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OXIDATIVE STABILITY OF SOYBEAN AND SUNFLOWER OILS ENRICHED WITH PIGMENT EXTRACTS OF THE BROWN SEAWEED *PHYLLARIA RENIFORMIS*

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ABSTRACT

The incorporation of bioactive additives such as natural pigments in food products offers many nutritional advantages, associated with functional properties in particular antioxidant effects. The aim of this work was to study the effect of adding natural pigments extracted from the brown seaweed *Phyllaria reniformis* on the oxidative stability of commercially available soybean and sunflower oils. *Phyllaria reniformis* pigment extract (200 and 1000 ppm) was dissolved in the two edible oils, and in comparison, a synthetic antioxidant butylated hydroxyanisole (BHA) was added. Experimental results showed that the addition of pigment extract had no significant effect on the quality parameters of vegetable oils: Free acidity (FA) and peroxide value (PV). Carotenoids were improved 2 and 1.5 times when adding 200 ppm of *pigment* extract to soybean and sunflower oils, respectively. While, when adding 1000 ppm, carotenoids were improved 3 times in comparison to the virgin oils. Similarly, chlorophylls contents in enriched sunflower oil with 200 or 1000 ppm of pigment extract increased 2 and 3 times in comparison to the control sample, however, in enriched soybean oil, this increase was interestedly higher achieving 10 and 33 times. Moreover, colour coordinates (a^*, b^* and L^*) in enriched oils changed to become greener and yellower. Antioxidant activities were improved compared to the control oils. The addition of pigment extract or BHA to soybean oil increased significantly ($p \leq 0.05$) its oxidative stability. Thus, these pigment extracts could be recommended as a potent source of natural antioxidants replacing synthetic ones for the protection of edible oils against oxidation.

Keywords: Oxidative stability, Edible oils, Brown seaweed, *Phyllaria reniformis*, Pigments

INTRODUCTION

During the past decade, consumer's interest and preference for natural substitutes of synthetic additives increased mainly for health benefic reason. Moreover, these natural additives are nontoxic, biodegradable and do not leave damaging residues, however, they showed lower efficiency compared to the synthetic ones **(Kim & Chojnacka, 2015; Scott et *al.*, 2020)**. Therefore, there is an urgent need to develop new and safe products of natural origin, with similar or better properties to the synthetic ones, in particular antimicrobial, antifungal, and antioxidative **(Kim & Chojnacka, 2015)**.The incorporation of natural bioactive additives into food products (i.e., beverages, bakery, oils and dairy products) is growing on worldwide market. Among commercial functional foods, enriched vegetable oils still take the major part in all the food categories **(Blasi & Cossignani, 2020; Lourenço et *al.*, 2019)**.

Edible vegetable oils, the ideal cooking media today, hold an important part of human diet for multiple viewpoints such as nutritional value, organoleptic characteristics and functional properties within the food matrix **(Czaplicki et *al.*, 2016; Hannachi & Elfalleh, 2020; Holt, 2016; Makni et *al.*, 2015)**. Soybean and sunflower oils belong to the popular vegetable oils utilized worldwide in food, cosmetic, and pharmaceutical industries because of their high fatty acids and liposoluble vitamin contents **(Kozłowska & Gruczyńska, 2018)**. However, lipid oxidation is a major factor affecting their nutritional and sensorial qualities **(Siraj et *al.*, 2019)**. Thus, enrichment of vegetable oils with antioxidants seems to be a solution to prevent the oxidation process **(Şahin et *al.*, 2017, Saoudi et *al.*, 2016)**. Various synthetic antioxidants were used within regulated limits to reduce deterioration, rancidity and oxidative discoloration in vegetable oils. Butylated hydroxyl anisol (BHA) and butylated hydroxyl toluene (BHT) are two widely used synthetic antioxidants, however, they are volatile and decompose easily at high temperatures **(Ammari et *al.*, 2012)**. Besides, recent reports revealed that these compounds may have harmful side effects **(Yao et *al.*, 2020)**. So far, many natural pigments were used as additives in food systems, they induced nutritional advantages in addition to an appealing colour associated with good functional properties in particular antioxidant effects **(Batista et *al.*, 2006; Gouveia et *al.*, 2007)**. In a previous study, **Ghaliaoui et *al.* (2020)** showed that Algerian coast is rich in brown seaweed such as *Phyllaria reniformis*, they showed that these seaweeds are renewable source of natural pigments such as chlorophylls and carotenoids with high antioxidant activities. They investigated the impact of freezing and drying preprocessing on pigments extraction from the brown seaweed *Phyllaria reniformis* and showed that the obtained extracts analysed by HPLC and UV-visible spectrophotometry, contained higher levels of carotenoids and chlorophyle with high antioxidant activities.

