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# Effect of *Cratoxylum formosum* Extract and Stripping on Soybean Oil Stability

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## ABSTRACT

Natural tocopherols in soybean oil have a favourable influence on oxidative stability. To study this impact, the antioxidant activity of ethanolic extract of *Cratoxylum formosum* (Jack) Dyer (*C. formosum*) was investigated in stripped and non-stripped refined soybean oils comparing to α-tocopherol and BHT. Peroxide value (PV), thiobarbituric acid reactive substances (TBARS) and oxidative stability index (OSI) values were measured. The results of PV, TBARS and OSI values of non-stripped and stripped oils containing *C. formosum* extract were significantly lower than that of control and sample containing α-tocopherol but higher than other containing BHT. The antioxidant activity of α-tocopherol (at 100 $mg.kg^{-1}$) was effective in stripped oil only. In addition, changes of the PV, TBARS and OSI values in non-stripped oil were slower than these in stripped oil. These results suggested that *C. formosum* can be a promising source for natural antioxidant. Moreover, natural tocopherols in refined soybean oil affect on the antioxidant property of studied compounds.


**Key words:** stability, soybean oil, strip, antioxidant.

## INTRODUCTION

In general, oils are susceptible to lipid oxidation which results in the loss of nutritional value of food and brings about undesirable changes in color, texture, sensory and other physiological properties (Iqbal and Bhanger, 2007). Due to these changes, consumers do not accept oxidized products while businesses suffer from economic loss. In order to overcome the stability problems associated with oils, fats and fatty foods, synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been used as food additives. Recent reports, however, suggest that these compounds may be implicated in health risks, including cancer and carcinogenesis (Spigno and De Faveri, 2007). Due to safety concerns, food scientists are aiming to replace synthetic antioxidants with natural ones, which are generally supposed to be healthier.

*C. formosum* is an indigenous Thai plant which is known as Tio Khao. Its fresh shoots and young leaves are traditionally consumed on their own. The plant tastes sour and is somewhat astringent due to the presence of phenolic components. *C. formosum* extract consists of 60% (W/W) chlorogenic acid (5-*O*-caffeoylquinic acid) (Maisuthisakul *et al.*, 2007). Its minor components are dicaffeoylquinic acid and ferulic acid derivatives. Chlorogenic acid is widely recognized to be active because of its free radical scavenging properties (Iwai *et al.*, 2004). It inhibits


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peroxidation of linoleic acid (Onishi *et al*., 1994) and acts as a cancer chemopreventive agent (Panzella *et al*., 2003). The high radical scavenging activity of extracts from *C. formosum* is due to the high content of phenolic compounds especially chlorogenic acid (Maisuthisakul *et al*., 2007).

Soybean oil is the most important vegetable oil in the world market because of its high quality and low cost. Soybean oil has a high degree of unsaturation and contains naturally occurring antioxidants (tocopherols) which are not completely removed during processing and also contribute to its stability. Tocopherols can also act synergistically with other added antioxidants. The aim of this study was to assess the effects of tocopherols, present in soybean oil on the oxidative stability following the addition of ethanolic *C. formosum* extract, and compare the data obtained with the stability observed when α-tocopherol and BHT are added. In order to achieve this purpose, stabilities of refined soybean oil (non stripped oil) and oil stripped of natural antioxidants (stripped oil) were investigated.

## MATERIALS AND METHODS

### Materials

α-Tocopherol, sodium thiosulfate, potassium iodide and butanol were purchased from Fluka Co. (Buchs, Switzerland). Ethanol was purchased from Sigma (Milwaukee, USA). BHT was purchased from BDH (Poole, United Kingdom). The other chemicals and solvents used in this experiment were reagent grade, and purchased from Sigma - Aldrich (Milwaukee, USA).

One batch of *C. formosum* leaves was purchased from a market place in Saraburi province during harvest season in April 2004. The leaves were cleaned immediately after harvesting. Sound quality leaves were hand picked for further extraction.

Soybean oil without antioxidants added was provided by Thai Oil Industry, Bangkok, Thailand. The oil contained α, γ and δ-tocopherol at concentrations of 115.7, 918.7 and 228.1 $mg.kg^{-1}$, respectively. The peroxide value determined by AOCS official method cd 8-53 (AOCS, 1990) was 0.70 ± 0.12 $meq.kg^{-1}$. The thiobarbituric acid reactive substances value (TBARS) determined according to McDonald and Hultin (1987), was 0.0001 $mmol.kg^{-1}$.

### Preparation of *C. formosum* extracts

The hand picked *C. formosum* leaves (80 g) were blended with 95% ethanol at -20°C for 1 min, and then flushed with nitrogen and shaken for 4.5 h in the dark at 25°C. The supernatant was filtered through a cheesecloth and Whatman No 4 filter paper. The solvent was removed using rotary evaporator and the residue left was freeze dried to be in powder form and stored in aluminum foil after flushing with nitrogen at -20°C.

### Preparation of stripped soybean oil

The method described by Yoshida (1993) was modified, to remove the tocopherols from soybean oil by column chromatography using alumina. Refined oil (200 g) was passed through a column containing activated aluminium oxide (140 g) dried at 200°C for 8 hours before use. The column was wrapped in aluminium foil to avoid oxidation. The oil was drawn through the column by suction. The oil collected was again passed through fresh alumina (140 g) to complete removal of the tocopherols. The oil was analyzed by HPLC to confirm the total removal of the tocopherols and stored at –70°C until use. Antioxidants were added to stripped and non-stripped soybean oils in the following quantities: 100 $mg.kg^{-1}$ of α-tocopherol, *C. formosum* extract and BHT.

