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Free-radical scavenging and antioxidative activities of some polysaccharides in emulsions

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Abstract

The antioxidant activity in linoleate emulsion systems, radical scavenging activity and inhibition of autoxidation in sunflower oil-in-water emulsions were studied in the presence of polysaccharide produced by *Rhizobium meliloti* (RPS), xanthan, curdlan, and carboxymethylcellulose (CMC) and compared to tertiary butylhydroxyquinone (TBHQ). The antioxidant activity in the linoleate emulsion was improved with increasing pH from 3 to 9 and concentration of polysaccharide from 20 to 60 mg/100 g emulsion, while it decreased with increase in storage temperature between 30 and 90 °C. The antioxidant activity of xanthan, curdlan, and RPS at concentration of 40 mg/100 g emulsion was equal to that of TBHQ at 20 mg/100 g emulsion. RPS showed the highest thermal stability and the lowest linoleic oxidation values compared to TBHQ, xanthan, and curdlan at 90 °C. The antioxidant activity of xanthan, curdlan, and RPS in linoleate emulsions at pH 3 and 5 was in the first order with significant ($P<0.05$) values compared to emulsion, prepared using TBHQ.

Curdlan and RPS were effective in radical scavenging being 60–90% at pH values ranging between 3 and 7. They showed an ability to inhibit lipid oxidation in sunflower oil emulsions during holding time for 50 h at 60 °C. In general, the polysaccharides RPS and curdlan can be used as food additives having many functions as stabilizers, radical scavengers, and antioxidants in emulsified foods such as mayonnaise, salad dressings, and cake products.

Keywords: *Rhizobium meliloti*; Curdlan; Xanthan; TBHQ; CMC; Oil-in-water emulsion

1. Introduction

Polysaccharides, which are widely distributed in animals, plants, and microorganisms, have been demonstrated to play an important role as dietary free-radical scavenger for the prevention of oxidative damage. There are increasing evidence indicating that reactive oxygen species produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects, such as causing DNA damage, carcinogenesis and cellular degeneration related to aging (Blander, Oliveira, Conboy, Haigis, & Guarente, 2003; Harman, 1993; Liu, Ooi, & Chang, 1997). Superoxide and hydroxyl radicals are the two most representative free radicals. Superoxide radical is normally formed first, and its effects can be magnified because it produces other kinds of free radicals and oxidizing agents. However, the damaging action of the hydroxyl radical is the strongest among free radicals. Many synthetic chemicals such as phenolic compounds are found to be strong radical scavengers, but they usually have side effects (Liu et al., 1997).

The preliminary research showed that *Misgurnus anguillicaudatus* polysaccharide was able to remove $O_2^{\bullet}$, $HO^{\bullet}$, H_2O_2 and other active compounds of oxygen and significantly protected DNA chains from being damaged by hydroxyl radicals (Chuanguang, Huang, & Xu, 2002). Free radicals and active oxygen can induce oxidant damage. Lipid peroxidation, which involves a series of free radical-mediated chain reaction processes, is also associated with several types of biological damage. The role of free radicals and active oxygen is becoming increasingly recognized in the pathogenesis of many human

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diseases, including cancer, aging, and atherosclerosis (Perry et al., 2000).

The butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylhydroxyquinone (TBHQ) were authorized as synthetic antioxidants for use in food (Abdalla, Tirzite, Tirzitis, & Roozen, 1999). The main concern about the safety of these synthetic compounds is related to their metabolism and possible absorption and accumulation in body organs and tissues. There are some serious problems concerning the toxicity of these compounds (Hayashi, Morimoto, Miyata, & Sato, 1993; Linderschmidt, Trylka, Goad, & Witschi, 1986). Therefore, the use of hydrocolloids, such as polysaccharides and proteins with mild antioxidative activity, is desirable to produce safe food products including lipids.

Polysaccharides play important roles as thickening, stabilizing and gelling agents in many foods. Also, for emulsion systems, polysaccharides are very often used to improve the emulsion stability and textural properties. Polysaccharides modify and control the rheological properties of aqueous systems, film-formers, lubricants, and friction reducers (Matsumura et al., 2003).

Rhizobium meliloti (EMCC-10011) polysaccharide is composed of 65.87% glucose, 17.65% galactose, 6.71% disaccharide, 5.35% galactouronic acid, and $<2\%$ rhamnose and its alcoholic form (Nagwa, Madkour, El-Mahdy, & Hanan, 1997). Curdlan (β-1,3 glucan) is a polysaccharide produced by microbial fermentation. It synthesized mostly by *Agrobacterium* sp. and *Alcaligenes faecalis* var. *myxogenes*. Curdlan is approved for food use in Japan, South Korea and Taiwan. In December 1996 the FDA approved curdlan as a direct food additive (Yotsuzuka, 2001).

