

## Antioxidant Activity of Phenolic Compounds in Bulk Camellia Oil and Corresponding Oil in Water (O/W) Emulsions

<sup>1</sup>Yong Zhu, <sup>1,2</sup>Qi-zhi Long, <sup>1,2</sup>Bo Zhou, <sup>3</sup>Paul D. Prenzler and <sup>1,2</sup>Hai-yan Zhong

<sup>1</sup>Faculty of Food Science and Engineering, Central South University of Forestry and Technology,

<sup>2</sup>Hunan key Laboratory of Deeply Processing and Quality Control of Cereals and Oils, Changsha 410004, Hunan, P.R. China

<sup>3</sup>School of Agricultural and Wine Sciences, EH Graham Centre for Agricultural Innovation, Charles Sturt University, Locked Bag 588, Wagga Wagga 2678, Australia

**Abstract:** The antioxidant activities of gallic acid, propyl gallate, (+)-catechin and quercetin in bulk camellia oil and corresponding emulsions were investigated using hydroperoxides and nonanal as indicators of oxidation. In bulk oil, these phenolic compounds showed antioxidant activities with the order of gallic acid>quercetin>propyl gallate> (+)-catechin when measured using hydroperoxides and gallic acid>quercetin>(+) catechin>propyl gallate using nonanal. In emulsions, propyl gallate and (+)-catechin showed antioxidant activities for the entire duration of the experiment, while quercetin and gallic acid displayed antioxidant activities for 10 days, and then pro-oxidant activities thereafter. Results suggested that in bulk oil, the antioxidant activity was regulated by phenol polarity and hydrogen atom donating ability too. In emulsions, the antioxidant activity of phenolic compounds seems to be related to their affinity toward the emulsifying agent rather than their polarity.

**Keywords:** Antioxidant activity, camellia oleifera, emulsion, interface, phenolics, polarity

### INTRODUCTION

Camellia oil (*Camellia oleifera*) is one of the four major tree-bearing oils (palm, olive and coconut oils) with health benefits, since it has abundant antioxidants, such as tocopherols, polyphenols etc. Data related to camellia oil polyphenol content have only become available in the last decade. Liu and Zhao (2002) used TLC and phenolic-specific spray reagents to detect phenols in *Camellia sinensis* seed oil. They suggested that polar antioxidant compounds played a major role in the stability of the oil. Zhong *et al.* (2006) measured total phenols and reported the phenolic profile of a water/methanol extract of cold-pressed camellia oil. The addition of exogenous phenols, caffeic acid and tyrosol, resulted in a decrease in peroxides and secondary oxidation products in bulk camellia oil during heating (Zhong *et al.*, 2007).

On the other hand, the performance of phenolic antioxidants in oil-in-water emulsions is of interest from numerous viewpoints. In human nutrition, it has been recently proposed that tea polyphenols can modify the emulsification of dietary lipids and this may change their digestion and absorption (Shishikura *et al.*, 2006) In food and health studies, there has been much interest

in antioxidant activity in emulsions due to the fact that lipids are more susceptible to oxidation in an emulsion due to the high surface/volume nature of the emulsion facilitating the interaction of pro-oxidants in the aqueous phase with high surface area dispersed lipids (Shahidi and Zhong, 2011). To date there have been no studies on the oxidation of camellia oil emulsions.

Individual polyphenols play different roles in bulk oil and oil-in-water emulsion due to different molecular structures. For several decades, the "Polar Paradox" theory has been the dominant theory to explain the different behaviors of antioxidants in heterogeneous phase (bulk oil and emulsions) (Porter *et al.*, 1989; Porter, 1993). It has been suggested that the activity of an antioxidant depends on its partition affinity between the oil-water and oil-air interfaces, where oxidation occurs. In bulk oil, hydrophilic antioxidants distributed at the oil-air interface can better protect lipid oxidation than lipophilic antioxidants dissolved in the lipid phase. In emulsions, lipophilic antioxidants, distributed at the oil-water interface, better protect lipid oxidation than hydrophilic antioxidants dissolved in the aqueous phase. Additionally, hydrogen bonding between hydrophilic antioxidants and water may reduce the ability of the phenolic compound to donate hydrogen

**Corresponding Author:** Hai-yan Zhong, Faculty of Food Science and Engineering, Central South University of Forestry and Technology Changsha 410004, Hunan, P.R. China, Tel.: +86-731-8562-3073; Fax: +86-731-8562-3240

atoms to inhibit oxidation (Frankel *et al.*, 1994, 1996; Schwarz *et al.*, 1996; Huang and Frankel, 1997; Pekkarinen *et al.*, 1999; Zhao and Hall.III, 2007; Mattia *et al.*, 2009).