Against this background, the aim of this study was to investigate and to improve oxidative stability of soybean and sunflower oils enriched with natural pigment extracts of the brown seaweed *Phyllaria reniformis*. To the best of our knowledge, the addition of *Phyllaria reniformis* pigment extracts as natural antioxidant was not reported to date.

MATERIAL AND METHODS

Seaweed collection

Fresh *Phyllaria reniformis*, was collected in June 2016 on the Tipaza coast, north center of Algeria (36° 35' 50" N / 2° 27' 10" E) (Figure 1). After a first rinse on-site with sea water, alga was taken to the laboratory in isothermal boxes. Alga species identification was made in the Laboratory of Biological Oceanography and Marine Environment, University of Science and Technology Houari Boumediene (USTHB), Bab Ezzouar, Algeria. Alga samples were washed for a second time with fresh tap water to remove sand, epiphytes, shells and any sediment, then a last wash by distilled water was achieved. Afterwards, alga was stored at -18°C until pigment extraction.

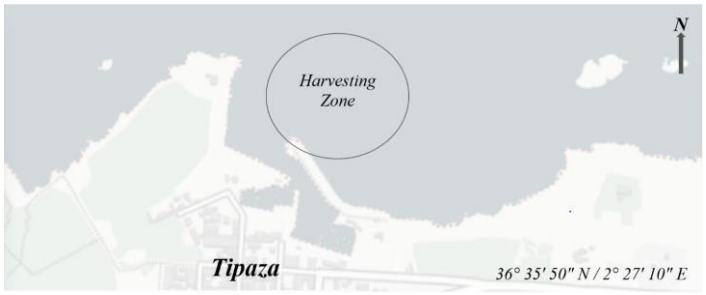

Figure 1 Harvesting location map of Alga

Extraction of seaweed pigments

Phyllaria reniformis pigments were extracted using ultrasound assisted extraction as previously described by **Ghaliaoui et *al.* (2020)**. First, *Phyllaria reniformis* alga was cut into small pieces and mixed with acetone at a ratio of 1/30 (w/v). the mixture was put in an ultrasonic bath (100W, 20 kHz, 24°C, 90 min) for pigments extraction. The temperature in ultrasonic bath was maintained below 25 °C. Obtained extract was filtered and concentrated using rotary evaporator at 28°C. The pigment extract was freeze-dried and then stored at – 20 °C in brown glass flasks. These steps were performed under low light intensity and as quickly as possible to prevent pigment decomposition.

Vegetable oils

Soybean oil (Labelle, Algeria) and refined sunflower oil (Lesieur, France) were purchased from a local market in 2 and 1 L packs, respectively. Both are edible vegetable oils extensively used in Algeria.

Preparation of enriched vegetable oils

Two concentrations of *Phyllaria reniformis* pigment extract (200 and 1000 ppm) were partially dissolved in sunflower or soybean oils and mixed vigorously using ultrasonic bath (Bioblock Scientific TS 540, Germany) at the following conditions: Power 100W and 20kHz for 30 min at 24°C to complete dissolution. 200 ppm of a synthetic antioxidant (BHA) was added to vegetable oils for comparison. Native and enriched oils were kept in amber glass bottles at 6°C for further analysis.

Free Acidity

Free acidity (FA) content was determined using the standard method **(ISO 660)**. 10 g of vegetable oil were weighed into a 250 mL glass Erlenmeyer, then 75 mL of neutralized ethanol and two to three drops of 1% (w/v) of phenolphthalein as indicator were added. The mixture was titrated with 0.1 N NaOH until pink colour appeared and persisted (10 s). The FA content was calculated as percentage of oleic acid according to the following formula:

$$FA\ \ (as\ oleic\ acid)\ (\%) = \frac{V \times N \times 28.2}{m}$$

Where:
V was the volume of NaOH consumed (mL);
N was the normality of NaOH;
m was the mass of the test sample (g).

Peroxide value

The primary oxidation compounds of oils were evaluated by the peroxide value (PV) using the standard method **(ISO 3960**) and briefly described in the following. 5 g of sunflower or soybean oils were weighed into a 250 mL glass Erlenmeyer, 12 mL of chloroform and 18 mL of acetic acid were added, then 1mL of saturated potassium iodide (KI) was incorporated into this solution. After 1 min of incubation in dark, 75 mL of distilled water were added with stirring. The mixture was titrated with 0.01 N of $Na_2S_2O_3$ in the presence of starch solution (1% (w/v)) until the solution was completely discoloured. PV is given by the following formula:

$$PV\ (meq.peroxide/Kg\ sample) = \frac{(V_1 - V_0) \times N \times 1,000}{m}$$

Where:
V_1 was the volume of $Na_2S_2O_3$ consumed (mL);
V_0 was the volume of $Na_2S_2O_3$ of the blank test;
N was the normality $Na_2S_2O_3$ solution used;
m was the mass of the test sample (g).

