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Investigation of phenol compounds and content of antioxidants in heated brown seaweed *(Ascophyllum nodosum)* extract

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الملخص:

ألاهتمام متزايد بخصوص مضادات الأكسدة ووجودها في كل من الصناعات الغذائية, كمواد الطبيعية لتحل محل المواد المضافة الاصطناعية في المواد الغذائية. ويتضح بأن الأعشاب البحرية لها دور في الحد من الاكسدة في المواد الغذائية وأن لها فوائد صحية متعددة نتيجة لاحتوائها علي مركبات الفينول. الهدف من هذة دراسة هو محاولة التعرف علي تأثير المعالجة الحرارية الغذائية, عند درجات حرارة 70، 90، 121 و 200 لمدة 15 و30 دقيقة بالتعون مع شركة تدعي seagreens ® بتوفيرحبيبات نقية 100٪ المجففة. تمت دراسة مضادات الاكسدة للمستخلص المثيلي واختبارها باستخدام ثلاثة فحوصات المضادة للأكسدة، DPPH جذري النشاط الكسح، Ferric الحد من القوة المضادة للأكسدة (FRAP) وإجمالي محتوى من الفينول (TPC). وأشارت النتائج الحالية أن إجمالي المحتوى الفينولي لم تتأثر بمعالجة الحرارة عند 70^C و 90^C لمدة 15 و 30 دقيقة. ومع ذلك فإنه انخفضت بشكل ملحوظ في 121^C و 200^Cلمدة 15 و 30 دقيقة. النشاط المضاد للأكسدة انخفض بشكل كبير عن طريق التسخين في 121^C و 200^c لمدة 15 و 30 دقيقة. وقد انخفضت قوة الحديد المختزل في مضادات الأكسدة عن طريق التسخين في جميع درجات الحرارة . يمكن أن نستنتج أنه من المفيد جدا اضافة عقدية عقدية لطهي الطعام في 70^C، 90^C كمصدر لمضادات الأكسدة بدلا من المواد المضادة للأكسدة الاصطناعية.

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Abstract

An increasing demand for natural antioxidants to replaces synthetic additives in the food industry. Seaweeds seem to prevent oxidation and proliferation of food and beneficial to health due to their specific phenolic compounds and antioxidant activity. The aim of the present work was to investigate the effect of food heat treatment at, 70, 90, 121 and 200^0C for 15 and 30 minutes on the antioxidant capacity and total phenolic contents in the brown seaweed Ascophyllum nodosum. Was tested using three antioxidant assays, DPPH radical scavenging activity, Ferric- reducing antioxidant power (FRAP) and total phenol content (TPC) .Was examined to estimate the antioxidant activity of Ascophyllum nodosum. Total phenolics were measured using the Folin-Ciocalteau method. FRAP was tested spectrophotometrically. The 2,2-Di- Phenyl-1-Picry-Hydrazl radical (DPPH) assay was used to determine Free-Radical Scavenging Activity. The present data indicated that the total phenolic content was not affected by heat processing at 70^0C and 90 ^{0}C for 15 and 30 min. However it was significantly decreased at 121 ^{0}C and 200 ^{0}C for 15 and 30 min. Scavenging antioxidant activity was significantly reduced by heating at 121 ^{0}C and 200 ^{0}C for 15 and 30 min, but it was not affected at 70 ^{0}C or 90 ^{0}C , for 15 and 30 min. Ferric reducing antioxidant power was decreased by processing at all heating temperatures. It can be conclude that it is beneficial adding Ascophyllum nodosum to food cooked at 70^0C, 90 as a potential source for the antioxidants rather than synthetic antioxidants.

Introduction

In this century, scientists extended their researches on antioxidants functions either in food production or for medical purpose. There many researchers discuss the benefits of antioxidants in protection of human life and food processing from harmful effects of free radicals and reactive species, which damage and cause impairment to the cells and biomolecules which result from peroxidation of lipid (1). Many scientific papers conducted on natural antioxidants in terrestrial plants and their application in food to prevent oxidation processes. Also studies show that, marine algae such as seaweeds have an important potential functions in prevention of oxidation process (2). This seaweed has a significant therapeutic feature due to their oxidative compounds (3). Recent studies showed that brown algae have a unique potential role against tumor induction, because they contain polysaccharides which have special action against cells apoptosis, inflammation, viral infections and promote immunological functions (4). Also in inhibition of growth of carcinogenic cells and metastasis (5). in addition is neuro protective effect (6).

