Stabilization of Soybean Oil with Heated Quercetin and 5-Caffeoylquinic Acid in the Presence of Ferric Ion

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The effect of heated quercetin (400 mg/kg of oil) or 5-caffeoylquinic acid (**5-CQA**) and the presence of ferric ion (2.2 mg/kg of oil) on the stability of soya oil oxidized in an oxidative stability index (OSI) instrument was investigated. After heating the phenolic at 100 °C or 150 °C, the OSI values of treated oils were not significantly ($p < 0.001$) different, whereas, at 200 °C, the values decreased significantly with addition of quercetin, but not with **5-CQA**. However, the antioxidative activity of quercetin remained significantly greater than that of **5-CQA**. The antioxidative potency of quercetin was reduced significantly by addition of ferric palmitate (**FP**), but that of **5-CQA** was not. Reaction between the ortho-dihydroxy groups of the quercetin and ferric ion may reduce the number of hydroxyls available to react with free radicals. Chelating action of **5-CQA** might be provided by ortho-dihydroxy grouping of the quinic acid moiety.

Keywords: *Soya oil stability; heating; quercetin; 5-caffeoylquinic acid; ion ferric*

INTRODUCTION

The rancidity of refined edible oils is caused by the presence of high amounts of polyunsaturated fatty acids (e.g., linoleic and linolenic acids) which oxidize rapidly (Sherwin and Lackadoo, 1970). Autoxidation by oxygen is the most common reaction that leads the oil to rancidity, and this occurs via a free-radical-chain mechanism which includes induction, propagation, and termination steps (Sherwin, 1976; Hamilton et al., 1997). The occurrence of pro-oxidant metals, mainly those containing two or more valence states, can accelerate the rate of oxidation even at low amounts in the oil (Paquette et al., 1985). As a consequence of the chain reaction, off-flavors and potentially deleterious products are formed (Paquette et al., 1985; Frankel, 1993).

Antioxidant phenolic compounds are added to oil to retard the appearance of rancidity (Sherwin, 1976; Shahidi, 1995). These components extend the induction period of the oil by acting as free-radical acceptors and as metal chelating agents and afford relatively stable free radicals and nonradical products (Hamilton et al., 1997; Meyer et al., 1998). Synthetic phenolic compounds, such as butylated hydroxyanisole, butylated hydroxytoluene, and *tert*-butylhydroquinone, are the most commonly used antioxidants in lipids (Dorko, 1995). However, some authors have demonstrated that these components are toxic to animals (Bran, 1975; Linderschmidt et al., 1986; Whysner et al., 1994). There is a trend to reduce the amount of chemically synthesized antioxidants in foods. As a result, naturally occurring phenolic compounds (e.g., flavonoids and phenolic acids from herbs, fruits, seeds, etc.) have drawn attention because they have been ingested for centuries and are assumed to be relatively safe for human consumption (Pokorny, 1991; Wanasundara and Shahidi, 1994; Zandi and Gordon, 1999).

Quercetin, a flavonoid compound, and caffeoylquinic acid (**5-CQA)**, a phenolic acid formed by caffeic acid esterification with quinic acid, are widely distributed in the plant kingdom. Previous work has shown that quercetin is effective in retarding rancidity in canola oil (Wanasundara and Shahidi, 1994) and flax oil emulsions (Chen and Ahn, 1998). Data from our laboratory have shown that addition of **5-CQA** extends the induction period of refined soya oil submitted to accelerated oxidation (Luzia et al., 1997, 1998). Furthermore, evidence concerning the protective effect of both quercetin and **5-CQA** against oxidative damage to low-density lipoprotein have been reported (Laranjinha et al., 1996; Meyer et al., 1998). The antioxidant effectiveness of **5-CQA** and quercetin has been mainly attributed to the presence of a 3′,4′-dihydroxy (ortho-dihydroxy) grouping in the aromatic ring (Figure 1) (Shahidi and Wanasundara, 1992; Brand-Williams et al., 1995). Both phenolic compounds with adjacent free hydroxyl groups could act as free-radical acceptors or as metal chelators.