Chlorophyll and carotenoid content in oil

Chlorophyll and carotenoid content in native and enriched oils were determined according to the procedure described by **Minguez-Mosquera *et al.* (1991)**. A sample of oil (7.5 g) was dissolved in 25 mL of cyclo-hexane. The amount of chlorophylls and carotenoids was measured using UV spectrophotometer (SPECORD 210 PLUS 623F1138, Germany) at 670 nm and 470 nm, respectively. The concentrations of total chlorophylls and total carotenoids in the enriched and native oils were expressed using the following equations:

$$Chlorophylls\ (mg/kg) = \frac{Abs_{670} \times 10^6}{613 \times 100 \times d}$$

$$Carotenoids\ (mg/kg) = \frac{Abs_{470} \times 10^6}{2000 \times 100 \times d}$$

Where:
Abs_{670} is the absorbance at 670 nm;
Abs_{470} is the absorbance at 470 nm;
d is the optical pathlength (1 cm);
613, 100, and 2000 are specific coefficients.

Colour measurement

The colour coordinates (*a**, *b** and *L**) of the native and enriched oils by seaweed pigment extracts or BHA were measured using a CR-10 colorimeter (Konica Minolta Cr-10 Tristimulus, Japan).

DPPH Radical Scavenging Activity of enriched oil

The DPPH radical scavenging activity was determined using the method of **Hazzit *et al.*, (2009).** 25 µL of soybean and sunflower oil samples enriched with *Phyllaria reniformis* pigment extract or BHA at different concentrations (0, 2.5, 5, 10, 20, 40, 60, 80, 100 µg/mL prepared using isooctane as solvent) were added to 975 µL of DPPH (2,2-diphenylpicrylhydrazyl) solution (60 µM), then incubated for 30 min in dark conditions. The absorbance was measured at 517 nm using a spectrophotometer (SPECORD 210 PLUS 623F1138). All measurements were repeated at least three times. The percentage of inhibition was estimated as:

$$\%\ \text{Inhibition} = (Abs_b - Abs_s\ /\ Abs_b) \times 100$$

Where Abs_s is the sample absorbance after 30 min and Abs_b is the sample absorbance before reaction. The IC_{50} (concentration that caused inhibition of 50% of DPPH) was calculated using the graph by plotting inhibition percentage against concentration.

Oxidative stability

The oxidative stability of the enriched and native oils was evaluated by measuring the induction time (IT), using a Rancimat apparatus (Metrohm, model 743, Switzerland). This method is based on the detection of the electrical conductivity in water caused by the volatile degradation compounds. The time taken to reach the conductivity inflection point was recorded and expressed as IT (h). In this study, 3 g of vegetable oil were heated at 100°C in a thermostated electric heating block and subjected to dried air at a flow rate of 10 L/h previously filtered and cleaned. IT was determined from the conductivity curve at the inflection point between the horizontal (conductivity, µS. min^{-1}) and vertical (time, h) tangents.

Statistical analysis

All the analysis (FA, PV, chlorophyll and carotenoid content, colour coordinates, IC50 and IT) were performed in triplicate and results were presented as average of replicates ± Standard deviation. An analysis of variance (ANOVA) was performed using the Statistical Analysis System R 4.0.2. **(R Core Team, 2020)**. ANOVA statistical tests were performed using Tukey's multiple comparison procedure with a 5% significance level.

RESULTS AND DISCUSSION

Free Acidity

Oil acidity expressed as FA is the most frequently used quality test for vegetable oils. However, FA increases with free fatty acids mainly formed during oxidation and triacylglycerol hydrolysis **(Neves *et al.*, 2020).** The vegetable oil oxidation process was induced by the reaction with moisture initially present or moisture formed during other deterioration reactions **(Al-Harbi & Al-Kahtani, 1993)**.
FA was determined, in order to assess the effect of *Phyllaria reniformis* pigment extracts addition in soybean and sunflower oils quality. Table 1 shows the FA content of supplemented or non-supplemented soybean and sunflower oils. The control oil sample without any additive and the enriched oil samples after the addition of 200 or 1000ppm pigment extract of *Phyllaria reniformis* or BHA (200ppm) were compared.
The control and enriched soybean and sunflower oils with BHA or pigment extract (200 ppm and 1000ppm) exhibited closer FA values which ranged from 0.42 to

0.63%. A slight increase of FA was observed in enriched soybean oils with BHA and with *Phyllaria reniformis* pigment extracts.

The addition of pigment extract to vegetable oils seemed not affecting FA, no significant differences (p>0.05) were observed in both vegetable oils (p =0.797 for soybean oil and p =0.401 for sunflower oil). Previously, similar results were obtained by **Sousa et *al.* (2015),** in their study about the effect of adding flavourings (hot chili peppers, laurel, oregano and pepper) to olive oils.

