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Antioxidant Activity and Emulsion-Stabilizing Effect of Pectic Enzyme Treated Pectin in Soy Protein Isolate-Stabilized Oil/Water Emulsion

Ping-Hsiu Huang,[†] Hao-Te Lu,[‡] Yuh-Tai Wang,*[,§] and Ming-Chang Wu*[,†]

[†]Department of Food Science and [‡]Department of Business Administration, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan

[§]Life Science Center, Hsing Wu Institute of Technology, No. 101, Sec.1, Fen-Liao Road, Lin-Kou, Taipei 244, Taiwan

ABSTRACT: The antioxidant activity of pectic enzyme treated pectin (PET-pectin) prepared from citrus pectin by enzymatic hydrolysis and its potential use as a stabilizer and an antioxidant for soy protein isolate (SPI)-stabilized oil in water (O/W) emulsion were investigated. Trolox equivalent antioxidant capacity (TEAC) was found to be positively associated with molecular weight (M_w) of PET-pectin and negatively associated with degree of esterification (DE) of PET-pectin. PET-pectin (1 kDa and 11.6% DE) prepared from citrus pectin after 24 h of hydrolysis by commercial pectic enzyme produced by *Aspergillus niger* expressed higher α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity, TEAC, and reducing power than untreated citrus pectin (353 kDa and 60% DE). The addition of PET-pectin could increase both emulsifying activity (EA) and emulsion stability (ES) of SPI-stabilized O/W emulsion. When the SPI-stabilized lipid droplet was coated with the mixture of PET-pectin and pectin, the EA and ES of the emulsion were improved more than they were when the lipid droplet was coated with either pectin or PET-pectin alone. The amount of secondary oxidation products (thiobarbituric acid reactive substances) produced in the emulsion prepared with the mixture of SPI and PET-pectin was less than the amount produced in the emulsion prepared with either SPI or SPI/pectin. These results suggest that PET-pectin has an emulsion-stabilizing effect and lipid oxidation inhibition ability on SPI-stabilized emulsion. Therefore, PET-pectin can be used as a stabilizer as well as an antioxidant in plant origin in SPI-stabilized O/W emulsion and thus prolong the shelf life of food emulsion.

KEYWORDS: lipid oxidation, antioxidant activity, emulsion, citrus pectin, commercial pectic enzyme, soy protein isolate

INTRODUCTION

Oil-in-water (O/W) emulsions are the basis of many food products. Proteins and polysaccharides play a key role in the structure formation and stabilization of food systems. Proteins are primarily used as emulsifying agents and polysaccharides as stabilizing agents.[1] The stability of O/W emulsions containing protein-coated droplets can be improved by adding polysaccharides under several conditions in which polysaccharide molecules are adsorbed onto the lipid droplet surfaces to form a protective layer.[2]

Moreover, the quality and shelf life of foods are greatly affected by the oxidative stability of lipids in O/W emulsions.[3,4] Therefore, antioxidants are usually added into emulsified foods to retard the peroxidation and degradation of unsaturated lipids and to avoid the accompanying bad smells and tastes related directly to the oxidative deterioration of food.[5,6] Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ) are commonly used in food formulations to prolong their storage stability. However, in relation to safety concerns, interest in natural antioxidants has intensified.[7]

Pectin is an inexpensive, nontoxic product extracted from citrus peels or apple pomaces. It has been used in the food and beverage industry as a gelling and stabilizing agent for many years.[8,9] Furthermore, pectin is also suggested to be used as an emulsifying agent and utilized in protein-stabilized O/W emulsions to increase the emulsifying activity (EA) and emulsion stability (ES).[1,2,10]

Pectin and its derivatives such as pectin hydroxamic acids were reported to exhibit antioxidant activity[10−13] and inhibit the lipid oxidation of Brij 35-stabilized menhaden oil in water emulsions.[4] The molecular weight (M_w) of pectin was suggested to have significant influence on the emulsifying ability of O/W emulsions. The optimum M_w of depolymerized pectin prepared from acid hydrolysis and heat treatment with the best emulsifying ability and emulsifying stability was 70 kDa.[10,14] Pectin with lower or higher M_w was not as effective. In addition, no investigation on the antioxidant activity of depolymerized pectin was discovered.