Some countries established seaweeds as daily food intake for example in china brown alga Hizikiafusi form is used as vegetable. The consumption of edible seaweeds has been traced back into fourth and sixth century in Japan and China respectively (7). Japanese are the main consumers of seaweed with an average of 1.4 kg (dry weight) per person (8). In the year 2004, the total global seaweed production was more than 15 million metric tons (9). In some kinds of foods such as meat exposure to oxidation process which leads to decomposition of proteins and lipids which result in deterioration in texture, flavor and color of fresh retail meat (10). Therefore In the food industry, carrageenans extracted from seaweeds are widely used as thickener and stabilizer to improve the texture of cottage cheese, to provide the required viscosity and texture of puddings and dairy desserts, and also it is utilized as binders and stabilizers in the meat-processing industry (11).

Today many types of artificial antioxidants, which have long been used as preservatives to delay the oxidation process and biochemical changes. Nevertheless, these synthetic antioxidants have been partially avoided in food processing as they are assumed carcinogenic (12). As a result, increase of public attention to natural antioxidants during the recent years, (13). The nature of survival atmosphere of seaweeds such as extreme light, shortage of nutrients, dehydration, and temperature fluctuation (14), all these characteristic environment have direct effect on seaweed to form antioxidant agents and different free radical species (15). Many characteristics attracted the biomedical scientists to use seaweeds species as natural alternative sources of human applications (1). For example study by Zubia et al., 2007 established that, macro algae can be stored for long time without any damage in component structure during processing and storage (15). The potential ant oxidative effects of seaweed is due to the presence of specific components such as: "Proteins with ant oxidative properties, phenolic compounds, such as flavonoids, to copherols, chlorophyll derivatives, amino acids and amines, as well as other compounds like carotenoids, ascorbic acid, glutathione macro algae" (1). The main aim of the present study was to investigate the phenol compounds and antioxidants in heated brown

seaweed Ascophyllum nodosum (*Family fucaceae*) extract by using in vitro antioxidant activity assays.

Materials and Methods. The present study was aimed to estimating the total phenol content and antioxidant activity of *Ascophyllum nodosum* species of the brown seaweeds (dry powder) as affected by different temperature degrees as that used during various cooking temperature and time at 70, 90, 121 and 200^{0}C for 15 and 30 minutes. These used degrees similar to that degrees people using in food processing bread, meat and other foods, and 121 ^{0}C for food pasteurization. Therefore the rationale behind use of these temperatures was to mimic the cooking temperature of these foods.

Materials. pure dried powder sample of brown seaweed species *Ascophyllum nodosum* was provided by a company called Seagreens® "Asco Fine Granules Demeter and Soil Association Standards. *Ascophyllum nodosum* was harvested from Norway's remote Lofoten Islands, air dried at 37 ^{0}C, then ground and packed immediately

Preparation of extracts. The 2g sample was heated for 15 or 30 minutes at 70, 90, 121 and 200 ^{0}C respectively. Antioxidants present in *Ascophyllum nodosum* were extracted with methanol according to study in 2007. After heating the samples, (control and heated) were extracted in triplicate (17).

Solvent for Extractions of Phenol Contents and TotalAntioxidants. Previous study found that, 70% ethanol and double distilled water which was used in their study showed that, they have beneficial effect for extraction of phenols and flavonoids from some varieties of grapes, possibly because ethanolic and aqueous extracts act on both oil-soluble and water-soluble antioxidants (18). Although study showed that, pure methanol extract provided a good result when it was used for determination of water soluble antioxidants capacity of 35 plants species (17). Therefore the solvent was selected pure methanol (100%) for extraction in present study.

Determination of Total Phenolic Content (TPC). The total phenol was assessed based on An aliquot (0.1 ml) of the extract or Gallic acid standard 7.5% ml of sodium carbonate (7.5 g NaCO3 method used by Waterman and Mole in 1994 (19). The solution was incubated for 2 hours at 25 ^{0}C, and the absorbance was measured by using a spectrophotometer (Cecil, CE 9500 Series, UK).

Phenol standard curve. A standard curve was created using varying concentrations of a standard phenolic compound, Gallic acid, versus absorption (Figure2 and 3). Results were then calculated as Gallic Acid Equivalents (GAE) of a sample using the standard curve. Gallic acid is used as the reference compound because it is only present in small amounts in the plant material, and it is a stable, pure, inexpensive substance (20).

Determination of Antioxidant Activity. The antioxidant activity of *Ascophyllum nodosum* crude methanolic extract was examined by different assays, first by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay based on the method used by Kumar et al.(2008) (21) and Turkmen et al. in 2007 (22). With a few modifications, the second assay Ferric Reducing Antioxidant Power (FRAP).FRAP

assay used in this study was adapted according to Escarpa and Gonzalez, (2001) method (23). The FRAP reagent working solution consisted of 400 ml (0.3M) Sodium acetate buffer, 40 ml (10 m M) TPTZ, 40 ml (20 m M) FeCl3. The absorbance was measured at 593 nm using a spectrophotometer (Cecil, CE 9500). FRAP standard curve was created by using different concentrations of a standard antioxidant compound, Ferrous Sulphate (FeSO4.7H2O) (Table1).