In opposition, other studies reported that the addition of natural additives led to increase significantly the vegetable oils FA. Thus, **Sousa et *al.* (2015)**, showed that the addition of garlic to olive oils induced an increase in FA values from 0.6 to 0.8%. In a similar study, **Gambacorta et *al.* (2007)** showed that FA results of extra virgin olive oils flavoured with herbs and spices were not affected after 7 months of storage. While, a significant increase was observed by **Ayadi et *al.* (2009)** in FA values of enriched oils by aromatic plants (rosemary, lavender, sage, lemon and thyme). Similar results were reported by **Ammar et *al*. (2017)** during their study of the effect of *Opuntia ficus indica* flowers addition to two virgin olive oils **(Ammar et *al*., 2017)**.

Peroxide value

PV is one of the most widely used quality parameters in food. It is measured to specify the concentrations of peroxides and hydroperoxides produced in the first stage of lipids oxidation **(Delfanian et *al*., 2016)**. PVs of soybean and sunflower oils before and after addition of *Phyllaria reniformis* pigment extract (200 ppm and 1000ppm) or BHA (200 ppm) were determined and shown in table 1.

PVs of soybean and sunflower oils obeyed the Codex Alimentarius limit for refined oils (≤10 mEq. O_2/kg) **(Codex Alimentarius, 1999).** When soybean oil was enriched with 200 or 1000 ppm of pigment extract or with 200 ppm BHA, PVs results varied from 3.96±0.03 to 4.95±1.00 mEq. O_2/kg. On the other hand, PVs of sunflower oil samples enriched with 200 or 1000ppm seaweed pigment extract were 8.92±1.01 and 8.94 ±0.97 mEq. O_2/kg, respectively. These values were lower than the control sample (10.47 ±1.47 mEq. O_2/kg) and the sunflower oil sample enriched with 200ppm BHA (10.38 ±0.44 mEq. O_2/kg). In all cases, in comparison to the two control oil samples, no significant changes ($p \leq 0.05$) were observed in PVs of enriched soybean and sunflower oils.

Hence, in the present study, addition of seaweed pigments or BHA to soybean and sunflower oils seems to not affect their PVs. Meanwhile, a recent study reported that the addition of *Opuntia ficus-indica* flowers induced a slight increase in the formation of peroxides of olive oil **(Ammar et *al*., 2017).** However, olive oils flavored with garlic and oregano exhibited lower PV comparatively to the control sample **(Sousa et *al*., 2015).**

Table 1 Free acidity (%), Peroxide values (mEq. O_2/kg of oil) and Colour coordinates (*a**, *b** and *L**) results of the enriched vegetable oils by *Phyllaria reniformis* pigment extracts.

	Soybean oil					Sunflower oil				
			Colour coordinates					Colour coordinates		
	Free acidity (%)	Peroxide value (mEq. O_2/kg of oil)	L*	a*	b*	Free acidity (%)	Peroxide value (mEq. O_2/kg of oil)	L*	a*	b*
Control	0.42 (0.14 $^{a)}$	3.96 (0.02 ^{a})	77.73 (0.70) ^{ab}	-4.2 (0.17) ^{b}	+18.46 (1.27) ^{a}	0.56 (0.00 ^{a})	10.47 (1.47 ^{a})	77.7 (1.15) ^{a}	-4.7 (0.43) ^{a}	+15.8 (0.43) ^{a}
BHA (200ppm)	0.56 (0.28 ^{a})	4.44 (0.48 ^{a})	75.3 (2.13) ^{ab}	-4.7 (0.17) ^{ab}	+17.9 (0.4) ^{a}	0.42 (0.14 ^{a})	10.38 (0.44 ^{a})	77.17 (3.08) ^{a}	-4.63 (0.20) ^{a}	+16.36 (0.32) ^{a}
Pigment extract (200ppm)	0.62 (0.08 ^{a})	3.96 (0.03 ^{a})	74.1 (1.47) ^{b}	-5.53 (0.64) ^{ab}	+20.63 (1.56) ^{a}	0.56 (0.00 ^{a})	8.92 (1.01 ^{a})	76.76 (3.21) ^{a}	-6.2 (1.73) ^{a}	+20 (0.7) ^{b}
Pigment extract (1000ppm)	0.63 (0.06 ^{a})	4.95 (1.00 ^{a})	69.23 (3.80) ^{a}	-6.03 (0.95) ^{a}	+46.8 (9.08) ^{b}	0.63 (0.07 ^{a})	8.94 (0.97 ^{a})	74.43 (1.55) ^{a}	-6.7 (0.78) ^{a}	+29.36 (2.47) ^{c}

Values between brackets design Standard Deviation (±SD), Mean values in the same column followed by different superscript letters are significantly different ($p \leq 0.05$ Tukey's HSD test).

Chlorophyll and carotenoids content

Carotenoids

Enriched soybean and sunflower oils with *Phyllaria reniformis* pigment extract exhibited richer contents of carotenoids than the control samples as shown in figure 2.

