control at different time intervals. Meanwhile, samples supplemented by different concentrations of DWGL gave lower TOTOX value and hence gave higher oxidative stability even more than sample with the same concentration of BHT (Fig. 3).

Table 2: Effect of addition of liposome of aqueous extracts compared with BHT to sunflower biodiesel on PV and *p*-AV during heating in oven test.

Time(h)	**a- PV(meq/kg oil)**						
	control	**DWGE**			**BHT**		
		500 ppm	**1000 ppm**	**1500 ppm**	**500 ppm**	**1000 ppm**	**1500 ppm**
0.5	19.21 ±1.1	16.3 ±2.4	13.66 ±1.4	10.33 ±2.3	19.28 ±0.7	18.82± 2.3	16.78± 1.4
1	22.94 ±4.1	17.12 ±2.0	13.54 ±2.4	11.4±2.4	22.81±0.5	22.64± 2.5	19.68± 1.4
2	25.03 ±3.0	14.01±0.2	10.72±0.02	9.91±0.18	23.25±2.7	21.37± 1.7	21.22± 2.1
3	26.34±2.0	17.82±4.1	12.17±1.6	9.93±0.05	24.48±1.7	22.22±0.8	21.79±0.5
4	26.62±0.9	20.62±2.1	19.06±3.0	13.74±2.9	25.7±3.7	24.6±2.4	23.26±1.3
6	30.21±1.7	23.32±2.2	22.34±0.2	24.18±1.1	23.3±2.7	20.61±3.3	17.85±2.3
b- *p*-AV							
0.5	12.11±	9.161±	7.73±	5.343±	11.37±	11.5±	11.6±
1	24.5±0.1	18.7±0.1	18.5±0.8	13.1±1.3	23±0.4	28.7±0.2	23.8±0.7
2	36.45±0.4	26.45±0.07	20.03±1.1	18.55±0.2	33.48±0.1	33.51±0.4	35.01±1.2
3	53.57±0.3	29.08±2.3	26.43±0.6	20.4±0.6	47.33±0.8	44.23±0.3	44.03±1.4
4	80.13±0.2	57.03±3.9	56.5±1.4	55.47±3.7	73.166±0.3	71.95±4.0	67.13±2.1
6	313.85±2.2	296.8±5.6	290.21±0.7	282.88±0.7	301.71±1.0	300.47±0.8	297.47±2.3

PV= peroxide value, *p*-AV = *p*-anisidine value, DWGE= defatted wheat germ water extract. BHT= butylatedhydroxytoluene

Table 3: Effect of addition of liposome of aqueous extracts compared with BHT to sunflower biodiesel on IO% during heating in oven test.

Time (h)	IO %					
	DWGE			BHT		
	500 ppm	1000 ppm	1500 ppm	500 ppm	1000 ppm	1500 ppm
0.5	23.47	35.87	51.5	9.48	11.64	21.22
1	25.37	40.97	50.3	0.56	1.3	14.21
2	44.02	57.17	60.4	7.11	14.62	15.22
3	32.34	53.79	62.3	7.06	15.64	17.27
4	22.53	28.39	48.38	3.45	7.58	12.62
6	22.8	26.05	19.96	22.87	31.77	40.91

IO %= the calculated inhibition of oil oxidation = [1-(PV increase of sample / PV increase of control) x 100],
BHT=butylated hydroxytoluene, DWGE= defatted wheat germ water extract.

Table 4: Effect of addition of liposome of aqueous extracts compared with BHT to sunflower biodiesel on TOTOX value during heating in oven test.

Time (h)	TOTOX value at 175 °C						
	Blank	DWGE			BHT		
		500 ppm	1000 ppm	1500 ppm	500 ppm	1000 ppm	1500 ppm
0	18.49	18.49	18.49	18.49	18.49	18.49	18.49
0.5	50.53	41.761	35.05	26.003	49.93	49.14	45.16
1	70.38	52.94	45.58	35.9	68.62	73.98	63.16
2	86.51	54.47	41.47	38.37	79.98	76.25	77.45
3	106.25	64.72	50.773	40.26	96.29	88.67	87.61
4	133.37	98.27	94.62	82.95	124.566	125.89	122.63
6	374.27	343.44	334.89	331.24	348.31	341.69	333.17

TOTOX value= total oxidation value, BHT=butylated hydroxytoluene, DWGE= defatted wheat germ water extract.

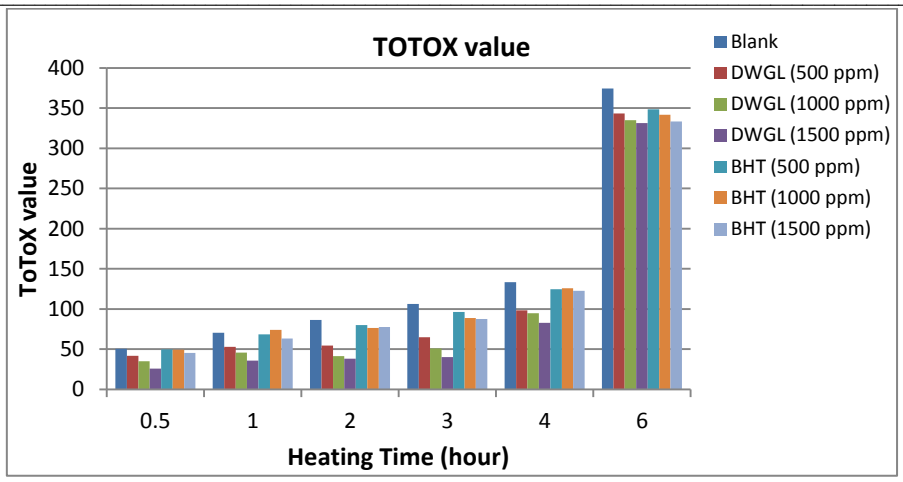


Figure 3: Chang in TOTOX value by heating during different time intervals

5. Conclusion

This study deals with the usage of different food industries by-products, such as WG and DWG from wheat industry as natural antioxidants to improve biodiesel stability. Water and its mixture with isopropanol or ethyl lactate were used as nontoxic solvents. The DWG loaded in liposome, to overcome the problem of its solubility in lipid, was added in different concentrations to sunflower biodiesel to improve its stability in comparison with BHT as synthetic antioxidant. The results indicated that aqueous extracts from WG and DWG have high TPC and TFC. This makes the extracts have high level of antioxidant activity. Loading DWG extract in liposome formulated a nanoparticle with PS < 100 nm and good PDI. Biodiesel stability was improved by addition of DWGL which gave promising results even better than BHT in accelerated oven test at about 175°C for 6h. This study provided new insight to use liposomes in the field of improving the lipid biodiesel stability using natural aqueous extracts that contain high level of polar bioactive compounds

6. Conflicts of interest.

The authors declare that they have no conflicts of interest in relation to this article.

7. References

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