Statistical Analysis All analysis were carried out in triplicate, and data were expressed as the means and standard deviation (SD). The excel program for PC was used for data analysis. Student T Test) was used to get level of significant difference with $p<0.05$.

Results: Total Phenol Content (TPC): Figure (1), illustrates the total phenol content (mg. of gallic acid equivalent /g. dry seaweed weight) extracted in the control and heated seaweed samples. Interestingly, there were no significant differences between control and each of these heated samples 70^0C, 15 min (18 ± 1 mg GE) 70^0C, 30 min (20 ±1 mg GE), 90^0C,15 min (22 ± 1 mg GE/ g), 90^0C, 30 (22 ± 1 mg GE) were $p > 0.05$ no significant difference between the control and each of these heated samples. This shows that heating process have no effect on phenol contents at these heating temperatures and times. However thermal treatment has significant effect on phenols compounds were $p<0.001$ between control and each of these treated samples, 121,^{0}C 15 min (14 ± 1 mg GE) 121^0C, 30 min (14 ±1 mg GE), 200^0C, 15 min (1.2 ± 0.72 mg GE/ g), 200^0C , 30 (0.3 ± 0.02 mg GE) This shows heating processing had no significant effect on TPC in samples heated below 121^0C , but had negative significant effect on those samples heated at 121^0C and 200^0C.

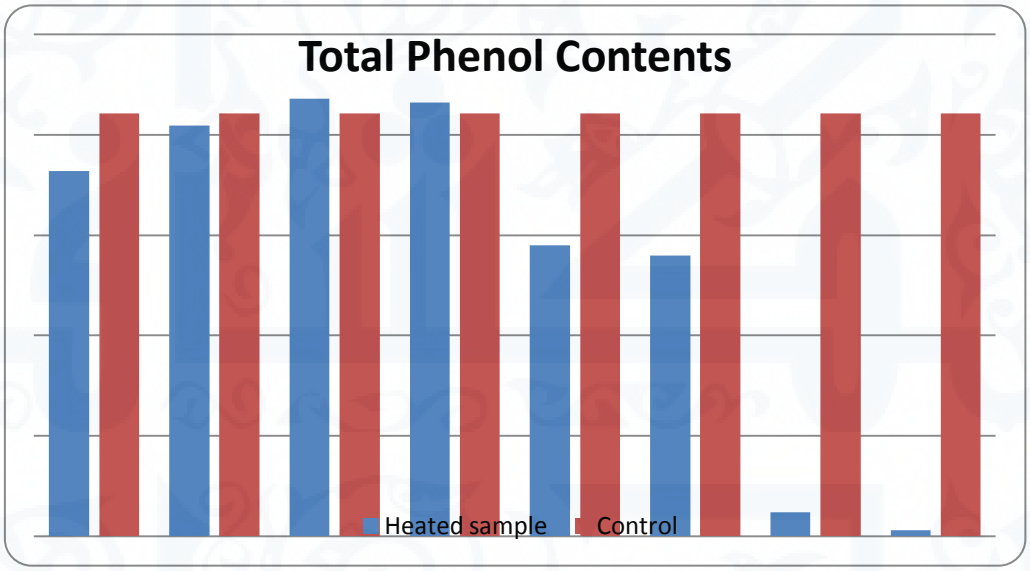


Figure 1: (TPC) of (100%) pure methanol extract in the control and heated samples.

All phenolic compound tested samples absorbance power were calculated, Gallic acid was used as reference compound. We created two standard curves, because TPC was

conducted in two different days on triplicate samples. The day 1 correlation coefficient value (R^2) was found to be 0.9867, as shown in phenol standard curve figure (2), and day 2 correlation coefficient (R^2) was 0.9284, as in Figure (3). Therefore the absorbance of day 1 samples were calculated by using correlation formula of day 1 Gallic standard curve as in (Figure.2), and the absorbance of day 2 samples were calculated by using correlation formula of day (2) Gallic standard curve as in (Figure 3).