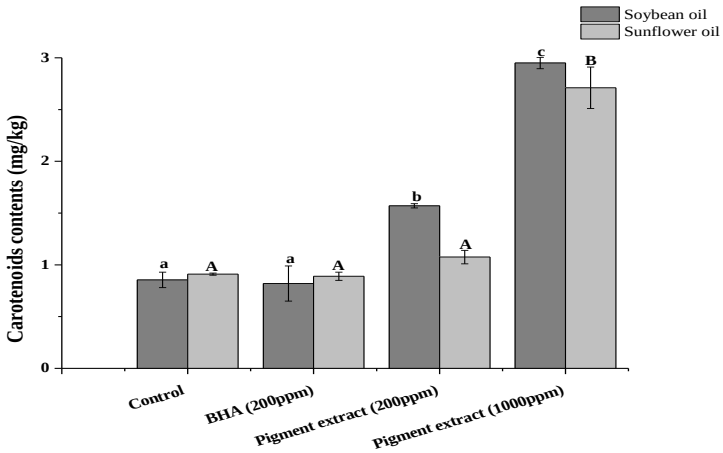

Figure 2 Carotenoids content of enriched and non-enriched edible oils (Mean ± SD). Different letters indicate significant differences ($p \leq 0.05$, Tukey's HSD test)

In comparison to both the control and the BHA soybean oil samples, total carotenoids content in the enriched soybean oil increased about 2 times (1.57± 0.02 mg/kg of oil) when adding 200 ppm and 3 times (2.95± 0.05 mg/kg of oil) when adding 1000ppm of seaweed pigment extract.

Statistical analysis showed a high significant difference (p=0.000293 ***) between total carotenoids content of enriched soybean oils with seaweed pigment extract and total carotenoids content of soybean oils without pigment extract.

The same results were observed with sunflower oil, where total carotenoids content in control sample (0.91± 0.01 mg/kg of oil) and BHA enriched sample (0.89 ±0.04 mg/kg of oil) were approximately 3 times lower than the enriched sample with 1000ppm of seaweed pigment extract (2.71± 0.2 mg/kg of oil). Results showed a highly significant difference (p=0.000701 ***) in the total carotenoids content of sunflower sample supplemented with 1000ppm compared to the remaining samples.

In the present study, total carotenoids content in soybean and sunflower oils increased significantly by increasing incorporation of seaweed pigment extract, particularly in soybean oil. This increase is probably due to the higher contents of total carotenoids in this pigment extract as shown in the previously published study of Ghaliaoui et al. (2020). These results revealed that the addition of seaweed pigment extract to soybean and sunflower oils may improve their oxidative stability.

Chlorophylls content

Chlorophylls content exhibited a similar trend to that of carotenoids. Figure 3 shows chlorophylls content of soybean and sunflower enriched oils.

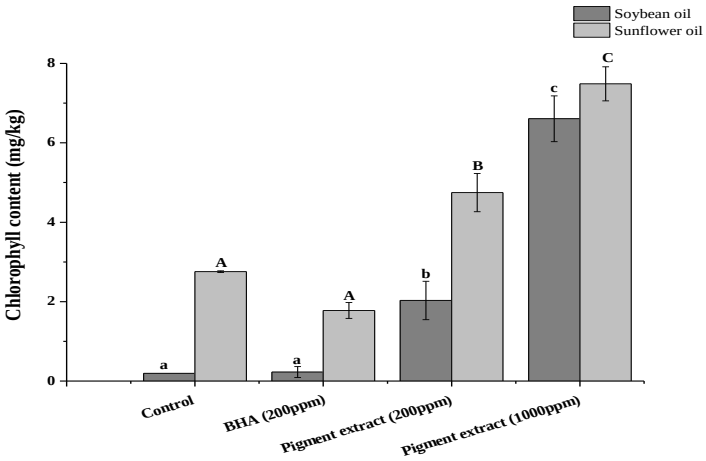

Figure 3 Chlorophylls content of enriched and non-enriched edible oils (Mean ± SD). Different letters indicate significant differences ($p \leq 0.05$, Tukey's HSD test).

The concentration of chlorophylls in soybean oils enriched with 200 or 1000ppm of *Phyllaria reniformis* pigment extract were 2.03±0.34 and 6.6±0.4 mg/kg of oil, respectively. These values were 10 and 33 times higher than the control (0.19 mg/kg) and the BHA enriched oil (0.22 ±0.09 mg/kg).
The analysis of variance showed a significant difference on chlorophylls content (p=0.000211 ***), between the supplemented soybean oil with 1000ppm seaweed pigment extract and the remaining studied samples.
The concentration of chlorophylls in sunflower oil enriched with *Phyllaria reniformis* pigment extract increased in comparison to the control and BHA samples. The highest total chlorophylls content was observed in 1000ppm pigment enriched sunflower oil (7.48 ±0.3 mg/kg) followed by that of 200ppm (4.74 ±0.34 mg/kg). High significant difference was observed (p=0.000278 ***) in the chlorophyll's contents in all samples of sunflower oil.
Results suggest that the carotenoids and chlorophylls contents in soybean and sunflower oils were deeply related to the concentration of seaweed pigment extracts incorporated and in the meantime the vegetable oils qualities may be improved with increasing concentration of the natural antioxidant additives such as *Phyllaria reniformis* pigment extract. The composition of *Phyllaria reniformis* pigments previously determined by our first published study **(Ghaliaoui et *al.*, 2020)** confirmed the efficiency of using this alga pigment as potential natural antioxidant in edible oils.

