

ANTIOXIDATIVE ACTIVITY OF TEA POLYPHENOL EXTRACTS IN SOYBEAN OIL

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ABSTRACT

Tea polyphenol extracts (TPE) (94.08% dry mater (DM) total polyphenols, 71.14% DM total catechins) at three concentrations (100 ppm, 200 ppm, and 400 ppm) were examined in soybean oil in accelerated oxidation conditions at 60°C. A sample without antioxidants and a sample with 100 ppm butylated hydroxyanisole (BHA) + 100 ppm butylated hydroxytoluene (BHT) were used as the negative and positive controls, respectively. The results showed that TPE was more effective than BHA+BHT for the stability of soybean oil at the same concentration (200 ppm). TPE was capable of the maintenance of the sensorial properties, and reductions of diene and peroxide formations as well as the secondary oxidative compounds (p-anisidine). Concerning the TPE concentration, 200 ppm of TPE was suitable to stabilize the soybean oil quality during storage.

Keywords: tea polyphenol extract, antioxidative activity, soybean oil quality

Hoạt tính kháng oxi hóa của polyphenol chè trong dầu đậu nành

TÓM TẮT

Chất chiết polyphenol chè (TPE) (hàm lượng polyphenol tổng số 94.08% chất khô (DM), catechin tổng số 71.14% DM) ở 3 nồng độ (100 ppm, 200 ppm, và 400 ppm) đã được thử nghiệm trong dầu đậu nành trong điều kiện thúc đẩy oxi hóa ở 60°C. Mẫu không bổ sung chất chống oxi hóa và mẫu được bổ sung 100 ppm butylated hydroxyanisole (BHA) + 100 ppm butylated hydroxytoluene (BHT) đã được sử dụng như các đối chứng negative và positive tương ứng. Kết quả chỉ ra rằng, TPE hiệu quả hơn hỗn hợp BHA+BHT đối với việc ổn định chất lượng dầu đậu nành ở cùng nồng độ (200 ppm). TPE có khả năng lưu giữ tốt các đặc tính cảm quan, làm giảm sự hình thành giá trị diene, peroxide cũng như các sản phẩm oxi hóa bậc 2 (p-anisidine) của dầu. Liên quan đến nồng độ xử lý TPE, 200 ppm là phù hợp để ổn định chất lượng của dầu đậu nành trong quá trình tàng trữ.

Từ khóa: Chất chiết polyphenol chè, chất lượng dầu đậu nành, hoạt tính kháng oxi hóa.

1. INTRODUCTION

Tea, which is processed from tea leaves (*Camellia sinensis* L.), is the cheapest and the most popular beverage in the world. Many studies have shown that polyphenolic compounds extracted from green tea leaves are good antioxidants that have potentialities against many diseases, including cancers (Yang, 2006), obesity (Lin and Shoei-Yn, 2006), atherogenesis (Osada *et al.*, 2001), and many others.

The chemical composition of tea is complex: polyphenols, caffeine, amino acids, carbohydrates, protein, chlorophyll, volatile compounds, fluoride, minerals, and other undefined compounds (Graham, 1992). Among these, the main constituents belong to the polyphenol group accounting for 30% on a dry weight basis (Chang *et al.*, 2000). Catechins (flavan-3-ols) are the principal composition of tea polyphenols and their major elements are: (+)-catechin (C), (+)-galocatechin (GC), (-)

epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCG) (Graham, 1992) (Figure 1). These catechins are also considered to be responsible for the pharmaceutical properties of tea, including antioxidant and antibacterial activities (Mendel, 2007).

During the last decades, many researchers have indicated that tea extracts/polyphenols can be used as a natural preservative in food matrices such as meats (McCarthy *et al.*, 2001; Tang *et al.*, 2001; Mitumoto *et al.*, 2005), fish (Lin and Lin, 2005; Seto *et al.*, 2005), and vegetables (Martin-Diana *et al.*, 2008). With regard to edible oil, Chen and Chan (1996) showed that a 200 ppm concentration of green tea catechins was more protective in terms of lipid oxidation than butylated hydroxytoluene (BHT) in canola oil heated at 95°C. In addition, Anna *et al.* (2006) evaluated the antioxidant activity of tea extracts against the oxidation (Rancimat test) of heated sunflower oil at

110°C. At set interval times, samples were taken out to test for antioxidant activity. Their results indicated that the highest antioxidant activity was at 1000 ppm green tea ethanol extract, and was comparable to α -tocopherol activity. However, Malheiro *et al.* (2012) showed that the tea extract only protected olive oil from oxidation in the first 3 min under a microwave cooking condition. Later on, the extract was pro-antioxidant.

