Antioxidant capacity of decarboxylated rice bran extract in bulk oil

Jung-Ah Shin¹ · Sang-Hwa Jeong¹ · Cai-Hua Jia¹ · Ki-Teak Lee¹

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Abstract Decarboxylation of ferulic acid would increase the solubility in oils. Rice bran extract (RBE) containing 29 mg ferulic acid/g RBE was decarboxylated to obtain decarboxylated rice bran extract (DRBE), and its antioxidant capacity in oil system was studied. After addition of DRBE (500 ppm), oxidation was monitored for 20 days at 60 °C under the dark. To compare the oxidation degree, 500 ppm of ferulic acid and well-known lipid soluble antioxidant, α -tocopherol, were used. Contents of conjugated dienes and aldehydes were measured using ¹H NMR as well as peroxide value (POV). On 7 days of oxidation, DRBE (539.0 meq/kg oil) showed lower POV than the control (819.7 meq/kg oil). Also, contents of total conjugated form and aldehydes were 194.60, and 5.94 mmol/L oil, which were lower than those of control (323.63 and 15.94 mmol/L oil). However, after 10 days of oxidation, antioxidant capacity of DRBE was not observed.

Keywords Antioxidant capacity · Ferulic acid · Rice bran extract · Decarboxylation · Bulk oil

Introduction

In oils, the degree of oxidation of unsaturated fatty acids is affected by external factors (such as oxygen, light, heat, and metal content). Because oxidation products cause the

qualities of bulk oils to deteriorate and can adversely affect health [1, 2], researches on the use of antioxidants to delay oxidation processes is needed.

In rice bran, ferulic acid is found in large quantities and has been reported to have beneficial effects, such as restricting heart diseases and reducing cholesterol levels, as well as having an antioxidant capacity that inhibits lipid oxidation [3, 4]. Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is one of hydroxycinnamic acid (i.e. *p*-coumaric, caffeic, ferulic, and sinapic acid etc.) having carboxyl, hydroxyl, and methoxyl group in its phenol structure. According to previous study [5], the radical scavenging activity in a bulk methyl linoleate system had an antioxidant capacity in the order caffeic acid > sinapic acid > ferulic acid > ferulic acid esters > *p*-coumaric acid, suggesting that different structural conformation of hydroxycinnamic acid affects the antioxidant capacity.

When hydroxycinnamic acid is decarboxylated, lipid-solubility would increase, changing its antioxidant capacity in oils because the presences of carboxyl along with hydroxyl group reduce the solubility in oils. Previous studies showed that decarboxylated sinapic acid had promising antioxidant capacity in bulk oil [6].

Rice bran is relatively inexpensive and has been obtained from a by-product of the rice milling process. The main phenolic compound of rice bran is known as a ferulic acid. Therefore, we used the rice bran powder as a source of ferulic acid. The rice bran extracts were used to obtain the crude 4-vinylguaiacol (4-VG) extract by decarboxylation that could be used as an antioxidant for lipid oxidation.

In general, primary (e.g. conjugated diene and hydroperoxides) and secondary (e.g. aldehydes) oxidation products are main parameters to determine the rate of oxidation in lipids. ¹H NMR spectroscopy has been widely used to determine simultaneously both primary (e.g.

Jung-Ah Shin and Sang-Hwa Jeong are co-first authors who contribute equally to this study.

✉ Ki-Teak Lee
klee@cnu.ac.kr

¹ Department of Food Science and Technology, Chungnam National University, Daejeon 34134, Republic of Korea

conjugated diene forms) and secondary (e.g. aldehydes) oxidation products in oils. This technique has proved to be very useful by several researchers through many published papers related to lipid oxidation [6–8]. This analysis is very simple and easy in preparing the sample and quickly obtains a lot of information within a few minutes.

In this study, 500 ppm of decarboxylated rice bran extract (DRBE) was added into the stripped soybean oil (SSBO) that was prepared to reduce the tocopherol content. In terms of antioxidant capacity, contents of conjugated dienes and aldehydes were measured using ^1H NMR along with peroxide value (POV) for 20 days at 60 °C under the dark. To compare the oxidation degree, each 500 ppm of ferulic acid and well-known lipid soluble antioxidant, α -tocopherol, was added in SSBO, respectively.