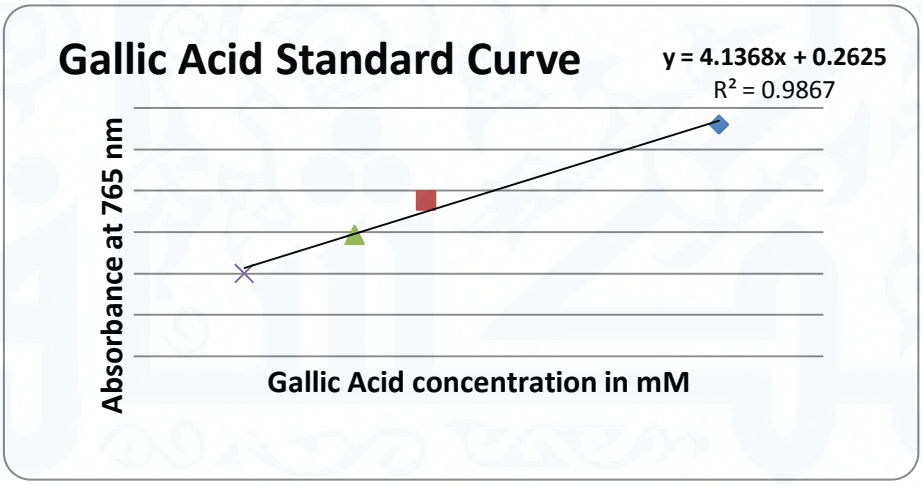


Figure 2: Gallic acid standard curve for calibration of Total Phenols Content (Day1)

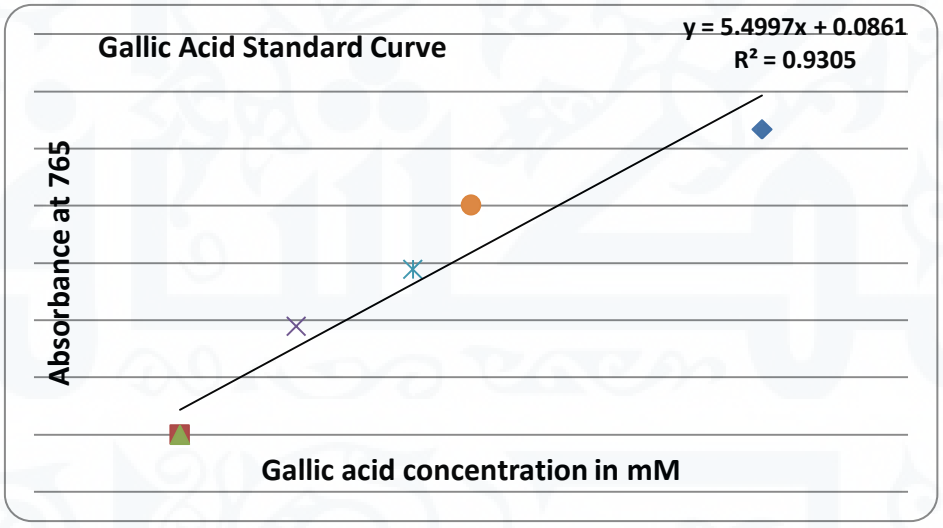


Figure 3: Gallic acid standard curve for calibration of total Phenols content (Day 2)

Antioxidants Activity.

Table 1: The percentage Inhibition of DPPH Radical Scavenging Activity in the Control and Heated Samples.

Heating temperature and time	% DPPH Inhibition
Control	65 ± 2.6
70 ^{0}C, 15 min	63± 2.1
70 ^{0}C, 30 min	61 ± 2.1
90 ^{0}C, 15 min	62 ± 2.7
90 ^{0}C, 30 min	60 ± 2.4
121 ^{0}C, 15 min	57±3.1
121 ^{0}C, 30 min	57± 3
200 ^{0}C, 15 min	41±2.1
200 ^{0}C, 30 min	13±2.6

Values were expressed as mean±standard deviation of triplicate experiment

DPPH Radical Scavenging Activity: Table.(1) showed the inhibition of DPPH radical scavenging activity of 100% dry methanolic extract of tested seaweeds. On the basis of our results, the control sample was found to contain the highest percentage of radical scavenging activity (65± 2.6%), followed by 70^{0}C, 15 min (63 ± 2%), > 90 ^{0}C, 15 min (62 ± 2.7%), > 70 ^{0}C, 30 min (61 ± 2.1%), > 90 ^{0}C, 30 min (60 ± 2.4%), > 121 ^{0}C, 15 min (57 ±3.1%), and >121^{0}C, 30 min (57±4.1%), respectively. The percentage of DPPH radical scavenging activity gradually decreased as the temperature and time increased, as showed in Table1. The antioxidant activity affected by thermal treatment in seaweed extracts was determined by calculations performed on the collected data. The T test show significant differences between the control and each of samples that were heated at 200^{0}C, 15 min (41±2.1%), and > 200 ^{0}C, 30 min (13 ± 2.6%), the P.value was <0.001. However, the differences were not significant between the control and each of the samples, as the P.value was >0.05. Apparently, thermal treatment had a significant negative effect on scavenging activity when the samples were heated at 200^{0}C for 15 and 30min.