In the oil industry, the addition of synthetic antioxidants, like butylated hydroxyanisole (BHA), BHT, and tertiary butylhydroquinone (TBHQ), to products has been a common practice over the years. However, the use of these types of antioxidants is controlled due to their toxic and carcinogenic potential (Chen *et al.*, 1992; Sun and Fukuhara, 1997). The aim of this study is to investigate the protective effect of tea polyphenol extracts (TPE) as a natural antioxidant on the oxidative stability of soybean oils that are currently popular.

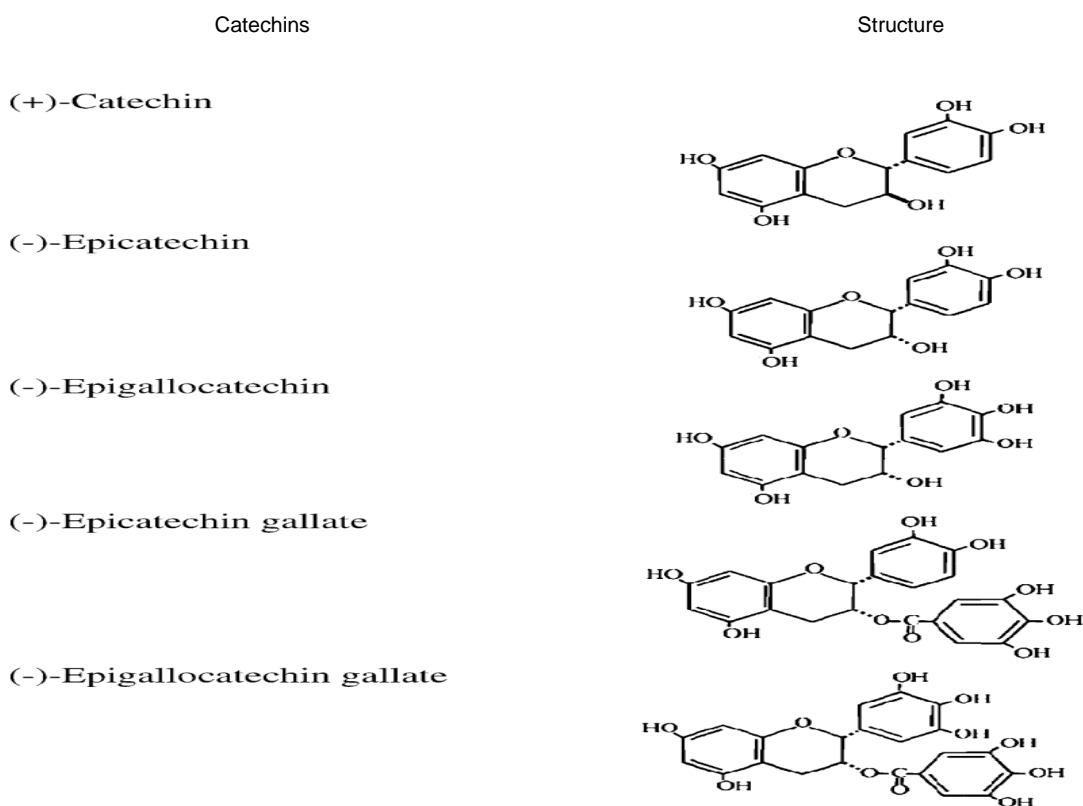


Figure 1. Chemical structures of major catechins found in tea (Yilmaz, 2006)

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Oil, tea polyphenols extract

In this study, commercial extra virgin soybean oil without preservatives was purchased from Vinacommodities (Pho Noi, Hung Yen, Vietnam).