Xanthan gum is produced by fermentation, using a pure culture of *Xanthomonas campestris*. It is a heteropolysaccharide made up of building blocks of D-glucose, D-mannose and D-glucuronic acid residues in the molar ratio of 2.8:3.0:2.0, grades currently available are primarily in the form of the potassium salt, the potassium is substituted onto the carboxyl groups. Xanthan gum was approved by the FDA for use as a stabilizer, emulsifier, thickener, suspending agent, bodying agent, or foam enhancer in foods (Rocks, 1971). Carboxymethylcellulose (CMC), which is more commonly called cellulose gum or CMC, is available in a variety of types (Dziezak, 1991).

Until now, studies on *R. meliloti* polysaccharide are mainly focused on the production of polysaccharide and its functions as a thickening, stabilizing and gelling agents, but its activities as radical scavenging and antioxidant have not yet been studied well. Therefore, this study was carried out to test exopolysaccharide produced by *R. meliloti* as a natural and economic source for polysaccharide and compared to curdlan, xanthan and CMC polysaccharides for their free-radical scavenging and antioxidative activities in comparison to TBHQ as synthetic antioxidant in emulsion systems.

2. Materials and methods

2.1. Materials

R. meliloti (EMCC-10011) was obtained from the Egyptian Microbial Culture Collection (EMCC), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Xanthan, curdlan and TBHQ were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) and Linoleic acid were obtained from Fluka Chemical Co. (Buchs, Switzerland). CMC (high viscosity) was purchased from El-Gomhoria Company for Drugs and Chemicals, Cairo, Egypt. Sunflower oil (refined, bleached and deodorized) was donated by Arma Food Industry Company 10th of Ramadan city, Egypt.

2.2. Methods

2.2.1. Production of Rhizobium polysaccharide

Rhizobium polysaccharide (RPS) was produced as exopolysaccharide by fermentation of *R. meliloti* (EMCC-10011) as described by Footrakul, Suyanandna, Amemura, and Harade (1981). A preculture was prepared by inoculate 80 ml of the yeast mannitol broth in 250 ml Erlenmeyr flasks with an activated bacterial strain and incubated for 48 h at 30 °C, 200 ml of the standard medium in 1000 ml flasks were inoculated with 20 ml of activated culture. The flasks were shaken at 120 strokes per min for 120 h at 30 °C. Standard medium and yeast mannitol broth were showed in Table 1.

Grown culture suspension was diluted with an equal volume of distilled water and centrifuged at 5000*g* for 1 h. Acetone (two volumes) was added to the supernatant with stirring, centrifuged at 5000*g* for 15 min and the precipitated polysaccharide was collected. The polysaccharide was

Table 1
Composition of standard medium and yeast mannitol broth

Component	Standard medium broth[a] (g)	Yeast mannitol broth[a] (g)
Mannitol	—	10.00
Glucose	30.0	—
Yeast extract	1.0	1.00
K_2HPO_4	—	0.50
KH_2PO_4	0.5	—
$MgSO_4 \cdot 7H_2O$	0.5	0.25
NaCl	1.0	0.10
$CaCO_3$	5.0	3.00
$(NH_4)_2HPO_4$	1.5	—
$MnCl_2 \cdot 4H_2O$	1.0	—
$FeCl_2 \cdot 7H_2O$	1.0	—
$ZnCl_2 \cdot 7H_2O$	0.1	—
$CuSO_4 \cdot 5H_2O$	0.1	—
$Na_2MoO_4 \cdot 2H_2O$	0.1	—
H_3BO_3	0.1	—

[a]pH 6.80 the previous mediums were sterilized at 121 °C for 20 min.

washed by water–acetone solution 1:2 (v/v) and dried at 40 °C under vacuum.

2.2.2. Emulsion preparation

Oil-in-water emulsions were prepared according to Gordon, Paiva-Martins, and Almeida (2001). The emulsions were made by mixing 10 g of sunflower oil, 0.66 g Tween 20 as emulsifier, 22.3 g phosphate buffer, pH 7.0 (0.1 mol/l) and the tested concentrations of polysaccharides or TBHQ (20 and 40 mg/100 g). The mixtures were emulsified in a waring blendor for 60 s.

2.2.3. Determination of antioxidant activity

Antioxidant activity was determined using a diene conjugated formation method according to Lingnert, Vallentin, and Eriksson (1979). The substrate consisted of 2.86 g of linoleic acid emulsified with an equal amount of Tween 20 in phosphate buffer, pH 7.0 (0.1 mol/l). The mixture was then homogenized at high speed for 1 min. Different concentrations of polysaccharides (20, 40 and 60 mg/100 g) were mixed with emulsions and incubated at 50 °C for 20 h. Absorbance was then measured at 234 nm.

The effect of pH and temperature on antioxidant activity of the polysaccharides were also determined. Phosphate–HCl buffer was used for pH 3 and 5, while phosphate–NaOH buffer was used for pH 7, 9 and 11. The autoxidation was examined at different temperatures of 30, 40, 50, 70 and 90 °C.