Materials and methods

Materials

Rice bran powder and soybean oil were purchased from a local market (Daejeon, Korea). Activated charcoal and silica gels used for the preparation of stripped soybean oil were obtained from Daejung Co. (Incheon, Korea) and Merck Co. (Darmstadt, Germany), respectively. Ferulic acid, α -tocopherol, and chloroform- D (CDCl_3 , 99.8 atom%D) were purchased from Sigma-Aldrich (St. Louis, MO, USA). N,N -Dimethylformamide (DMF) was from Tokyo Chemical Industry Co. (Tokyo, Japan). Organic solvents used for high-

pressure liquid chromatography (HPLC) and ^1H NMR were all of HPLC grade purity.

Preparation of stripped soybean oil (SSBO)

Tocopherols were removed from soybean oil as followings: soybean oil (20 g) and n -hexane (10 mL) were mixed and passed through a column (3.5 cm diameter, 20 cm length) packed sequentially from the bottom with 1 g of anhydrous sodium sulfate, 6 g of activated charcoal and another 20 g of silica gel [6]. The tocopherol contents (ppm) were determined using a normal-phase HPLC system (Agilent Technologies, Little Falls, DE, USA) as described previously [9]. Quantification was carried out using an external standard curve. After stripping the oil, tocopherol content in stripped soybean oil (SSBO) was around 38 ppm.

Preparation of decarboxylated rice bran extract (DRBE)

Rice bran extraction proceeded as shown in Fig. 1, to obtain rice bran extract (RBE) containing a large proportion of ferulic acid. Defatted rice bran powder was prepared by extraction with n -hexane at 55 °C of a shaking water bath (Fig. 1). Defatted rice bran (15 g) was placed into an Erlenmeyer flask (250 mL) and extracted twice with 80% ethanol (EtOH, 200 mL) solution for 24 h in a shaking water bath set at 55 °C and 180 rpm. The extract was filtered through Whatman No. 1 filter paper to separate the rice bran solid, and the solvents in the rice bran extract were

Fig. 1 Scheme for obtaining decarboxylated rice bran extract (DRBE)

completely removed using a vacuum evaporator and N₂ gas. In order to removal of large molecules (i.e. sugars and their derivatives) and to increase the purity of the extracts, HP-20 resin column was prepared as followings: fifty gram of HP-20 resin dispersed in 150 mL ethanol was packed into a glass column (3.5 cm diameter, 20 cm length). For adsorption chromatography, the extract was dissolved in about 4 mL of 80% EtOH, and it was loaded on the prepared HP-20 resin column. The column was sequentially eluted with water (100 mL), 30% EtOH (100 mL), and 50% EtOH (100 mL) after which three fractions were collected.

The collected fractions (300 mL) were hydrolyzed to yield free ferulic acid as following process. The fractions placed in an Erlenmeyer flask and stirred at 500 rpm with 4 M NaOH solution (100 mL) to initiate a reaction by blowing over nitrogen gas for 2 h at room temperature. Next, the pH of the hydrolyzed product was adjusted to 7 using 1 M HCl solution. The hydrolyzed product was extracted three times with a solvent mixture of diethyl ether/ethyl acetate = 1:1 (v/v, 250 mL). Solvent in the combined extracts was completely removed using a vacuum evaporator and nitrogen gas. The ferulic acid content of RBE was quantified using an external standard curve by analysis of reversed-phase HPLC [10] with slight modifications, showing 29 mg/g RBE of ferulic acid. Decarboxylation of ferulic acid in RBE was performed with some modifications [11]. RBE (85 mg) was weighted in a screw-capped test tube. The DMF (2 mL) and a sodium acetate (20 mg as a catalyst) were added into the test tube. After sealing the test tube completely by Teflon type, the mixture was reacted at 130 °C for 1 h. After the reaction, saturated NaCl solution (20 mL) was added and the decarboxylated ferulic acid was extracted with diethyl ether (2 mL × 5 times). The solvents were removed using a vacuum rotary evaporator. Finally, the decarboxylated rice bran extract (DRBE) composed of mainly 4-VG was obtained.

Lipid oxidation study

Evaluation of the DRBE, ferulic acid, and α -tocopherol on lipid oxidation was performed using the stripped soybean oil placed in a petri dish (3.5 cm diameter, 1 cm height). Their activity on lipid oxidation was compared to the control (SSBO) with no antioxidant. Each SSBO sample was added with antioxidant at the level of 500 ppm (6 mg/12 g SSBO), then they were homogenized completely using an ultrasonic homogenizer. Lipid oxidation of control (SSBO) and three antioxidants was carried out during a storage of 20 days under a dark in an air-ventilated oven set at 60 °C.