The TPE was produced as follows: TPEs were extracted three times from dry tea leaves (0.5 - 1 mm diameter) at 45°C for 2.3 h using 56% acetone. The infusion was cooled to room temperature and centrifuged at 4500 rpm for 15 min. The supernatant was added to dichloromethane with a 1:1.5 ratio of supernatant to dichloromethane in order to remove the caffeine and pigments. The remaining aqueous layer was extracted by adding ethyl acetate at a 1:3 ratio of tea extract to ethyl acetate. The ethyl acetate was then removed from the extract by using a rotary evaporator under vacuum. The concentrate was then lyophilized under vacuum to 5% humidity. The final TPE had a content of 94.08% DM total polyphenols and 71.14% DM total catechins.

2.1.2. Chemicals

Acetic glacial acid, para-anisidine, n-hexane, chloroform, and iso-octan were purchased from Sigma-Aldrich (Singapore). The other chemicals were analytical grade (China).

2.2. Methods

2.2.1. Preparation of the oil sample and storage conditions

The experiment, which was conducted in the condition of accelerated oxidation at 60°C (AOCS Recommended Practice Cg 5-97), was composed of 5 formulas with triplicate experiments: F1 - oil without antioxidant as a negative control; F2 (positive control) - addition of 100 ppm of BHT + 100 ppm of BHA; F3 - addition of 100 ppm of TPE; F4 - addition 200 ppm of TPE; and F5 - addition 400 ppm of TPE. After treatment with antioxidants, the oil was contained in dark brown vials of 100 ml (uncovered) and placed in

an incubator at 60°C. The sensorial properties and oxidative stability of the oil were evaluated at 0, 3, 6, and 12 days of storage.

2.2.2. Analytic methods

Sensorial analyses

The sensorial properties (color, odor, clearness) of the oil were evaluated according to the TCVN 2627: 1993.

Free fatty acids

Free acidity of the oil was determined following the TCVN 6127: 2007.

Diene value

Diene value was determined according to the standard method IUPAC (Paquot, 1979).

Peroxide value

Peroxide value of the oil was evaluated according to the analytical methods described in the AOCS Official Method Cd 8-53 (acetic acid-chloroform method).

Para-anisidine value

p-anisidine value was determined based on the AOCS Official Method Cd 18-90.

2.2.3. Statistical analysis

Statistical analyses were conducted with SAS 9.1 using the procedure for ANOVA. The experimental results were expressed as means of triplicates. Analysis of variance was performed by the one-way ANOVA procedure. Significant differences between means were determined by Fisher's least significant difference (LSD) test. Differences were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of TPE on the sensorial properties of soybean oil after 12 days of accelerated oxidation

The sensorial quality of oil relates directly to product acceptability by consumers. Results showed that there was not any change of sensorial properties when adding antioxidants (BHT, BHA, and TPE) to the oil at bday 0. The oil still maintained an original slightly yellow, specific odor, and clear state.

Table 1. Effect of TPE on the sensorial properties of soybean oil after 12 days of accelerated oxidation

Formula	Color	Odor	Clearness
F1	Dark yellow	Extremely rancidness	Clearness, sans sediments
F2	Dark yellow	Rancidness	Clearness, sans sediments
F3	Yellow	Slightly rancidness	Clearness, sans sediments
F4	Slightly yellow	Sans curious odor	Clearness, sans sediments
F5	Slightly yellow	Sans curious odor	Clearness, slight sediments of TPE

Note: F1 - without antioxidant, F2 - 100 ppm BHT + 100 ppm BHA, F3 - 100 ppm TPE, F4 - 200 ppm TPE, F5 - 400 ppm TPE

However, after 12 days of accelerated oxidation at a high temperature (60°C), in two formulas (F1-without antioxidant and F2 - BHT+BHA), the oil color changed to dark yellow, and the odor was extremely rancid for F1 and a lower rancidity level for F2 (BHT+BHA). In contrast, a slight rancidity appeared only for the treated oil with 100 ppm of TPE, and absolutely no rancidity occurrence in the treated oil samples at higher TPE concentrations (200 ppm and 400 ppm). In addition, these samples (F3, F4) maintained an original slight yellow color. However, there was a little TPE sediment in the formula which was introduced from 400 ppm of TPE.