Despite the popularity of the polar paradox theory, there is recognition that it may not account for all situations of antioxidant, lipid, emulsifier, etc. In fact the interfacial particle size of droplet (surface/volume ratio) and type of emulsifier (cationic, anionic, and neutral) may affect the oxidative stability of emulsions. These additional factors are now being considered in response to studies showing nonlinear relationships between polarity and antioxidant activities in emulsions (Laguerre *et al.*, 2010; Shahidi and Zhong, 2011) and some phenolic compounds acting as pro-oxidants in emulsions (Huang and Frankel, 1997; Mattia *et al.*, 2009). More comprehensive factors (i.e., emulsifier, concentration of antioxidants, lipid character, surface/volume ratio) may be responsible for antioxidant activity, apart from polarity of the antioxidant.

In the present study, the antioxidant activity of phenolic compounds in bulk camellia oil and corresponding emulsions was investigated and some mechanistic interpretations will be discussed. Antioxidant activities of individual phenolic compounds were evaluated by determining hydroperoxides (Huang and Frankel, 1997) and nonanal (Hall *et al.*, 2005) which represented the primary and secondary oxidative products, respectively.

## MATERIALS AND METHODS

**Materials:** Nonanal, dodecane, gallic acid, propyl gallate, (+)-catechin, quercetin were purchased from Sigma-Aldrich® (St Louis, MO, USA). Commercially refined camellia oil was obtained from Xuefengshan Camellia Oil Co. (Hunan, China).

**Preparation of bulk oil and oxidation:** 0.8 mL of 2 mM gallic acid, propyl gallate, (+)-catechin and quercetin methanol solution was added into 50 mL vial, respectively, and purged under nitrogen prior to addition of 20 mL of oil. Sample without phenolic compound addition was used as control. All samples were placed randomly in an incubator shaker (ZHWHY-2102C, Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd, China) in the dark at 60°C (Zhao and Hall.III, 2007). Oxidated oils were collected after 0, 2, 4, 6, 8 days for further determination.

**Preparation of emulsions and oxidation:** The emulsions (40%, w/w) were prepared using the modified method described by Frankel *et al.* (1996). Forty percent of camellia oil, 59% of deionized water and 1% of 20 were transferred into an Erlenmeyer flask.

Emulsification was carried out using a sonicator (Model W-10, Inc., and New York). 2.4 µmol of gallic acid, propyl gallate, (+)-catechin and quercetin was added into 40 mL emulsion, respectively. All samples were randomly placed in an incubator shaker in the dark at 60°C (Zhao and Hall.III, 2007). The particle sizes of emulsions were determined by microscope (Olympus BX 51, Japan). Oxidated emulsions were collected after 0, 2, 4, 6, 8, 10, 12, 14 days for further determination.

**Determination of oxidised products:** The content of hydroperoxide was determined by the method from Crowe and White (2001). The nonanal was measured by headspace solid phase microextraction-gas chromatography (SPME-GC). Oil (0.19 g), emulsions (0.19 g) was added to vial (15 mL, supelco) which was contained dodecane (IS), respectively, and sealed with Teflon lined septum, the sealed vials were placed in incubator (IKA, RCT basic, Germany) at 95°C, The SPME needle (DVB/CAR/PDMS, 50/30 µm, 1 cm, Supelco) was inserted into the headspace of vial and left exposed for 10 min, removed and immediately desorbed at the GC (Shimadzu-2014) injector at 250°C with splitless mode. Separation was achieved on a SP 2340 column (60 m×0.25 mm×0.2 µm film thickness, Supelco) using nitrogen as carrier gas with flame ionization detection (FID, 250°C). The oven program started at 40°C for 2 min, then at the rate of 3.00 °C/min to 160°C for 2 min, increasing at 12°C/min to 220°C with final isothermal period of 5 min.

**Statistical analysis:** Data analysis was done using IBM SPSS statistics 19.0. All analyses were performed in triplicate.

## RESULTS AND DISCUSSION

**Antioxidant effect of phenolic compounds on bulk camellia oil:** On the basis of hydroperoxides formation, gallic acid, propyl gallate, (+)-catechin and quercetin showed antioxidant activities compared with control ( $p < 0.05$ ), respectively. While, individual phenolic compound represented different antioxidant efficiency, the antioxidant activities were ranked as the following order: gallic acid > quercetin > propyl gallate > (+)-catechin > control (Table 1). Among them, gallic acid was the best antioxidant compared with others ( $p < 0.05$ ). On the basis of nonanal formation, the rank of antioxidant activities was similar to hydroperoxides formation except that (+)-catechin played a better role than propyl gallate ( $p > 0.05$ ) after the 4<sup>th</sup> day. Therefore, the sequence of antioxidant activities using nonanal indicator was: gallic acid > quercetin > (+)-catechin > propyl gallate > control (Table 2).