Colour measurement

Colour, is an important factor for consumer appeal and acceptability **(Gouveia et *al.*, 2007)**.The enrichment of soybean and sunflower oils with *Phyllaria reniformis* pigment extract influenced their colour and gave them more greenness. However, for more detailed insight into this colour change, colours parameters L^* (lightness), a^* (redness) and b^* (yellowness) of analysed samples were obtained by colorimetric measurements. Results of colours (a^*, b^* and L^*) measurements are shown in table 1.
The L^* value, which indicates the lightness was higher in native soybean oil without additives than the remaining supplemented samples. Enrichment of soybean oil caused a slight decrease of L^* from 77.73± 0.70 to 69.23±3.80. The obtained value of a^* were all negative corresponding to the green zone, they decreased from -4.2±0.17 in the control to -6.03±0.95 in enriched soybean oil with 1000ppm of pigment extract. The b^* values were all positive indicating the yellowness. The highest value of b^* was obtained in soybean oil sample supplemented with 1000ppm (+46.8 ±9.08), and the lowest b^* value was observed in the control (+18.46±1.27). For sunflower oil samples, the same observation was found where L^* decreased from 77.7±1.15 (control) to 74.43 ± 1.55 (sample supplemented with 1000 ppm of pigment extract). a* value decreased from -4.7±0.43 (control) to -6.7± 0.78 (sample supplemented with 1000ppm), while b^* increased from +15.8±0.43 (control) to +29.36± 2.47 (sample supplemented with 1000ppm).
Results of statistical analysis showed significant difference ($p \leq 0.05$) between the enriched soybean oil by 1000 ppm of pigment extract and the remaining oil samples in all determined colour coordinates. While in sunflower oil, no significant difference ($p > 0.05$) was observed between samples for the two colour coordinates L^* and a^*, whereas, b^* values of enriched sunflower oil at the concentration of 200, whereas when adding 1000ppm of pigment extract to sunflower oil a significant difference was found compared to the enriched BHA oil and the control. Hence, the addition of *Phyllaria reniformis* pigment extract to both oils caused a notable change in the colour coordinates (a^*, b^* and L^*) especially soybean oil. Consequently, the oil enriched with pigment extract became less luminous, greener and yellower. This interesting colour variation could be attributed to the high content of chlorophylls and carotenoids in the pigment extract. According to **Corbu et *al.* (2020)**, the improvement of the colour parameters of oils may increase consumer attractiveness.

DPPH Radical Scavenging Activity of enriched oil

Among several methods for vegetables oils antioxidant activity evaluation, the DPPH radical scavenging procedure was the most common used. In this study, the effect of adding *Phyllaria reniformis* pigment extract on antioxidant capacity of soybean and sunflower oils was assessed.
Figure 4 shows the IC50 values of native or enriched oils. The obtained results showed that DPPH activity of the enriched soybean oil was improved when the concentration of pigment extract increased from 200 to 1000ppm. The highest DPPH radical-scavenging capacity was observed in the soybean oil sample containing 1000 ppm of *Phyllaria reniformis* pigment extract with the lowest IC50 (5.23±0.10 mg/mL), followed by those enriched with 200 ppm of BHA or 200ppm pigment extract with IC50 of 5.58±0.11 and 5.75±0.03mg/mL, respectively. The control oil showed the lowest activity (IC50= 6.20±0.03 mg/mL). Significant difference (p=0.00312 **) was noted between the enriched soybean oil and the control oil.
Concerning sunflower oil, the control sample was characterized by the lowest DPPH radical-scavenging capacity with IC50 of 10.06±0.31mg/mL, followed by 200ppm pigment extract enriched oil (IC50 = 9.58±0.09mg/mL) then 1000ppm (IC50 = 9.21±0.13mg/mL). However, BHA as a synthetic antioxidant, exhibited the best efficiency ($p \leq 0.05$) with IC50 value of 7.56±0.16mg/mL. In all cases DPPH antioxidant capacity before enrichment of oils with pigment extract or BHA was lower than the control sample. Therefore, the enrichment of soybean or sunflower oils led to increasing their antioxidant capacities, in particular when adding 1000ppm of seaweed pigments of soybean oil.