Therefore, the aim of this study was to evaluate the antioxidant activity of pectic enzyme treated pectin (PET-pectin) prepared from citrus pectin by enzymatic hydrolysis and its potential use as a stabilizer for SPI-stabilized O/W emulsions. Also, the effect of PET-pectin on the oxidative stability of SPI-stabilized O/W emulsions was investigated.

MATERIALS AND METHODS

Materials. Commercial pectic enzyme Peclyve CP (CPE) containing 133.5 U/mL pectin lyase (PL) activity, 50.6 U/mL pectin methyl esterase (PME) activity, and 22.4 U/mL polygalacturonase (PG) activity produced by *Aspergillus niger* was purchased from Lallemand Australia Pty Ltd. (Underdale, SA, Australia). Citrus pectin with 60% degree of esterification (DE) was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Thiobarbituric acid (TBA), 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABST), α,α-diphenyl-β-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), DL-*all-rac*-α-tocopherol, and 1,1,3,3-tetraethoxypropane were purchased from Sigma (St. Louis, MO). Soy protein isolate Supro 545 (SPI) was purchased from Protein Technologies International (St. Louis,

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MO). Soybean oil was purchased from a local grocery. Analytical grade reagents were used.

Preparation of Pectic Enzyme Treated Pectin. PET-pectin was prepared from 1 L of solution composed of citrus pectin (1%) and CPE (containing 0.13 U/mL PL activity, 0.05 U/mL PME activity, and 0.02 U/mL PG activity). After incubating at 45 °C for 0−72 h, the pectin solution was heated in a boiling water bath for 10 min, cooled to room temperature, and then freeze-dried.

Determination of Pectin. The content of pectin or PET-pectin was determined according to the method of Jiang et al.[15] In brief, a pectin solution of pectin (5 mg), precooled H_2SO_4 (2 mL), and distilled water (15 mL) was magnetically stirred in an ice bath for 1 h. Subsequently, an aliquot of the pectin solution (0.5 mL) was mixed with 3 mL of 12.5 mM sodium tetraborate solution (in sulfuric acid) in an ice bath and heated in a boiling water bath for 5 min. After cooling, the reaction mixture was mixed with 0.05 mL of 0.15% *m*-hydroxydiphenyl solution (in 0.5% NaOH) and then allowed to stand at room temperature for 5 min. The absorbance of the solution at 520 nm was recorded. D-Galacturonic acid was used to construct the standard curve for the calculation of pectin content in the samples.

Determination of DE. The DE of pectin or PET-pectin was determined according to the method described by Jiang et al.[16] An adequate amount of pectin or PET-pectin (10 mg) was added to 10 mL of 0.5 N KOH, followed by the processes of incubation (at ambient temperature for 1 h), filtration, and neutralization (with dilute phosphoric acid to pH 7.5), and then the volume was brought to 25 mL using 0.05 M phosphate buffer (pH 7.5). Subsequently, gas chromatography was used to analyze the methanol content of the thusly obtained solution. The DE of pectin was calculated with the following equation:

$$\text{DE}\ (\%) = [\text{methanol content} \times (31/32) \div 16.32 \div \text{pectin content}] \times 100\%$$

Determination of Molecular Weight. The M_w of pectin or PET-pectin was measured using size exclusion chromatography in a high-performance liquid chromatographic system. Sample (20 μL) was filtered through a 0.22 μm syringe filter prior to injection, separated on a TSK-Gel G5000 PWXL column (Tosoh, Tokyo, Japan) under isocratic temperature (40 °C) and flow (0.6 mL/min) conditions with double-distilled water as mobile phase, and then detected using a Hitachi L-2490 refractive index detector (Hitachi, Tokyo, Japan). The acquired data were analyzed using HPLC System Manager (HSM) software, version 2.0 (Hitachi). A dextran standard series (1, 50, 150, 270, 410, and 750 kDa; Sigma) was used as marker for M_w calculation.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. Total antioxidant capacity of pectin or PET-pectin was measured using the ABTS assay according to the method described by Rice-Evans and Miller.[17] In brief, an aliquot (2 μL) of sample was added to 20 μL of ethanol solution and then mixed with 180 μL of ABTS radical solution. The absorbance was read at 734 nm at 10 min after mixing. The percentage inhibition was calculated against trolox standard curve prepared using trolox at different concentrations, and the results were expressed as millimolar trolox equivalents.