Peroxide value (POV)

The peroxide value of oxidized oils was measured according to the IDF method [12]. UV/Vis

spectrophotometer (UV 1700, Shimadzu, Kyoto, Japan) was used and the absorbance was set at 500 nm. The results were expressed as meq/kg oil.

¹H-NMR analysis

The conjugated diene form and aldehyde contents in oxidized SSBO were measured by ¹H-NMR (BRUKER AVANCE III 600, Bruker, Germany) [8] with little modification. Oxidized oil (around 50 mg) was dissolved in 700 μ L of chloroform-D with 0.1% tetramethylsilane (TMS) as an internal reference. NMR analysis was performed at a spectral width of 12,335.5 Hz, scanning for 16 rounds with an acquisition time of 2.656 s. All chemical shifts (δ) were set relative to TMS at δ = 0 ppm. The unit for contents of conjugated diene forms (Z,E-, and E,E-forms, -CH=CH-CH=CH-) and aldehydes (-CHO) measured by ¹H-NMR was expressed as mmol/L oil [6, 8]. First, the unit was calculated as mol/mol oil by relative molar proportions based on normalization of the signal area to β hydrogen atom (reference = 1) in a triacylglycerol molecule. Then, the amounts were converted into mmol/L oil by considering the molecular weight of oil used.

Statistical analysis

Significant differences among experimental treatments were evaluated by using Duncan's multiple range test in SAS statistical software, version 9.2 (SAS institute, Inc., Cary, NC, USA). Statistical significance was calculated at 5% significance level ($p < 0.05$).

Results and discussion

POV of stripped soybean oil (SSBO) containing antioxidant

Generally, ferulic acid is present in its free form in nature, but is mostly bound to a sugar or sterol as γ -oryzanol [13]. Therefore, free form of ferulic acid was present in the RBE, while decarboxylated ferulic acid would be found in DRBE after decarboxylation (Fig. 1).

SSBO in which each of 500 ppm of DRBE, ferulic acid, and α -tocopherol were added as antioxidant was oxidized in the dark exposed to air at 60 °C for 20 days to measure hydroperoxide value (POV) (Fig. 2). When oil is oxidized, hydroperoxide (the primary oxidation product) is produced quickly and then decomposes into a secondary oxidation product. Therefore, POV that measures hydroperoxide at the primary stage of oxidation initially increases followed by a decrease [14]. In Fig. 2, on 3 days of oxidation, the POVs of SSBO with α -tocopherol, ferulic acid, and control were 42.9,

Fig. 2 Peroxide value of stripped soybean oil (control) and other treatments [500 ppm of ferulic acid, decarboxylated rice bran extract (DRBE) and α -tocopherol]. The different letters (*a–d*) within same storage period are significantly different at $p < 0.05$

112.1, and 92.3 meq/kg oil, respectively. Such values were significantly higher than that of DRBE (26.3 meq/kg oil) ($p < 0.05$). Furthermore, on 5 days of oxidation, the POV of the SSBO containing DRBE (136.3 meq/kg oil) was lower than those of ferulic acid (195.3 meq/kg oil) and control (372.0 meq/kg oil). However, DRBE showed significantly higher POV than that of α -tocopherol (74.9 meq/kg oil) ($p < 0.05$). After 5 days, the oxidation of the SSBO containing DRBE was faster than those with ferulic acid and α -tocopherol, yet the POV of DRBE was lower than the control before 10 days of oxidation. When POVs were measured on 10 days of oxidation, those of DRBE and the control were 1102.5 and 1130.2 meq/kg oil ($p > 0.05$), respectively. Meanwhile, the maxima POVs of SSBO containing ferulic acid, and α -tocopherol reached after 15 days of oxidation as 1026.3, and 1254.6 meq/kg oil, respectively, and then decreased afterwards.

Therefore, comparison of POV during the 20 days of oxidation concluded that addition of 500 ppm DRBE retarded the oil oxidation before 10 days, thereafter antioxidant capacity was not observed in our experiment condition.

Conjugated forms and aldehydes of stripped soybean oil (SSBO) containing antioxidant

Generally, oxidation is triggered by removing hydrogen located between the double bonds of fatty acids containing more than two double bonds [14]. During this process, the

location of the double bond is changed, resulting in the *Z,E*-conjugated (*cis-trans*) and *E,E*-conjugated (*trans-trans*) forms. If oxidation progresses further, various aldehydes such as *n*-alkanals, 4-hydroperoxy-(*E*)-2-alkanals, 4-hydroxy-(*E*)-2-alkanals, 4,5-epoxy-(*E*)-2-alkanals, (*E,E*)-2,4-alkadienals and (*E*)-2-alkanals are produced as secondary oxidation products. The contents of conjugated forms (*Z,E*- and *E,E*-conjugated form) and aldehydes produced during oxidation were deduced using $^1\text{H-NMR}$ [6–8, 15].