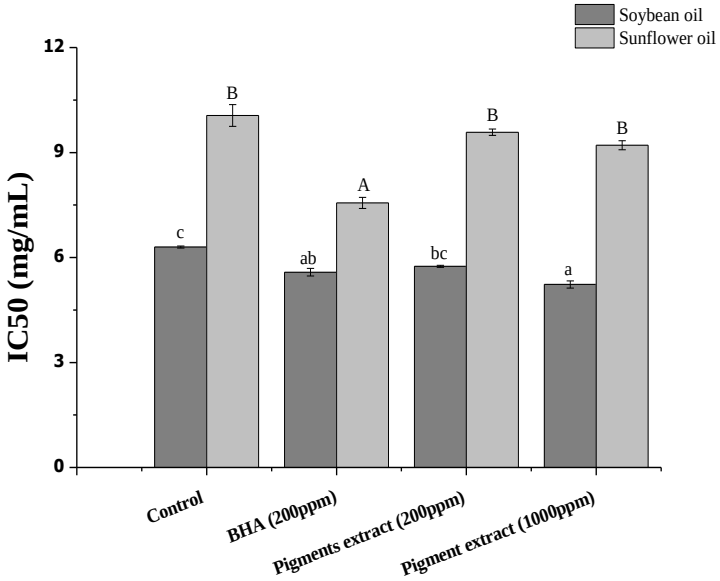

Figure 4 DPPH (IC50) values of soybean and sunflower oils enriched by BHA and pigment extract obtained from the brown alga *Phyllaria reniformis* (Mean ± SD). Different letters indicate significant differences ($p \leq 0.05$, Tukey's HSD test).

In previous study, **Gouveia et *al.* (2007)** evaluated the stability of soybean oil containing pigment extract of a microalga *Chlorella vulgaris*. They reported that pigments could contribute to oil stability due to their antioxidant effect. A similar result was found when using xanthophylls isolated from orange peel as antioxidant additive in soybean oil **(Yen & Chen, 1995)**.
Yao *et al.* (2020) evaluated the antioxidant capacity of zeaxanthin in soybean oil. They proved that the addition of zeaxanthin enhanced the ability of soybean oil to scavenge the free radical. In another study, supplementation of commercial oils (olive, sunflower and palm oils) by adding olive leaf extract contributed to the increase of radical scavenging activity **(Salta et *al.*, 2007)**. More recently, **Tenillo & Lante (2020)** reported that the antioxidant activity of soybean oil increased after adding ginger and turmeric freeze dried powders. Pigments extract of *Phyllaria reniformis* could be a food and safe antioxidant additive to vegetable oils or other food product.

Oxidative stability by Rancimat test

The oxidative process of vegetable oils could be accelerated by the Rancimat test. This method is usually used to assess the oxidative stability of edible oils. In this study, the Rancimat analysis was performed at 100°C and the IT (h) was evaluated for soybean and sunflower oils enriched with *Phyllaria reniformis* pigment extract or with BHA or without enrichment as control oil sample.

Figure 5 illustrates the oxidative stability of soybean and sunflower oils expressed by the IT values. Results showed that both BHA and pigment extract had a strong antioxidant activity in soybean oil compared to the control one (8.95± 0.54 h). Thus, the presence of pigment extract retarded the oxidation of soybean oil. The highest stability was observed in soybean oil sample containing 1000 ppm of extract (12.95± 0.43h) and those containing 200 ppm of pigment extract (12.06± 0.7 h), the synthetic antioxidant BHA gave similar IT values (12.47± 0.12 h). The addition of pigment extract or BHA to soybean oil increased significantly ($p \leq 0.05$) the IT compared to the control sample.

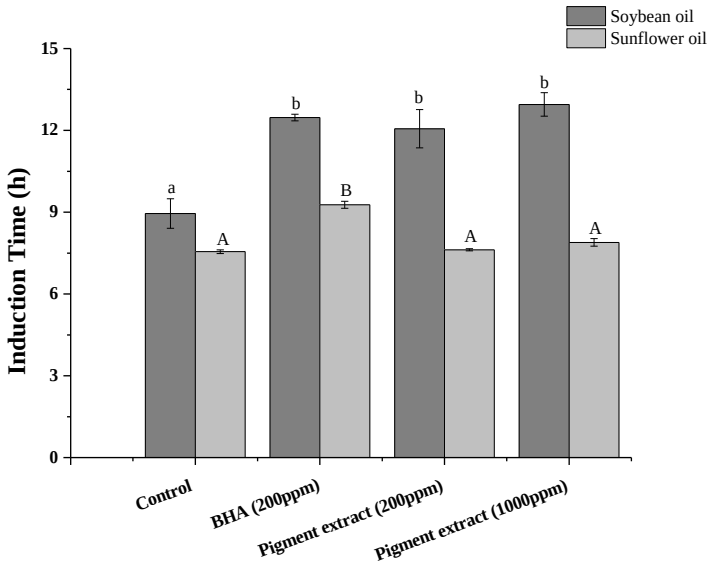

Figure 5 Induction time of the control and enriched soybean and sunflower oils (Mean ± SD). Different letters indicate significant differences ($p \leq 0.05$, Tukey's HSD test).