DPPH Radical Scavenging Assay. The DPPH radical scavenging activity of pectin or PET-pectin was evaluated according to the method described by Chen et al.[18] with some modification. Briefly, the sample solution (4 mL) was mixed with 1 mL of 0.1 mM DPPH methanol solution. After 30 min of incubation at room temperature in the dark, the absorbance of the solution at 517 nm was measured. The DPPH radical scavenging activity was calculated according to the following formula:

$$\text{DPPH radical scavenging activity}\ (\%) = [1 - (\text{absorbance of sample}/\text{absorbance of control})] \times 100\%$$

Determination of Reducing Power. The reducing power of pectin or PET-pectin was determined according to the method of Shona et al.[19] Sample solution (1 mL) was mixed with 2.5 mL of 200 mM phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture, followed by centrifugation at 1000*g* for 10 min. After an aliquot (5 mL) of the upper layer had been mixed with 5 mL of deionized water and 1 mL of 0.1% of ferric chloride, the absorbance of the solution at 700 nm was measured. The reducing power was expressed as the absorbance value. Higher absorbance of the reaction mixture indicated higher reducing power.

Metal Ion Chelating Assay. The ferrous ion chelating ability was measured by the ferrous iron−ferrozine complex at 562 nm according to the method described by Shimada et al.[20] Briefly, sample solution (25 μL) was mixed with 25 μL of 2 mM $FeCl_2$, 50 μL of 5 mM ferrozine, and 0.9 mL of methanol. After mixing and 10 min of incubation at room temperature, the absorbance of the mixture was measured at 562 nm. Metal ion chelating ability was calculated according to the following formula:

$$\text{metal ion chelating ability}\ (\%) = [1 - (\text{absorbance of sample}/\text{absorbance of control})] \times 100\%$$

Preparation and Characterization of SPI-Stabilized O/W Emulsions. SPI-stabilized O/W emulsion was prepared and characterized as described by Yasumatsu et al.[21] with some modification. After the addition of 20 mL of soybean oil to the SPI−pectin solution constructed of 80 mL of 1 g SPI solutions and 0−2 g of pectin (or PET-pectin) in 10 mM citric acid buffer (pH 3), the resulting mixture was placed into an Oster blender model 6642 blender (Oster, Miami, FL) jar. Emulsification was performed by blending at maximum speed for 2 min with four 30 s intervals.

For the measurement of EA and ES, an aliquot (10 mL) of formed emulsion was immediately transferred into a screw-capped Falcon conical centrifuge tube (BD Biosciences, San Jose, CA) and centrifuged at 2000*g* for 20 min. The height of the emulsion layer was noted in the graduated tube and calculated for EA according to the following formula:

$$\text{EA}\ (\%) = (\text{height of the emulsified layer}/\text{total height of mixture in tube}) \times 100\%$$

ES was determined in a similar manner as above, but it involved heating the emulsion before centrifugation at 80 °C for 30 min in a water bath. The emulsion-containing tube was kept for cooling under running water for 15 min and then centrifuged at 2000*g* for 20 min. ES was expressed as the percentage of the emulsifying activity remaining after heating and calculated according to the following formula:

$$\text{ES}\ (\%) = (\text{height of the emulsified layer after heating}/\text{total height of mixture in tube}) \times 100\%$$