Ferric-Reducing Antioxidant Power (FRAP): In the FRAP assay, antioxidant activity was determined based on the ability of the antioxidant compounds in the samples to act as reducer and reducing ferric (III) to (II) in a redox-linked colorimetric reaction that involves single electron transfer (23). Table(2), show the FRAP values (m mol FRAP/g dry seaweed) of 100% methanolic extracts of the tested samples including the control and heated samples. The results showed that the highest FRAP value was found in the control (52±0.75 mmol/g) followed by As 70^{0}C, 15min (40 ±3 mmol/g), 70^{0}C, 30 min (37±2.5 mmol/g), 90 ^{0}C, 15 (37 ± 1.4 m mol/g), 90^{0}C, 30min (36 ± 0.82 m mol/g), 121 ^{0}C, 15 (34 ± 2.5 m mol/g), and 121 ^{0}C, 30 (33 ± 2.5 m mol/g) respectively. However the lowest FRAP values were found in 200^{0}C, 15 min (29 ± 2.4 m mol/g) and 200 ^{0}C, 30 min (17 ± 1.4 m mol/g) respectively. Interestingly,

the FRAP values of the heated samples in this assay significantly decreased as the thermal treatment and time increased.

Table.(2): FRAP values as (mmol/g dry seaweed)

Temperature, time	FRAP values (m mol/g dry seaweed)
Control	(52 ± 0.8)
70 ^{0}C, 15 min	(40± 3)
70 ^{0}C, 30 min	(37± 2.5)
90 ^{0}C, 15 min	(37± 1.4)
90 ^{0}C, 30 min	(36± 2.5)
121 ^{0}C, 15 min	(34± 2.5)
121 ^{0}C, 30 min	(33± 2.5)
200 ^{0}C, 15 min	(29±1.4)
200 ^{0}C, 30 min	(17± 2.4)

Values are expressed as mean±standard deviation of triplicate experiment.

The ferric-reducing antioxidant power of heated samples was negatively affected by heating processing. Therefore, the results of the T test showed significant differences between the control and each thermal-treated sample where the P.value was < 0.001. Therefore all heated samples had lower FRAP than the control. On the basis of our results, the heat-processing appears to have had a negative effect on *Ascophyllumnodosum*and seems to have decreased FRAP values. However, there were significant differences due to the thermal processing for these heated samples. There were significant differences between the control and each heated sample but not within heated seaweed samples as shown in Table (2).

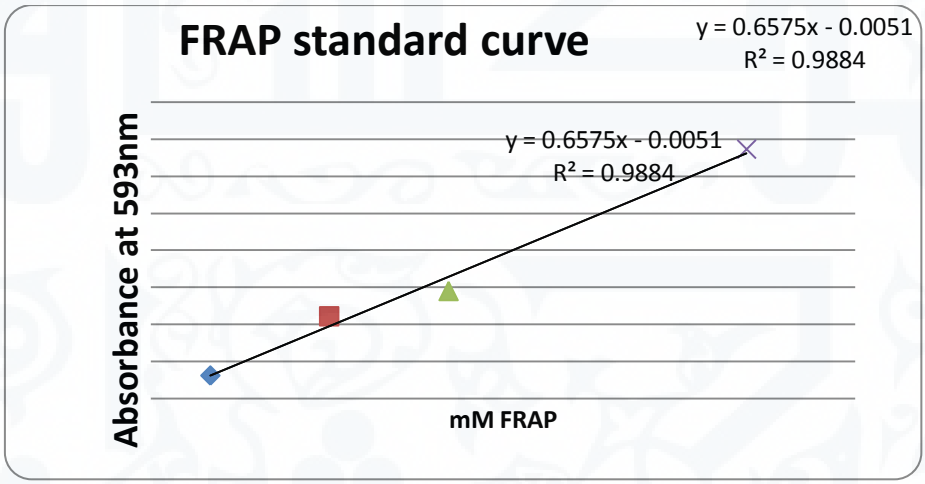


Figure (4): Standard Curve for the FRAP Assay

For calibration of the FRAP assay, aqueous solutions of Fe (II) (FeSO4.7H2O) of known concentration in the range of 100, 300, 500 and 1000 µ mol were used and tested. The results of analysis found that Fe (II) concentrations had R-squared value (R2) = 0.9884. (Figure4)

Correlation between Total Phenol Contents and Antioxidants.