After 7 days of oxidation, the total conjugated forms in the control was 323.63 mmol/L oil, followed by a decrease to 125.10 and 90.19 mmol/L oil on 15 and 20 days of oxidation, respectively. Furthermore, as the oxidation was prolonged, the ratio of the *E,E*-conjugated form in the total conjugated forms increased from around 42% (on day 7) to around 60% (on day 20) (Table 1). When α -tocopherol was added, the total conjugated forms on day 7 (41.76 mmol/L oil) were lower than in other samples. In particular, the addition of DRBE produced a higher total content of conjugated products on 7 days of oxidation than ferulic acid. Nevertheless, DRBE resulted in less total content of conjugated products (194.60 mmol/L oil) than the control (323.63 mmol/L oil) on 7th day.

Aldehydes content also gradually increased during oxidation for 20 days in all the samples (Table 1 and Fig. 3). After 7th days, aldehydes were not detected in the SSBO containing ferulic acid and α -tocopherol. However, aldehyde contents in the SSBO containing DRBE and the

Table 1 Amount of *Z,E*-, *E,E*- and total conjugated forms, and aldehydes in stripped soybean oils (SSBO) with 500 ppm of ferulic acid, decarboxylated rice bran extract (DRBE), and α -tocopherol during oxidation process of 20 days at 60 °C in the dark

	Day	<i>Z,E</i> -Conjugated forms (mmol/L oil)	<i>E,E</i> -Conjugated forms (mmol/L oil)	Total conjugated forms (mmol/L oil)	Aldehydes ^a (mmol/L oil)
Ferulic acid	0	ND	ND	ND	ND
	7	46.22	64.06	110.28	ND
	15	94.36	138.42	232.78	30.78
	20	46.67	87.28	133.96	67.33
DRBE	0	ND	ND	ND	ND
	7	101.86	92.74	194.60	5.94
	15	45.18	91.98	137.16	77.39
	20	27.06	43.80	70.86	73.96
α -Tocopherol	0	ND	ND	ND	ND
	7	27.98	13.77	41.76	ND
	15	100.66	139.93	240.58	35.76
	20	48.92	99.35	148.27	74.50
Control	0	ND	ND	ND	ND
	7	190.68	132.95	323.63	15.94
	15	50.46	74.64	125.10	79.27
	20	36.00	54.19	90.19	85.68

ND not detected; DRBE decarboxylated rice bran extract

^aAldehydes are *n*-alkanals, 4-4-hydroperoxy-(*E*)-2-alkenals, 4-hydroxy-(*E*)-2-alkenals, 4,5-epoxy-(*E*)-2-alkenals, (*E,E*)-2,4-alkadienals and (*E*)-2-alkenals **Fig. 3** ¹H-NMR spectra of aldehydes (9.50–9.75 ppm) and conjugated forms (5.45–6.55 ppm) in stripped soybean oil (control, 0 and 20 day storage) and the treated oils (20-day storage) with α -tocopherol, ferulic acid, and decarboxylated rice bran extract (DRBE), respectively

control were 5.94 and 15.94 mmol/L oil, respectively. On the 15 days of oxidation, aldehydes in the samples containing DRBE abruptly increased to 77.39 mmol/L oil. However, for ferulic acid and α -tocopherol, these values were lower, showing 30.78 and 35.76 mmol/L oil, respectively.

Under the 20 days of oxidation in this experimental condition, 500 ppm addition of DRBE significantly retarded lipid oxidation in the early period (0–7 days) when compared to the control, yet the antioxidant capacity was less than the same amount of ferulic acid and α -tocopherol.

In conclusion, ferulic acid is found in large quantities in rice bran, and has been known as a natural antioxidant. To increase the lipid-solubility of ferulic acid, decarboxylated rice bran extract (DRBE) was prepared and its antioxidant capacity was studied in the bulk oil system. When compared to the control (without antioxidant), addition of 500 ppm DRBE significantly reduced POV, and content of conjugated diene and aldehydes in the early period of 20 days of oxidation. However, when the same amount (500 ppm) in oil was compared, DRBE was believed to have a lower antioxidant capacity than ferulic acid.

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