Evaluation of Oxidative Stability of SPI-Stabilized O/W Emulsions. Oxidative stability was evaluated by the determination of secondary oxidation products, which were produced from lipid oxidation and monitored with the thiobarbituric acid reactive substances (TBARS) method using the procedure described by Tong et al.[22] Emulsion (30 mL) was placed in screw-capped Falcon conical centrifuge tubes and held at 50 °C in an oven for 10 days. At each incubation point, an aliquot (0.1 mL) of the stored emulsion was mixed with 0.9 mL of distilled water and 2.0 mL of TBA reagent containing 15% (w/v) tricholoracetic acid and 0.375% (w/v) TBA in 0.25 N HCl in a screw-capped tube. Sample was then heated in a boiling water bath for 15 min, cooled at room temperature for 10 min, and centrifuged at 1000*g* for 15 min. The absorbance at 532 nm of the supernatant was measured. Concentrations of TBARS were determined from a standard curve constructed by 1,1,3,3-tetraethoxypropane.

Table 1. Multiple Regression Model for Trolox Equivalent Antioxidant Capacity with Degree of Esterification (DE) and Molecular Weight (M_w) of Pectin after Commercial Microbial Pectic Enzyme (CPE) Treatment[a]

variable	parameter estimate	standard error	p value
intercept	6.47	0.17	<0.0001
DE	−0.22	0.01	<0.0001
M_w	0.000021	0.000001	<0.0001

$R^2 = 0.95$ ($p < 0.0001$)

[a] The reactions of citrus pectin (1%) and CPE were carried out at pH 4 and 45 °C for 0−72 h.

Statistical Analysis. All measurements were carried out in triplicate. Values given in the tables and figures are expressed as means ± standard deviation (SD) of triplicate measurements. Statistical analysis was accomplished using SAS Statistical Software, for Windows, version 9.1 (SAS, Cary, NC). The statistical significance of differences among means was evaluated using Duncan's multiple-range tests at a significant level of 0.05. In multiple regression analysis, TEAC was used as dependent measurement. DE and M_w were used as independent variables.

RESULTS AND DISCUSSION

Relationship between TEAC of Pectin after CPE Treatment and Its Characteristics. After different time course CPE treatments, the TEAC, DE, and M_w of the enzymatically hydrolyzed pectin (PET-pectin) were evaluated. The TEAC value of CPE-treated pectin increased continuously and reached a plateau at 24 h with a TEAC value of 4.5 mM trolox. The relationship between TEAC of PET-pectin and its characteristics (DE and M_w) was analyzed using multiple regressions. The result is summarized in Table 1. TEAC was positively associated with M_w of PET-pectin but negatively associated with DE of PET-pectin. According to the DE parameter (−0.22), it is obvious that TEAC is DE related.

The DPPH radical scavenging activity was galacturonic acid content related in the pectin from *Cicer arietinum* L.[13] and positively correlated with the corresponding DE values (DE94, DE65, and DE25) of original pectin in pectin hydroxamic acid derivatives; however, no significant relationship was found between DPPH radical scavenging activity and DE in pectin without hydroxylamine treatment.[11] Chen et al.[4] revealed that there is no significant difference in peroxyl radical scavenging activity between low-methoxyl (LM) pectin and high-methoxyl (HM) pectin. Furthermore, Chen et al.[23] proposed that a relatively low molecular weight and a high uronic acid content in tea polysaccharides appeared to increase the antioxidant activity. Nevertheless, the relationship between antioxidant activity and M_w of pectin is unclear. Especially, according to the result of the present study, we found that TEAC of pectin after CPE treatment was positively associated with M_w and negatively associated with DE of PET-pectin (Table 1). On the basis of these findings, we speculate that the effect of DE on the antioxidant activity of pectin can be enhanced by chemical modification (hydroxylamine treatment) and enzyme modification (CPE treatment).