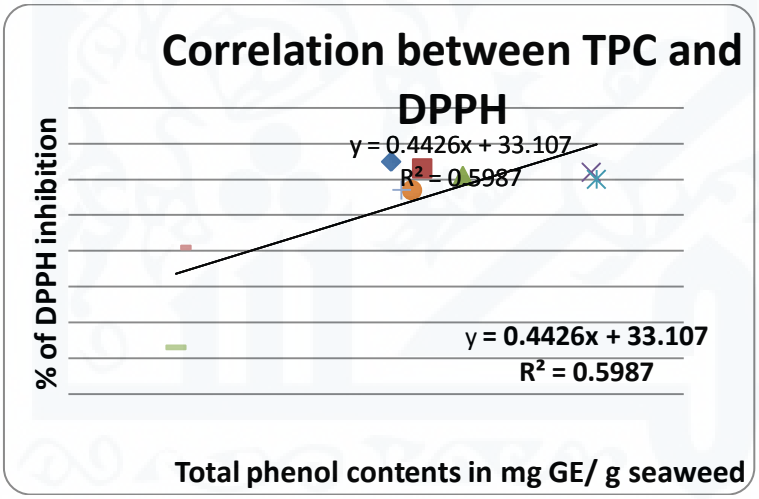
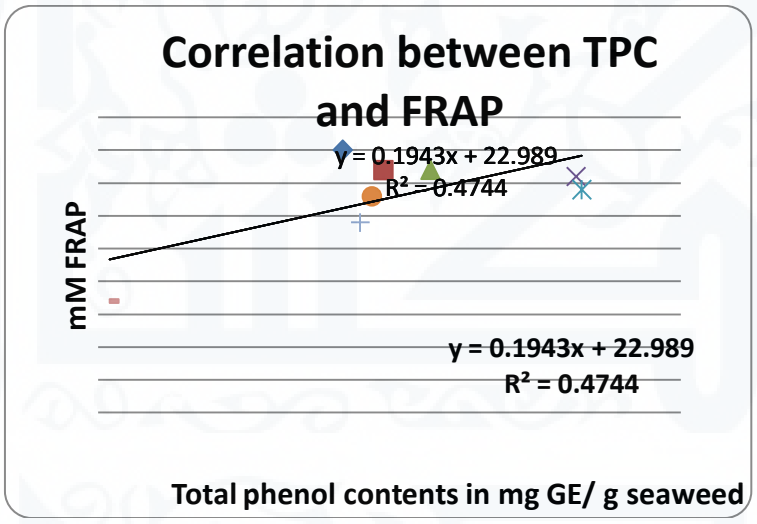


Figure (5): Correlation between Total Phenol Contents and DPPH



Figure(6): Correlation Coefficient between Total Phenols Content and FRAP

6.Discussion. The present study was carried out to determine the effect of heat processing at 70, 90, 121, and 200^{0}C for 15 and 30 minutes on total phenol content and antioxidant activity of a brown seaweed species (Ascophyllumnodosum) using three assays: TPC, DPPH, and FRAP, we could say.

6.1. Extraction Solvent Selection and Extraction Efficiency (EE). Methanol was chosen as an appropriate solvent in order to yield better extraction. Many studies have found that methanol shows better extraction (24) and it is proved to be the most effective solvent in extracting phenolic compounds (25). Yen et al, reported that methanol showed a good recovery rate (88-116%) in the extraction of phenols in fresh tea compared with chloroform, ethyl acetate and water. 80% methanol (v/v) was found to be the most efficient solvent to extract antioxidant-containing phenolic constituents (24). There are many data available about the correlation between the extracts of phenol compounds and antioxidant activity with solvent extractants. In particular the DPPH radical scavenging activity of methanol extracts of some brown algae has been reported (26). And they have been shown to give the highest antioxidant activity in many seaweed species(17).

6.2 Total Phenol Content (TPC). The Folin–Ciocalteu assay is one of the oldest methods to be commonly used for assessing total phenol content, based on its reduction ability and the transfer of electrons (19). Previous studies revealed that marine seaweed extracts, especially their polyphenols, have antioxidant activity (27). Using this assay on control and heated samples of *Ascophyllum nodosum*, we found, by using the T test, that there were no significant differences in phenol content between control and heated samples at p.value >0.05. This shows that the heating process has no effect on phenol content at these temperatures and times. However thermal treatment has a significant effect on phenol compounds in each of the treated samples at p.values < 0.001.This shows heating processing had no significant effect on TPC in samples heated below 121^0C, but had negative significant effect on those samples heated at 121^0C and 200^0C. Control test samples had a low phenol content (20±1 mg GE/ g, i.e. 2% DW phenols). This disagreed with previous studies, where A. nodosum had the highest average phenol level (~ 6% DW) more than other brown seaweeds such as B.bifurcata (~4% DW), and P.canaliculate had the lowest average phenol level (~ 3% DW) (28). This could be because a nodosum samples were collected from new blades which had lower TPC values compared to old blades. As reported in earlier studies, Connan et al. (2006) found that there was a high correlation between the age of the tissues and TPC for brown seaweed (29). In particular, we have no idea about the collection procedure and the age of the tested seaweed species. When the samples were heated at 70^0C and 90^0C for 15 and 30 min, phenol content was not significantly affected. This obtained results which were in complete agreement with previous research by Larraui et al. (1997) (30). This stated that the polyphenols of the grape pomace peel samples were not significantly affected when dried at 60^0C. However the results were in disagreement with Jimenez-Escrig et al., (2001) (31) who found that dry processing at 50^0C for 48 hours has a negative effect on the total phenolic content in brown seaweed methanolic extracts.There are no significant differences between the two samples heated at 70^0C and 90^0C for 15 and 30 min and control, but there is variation between the two samples, 90 had higher phenols. This in agreement with (Anese et al., 1999) (32) who found that, heat processing at 95^0C for more than 10h more beneficial than that at 75^0C in rising tomato juice antioxidant capacity. This rising in TPC could be due to the antioxidant activity of botanical materials sometimes may improve by thermal processing or storage because of the production other antioxidative contents (Anese et al., 1999)