2.2.4. Radical scavenging ability

The radical-scavenging ability of the polysaccharides was tested by the method of Paiva-Martins and Gordon (2001). Two milliliters of aqueous polysaccharides or TBHQ solution (20 mg/100 g) were added to 1 ml of methanolic DPPH$^{\bullet}$ solution (0.128 g/l methanol). The decrease in absorbance was determined at 515 nm after 10, 60 and 240 min using spectrophotometer Shimadzu UN-1201 (Shimadzu Co., Ltd., Kyoto, Japan). The scavenged percent of DPPH$^{\bullet}$ in the reaction was calculated from a calibration curve using the following equation:

$$Y = 0.0847 + 6972.06x, \quad r^2 = 0.9988.$$

2.2.5. Oxidation experiments

The prepared emulsions were oxidized in the dark at 60 °C for 50 h. Samples were taken at zero time and after 5 h interval till 50 h for analysis. Isolation of oil from emulsions for analysis was by freezing, thawing, and centrifugation. Progress of oxidation was monitored by determination of the peroxide value (PV) according to AOAC (2000), conjugated dienes (CD) as adopted by AOCS (1989), *p*-anisidine value (AnV) by the method described by IUPAC (1987) and total oxidation value (Totox V) calculated by the formula as reviewed by Rossell (1983):

$$\text{Totox V} = 2\,\text{PV} + \text{AnV}.$$

2.2.6. Statistical analysis

The obtained data were exposed to analysis of variance. Duncan multiple range at 5% level of significance was used to compare between means. The analysis was carried out using the PROC ANOVA procedure of Statistical Analysis System (SAS, 1996).

Contour plot was used as a method to study the response surface of radical scavenging activity as dependent variable with pH values, and time as independent variable. The response surface method was applied using Harvard ChartXl software version 2.0 with TBHQ, xanthan, curdlan, RPS and CMC to locate the optimum conditions of reaction and to identify the best compound as a radical scavenging.

3. Results and discussion

3.1. Effect of concentration

Effect of the concentration of TBHQ, xanthan, curdlan, RPS and CMC on oxidation of linoleic acid at pH 7 and at 40 °C is shown in Table 2. Increasing the concentration of TBHQ and the polysaccharides up to 20 mg/100 g emulsion resulted in a significant ($P<0.05$) increase in antioxidant activity in all samples. The antioxidant activity of xanthan, curdlan and RPS at concentration of 40 mg/100 g emulsion recorded the same effect of TBHQ at 20 mg/100 g emulsion. CMC showed slight inhibitory effect on linoleic acid oxidation compared to xanthan, curdlan and RPS. This result is similar to that of Matsumura et al. (2003) who demonstrated that the antioxidant activity increased when the concentration of gum arabic and soybean polysaccharides was increased in linoleate emulsion.

3.2. Effect of temperature

The antioxidant activity of TBHQ, xanthan, curdlan, RPS and CMC in linoleate system incubated at 30, 40, 50, 70 and 90 °C (at concentration 20 mg/100 g emulsion) is shown in Table 3. Oxidation of linoleic acid was

Table 2
Effect of concentration of TBHQ, xanthan, curdlan, RPS and CMC on antioxidant activity in linoleic acid emulsion system at pH 7 and at 40 °C

Compound	Antioxidant activity at different concentration (mg/100 g emulsion)		
	20	40	60
TBHQ	0.232^{c}	0.188^{c}	0.171^{d}
Xanthan	0.253^{b}	0.245^{b}	0.226^{b}
Curdulan	0.251^{b}	0.236^{b}	0.199^{c}
RPS	0.253^{b}	0.228^{b}	0.213^{bc}
CMC	0.299^{a}	0.279^{a}	0.252^{a}

TBHQ, tertiary butylhydroxyquinone; RPS, rhizobium polysaccharides; CMC, carboxymethylcellulose.
Different alphabets within the same column are significantly ($P<0.05$).

Table 3
Effect of different incubation temperature on antioxidant activity of TBHQ, xanthan, curdlan, RPS and CMC in linoleic acid emulsion system at pH 7 and concentration of 20 mg/100 g emulsion

Compound	Antioxidant activity at different temperature (°C)				
	30	40	50	70	90
TBHQ	0.176^{bc}	0.232^{c}	0.360^{c}	0.450^{b}	0.460^{ab}
Xanthan	0.198^{ab}	0.253^{b}	0.397^{bc}	0.457^{b}	0.477^{ab}
Curdulan	0.172^{c}	0.251^{b}	0.411^{abc}	0.441^{b}	0.456^{b}
RPS	0.191^{bc}	0.253^{b}	0.437^{ab}	0.450^{b}	0.421^{c}
CMC	0.217^{a}	0.299^{a}	0.452^{a}	0.482^{a}	0.480^{a}

TBHQ, tertiary butylhydroxyquinone; RPS, rhizobium polysaccharides; CMC, carboxymethylcellulose.
Different alphabets within the same column are significantly ($P<0.05$).