Guillot et al. (1996) and Stadler et al. (1996) have reported the identification of antioxidants (e.g., tetraoxygenated phenylindan isomers) derived from caffeic acid pyrolysis. However, the effect of heating on the antioxidative potency of quercetin and **5-CQA** has not been well studied. It is important to investigate the antioxidative potency of the antioxidant under conditions of heating used in conventional cooking processes. On the other hand, the occurrence of pro-oxidant metals in soya oil could influence the antioxidative activity of natural polyphenols. Among transition metals, iron (maximum of 0.76 mg/kg of oil) is the element found in the greatest amount in soya oil (Garrido et al., 1994). The present work investigates the effect of different conditions of heating and the addition of ferric ion on the antioxidative potency of quercetin and **5-CQA**.

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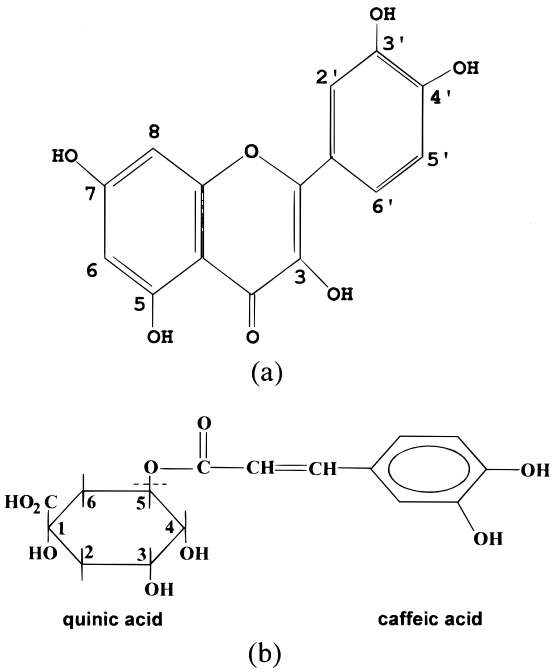

Figure 1. Chemical structures of polyphenols. (a) quercetin, (b) 5-caffeoylquinic acid (**5-CQA**).

Additives were added to fresh refined soya oil that was submitted to forced oxidation in an oxidative stability index (OSI) instrument.

MATERIAL AND METHODS

Materials. Fresh refined soya oil was obtained from Cargill Agrícola S. A. (Brazil). Quercetin and **5-CQA** were from Sigma (EUA). All other chemicals were from Merck (Brazil).

Synthesis of Ferric Palmitate. The ferric palmitate (**FP**), a lipid-soluble compound, was added to fresh refined oil to increase the iron concentration. The synthesis of **FP** was as described in the following (Gordon and Weng, 1992).

Palmitic acid was added to a beaker containing 100 mL of an aqueous sodium hydroxide solution (4.2%, p/v) and stirred for 5 min. The mixture was diluted with distilled water (400 mL) and shaken at 90 °C. The supernatant (aqueous sodium palmitate) was poured into a beaker containing ferric chloride hexahydrate (20 g), and the mixture was shaken at 80 °C. A brown solid was removed by filtration after 20 min and washed three times with hot distilled water at 80 °C. The solid was dried in a vacuum oven at 80 °C for 8 h to yield a chloroform-soluble dry solid melting at 85−89 °C. Analysis by spectrometry (AOAC, 1984) indicated that the solid contained 67 g of Fe per kg of **FP**, similar to the theoretical value for **FP** (68 g of **Fe** per kg of **FP)**.