(32) and increasing the components of bioaccessible antioxidants (33). It also reported that antioxidant capacity may increasing as a result of the formation of Maillard reaction products (30).

In our result TPC was significantly decreased at 121^{0}C and 200^{0}C for 15 and 30 min. This in agreement with study conducted by Shi and Le Maguer (2002) (34) showed that antioxidant in tomato was lost when the temperature was increased from 90 to 150^{0}C, as well as agreeing with Larrauri et al.,(1997) (30) who reported that dried processed food at 100 and 140^{0}C found to be significantly reduced the natural antioxidants activity due to loss of polyphenols content. Also in agreement with Ragan and Glombitze (1986) (35) who found phenolic compounds are rapidly degraded by the drying of samples at temperatures above 40^{0}C. Also Connan et al. (2007) found that phenol levels in a nodosum were correlated significantly with air temperature (28). The fact that the samples at 200^{0}C for 15 and 30 min lost their phenols could be explained by the thermal degradation of polyphenols by oxidative enzymes such as polyphenol-oxidases and peroxidases (36). These enzymes were found to be not immediately deactivated by oven-drying (37). Also the bioactivity of phenolic compounds is likely to be denaturated and hampered by the heat produced by an oven or UV radiation(38). Also these current findings were in agreement with a study conducted by Wong and Cheung. (39). They found that the total phenol content was reduced in plant or seaweed samples when nfxsdtsome environmental factors such as light, air and sea-water temperatures (40).

Antioxidant activity

DPPH. The antioxidant activity of the samples was also tested using DPPH radical scavenging and FRP assays. These two assays test different mechanisms of antioxidant action. DPPH has been employed extensively as a free radical reagent to assess reducing substances (41), and it is considered to be a useful reagent for investigating the free radical scavenging activities of compounds (42). DPPH compounds possess free radical-scavenging activity.This indicates that their mechanism of action is as a hydrogen donor and they terminate the oxidation process by converting free radicals to more stable products; whereas a compound exhibiting a positive result in the FRAP assay was an electron donor and it terminated the oxidation chain reaction by reducing the oxidized intermediates into the stable form (43).Also a positive correlation has been recognized between total phenolic content and the antioxidant capacity of different seaweed extracts (1). This seems to agree with Oki et al. (2002), who found that the radical scavenging activity increased with the increase of phenolic compound content (44). On the basis of our study, we found that the highest DPPH radical scavenging activity for the tested seaweed samples was in the control samples (65% ± 2.6). The inhibition percentage of DPPH of all the heated samples gradually decreased as the heating process increased, but this decrease was not significantly different compared with the unheated sample (Table1).

The effects on the scavenging activity by the thermal treatment of seaweed extracts were determined by T test. The T test was employed to ascertain if significant differences existed in the radical scavenging activity % before and after heat-processing. There were significant differences between the control and each of the samples that were heated at 200^{0}C, 15 min and 200^{0}C, 30 min where the P value was

<0.001. On the other hand, the differences were not significant between the control and each one of the rest of the samples at 70^0C, 15 min, 70^0C , 30 min, 90 , 15 min, and 90^0C , 30 min as the P value was >0.05. Apparently, thermal treatment had a significant negative effect on scavenging activity when the samples were heated at 200^0C, 15 min and 200 ^{0}C, 30 min. However the scavenging activity was not affected in the rest of the heated samples. Thus scavenging activity % significantly decreased in the samples at 200^0C, 15 and 30 min, and there was no significant change in the rest of the samples. A study by Escarpa, and Gonzalez. (2001) (23) reported that the radical scavenging activity of a brown alga was decreased 98% by dry processing at 50^0C for 48 hours. There was disagreement with the results of the present study that heating processes had no significant effect on the antioxidant activity in the seaweed tested at 70, 90, 121^0C, for15 & 30 min. On the other hand it was totally in agreement with that thermal treatment at 200^0C, for 15 & 30min, had a significant negative effect on scavenging activity. This could be due to the thermal effect especially since the heat is known as the most destructive factor.