Table 4
Effect of different pH values on antioxidant activity of TBHQ, xanthan, curdlan, RPS and CMC at concentration of 20 mg/100 g emulsion in linoleic acid emulsion system at 40 °C

Compound	Antioxidant activity at different pH values				
	3	5	7	9	11
TBHQ	0.509^{a}	0.492^{a}	0.232^{c}	0.092^{b}	0.046^{c}
Xanthan	0.371^{c}	0.381^{b}	0.253^{b}	0.128^{a}	0.088^{a}
Curdulan	0.367^{c}	0.347^{c}	0.251^{b}	0.106^{b}	0.055^{bc}
RPS	0.382^{bc}	0.378^{b}	0.253^{b}	0.105^{b}	0.062^{b}
CMC	0.398^{b}	0.370^{b}	0.299^{a}	0.134^{a}	0.096^{a}

TBHQ, tertiary butylhydroxyquinone; RPS, rhizobium polysaccharides; CMC, carboxymethylcellulose.
Different alphabets within the same column are significantly ($P<0.05$).

accelerated with increase in incubation temperature from 30 to 90 °C. The closely significant antioxidant activity values were observed with addition of xanthan, curdlan, RPS compared to TBHQ at temperatures less than 90 °C. RPS showed a higher thermal stability with lower linoleic oxidation values compared to TBHQ, xanthan and curdlan at 90 °C. Conversely, CMC appeared to have significantly ($P<0.05$) higher values with a lower antioxidant activity compared to tested polysaccharides. It could be noticed that RPS is a good emulsion stabilizer, besides its higher activity against oil autoxidation in food emulsions.

3.3. Effect of pH

Antioxidant activity of studied polysaccharides can be seen in Table 4. The antioxidant activity of the different polysaccharides increased with increasing pH values from 3 to 11, indicating strong dependence on the pH of the system. This is similar to the study done by Azizah, Nik Ruslawati, and Swee Tee (1999), who reported that an extract from coca by-products showed high antioxidant activity with increasing pH values in the linoleic acid system. The antioxidant activity of xanthan, curdlan and RPS emulsions at pH 3 and 5 were in the first order with significant ($P<0.05$) values compared to TBHQ, which was in the second order. On the other hand, CMC showed intermediate activity in breaking down the linoleic acid oxidation in the emulsion. At pH 9 and 11 the antioxidant activity of curdlan and RPS were closed to that of TBHQ without significant difference ($P>0.05$). It could be suggested that xanthan, curdlan and RPS can be used in acidic foods; curdlan and RPS in foods with pH more than 7, to delay the oxidation in oil. These results agree with those of Shimada, Fujikawa, Yahara, and Nakamura (1992), who reported that xanthan can be used as an emulsion stabilizer to inhibit strongly the autoxidation of soybean oil in emulsion system. The weak antioxidant activity of polysaccharides at low pH values may be due to their insoluble form according to the reported data of Tolstoguzov (1986), who had shown that at low pH values (<6.0) the polysaccharide carries net opposite charges. At this pH region, polysaccharides were in the insoluble form.

3.4. Radical scavenging ability

The contour plot in Fig. 1 shows the response surface of radical scavenging activity as observed in the presence of TBHQ, xanthan, curdlan, RPS and CMC with the stable DPPH$^{\bullet}$ radical at pH values 3, 5, 7, 9 and at different periods of 10, 60 and 240 min. Radical scavenging activity increased with increasing pH values from 3 to 7, whereas at pH 9.0, it decreased in all cases. After 10 min at pH values ranging between 3 and 7, TBHQ demonstrated significantly ($P<0.05$) the strongest activity of radical scavenging, followed by curdlan and RPS, whereas, CMC and xanthan showed the lowest scavenging activities. Opposite status was observed after 240 min at all pH values, which was attributed to the change in radical scavenging activity with time according to the kinetic behavior of reaction (Paiva-Martins & Gordon, 2001).

Table 5 shows the optimum values of radical scavenging at reaction times and different pH values; these data were extracted from the study of the response surface by contour plot of pH values, times and radical scavenging activity. It can be seen that the curdlan was effective in radical scavenging at pH between 3 and 7 this reached from 60% to 90%, while RPS was effective in radical scavenging at pH values of 7 and 9 reaching 90% and 60%, respectively. A comparable results were observed previously by Sun, Wang, Fang, Gao, and Tan (2004). They indicated that exopolysaccharide produced by a fungus *Keissleriella* sp. YS 4108 were effective in scavenging of superoxide radical.