Antioxidant Activity of PET-Pectin. As shown in Table 2, DPPH radical scavenging activity, TEAC, and reducing power of citrus pectin (10 mg/mL) at 353 kDa and 60% DE were lower than those of the PET-pectin at 1 kDa and 11.6% DE prepared from CPE 24 h hydrolyzed citrus pectin. DPPH radical scavenging activities of pectic acid (1 mg/mL) from soybean byproduct okara, pectin (1 mg/mL) from *C. arietinum* L., and commercial pectin (1 mg/mL) with different DE (25, 65, and 94%) were 60, 29, and 10%, respectively.[11−13] The DPPH radical scavenging activity of citrus pectin (10 mg/mL) with DE 60% was 1% in the present study (Table 2). Nevertheless, Yang et al.[11] revealed that there was no significant relationship between DPPH radical scavenging activity and DE in pectin. We suggest that the differences in DPPH radical scavenging activity among various pectin solutions might be due to the variation in M_w and structure of pectin.

The antioxidant activities of BHT and α-tocopherol were much higher than thatof PET-pectin. The value of DPPH radical scavenging activity of 10 mg/mL PET-pectin (59.1%) was similar to the values of 0.1 mg/mL BHT (49.2%) and 0.01 mg/mL α-tocopherol (46.1%). The value of TEAC of 10 mg/mL PET-pectin (4.3 mM) was similar to the value of 0.1 mg/mL α-tocopherol (3.5 mM). The value of reducing power of 10 mg/mL PET-pectin was in the range of 0.01−0.1 mg/mL of BHT and 0.01−0.1 mg/mL of α-tocopherol. The concentrations required for 50% inhibition (EC_{50}) of DPPH radicals were 0.56 mg/mL for methanolic extract of rice bran, 0.10−0.22 mg/mL for methanolic extract of black bran,[24] 30.6−48.6 mg/mL for methanolic extract of potato,[25] and 9.17 mg/mL for PET-pectin (Table 2). EC_{50} values of BHT and α-tocopherol were 0.1 and 0.02 mg/mL, respectively. The reducing power was 0.04−0.11 for 10 mg/mL methanolic extract of potato,[25] 1.1 for 10 mg/mL water extract of broccoli flower,[26] and 1.1 for 10 mg/mL PET-pectin (Table 2). The extracts of rice bran and black bran expressed higher antioxidant activity; however, the higher content of total phenolic acids (2−3 mg/mL) and pigment (0−3 mg/mL anthocyanin) might limit their application.[24] Pectin has been widely used in the food and beverage industry. Our results suggested that PET-pectin produced from citrus pectin to possess antioxidant activity could be a valuable source of antioxidant from plant origin for use in food formulation.

Khasina et al.[27] suggested that pectin interacts directly with oxidants and free radicals. However, the mechanism of free radical scavenging of polysaccharides is still not fully understood.[23] Metal ion chelation of polysaccharides was also reported to inhibit lipid oxidation.[28] Chen et al.[4] revealed that LM pectin bound more iron than HM pectin; however, there is no significant difference in peroxyl radical scavenging activities between LM pectin and HM pectin. In the present study, the iron ion chelation ability of CPE-treated PET-pectin was lower than that of untreated pectin (data not shown). It is not metal ion chelating ability related that the level of antioxidant activity in PET-pectin was higher than the level in untreated pectin (Table 2).

In this study, according to the conclusion of Laokuldilok et al.,[24] the results of antioxidant activity reveal that PET-pectin acts as an electron donor and is capable of scavenging free radicals. The radical chain reaction was thus terminated.

Emulsification Properties of PET-Pectin. The emulsification properties of SPI-stabilized O/W emulsions were measured at pH 3 as a function of pectin and PET-pectin amounts. Results of variance analysis for the effects of pectin and PET-pectin on the EA and ES of the emulsions are shown in Table 3. EA and ES of SPI-stabilized O/W emulsions increased at the addition of pectin with or without PET-pectin except 2% pectin addition. ES decreased in the presence of 2% pectin in SPI-stabilized O/W emulsion. Adding higher amounts of PET-pectin did not enhance EA and ES. The higher degree of syneresis caused by centrifugation was due to a weak emulsion structure of the continuous phase.[29] Our results revealed that the addition of pectin with or without PET-pectin at 1−2% concentration could strengthen the structure of SPI-stabilized O/W emulsion.