6.4. FRAP Assay. FRAP assay is used to investigate the antioxidant capacity based on the ability of antioxidant contents in tested samples to reduce iron (III) to iron (II) (43) in a redox-linked colorimeteric reaction that involves single electron transfer (45). The results showed that the highest FRAP value was found in the control (52 ± 0.8 m mol/g). The FRAP values in all heated samples decreased as the heat increased and there were significant differences between the control and each thermal treated sample where the P value was < 0.001. Therefore the heating process had a significant effect on all the heated samples (as shown in table2).

The negative effect of heat processing on the seaweed extracts was in disagreement with a study conducted by Wachtel-Galor et al. (2008) (46). They found that boiling broccoli and cauliflower for 10 minutes led to apparent increases in antioxidant activity. This lack of agreement could be due to the production of secondary metabolites or breakdown products. The main factor that lies behind the decreased reducing power in heated samples may be arising from different parameters which have a considerable effect on the antioxidant capacity. Duan et al., (2006)reported that antioxidant activity correlated significantly with total phenols (42). This did not agree with current findings, because the ferric reducing power decreased at all heating temperatures, however TPC was decreased at 200^0C.

In this study, there are some knowledge gaps, especially because most of data were available about phenol content and antioxidants in unheated seaweed. Also sample preparation and extraction methods used in the current study were different from those of previous studies. Therefore direct comparison of current results on the effect of heat processing on total phenol content and antioxidant activity of seaweed extracts with other studies does not have good justification. Furthermore, the methods which were used in this study (DPPH assay and FRAP assay) differ in terms of their assay principles and experimental conditions; consequently, in different methods antioxidants in particular make varying contributions to total potential antioxidant (47).

6.3. Correlation between Total Phenol Contents and Antioxidants. The results of the current study showed that TPC not significantly changed in the samples at 70^0C, 15min (18 ± 1 mg GE) 70^0C, 30 min (20 ±1 mg GE), 90^0C , 15 min (22 ± 1 mg GE/ g), 90^0C, 30 (22 ± 1 mg GE), and decreased in the sample at121^0C, 15 min (14 ± 1 mg GE) 121^0C, 30 min (14 ±1 mg GE), 200^0C, 15 min (1.2 ± 0.72 mg GE/ g), 200^0C, 30 (0.3 ± 0.02 mg GE). Similarly, the DPPH decreased in the sample at 200^0C, 15 min (41 ± 2.1 mg GE) and 200^0C, 30 min (13 ± 2.6 mg GE); but it did not significantly change in the rest of the heated samples. In FRAP, the heating process had a significant negative effect on all the heated samples. From these points TPC and DPPH values correlated to each other except in the sample at 90^0C, 15 min where they are different: among these results the correlation between TPC and DPHH was r= 0.5987 and between TPC and FRAP was r = 0.4;. From this point we may conclude that the results do not seem to agree with what was previously mentioned about the correlation between TPC and radical scavenging activity, where r=0.97. This lack of agreement may be due to the seaweed extracts including other materials, such as small molecular weight proteins or peptides, polysaccharides, pigments (48), ascorbic acid and vitamin E, which act as main contributors to the increase in the antioxidant activity for A. nodosum andthis is in agreement with a study done by Coulter , et al in 2005, who reported that the radical scavenging activity of C. racemosa could be due to the presence of other antioxidants such as folic acid, thiamine and ascorbic acid (49).

Conclusion . This study dealt with the effect of heat processing on total phenolic content and antioxidant activity of Ascophyllum nodosum at different food processing temperatures. Heating processes at 70^0C and 90^0C for 15 and 30min had no significant effect on Ascophyllum nodosum total phenol content. However, TPC was significantly decreased at 121^0C and 200 ^{0}C for 15 and 30min. Therefore, it is beneficial to add Ascophyllum nodosum to food that is processed at 70^0C, 90 ^{0}C, for 15 and 30min. in order to increase or maintain the TPC. Scavenging antioxidant activity was significantly reduced by cooking processes at 200 ^{0}C for 15 and 30min, and it was not affected at 70^0C, 90^0C, and 121^0C for 15 and 30min. So it is worth adding A Nodosum to food cooked at these temperatures but not adding it to food cooked at 200^0C. However all these cooking degrees reduced the ferric-reducing antioxidant power. Therefore, to maintain or increase and avoid any loss of TPC, antioxidants, adding Nodosum to food cooked at 70^0C and 90^0C and not worth adding it to food cooked at 121^0C and 200 ^{0}C.

Recommendations. The findings of our study provided helpful information for further studies which should be carried out in order to confirm if the heat processing can maintain and enhance TPC, AOA. Anodosum had excellent phenol content and antioxidant capacity. Furthermore, it should be used extensively in Food Technology to support health and minimize the risk of modern life disease.

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