Polysaccharide extracts from mushroom (Liu et al., 1997) and *Keissleriella* sp. YS 4108 exopolysaccharide (Sun et al., 2004) were also reported to have free-radical scavenging effects related to its affinity to the radical in the specific site. However, the mechanism of free-radical scavenging of polysaccharides is still not fully understood

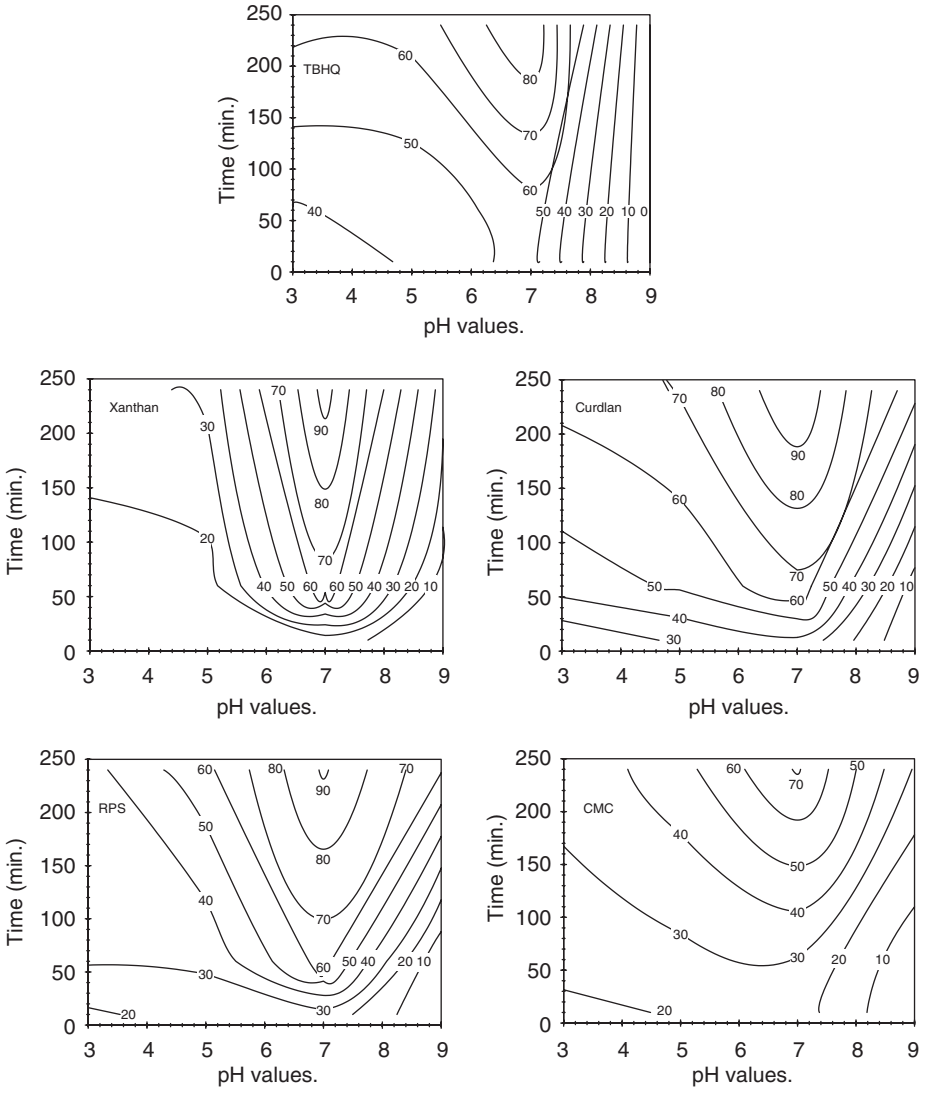

Fig. 1. Contour plot of radical scavenging (%) of TBHQ, xanthan, curdlan, RPS and CMC at different times and pH values.

(Liu et al., 1997). It is known that phenolic compounds from the plant *Pedicularis alashanica*, such as phenylpropaniod glycosides, may react with superoxide radical by a one-electron transfer mechanism or hydrogen abstraction mechanism to form the semiquinones. Therefore, the scavenging activity of phenylpropaniod glycosides for superoxide radical may be due to their reduction activities, which may be related to the number of phenolic hydroxyl groups and the conjugated system (Wang et al., 1996). However, it was not clear whether the mechanisms of radical scavenging by Cardulan and RPS were similar to that of plant phenolic compounds.

3.5. Effects on oxidation parameters

3.5.1. Conjugated diene

In regard to Fig. 2, the CD values of extracted sunflower oil from prepared emulsions increased from 0.181 before storage reaching their maximal values of 1.556, 0.667, 1.186, 0.649, 0.649 and 1.166 of control, TBHQ, xanthan, curdlan, RPS and CMC emulsions, respectively, in the end of storage period at 60 °C. Curdlan and RPS showed a lower CD values in its emulsions followed by TBHQ. The first steps in oxidation were CD formation by the shift in position of double bonds. This formation occurred specially in oils having linoleate (C18:2) or higher polyunsaturated fatty acids. The shift occurs as one hydrogen is lost from the methylene group positioned between two double bonds (White, 1995). The antioxidant effect of curdlan and RPS against CD formation may be due to its hydrogen donating activity according to Shimada et al. (1992). Apparently, curdlan and RPS are more effective in preventing CD formation than xanthan and CMC.