Table 2. Antioxidant Activity, Degree of Esterification (DE), and Molecular Weight (M_w) of Pectin and Pectic Enzyme Treated Pectin (PET-Pectin)[a]

	content (mg/mL)	DE (%)	M_w (kDa)	DPPH radical scavenging activity (%)	TEAC (mM trolox)	reducing power
pectin	10.00	60.0	353	1.2 ± 0.0 f	0.2 ± 0.0 d	0.4 ± 0.0 d
PET-pectin	10.00	11.6	1	59.1 ± 0.4 b	4.3 ± 0.1 a	1.1 ± 0.1 c
BHT	0.01			5.2 ± 0.1 e	0.4 ± 0.0 d	0.5 ± 0.0 d
	0.10			49.2 ± 2.9 c	1.3 ± 0.2 c	1.7 ± 0.1 b
α-tocopherol	0.01			46.1 ± 0.4 d	0.5 ± 0.0 d	0.6 ± 0.0 d
	0.10			96.8 ± 0.5 a	3.5 ± 0.1 b	2.5 ± 0.0 a

[a] Pectin: no enzyme treatment. PET-pectin was prepared from citrus pectin (1%) and commercial microbial pectic enzyme and reacted at pH 4, and 45 °C for 24 h. The full terms of DPPH, TEAC, and BHT are α,α-diphenyl-β-picrylhydrazyl, trolox equivalent antioxidant capacity, and butylated hydroxytoluene, respectively. Data are expressed as the mean ± SD; those with the same letter in the same column are not significantly different ($p > 0.05$).

Table 3. Effects of Pectin and Pectic Enzyme Treated Pectin (PET-Pectin) on the Emulsion Properties of Soy Protein Isolate (SPI)-Stabilized Oil in Water (O/W) Emulsions[a]

SPI (%)	pectin (%)	PET-pectin (%)	emulsifying activity (%)	emulsion stability (%)
1	0	0	34.3 ± 1.3 f	39.7 ± 0.5 e
1	1	0	60.7 ± 0.8 d	50.1 ± 1.8 c
1	0	1	36.5 ± 1.7 e	49.3 ± 1.4 c
1	1	1	98.4 ± 0.8 a	78.2 ± 1.0 a
1	1	2	65.1 ± 1.9 c	62.0 ± 1.6 b
1	2	0	75.2 ± 3.6 b	35.8 ± 2.2 f
1	0	2	37.4 ± 0.9 e	46.1 ± 1.2 d

[a] SPI-stabilized O/W emulsions containing 20% (v/v) soybean oil, 1% (w/v) SPI, and 0−2% (w/v) pectin (or PET-pectin) in 10 mM citric acid buffer (pH 3) were prepared for the determination of emulsion properties. Data are expressed as the mean ± SD; those with the same letter in the same column are not significantly different ($p > 0.05$).

The EA of pectin increased according to pectin content, whereas the ES decreased in the same manner. PET-pectin showed higher antioxidant activity than pectin but expressed lower EA. We speculate that the small M_w PET-pectin might have an ability to embed into the structure of SPI−pectin emulsion and thus increase the stability of the emulsion. As expected, EA increased from 60.7% (1% SPI/1% pectin) to 98.4% (1% SPI/1% pectin/1% PET-pectin) and ES increased from 50.1% (1% SPI/1% pectin) to 78.2% (1% SPI/1% pectin/1% PET-pectin) when 1% PET-pectin was present in the emulsion formula. EA and ES did not further increase while more PET-pectin (1% SPI/1% pectin/2% PET-pectin) was added.