Gallic acid, propyl gallate, (+)-catechin and quercetin (Fig. 1) involve in different affinity between lipids and aqueous phase. Gallic acid has a great affinity with aqueous phase due to the abundant of

Table 1: Effect of individual phenolic compounds on oxidative stability of bulk camellia oil by monitoring hydroperoxides formation (mmol/kg oil) at 60°C

Phenolic compounds	Oxidation time (day)				
	0	2	4	6	8
Gallic acid	10.44±0.32	15.24±0.32d	34.11±1.33d	75.79±0.38e	127.61±1.11d
Propyl gallate	10.44±0.32	16.40±0.38c	44.74±0.92c	85.53±1.03c	138.91±1.73c
(+)-catechin	10.44±0.32	17.38±0.64b	47.03±0.53b	90.36±1.27b	142.01±1.75b
Quercetin	10.44±0.32	17.87±0.20b	45.77±1.60bc	81.48±1.14d	129.80±1.06d
Control	10.44±0.32	37.07±0.73a	76.33±1.22a	115.27±1.04a	150.37±0.64a

The values are represented by means±SD, n = 3. Values within a column by no letter or the same letter are not significantly different (p>0.05)

Table 2: Effect of individual phenolic compounds on oxidative stability of bulk camellia oil by monitoring nonanal formation (µmol/kg oil) at 60°C

Phenolic compounds	Oxidation time (day)				
	0	2	4	6	8
Gallic acid	10.63±0.96	12.77±0.67e	40.64±1.43c	75.970±4.320c	178.41±22.29c
Propyl gallate	10.63±0.96	15.86±0.50d	69.75±2.14b	133.76±12.95b	285.39±9.84b
(+)-catechin	10.63±0.96	24.78±1.21b	67.53±1.34b	95.030±6.620c	267.65±13.65b
Quercetin	10.63±0.96	19.82±1.17c	60.82±5.38b	77.410±5.920c	245.36±21.31b
Control	10.63±0.96	40.88±1.35a	100.18±15.30a	161.16±20.17a	474.32±33.03a

The values are represented by means±SD deviation, n = 3. Values within a column by no letter or the same letter are not significantly different (p>0.05).

Table 3: Effect of individual phenolic compounds on oxidative stability of emulsions (o/w) by monitoring hydroperoxides formation (mmol/kg oil) at 60°C

Phenolic compounds	Oxidation time (day)							
	0	2	4	6	8	10	12	14
Gallic acid	3.85±0.02	2.81±0.02d	4.15±0.14c	6.30±0.07b	9.15±0.10a	13.37±1.03a	13.38±0.89b	41.75±1.760b
Propyl gallate	3.85±0.02	3.78±0.11b	3.49±0.22d	5.26±0.05d	6.48±0.21d	6.53±0.28c	6.79 ± 0.31e	7.960±0.660d
(+)-catechin	3.85±0.02	2.14±0.01e	5.06±0.11b	5.58±0.30c	8.39±0.20b	8.29±0.29b	8.40 ± 0.21d	8.580±0.090d
quercetin	3.85±0.02	3.44±0.11c	4.04±0.06c	5.35±0.08cd	7.49±0.13c	9.27±0.18b	14.26 ± 0.44a	46.810±1.55a
Control	3.85±0.02	5.61±0.20a	7.14±0.15a	7.28±0.17a	9.45±0.28a	9.24±0.30b	12.02 ± 0.17c	17.050±0.17c

The values are represented by means±SD, n = 3. Values within a column by no letter or the same letter are not significantly different (p>0.05)

Table 4: Effect of individual phenolic compounds on oxidative stability of emulsions (o/w) by monitoring nonanal formation (µmol/kg oil) at 60°C