3.5.2. Peroxide value

Fig. 3 indicated that the PV in extracted sunflower oil from emulsions was gradually increased versus storage period reaching its maximal values after 50 h. In control emulsion, which prepared without any tested samples, it recorded significantly the highest value compared to those

Table 5
Optimum values of radical scavenging (%) of TBHQ, xanthan, curdlan, RPS and CMC at different pH values and times

Compound	PH	Time (min)	%
TBHQ	3	218.3	60
	5	209.1	60
	7	187.8	80
	9	240.0	0.0
Xanthan	3	140.8	20
	5	207.0	30
	7	213.4	90
	9	194.5	20
Curdlan	3	207.8	60
	5	233.4	70
	7	188.3	90
	9	190.3	40
RPS	3	56.4	30
	5	187.4	50
	7	231.4	90
	9	237.3	60
CMC	3	167.3	30
	5	178.7	40
	7	235.2	70
	9	240.0	30

TBHQ, tertiary butylhydroxyquinone; RPS, rhizobium polysaccharides; CMC, carboxymethylcellulose.

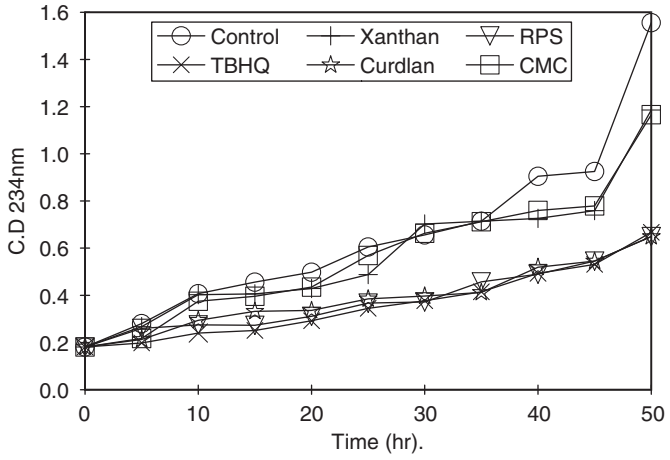

Fig. 2. Conjugated diene (CD) of oil extracted from emulsions prepared using TBHQ and different polysaccharides and stored at 50 °C.

of other emulsions. It could be noticed that the PV of extracted oil from emulsions prepared using RPS, curdlan and TBHQ were significantly lesser than those prepared using CMC and xanthan and showed no going down with advancing storage period. From these results, the inhibitory effect of RPS and curdlan may be due to their activity in decreasing the oxygen consumption rate by means of spreading these polysaccharides in interfacial surfaces. These observation agree with Matsumura et al. (2003) who reported that soybean polysaccharides were effective in decreasing oxygen consumption.

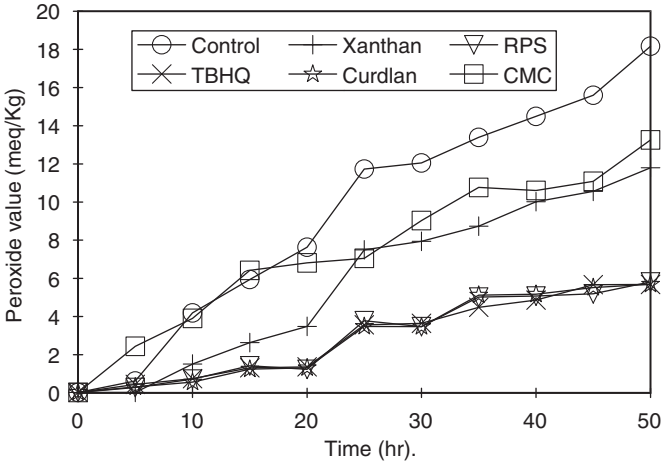

Fig. 3. Peroxide values of oil extracted from emulsions prepared using TBHQ and different polysaccharides and stored at 50 °C.

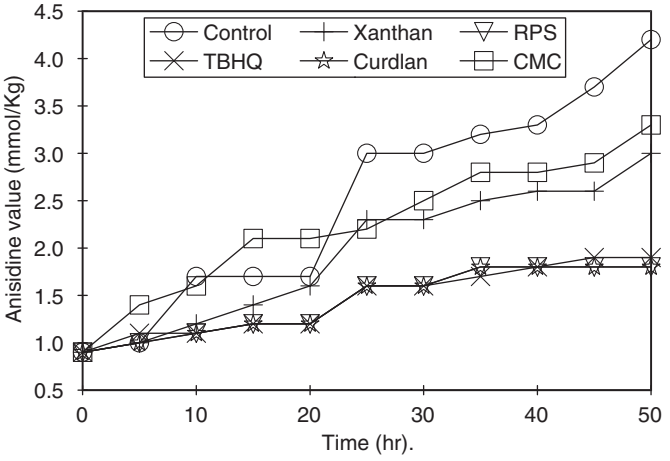

Fig. 4. Anisidine values of oil extracted from emulsions prepared using TBHQ and different polysaccharides and stored at 50 °C.