Cho and McClements[30] revealed that the emulsion consisting of a protein-coated lipid droplet surrounded by a pectin layer could form a stable emulsion at certain droplet and became unstable to bridging flocculation and depletion flocculation at higher pectin concentration. Hence, the highest EA and ES in 1% SPI/1% pectin/1% PET-pectin stabilized emulsion indicating that pectin and PET-pectin interacted with SPI and gave a better stable lipid droplet coating layer at the ratio of 1% SPI/1% pectin/1% PET-pectin and 20% (v/v) oil (Table 3). The reduction of ES at higher concentration of pectin or PET-pectin might be caused by the presence of nonadsorbed pectin or PET-pectin molecules in the aqueous phase as described by Cho and McClements.[30]

Depolymerized pectin was reported to exhibit good emulsifying ability and emulsion stability at 50−80 kDa. Depolymerized pectins with lower or higher M_w were not as effective.[10,14] The effect of PET-pectin (1 kDa) on the emulsifying activity of SPI-stabilized O/W emulsions was less than that of untreated pectin (353 kDa). Nevertheless, in considering the antioxidant activity of enzymatically hydrolyzed pectin, PET-pectin at 1 kDa was used in the formula of SPI-stabilized O/W emulsion as a stabilizer for the following study.

Effect of Pectin and PET-Pectin on the Oxidative Stability of SPI-Stabilized O/W Emulsion. To identify the effect of PET-pectin on the oxidative stability of SPI-stabilized O/W emulsion, the ability of PET-pectin to inhibit the lipid oxidation of emulsion was further evaluated by monitoring the increase of secondary oxidation products with TBARS (Figure 1). It was found that the addition of pectin or PET-pectin showed beneficial effects in inhibiting the increase of TBARS. Comparatively, PET-pectin was more effective in inhibiting lipid oxidation of the emulsion than pectin at the same concentration under accelerated storage. Pectin was not an effective antioxidant in the O/W emulsion. This result could be due to the antioxidant activity of PET-pectin being higher than that of pectin. In addition, the ability of lipid oxidation inhibition by PET-pectin in SPI-stabilized O/W emulsion was not concentration related.

The major difference between pectin (353 kDa and 60% DE) and PET-pectin (1 kDa and 12% DE) used in this research is the level of DE and M_w. PET-pectin exhibited more DPPH radical scavenging activity, TEAC, and reducing power than pectin. TEAC was found to be significantly associated with the DE and weakly associated with the M_w of PET-pectin (Table 1). No relationship between lipid oxidation inhibition ability of pectin on Brij 35-stabilized O/W emulsion and the peroxyl radical-scavenging activity of pectin was found.[4] The inhibition ability of pectin on lipid oxidation of Brij 35-stabilized O/W emulsion was likely due to the higher iron-binding capacity in LM pectin (37% DE) as compared with HM pectin (70% DE). PET-pectin exhibited higher radical scavenging activity (4.3 mM trolox) than pectin (0.2 mM trolox) in the present study, suggesting that PET-pectin acted as an electron donor to terminate the free radical chain reactions (Table 2). Hence, the higher inhibition

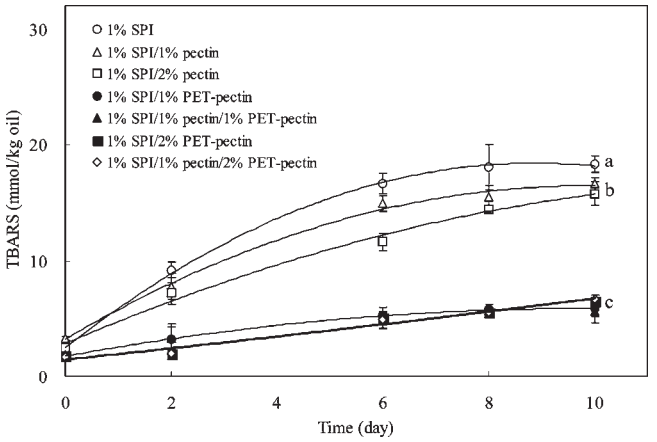

Figure 1. Effects of pectin and pectic enzyme treated pectin (PET-pectin) on the formation of thiobarbituric acid reactive substances (TBARS) in soy protein isolate (SPI)-stabilized oil in water (O/W) emulsions under accelerated storage. PET-pectin was prepared from citrus pectin (1%) and commercial microbial pectic enzyme and reacted at pH 4 and 45 °C for 24 h. SPI-stabilized O/W emulsions were prepared from 20% (v/v) soybean oil, 1% (w/v) SPI, and various concentrations of pectin (or PET-pectin) in 10 mM citric acid buffer (pH 3) and then stored at 50 °C for 10 days. Data are expressed as the mean ± SD; those with the same letter are not significantly different ($p > 0.05$).

ability of PET-pectin on lipid oxidation SPI-stabilized O/W emulsion might be due to the higher radical scavenging ability and lower DE value in PET-pectin.