These results showed that the TPE limited sensorial modifications of the oil better than BHT and BHA during accelerated oxidation at a high temperature. However, it is necessary to

obtain further observations such as the reduction of diene and peroxide formations.

3.2. Effect of TPE on the free fatty acid content of soybean oil during accelerated oxidation

The results of acidity values along the exposure time, with or without antioxidants, are presented in Table 2. There was not a remarkable change throughout the exposure time between control samples, the BHT+BHA sample and the added tea extracts samples. This fact could be explained by the nearly inexistence of water in the soybean oil. For this reason, under these conditions, hydrolysis is usually negligible. This result is in agreement with the research of Malheiro *et al.* (2012), in which tea extract was added to olive oil in microwave heating condition.

Table 2. Effect of TPE on the free fat acids content of soybean oil during accelerated oxidation

Formula	Period of accelerated oxidation (days)			
	0	3	6	12
F1	0.467 ^{Ab}	0.503 ^{Aa}	0.500 ^{Aa}	0.479 ^{Ab}
F2	0.465 ^{Ac}	0.483 ^{Bb}	0.497 ^{Aa}	0.487 ^{Abc}
F3	0.463 ^{Abc}	0.474 ^{Cba}	0.483 ^{Ba}	0.461 ^{Bc}
F4	0.468 ^{Aa}	0.469 ^{Ca}	0.473 ^{Ca}	0.464 ^{Ba}
F5	0.467 ^{Aa}	0.468 ^{Ca}	0.470 ^{Ca}	0.441 ^{Cb}

Note: F1 - without antioxidant, F2 - 100 ppm BHT + 100 ppm BHA, F3 - 100 ppm TPE, F4 - 200 ppm TPE, F5 - 400 ppm TPE. Means in a column (A-C across formulas) not having a common letter are different ($p < 0.05$). Means in the row (a-c across periods) not having a common letter are different ($p < 0.05$).

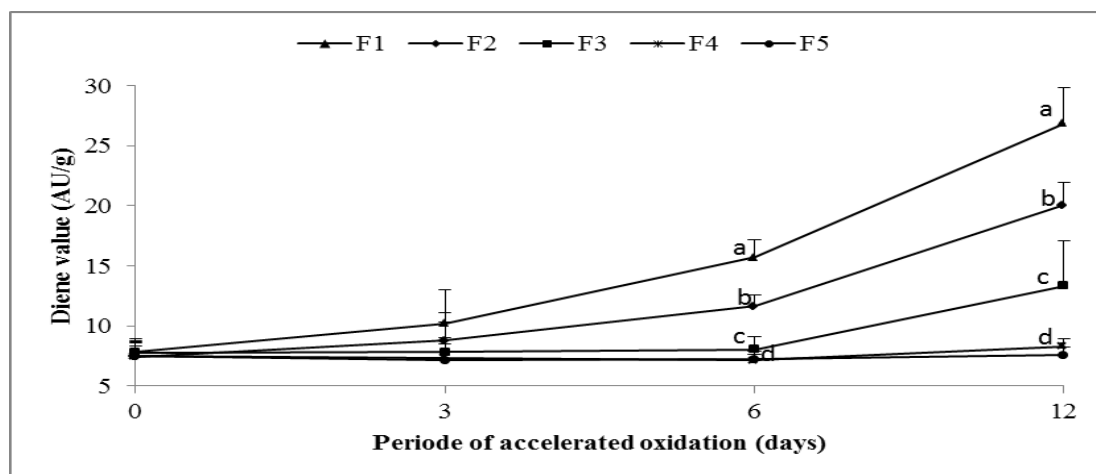


Figure 2. Effect of TPE on the dienes value of soybean oil during accelerated oxidation

Note: F1 - without antioxidant, F2 - 100 ppm BHT + 100 ppm BHA, F3 - 100 ppm TPE, F4 - 200 ppm TPE, F5 - 400 ppm TPE. According to day, means with no common letters differ significantly ($P < 0.05$).

However, at the same observation time, the values in the samples with added TPE were always lower than the control sample or the sample with added BHT+BHA ($\alpha = 0.05$). It could be assured that the TPE is capable of limiting the hydrolysis of lipid even at a high temperature.