Phenolic compounds	Oxidation time (day)								
	0	2	4	6	8	10	12	14	
Gallic acid	4.73±0.17	2.63±0.14a	2.09±0.06e	3.77±0.24b	5.96±0.16b	6.46±0.10b	19.21±1.36b	100.28±7.13b	
Propyl gallate	4.73±0.17	2.60±0.18a	2.55±0.05c	1.63±0.09d	3.92±0.03c	4.59±0.25c	5.72±0.42c	7.25 ±1.03c	
(+)-catechin	4.73±0.17	1.68±0.11b	2.76±0.08b	3.22±0.15c	3.84±0.16c	6.21±0.05b	8.28±0.69c	11.53±0.60c	
Quercetin	4.73±0.17	0.93±0.04c	2.31±0.10d	3.65±0.04b	7.56±0.64a	8.78±0.94a	36.90±5.43a	425.08±45.23a	
Control	4.73±0.17	2.66±0.04a	3.49±0.05a	4.40±0.19a	7.45±0.46a	7.76±0.99a	17.80±1.72b	28.49±4.09c	

The values are represented by means±SD, n = 3. Values within a column by no letter or the same letter are not significantly different (p>0.05)

hydroxyl group which endow the molecular structure a high polarity (Mattia *et al.*, 2009). Propyl gallate consists of propoxy and gallic acyl groups which has affinity with lipids and aqueous phase, respectively, hence, it can play some interfacial properties. (+)-Catechin has been considered an amphiphilic compound due to its two benzenic rings and a central eterocyclic oxygenated ring structure (Mattia *et al.*, 2009). Quercetin belongs to a lipophilicity compound and is the less polar among these phenolic compounds (Burda and Oleszek, 2001) which characterized by its pyrone ring structure. As described above, the polarity of these phenolic compounds were as the following order: gallic acid>catechin, propyl gallate>quercetin (Mattia *et al.*, 2009).

According to the “polar paradox”, hydrophilic antioxidants were more effective than lipophilic antioxidants in bulk oil. The sequence of antioxidant activity of this research for individual phenolic compounds (see above) was not following the “polar

paradox” absolutely. It was supposed that polarity is not the only factor to determine efficiency of antioxidants. Mattia *et al.* (2009) reported that the ability (amount) of hydrogen atoms donating of these antioxidants following the order of quercetin>catechin>gallic acid, propyl gallate. Additionally, gallic acid was faster than quercetin for donating hydrogen atoms. Regarding of the inhibition of nonanal formation, (+)-catechin was a better antioxidant than propyl gallate, it was suggested that catechol structure was more effective in prohibition of hydroperoxides decomposition than that of pyrogallol structure.

**Antioxidant effect of phenolic compound on oil in water emulsions:**

In contrast to bulk oil, the antioxidant activity of phenolic compound in emulsion was significantly different. The antioxidant activity was ranked as the following order: propyl gallate> (+)-catechin>control>gallic acid> quercetin (Table 3 and 4). Interestingly, the emulsion oxidation was accelerated

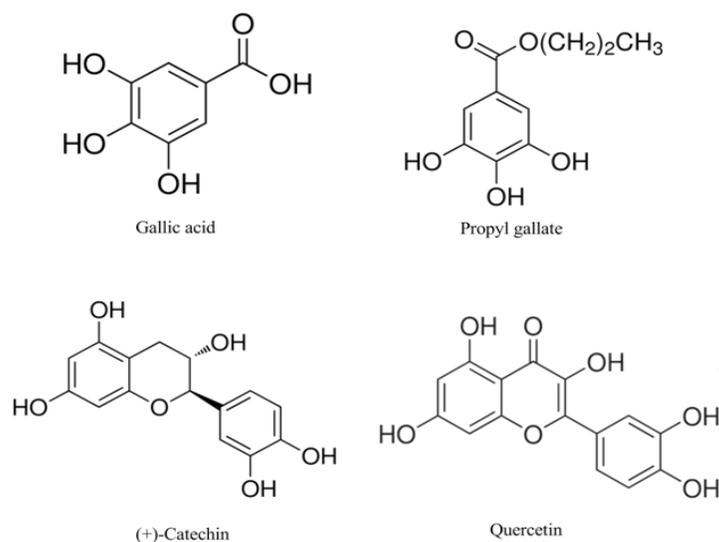


Fig.1: Molecular structure of gallic acid, propyl gallate, (+)-catechin and quercetin