The addition of PET-pectin was able to inhibit lipid oxidation at concentrations that did not cause the physical destabilization of the emulsion. However, further increasing PET-pectin concentrations to 2% did not inhibit the TBARS formation after 10 days of storage. This revealed that the concentration of PET-pectin did not influence the oxidative stability of SPI-stabilized O/W emulsion. It was also found in Brij 35-stabilized O/W emulsion that the antioxidant activity of pectin (both low-methoxyl pectin and high-methoxyl pectin) was lost at higher concentrations.[4] The lipid oxidation of Brij 35-stabilized O/W emulsion was accelerated by polysaccharides including alginate, carrageenan, and LM pectin at higher concentrations.

Comparatively, PET-pectin, pectin, and SPI formed a stable lipid droplet coating layer in the present study. No difference in the oxidative activity was observed in PET-pectin added group. Higher concentrations of PET-pectin might produce nonadsorbed PET-pectin molecules in the aqueous phase and thus reduce the ES of emulsion as described by Cho and McClements.[30] Liu et al.[31] revealed that the presence of the nonadsorbed HM pectin in sodium caseinate/HM pectin-stabilized emulsions resulted in spatial rearrangement of the oil droplets. Hence, the loss of antioxidant activity at higher concentrations of PET-pectin might be due to the spatial interaction of nonadsorbed PET-pectin molecule with the SPI-coated lipid droplet. Fortunately, the M_w of PET-pectin was too small to change the structure of the SPI-coated layer; hence, the lipid oxidation of SPI/PET-pectin-stabilized O/W emulsion was not accelerated at higher PET-pectin concentrations.

PET-pectin at 1 kDa and 11.6% DE expressed more antioxidant activity (DPPH radical scavenging activity, TEAC, and reducing power) than untreated citrus pectin (353 kDa and 60% DE). In addition, TEAC was found to be positively associated with M_w of PET-pectin and negatively associated with DE of PET-pectin. The addition of PET-pectin could increase the EA, ES, and oxidative stability of SPI-stabilized O/W emulsion. When the SPI-stabilized lipid droplet was coated with the mixture of PET-pectin and pectin, EA and ES of the emulsion were improved more than than they were when the droplet was coated by either pectin or PET-pectin alone. The amount of TBARS produced in the emulsion prepared with the mixture of SPI and PET-pectin was less than the amount of TBARS produced in the emulsion prepared with either SPI or SPI/pectin. In other words, the lipid oxidation of SPI-stabilized O/W emulsion was inhibited by adding PET-pectin to the surface of SPI-stabilized O/W emulsion droplets and thus decreased interactions between aqueous phase prooxidants and lipids.

These results suggest that PET-pectin has an emulsion-stabilizing effect and a lipid oxidation inhibiting ability on SPI-stabilized emulsion. SPI and its individual proteins have been widely used as emulsifiers or emulsion stabilizers in several food products, ranging from meat emulsions and baby foods to liquid diet formulations.[32,33] Therefore, PET-pectin can be used as both a stabilizer and an antioxidant of plant origin in SPI-stabilized O/W emulsion and thus prolong the shelf life of food emulsion.

AUTHOR INFORMATION

Corresponding Author

*(Y.-T.W.) Phone: +886-2-26015310, ext. 2481. Fax: +886-2-26010349. E-mail: yuhtai@yahoo.com. (M.-C.W.) Phone: +886-8-774-0240. Fax: +886-8-774-0378. E-mail: mcwu@mail.npust.edu.tw.

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