3.5.3. Anisidine value

Formation of secondary oxidation products considered responsible for off-flavor development in oils and oil products. The AnV determine the level of 2-alkenals present in the oil as an indication of the formation of secondary oxidation compounds (White, 1995). As shown in Fig. 4, the AnV of sunflower oil separated from prepared emulsions using TBHQ (20 mg/100 g emulsion); xanthan, curdlan, RPS and CMC (40 mg/100 g emulsion) and stored at 60 °C for 50 h were gradually and markedly stepped up from 0.9 with advancing storage period reaching its maximal values of 4.2, 1.9, 3, 1.8, 1.8 and 3.3 mmol/Kg at the end of storage period for control emulsion and others prepared using TBHQ, xanthan, curdlan, RPS and CMC, respectively. Data indicated that using curdlan and RPS were more effective than TBHQ in preventing the decomposition of primary oxidation products to 2-alkenals. While, the control emulsion and emulsions prepared by adding xanthan and CMC characterized by the highest AnVs overall the storage periods.

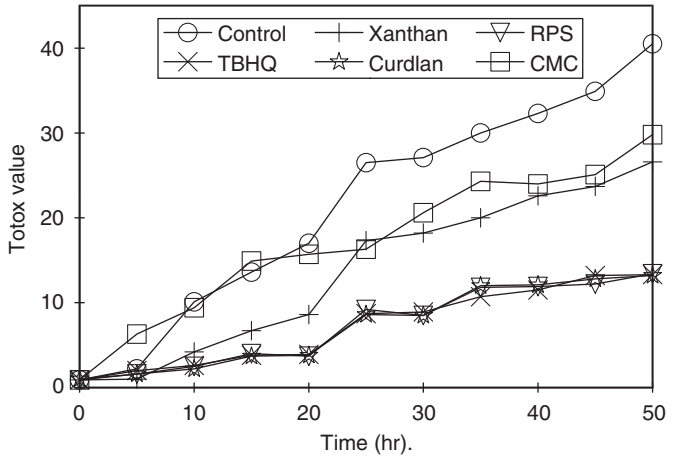

Fig. 5. Totox values of oil extracted from emulsions prepared using TBHQ and different polysaccharides and stored at 50 °C.

3.5.4. Totox value

Fig. 5 shows that the totox values of extracted sunflower oil from prepared emulsions had the same trend of peroxide and AnVs. Totox value increased from 0.9 before storage reaching its maximal values of 40.5, 13.3, 26.6, 13.2, 13.4 and 29.8 at the end of storage period for control emulsion and other emulsions, which was prepared using TBHQ, xanthan, curdlan, RPS and CMC, respectively, at 60 °C. The increase of totox value in emulsions prepared using xanthan and CMC may be attributed to higher oxidation rate in their systems as a result of higher moisture content. On the other hand, lower totox values in emulsions prepared using curdlan and RPS can be attributed to the highest hydrogen donating activity and its ability in lessening the oxygen consumption at interfacial surface between oil and water.

4. Conclusion

Curdlan and RPS can be used as emulsion stabilizers to inhibit strongly the autoxidation of oil in emulsion systems at wide range of pH, especially at acidic and alkaline pH values of 3–5 and 7–9. They can be substitute TBHQ in emulsion systems at concentration of 40 mg/100 g emulsion without significant difference ($P > 0.05$) in antioxidant activity. In addition, curdlan and RPS appeared with high radical scavenging activities. Curdlan was effective in radical scavenging at pH ranging between 3 and 7, while RPS was effective in radical scavenging at pH values 7 and 9 more than TBHQ.

References

Abdalla, A. E., Tirzite, D., Tirzitis, G., & Roozen, J. P. (1999). Antioxidant activity of 1,4-dihydropyridine derivatives in β-carotene-methyl linoleate, sunfower oil and emulsions. *Food Chemistry*, *66*, 189–195.

AOAC. (2000). *Official methods of analysis* (17th ed.). Gaithersburg, MD, USA: Association of Official Analytical Chemists.

AOCS. (1989). *Official and tentative methods* (4th ed.). Champaign, IL, USA: American Oil Chemists' Society.

Azizah, A. H., Nik Ruslawati, N. M. N., & Swee Tee, T. (1999). Extraction and characterization of antioxidant from coca by-products. *Food Chemistry*, *64*, 199–202.

Blander, G., Oliveira, R. M., Conboy, C. M., Haigis, M., & Guarente, L. (2003). Superoxide dismutase 1 knock-down induces senescence in human fibroblasts. *The Journal of Biological Chemistry*, *278*, 38966–38969.