3.3. Effect of TPE on the diene value of soybean oil during accelerated oxidation

The first stage in lipid peroxidation is an abstraction of hydrogen from a molecule of polyunsaturated fatty acid and formation of conjugated dienes. The diene value is used to verify the degree of oil oxidation, complementing the observations of the peroxide value. The results are presented in Figure 2.

It is clear that the diene concentration in all samples increased according to the accelerated oxidation time, but it was very different between the formulas. The value of the negative control sample (from 7.8 AU/g oil to 26.9 AU/g oil after 12 days) increased the highest compared to the sample with added BHA+BHT (20.1 AU/g after 12 days). The TPE exhibited an important protection effect against diene formation in the oil during the accelerated oxidation process, in particular at the 200 and 400 ppm concentrations. At the 200 ppm and 400 ppm TPE concentrations, after 12 days of

accelerated oxidation, the diene concentration was only 30.9% and 41.3%, respectively, in comparison with these values in the control and BHA+BHT samples. Our results are similar to the research of Chen and Chan (1996) who indicated that green tea extract is more protective than BHT against lipid oxidation (oxygen consumption test) in canola oil under the same conditions.

3.4. Effect of TPE on the peroxide value of soybean oil during accelerated oxidation

Peroxide amounts are commonly used for an estimation of oxidative degradation. The results are presented in Figure 3.

The results showed that before heat incubating, there did not exist a significant difference between the control sample (without antioxidant) and the added antioxidants samples (0.8 meq. O_2 /kg oil). However, throughout the exposure time, a strong increase in these values was observed for F1 and F2 (control, BHA+BHT), whereas this change was very little within the added TPE samples, in particular the F4 and F5 formulas. Concretely, after 12 days of accelerated oxidation, the peroxide value varied from 0.8 meq. O_2 /kg oil to 150.8 meq. O_2 /kg oil for the control (F1) and to 123.7 meq. O_2 /kg oil for added oil BHA+BHT

(F2), whereas this value was only to 11.7 meq.O₂/kg oil for the 200 ppm of TPE sample and 7.8 meq.O₂/kg oil in the sample with 400 ppm TPE added. In addition, we remarked that in general, the peroxide values of samples F4 and F5 corresponded with the legal limit for vegetable oil after 12 days of exposure, a value of 10 meq.O₂/kg oil (Commission Regulation (EC) No, 1989/2003). Similarly, the TPE inhibited peroxide formation in the oil during

accelerated oxidation at a high temperature. These results are consistent with the research of Chen and Chan (1996) who indicated that green tea polyphenol extract containing 51.2% of EGCG, 18.7% of EGC, 11.8% of ECG, and 12.3% of EC exhibited a significant protection to canola oil from lipid oxidation after 23 h of heating at 95°C. However, there was not a pro-antioxidant activity of TPE as observed by Malheiro *et al.* (2012).

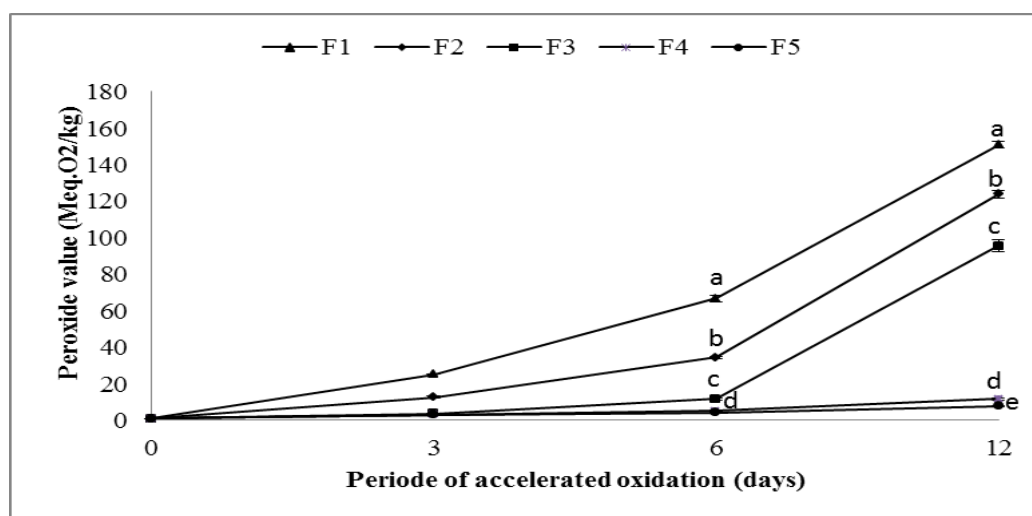


Figure 3. Effect of TPE on the peroxide value of soybean oil during accelerated oxidation