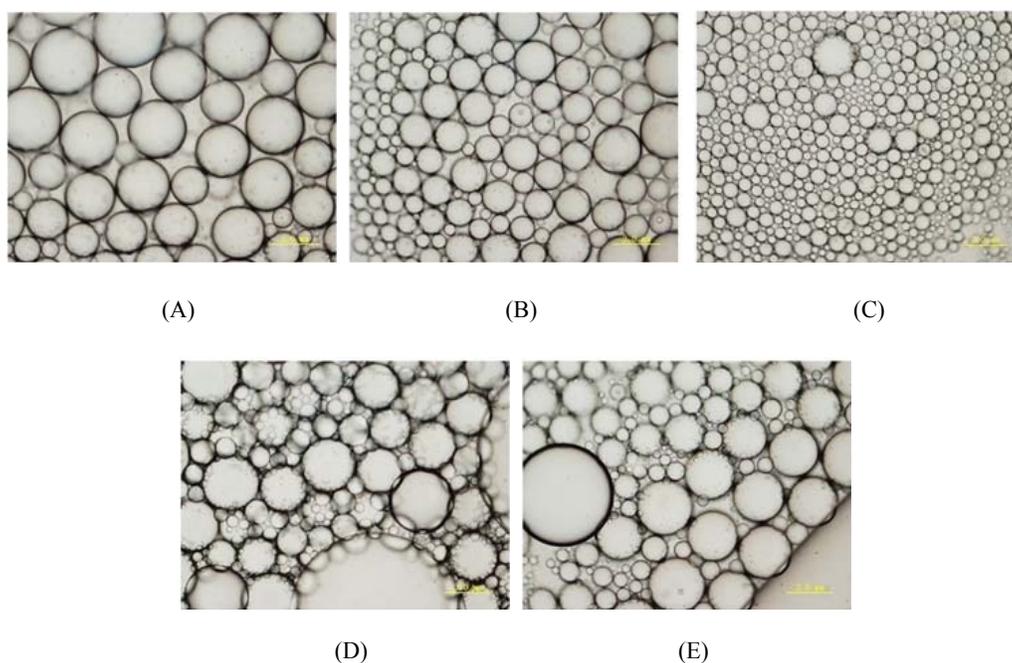


Fig. 2: Particle size of droplets ( $\times 100$ ) in emulsions with added gallic acid (A), Propyl gallate (B), (+)-catechin (C), quercetin (D) and control (E)

by addition of gallic acid and quercetin after the 10th day compared with control ( $p < 0.05$ ). While, propyl gallate and (+)-catechin represented antioxidant activities during the whole period of the oxidating compared with control ( $p < 0.05$ ).

Regarding of the pro-antioxidant role for quercetin and gallic acid after the 10th day in the emulsion, the reason should be the occupational competition on the interface of oil in water emulsion for antioxidants and oxidated compounds. Interface is the site where oxidation occurred, therefore, the interface can also be

colonised by numbers of radicals and hrdroperoxides which produced from the interface oxidation, these colonists may be involved in the mixture that could catalyse lipids further oxidation at interface with water, emulsifier and phenolic compounds (Huang and Frankel, 1997). This may be responsible for the results of quercetin, gallic acid played a pro-oxidant activity in emulsions. However, Škerget *et al.* (2005) suggested that quercetin can exert an antioxidant activity in linoleic acid emulsion, it may be related to the type of lipids in emulsion system.

Propyl gallate and (+)-catechin were located at the oil-water interface in emulsions due to its high affinity toward tween 20 (Huang *et al.*, 1997). Hence, the interface oxidation was inhibited by these interface protectors, then it was difficult for the interface colonists producing, therefore, further oxidative reaction was prohibited. As regards the antioxidant order of propyl gallate > (+)-catechin, it may be related to the difference of affinity toward tween 20.

According to “polar paradox”, interface is the site where oxidation occurred in emulsion. Hence, high surface/volume ratio of emulsion makes it more susceptible to oxidation. In this study, propyl gallate, (+)-catechin showed antioxidant activity in emulsion with a smaller particle sizes (high surface/volume ratio) than others (Fig. 2). It seems to have a contradictory with the theory of “polar paradox”. This phenomenon should be explained that the concentration of propyl gallate or (+)-catechin may be enough for colonising the interface. Under this circumstance, the surface/volume ratio is not the dominant factor in emulsion oxidation.

## CONCLUSION

In bulk camellia oil gallic acid, propyl gallate, (+)-catechin and quercetin showed different positive efficiency in inhibiting lipid oxidation. While in camellia oil emulsion gallic acid, quercetin showed antioxidant efficiency during the initial 10 oxidating days, then pro-oxidant activity after the 10<sup>th</sup> day of oxidation; Propyl gallate, (+)-catechin played antioxidant roles during the whole period of the oxidating. These factors confirm that interface is the site where oxidation occurred in bulk oil and emulsions. In bulk oil, the antioxidant activity was regulated by polarity and ability, rapidity of donating hydrogen atoms of phenolic compounds. In emulsions, the antioxidant activity of phenolic compounds prefer to the affinity toward emulsifier (tween 20) rather than their polarity.

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