Chuanguang, Q., Huang, K., & Xu, H. (2002). Protective effect of polysaccharide from the loach on the in vitro and in vivo peroxidative damage of hepatocyte. *Journal of Nutritional Biochemistry*, *13*, 592–597.

Dziezak, J. D. (1991). A focus on gums. *Food Technology*, *45*, 116–130.

Footrakul, P., Suyanandna, P., Amemura, A., & Harade, I. (1981). Study of extracellular polysaccharides of *Rhizobium*. In H. Taguchi (Ed.), *Microbial utilization of renewable resources*, Vol. 2 (pp. 141–146). Osaka Univ. Suitashi, Osaka, Japan: Institute of scientific and industrial research.

Gordon, M. H., Paiva-Martins, F., & Almeida, M. (2001). Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. *Journal of Agriculture and Food Chemistry*, *49*, 2480–2485.

Harman, D. (1993). Free radical involvement in aging, pathophysiology and therapeutic implications. *Drugs and Aging*, *3*, 60–80.

Hayashi, Y., Morimoto, K., Miyata, N., & Sato, H. (1993). Quantitative cancer risk analysis of BHA based on integration of pathological and biological/biochemical information. *Toxicology and Industrial Health*, *9*, 243–249.

IUPAC. (1987). *Standard methods for the analysis of oils, fats and derivatives* (7th ed.). Pergamon Press, New York, USA: International Union of Pure and Applied Chemistry.

Linderschmidt, R., Trylka, A., Goad, M., & Witschi, H. (1986). The effects of dietary butylated hydroxytoulene on liver and colon tumor development in mice. *Toxicology*, *38*, 151–160.

Lingnert, H., Vallentin, K., & Eriksson, C. E. (1979). Measurement of antioxidative in model system. *Journal of Food Processing and Preservation*, *3*, 87–103.

Liu, F., Ooi, V. E. C., & Chang, S. T. (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life Science*, *60*, 763–771.

Matsumura, Y., Egami, M., Satake, C., Maeda, Y., Takahashi, T., Nakamura, A., & Mori, T. (2003). Inhibitory effects of peptide-bound polysaccharide on lipid oxidation in emulsions. *Food Chemistry*, *83*, 107–119.

Nagwa, M. H. R., Madkour, M. H. F., El-Mahdy, R. M., & Hanan, M. A. A. (1997). Production of food grade exopolysaccharide from *Rhizobium meliloti*. II: Characterization of polysaccharide. *Annals of agriculture science, Moshtohor*, *35*, 2241–2262.

Paiva-Martins, F., & Gordon, M. H. (2001). Isolation and characterization of the antioxidant component 3, 4-dihydroxyphenylethyl 4-formyl1-3-formylmethyl1-4-hexenoate from olive (*Olea europaea*) leaves. *Journal of Agricultural and Food Chemistry*, *49*, 4214–4219.

Perry, G., Raina, A. K., Nonomura, A., Wataya, T., Sayre, L. M., & Smith, M. A. (2000). How important is oxidative damage? Lessons from Alzheimer's disease. *Free Radical Biology and Medicine*, *28*, 831–834.

Rocks, J. K. (1971). Xanthan gum. *Food Technology*, *25*, 476–482.

Rossell, J. B. (1983). Measurment of rancidity. In J. C. Hllin, & R. J. Hamilton (Eds.), *Rancidity in foods* (pp. 26–28). Essex, UK: Applied Science Publishers Ltd.

SAS Program. (1996). *SAS/STAT user's guide release 6.12 edition*. Cary, NC, USA: SAS Inst. Inc.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidtive properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, *40*, 945–948.

Sun, C., Wang, J. W., Fang, L., Gao, X. D., & Tan, R. X. (2004). Free radical scavenging and antioxidant activities of EPS2, an exopolysaccharide produced by a marine filamentous fungus *Keissleriella* sp. YS 4108. *Life Science*, *75*, 1063–1073.

Tolstoguzov, V. B. (1986). Functional properties of protein–polysaccharide mixtures. In J. R. Mitchell, & D. A. Ledward (Eds.), *Functional properties of food macromolecules* (pp. 385–415). London: Elsevier Applied Science Publishers.

Wang, P., Kung, J., Zheng, R., Yung, Z., Lu, J., Guo, J., & Jiu, Z. (1996). Scavenging effects of phenylpropanoid glycosides from pedicukk on superoxide anion and hydroxyl radical by the spin trapping method (95) 02255-4. *Biochemical Pharmacology*, *51*, 491–687.

White, P. J. (1995). Conjugated diene, anisidine value, and carbonyl value analyses. In K. Warner, & N. A. M. Eskin (Eds.), *Methods in assess quality and stability of oils and fat-containing foods* (p. 159). Champaign, IL, USA: AOCS.

Yotsuzuka, F. (2001). Curdlan. In S. S. Cho, & M. L. Dreher (Eds.), *Handbook of dietary fiber* (pp. 737–757). New York: Marcel Dekker Inc.