Note: F1 - without antioxidant, F2 - 100 ppm BHT + 100 ppm BHA, F3 - 100 ppm TPE, F4 - 200 ppm TPE, F5 - 400 ppm TPE. According to day, means with no common letters differ significantly ($P < 0.05$).

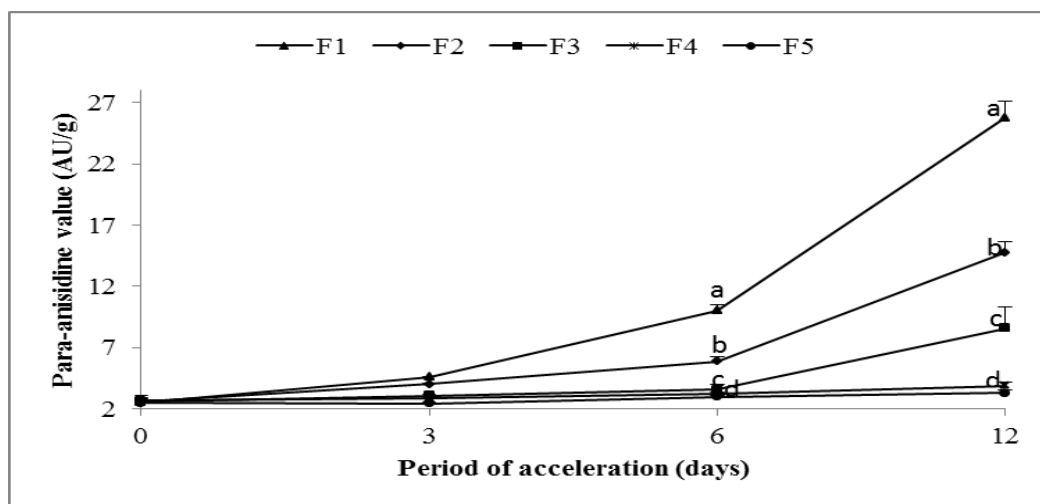


Figure 4. Effect of TPE on the para-anisidines value of soybean oil during accelerated oxidation

Note: F1 - without antioxidant, F2 - 100 ppm BHT + 100 ppm BHA, F3 - 100 ppm TPE, F4 - 200 ppm TPE, F5 - 400 ppm TPE. According to day, means with no common letters differ significantly ($P < 0.05$).

3.5. Effect of TPE on the p-anisidine value of soybean oil during accelerated oxidation

Similar to the TBARS test, the p-anisidine value is often used to determine a secondary lipid oxidation marker. The results of this determination are presented in Figure 4.

Just as with the peroxides value, the p-anisidines amount increased rapidly after just 3 days of accelerated oxidation at 60°C for the control and the BHA+BHT samples. However, this value increased inconsiderably for samples with added TPE, especially for the samples with 200 ppm and 400 ppm TPE. Concretely, after 12 days of oxidation, the p-anisidine concentration increased from 2.5 AU/g to 25.8 AU/g oil in the control (F1) and to 14.8 AU/g in the BHA+BHT sample (F2), whereas it increased to only 3.9 AU/g and 3.3 AU/g in the F4 and F5 samples, respectively. Similarly, the TPE was significantly effective in reducing secondary lipid oxidation in oil.

4. CONCLUSIONS

Tea polyphenols extracts containing 94.08% DM total polyphenols and 71.14% DM total catechins have strong antioxidative properties in lipid systems. Research showed that the TPE was more abundantly effective than BHA+BHT for the stability of soybean oil at the same concentration (200 ppm). TPE is capable of the maintenance of the sensorial properties, and of the reduction of diene and peroxide formations as well as the secondary oxidative compounds (p-anisidine). Concerning the TPE concentration, 200 ppm of TPE is suitable to stabilize soybean oil quality during storage.

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