IT of enriched sunflower oil with 200 ppm of BHA was significantly higher (9.27± 0.13 h) than all remaining samples. Enriched sunflower oil with *Phyllaria reniformis* pigment extract led to slightly increase IT compared to the control sample as shown in Figure 4. Statistical analysis showed a high significant difference ($p \leq 0.05$) between sunflower oil sample enriched with BHA and the remaining samples.

Interestingly, the obtained results showed that oxidative stability of soybean and sunflower oils enriched with *Phyllaria reniformis* pigment extract was improved compared to control oil samples. The oxidative stability of both oils tended to increase with the addition of BHA or pigment extracts. Consequently, the ability of an additive to stabilize vegetable oils may depend on the nature of oil, the type and concentration of additives.

Due to the lack of published works on the effect of enrichment of vegetable oils with seaweed pigment extracts on their oxidative stability, it was very difficult to compare the obtained data with other studies; nevertheless, we tried to compare our results to other natural additives enrichment of vegetable oils.

According to **Shadyro et *al.* (2020)**, the addition of carotenoids (*β*-carotene, lutein, zeaxanthin) in flaxseed oil showed an increase of the IT values compared to the oil without additives (3.8 - 5.6 h), whereas, at concentration higher than 10 mg of carotenoids per 100 g of flaxseed oil, a pro-oxidant effect appeared and then decreased the oxidation stability of flaxseed oil. In another study, **Le Tutour (1990)** studied the antioxidant effect of the methanol-chloroformic extract of seven species of seaweed on the oxidation stability of sunflower oil. Results showed that within the seven-seaweed species, *Laminaria digitata* and *Himanthalia elongata* extracts were the most effective in extending the IT of the enriched oil. Similarly, in more recent studies, lipidic oxidation was inhibited by the addition of the brown seaweed *Fucus vesiculosus* ethanolic or acetonic extracts to fish oil enriched granola bars **(Karadağ et *al.*, 2017)**, milk or mayonnaise **(Hermund et *al.*, 2015; Honold et *al.*, 2016)**. Likewise, **Alavi and Golmakani (2017)** reported that the supplementation of olive oil by increasing percentage of microalga *Spirulina* powder (0.5-1.5%) improved the oxidative stability from 12.69 to 22.24 % in comparison to the control sample and then extended the shelf life of olive oil.

Furthermore, the effectiveness of adding various herbal plant extracts (marjoram, thyme, oregano, *Bifurcaria bifurcata*, beetroot, carrot, tomato, swede, ginger, turmeric, and *Opuntia ficus-indica*) on oxidative stability of edible oils (sunflower, soybean, canola, rapeseed, and olive oil) was evaluated in many studies **(Agregán et *al.*, 2017; Ammar et *al.*, 2017; Kozłowska & Gruczyńska, 2018; Salta et *al.*, 2007; Tinello & Lante, 2020; Tundis et *al.*, 2017).**

Delfanian et *al.* (2016) showed that *Eriobotrya japonica* skin extracts could retard the oxidation of soybean oil, where the highest IT was observed in oils containing 400 or 1000ppm with IT values of 4.69 and 4.49 h, respectively. While the control oil exhibited only 3.32 h. The IT values obtained in the present study were two to three times higher, this may indicate that enrichment of soybean or sunflower oils with *Phyllaria reniformis* pigment extracts allowed more oxidative stability than *Eriobotrya japonica* skin extracts. Therefore, plant extracts **(Taghvaei & Jafari, 2015; Yanishlieva & Marinova, 2001)** and particularly seaweed pigment extracts could be recommended as a potent source of natural antioxidants replacing synthetic antioxidants for protection of edible oils against oxidation.

CONCLUSION

Food industry interest on natural pigments highly increased, for that reason, seaweed pigments as renewal, safety and biodegradable natural dye could find many applications in food products. The present study was an opportunity to highlight the effectiveness of *Phyllaria reniformis* pigment extracts on reducing soybean and sunflower oils oxidation. *Phyllaria reniformis* pigment extract was added as natural antioxidant and colorant additive to these two edible oils extensively used in Algeria. Results indicated that adding pigment extract increased IT values of both vegetable oils and then their stability against oxidation without affecting their physicochemical proprieties (Acidity and peroxide contents) and in the meantime enhanced the carotenoid and chlorophyll contents. This study could be a starting point, to increase the use of coloured functional extracts from *Phyllaria reniformis* as natural antioxidants preservative for enrichment of edible oil.

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