Evaluation of the Oxidative Stability of Soya Oil Samples. Soya oil (5 g) was incinerated in a furnace at 550 °C and iron content was determined based on AOAC (1984). Quercetin or **5-CQA** (ca. 10 mg) was weighed in a dish glass (100-mm diameter) and subjected to heating in an oven (Heraeus, Germany) at 100, 150, or 200 °C for 15 or 30 min. Six independent replicates were made for each experiment. Each heated phenol was cooled at room temperature, and dissolved (8 mg) in 1 mL of absolute ethanol. Each solution was then added to the oil (20 g) and the mixture thoroughly homogenized. The concentration of antioxidant in both oil samples was 400 mg/kg of oil. The same assays were carried out with addition of 2.2 mg of Fe per kg of oil (32.3 mg of **FP** per kg of oil). Control samples containing 1 mL of absolute ethanol (no additives) or nonheated additives were prepared.

OSI Method. Each oil sample (5 g) was submitted to forced oxidation in an OSI instrument (model OSI 8, Omnion, USA). The OSI values were determined at 110 °C with an air flow rate of 9L/h for all analyses. The OSI value is defined mathematically as the maximum of the second derivative of the electrical conductivity with respect to time. This time-based end is determined by software coupled with the OSI Instrument. All determinations of OSI values are the means from six independent experiments. Standard error and one-way analysis of variance ($p < 0.001$) were performed using a statistical graphics system (STSC, 1986).

Table 1. Effect of Heated Quercetin (QUE) and 5-CQA on the Fresh Refined Soya Oil Oxidative Stability When Subjected to Forced Oxidation at 110 °C[a]

treated samples	OSI (h)	treated samples	OSI (h)
no additive	6.2 ± 0.10^{a}	no additive	6.1 ± 0.05^{a}
nonheated **QUE**	15.5 ± 0.08^{b}	nonheated **5-CQA**	8.9 ± 0.16^{e}
nonheated **QUE** + **FP**	9.8 ± 0.09^{c}	nonheated **5-CQA** + **FP**	8.8 ± 0.10^{e}

heated **QUE**[a]		heated **5-CQA**[a]	
100 °C	15.3 ± 0.11^{b}	100 °C	9.0 ± 0.12^{e}
150 °C	15.6 ± 0.16^{b}	150 °C	8.9 ± 0.09^{e}
200 °C	14.4 ± 0.13^{d}	200 °C	8.8 ± 0.15^{e}

heated **QUE** + **FP**[a]		heated **5-CQA** + **FP**[a]	
100 °C	9.6 ± 0.16^{c}	100 °C	8.8 ± 0.11^{e}
150 °C	9.1 ± 0.11^{e}	150 °C	8.8 ± 0.13^{e}
200 °C	7.6 ± 0.11^{f}	200 °C	7.8 ± 0.11^{f}

[a] Heated oil samples were heated for a time period of 30 min. Means with different superscripts are significantly different at $p < 0.001$. Mean and standard deviation of $n = 6$. OSI, Oxidative Stability Index; **QUE**, quercetin (400 mg/kg of oil); **5-CQA**, 5-caffeoylquinic acid (400 mg/kg of oil); **FP**, ferric palmitate (32.3 mg/kg of oil).

RESULTS AND DISCUSSION

The OSI instrument allows determination of the induction time (OSI value), which is the time before rapid oxidation of the oil occurs. The method is applicable in general to all oils and fats, and it is based on the change in electrical conductivity of the water caused by the formation of volatile organic acids (mainly formic acid) produced during oxidation.

The OSI values of soya oil samples treated with quercetin or **5-CQA** are given in Table 1. Addition of nonheated quercetin or **5-CQA** significantly ($p < 0.001$) increased the OSI value of the oil. However, the extension of the OSI value by nonheated quercetin was 3.3 times that of the nonheated **5-CQA**. According to previous work (Hudson and Lewis, 1983; Cuvelier et al., 1992; Shahidi and Wanasundara, 1992), phenolic compounds possessing multiple hydroxyl groups as substituents in the benzene ring are generally the most efficient antioxidants for edible oils. Indeed, quercetin contains 4 hydroxyl groups in the aromatic ring, whereas **5-CQA** has just two (Figure 1).

Both natural phenols were subjected to heat at 100, 150, or 200 °C for 15 or 30 min before they were added to oil. There were not significative differences ($p < 0.001$) between OSI values from samples heated for time periods of 15 min and those heated for 30 min. Thus, only OSI values from samples heated for a time period of 30 min are shown in Table 1. At 100 or 150 °C, the OSI values of treated oils were not significantly changed. At 200 °C, there was a small but significant difference between the OSI values of oil samples containing heated quercetin and those treated with nonheated quercetin (Table 1). However, antioxidative potency of quercetin remained significantly greater than that of **5-CQA**. The antioxidative activity of **5-CQA** was not affected by heating at 200 °C.

According to results of Gordon and Weng (1992) and Luzia et al. (1998), ferric ions functioned as pro-oxidants in edible oils. Because of the presence of low amounts of iron (0.5 mg/kg of oil) in the soya oil analyzed, samples were treated with 2.2 mg of Fe per kg of oil (32.3 mg of **FP** per kg of oil). Thus, the effect of ferric ions on the antioxidative activity of quercetin or **5-CQA** could be better assessed. As shown in Table 1, the OSI values of oil samples containing quercetin together with **FP** were significantly ($p < 0.001$) lower than those treated with quercetin only. Our findings indicated that addition of ferric ions (concentration of 2.2 mg/kg of oil) significantly decreased the antioxidative potency of quercetin. These results could be because of the formation of a complex between the ortho-dihydroxy grouping of the quercetin and ferric ions which may reduce the number of hydroxyl groups available to react with free radicals. However, since iron is a pro-oxidant, the effect could be simple kinetics due to the two competing reactions. According to the reports of Cuvelier et al. (1992), and Shahidi and Wanasundara (1992), the occurrence of an ortho-dihydroxy grouping in the B-ring of flavonoids was capable of providing not only free-radical inhibition but also metal chelation due to stabilization of the aryloxylradical and *o*-quinone formation.

The OSI values of samples treated with **5-CQA** and **FP** paralleled those containing **5-CQA** (Table 1). Caffeic acid moiety of the **5-CQA** contains an ortho-dihydroxy grouping which is responsible by its antioxidative action (Figure 1). However, it is known that **5-CQA** is less effective than free caffeic acid in retarding the oxidation process (Cuvelier et al., 1992; Chen and Ho, 1997). It was due to the esterification of caffeic acid by a sugar moiety (quinic acid) which decreased its primary antioxidative efficiency (Cuvelier et al., 1992). On the other hand, Chen and Ahn (1998) reported that caffeic acid was considerably less effective than quercetin in delaying ferrous ion-induced lipid oxidation, while data from our laboratory (Luzia et al., 1998) showed that **5-CQA** was an efficient chelator of heavy metals. We suggested that ortho-dihydroxy grouping of the quinic acid moiety might help in the chelating action by **5-CQA** on ferric ions. Thus, ortho-dihydroxy grouping of the caffeic acid moiety would be available to react with free radicals produced during forced oxidation of the oil. This could explain the reason addition of ferric ion did not alter the antioxidant potency of **5-CQA**. Further investigation should address this probability.

Although antioxidative effectiveness of quercetin was reduced significantly ($p < 0.001$) by addition of ferric ions, it remained significantly greater than that of **5-CQA**. Similar results were obtained for antioxidants heated at 100 °C or 150 °C. At 200 °C, the OSI values of samples treated with quercetin and **FP** were similar to those containing **5-CQA** and **FP**. In general, antioxidative effectiveness of quercetin and **5-CQA** was not influenced substantially by heating under the conditions examined.

Isolation of the complex formed by reaction of **5-CQA** with ferric ions and selective blockade of the hydroxyl groups from quinic acid moiety would be an interesting approach to unravelling the possible mechanism involved in the **5-CQA** chelating action on ferric ion.

Abbreviations Used. 5-CQA, 5-caffeoylquinic acid; OSI, oxidative stability index; FP, ferric palmitate; Fe, iron.

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