### Oxidation and analysis

Stripped and non-stripped soybean oil samples with or without antioxidants added were transferred to screw-capped sample vials with

aluminium foil wrapping and held in an oven at 60°C for 12 days. The lids were only screwed loosely on the vials, therefore the air could pass in and out of the headspace above the samples. Aliquots (10 g) in separated vials were removed every 3 days for analysis. The oxidative state of sample was monitored by analysis of the peroxide value (PV), thiobarbituric acid reactive substances (TBARS) and oxidative stability index (OSI).

The peroxide value of soybean oil samples was determined according to AOCS official method Cd 8-53 (1990) using an automatic titrator (Mettler-Toledo model DL 5X, Switzerland) equipped with stirrer and redox electrode. The solution was titrated against standard sodium thiosulfate (0.01 M). PV was calculated and expressed as milliequivalent peroxide per kg of sample:

$$\text{PV (meq.kg}^{-1}) = \frac{(S - B) \times N \times F}{W} \times 100$$

where S is the titre (ml) for the sample; B is the titre (ml) for the blank, N is the normality of the sodium thiosulfate solution, F is the factor from standardization with potassium dichromate and W the sample weight (g).

TBARS values of stripped and non-stripped soybean oils were determined using the method described by Mcdonald and Hultin (1987). Oil (0.1 ml) was mixed with water (0.9 ml) and TBA reagent (2.0 ml, 15% w/v trichloroacetic acid and 0.375% w/v thiobarbituric acid in 0.25 M HCl) in test tubes and heated in water bath for 15 min. The tubes were cooled to room temperature for 10 min and then centrifuged (1000 × g) for 15 min. The absorbance was measured at 532 nm. Concentrations of TBARS were evaluated from a standard curve prepared using 1, 1, 3, 3-tetraethoxypropane.

OSI of oil samples was evaluated by using rancimat (Metrohm model 743, switzerland). A stream of air was bubbled into 5 g of oil contained in a reaction tube placed in an electric heating chamber. The outlet air containing volatile organic acids generated from the oil was collected in another tube containing deionized water (50 ml). The conductivity of the water as oxidation proceeded was measured automatically. Air flow rate was set at 150 ml/ min and OSI values were measured at 90°C.

**Statistical analysis**

Each experiment, from sample preparation to analysis, was repeated in triplicate, and the data were analyzed by SPSS software program (SPSS Inc., Chicago, IL, USA). The general linear model procedure was applied and Duncan's multiple range test was used to compare the mean values at $p < 0.05$. Mean values and pooled standard error of the mean (SEM) were then estimated.

## RESULTS AND DISCUSSION

**Effect of *C. formosum* extract on soybean oil stability**

Stored samples were analyzed periodically for PV and TBARS to allow both hydroperoxides and hydroperoxide degradation products to be monitored. Volatile short chain acid degradation product, which is tertiary oxidation product, was also evaluated by OSI methods. The degradation products contribute oxidative off-flavours to foods, and consequently it is important to monitor both precursors of these off-flavours as well as the degradation products themselves.

Peroxide value changes of non-stripped soybean oil containing *C. formosum* extract compared with oil containing individual antioxidants were investigated (Figure 1a). The PV and TBARS of oil containing *C. formosum* extract were substancially lower than these of control. *C. formosum* extract treatment showed higher antioxidant activity than that of α-tocopherol but less than that of BHT. The order of antioxidant activity assessed on the basis of OSI values (Table 1) are consistent with those deduced by measuring

by PV and TBARS value (Figure1). It is noteworthy that α-tocopherol at 100 mg.kg$^{-1}$ was not proved to be an effective antioxidant in non-stripped soybean oil. This suggests that the addition of α-tocopherol at 100 mg.kg$^{-1}$ to soybean oil which already contained 115.7 mg.kg$^{-1}$ of natural α-tocopherol, did not influence the overall antioxidant capacity. This observation is consistent with the study of Isnardy *et al*. (2003), who reported that α-tocopherol at concentrations of 100 and 500 mg.kg$^{-1}$ showed no difference in antioxidant activity in purified rapeseed oil.

The change in oxidative stability of stripped soybean oil with and without antioxidants added was determined by measuring the PV and TBARS (Figure 2) and OSI values (Table 2). All antioxidants remained effective over a specific period of time, and with the passage of time, their effectiveness decreased to a point where they finally become ineffective (Laandrault *et al*., 2001). Such antioxidants end, or at least interrupt, oil and fat deterioration in the early stages, and thus delay the onset of oxidation. It may be hypothesized that phenolic antioxidants inhibit lipid peroxidation by undergoing degradation themselves over a period of time (Iqbal and Bhanger, 2007).

The stripped oil containing *C. formosum* extract showed stronger antioxidant capacity than that of α-tocopherol (Figure 2 and Table 2). This may be attributed to the differences between the antioxidant mechanisms of α-tocopherol and phenolic components in *C. formosum* extract. Tocopherols impart high stability to lipids due to their ability to inhibit free radical propagation by donating hydrogen from their phenolic group to peroxyl radicals in order to stabilize those (Isnardy *et al*., 2003). Therefore, tocopherols can inhibit

**Table 1** Oxidative stability index (OSI) of non-stripped soybean oil.

| Antioxidants | OSI value (h) |
|---|---|
| No antioxidant | 29.61 ± 0.09 [a] |
| ∝-tocopherol 100 mg.kg$^{-1}$ | 29.65 ± 0.27 [a] |
| *C. formosum* extract 100 mg.kg$^{-1}$ | 33.19 ± 0.07 [b] |
| BHT 100 mg.kg$^{-1}$ | 37.23 ± 0.12 [c] |

Note : OSI data followed by different letters are significantly different according to Duncan's multiple range test at $p < 0.05$. The data represent means ± standard deviation derived from triplicate measurement.

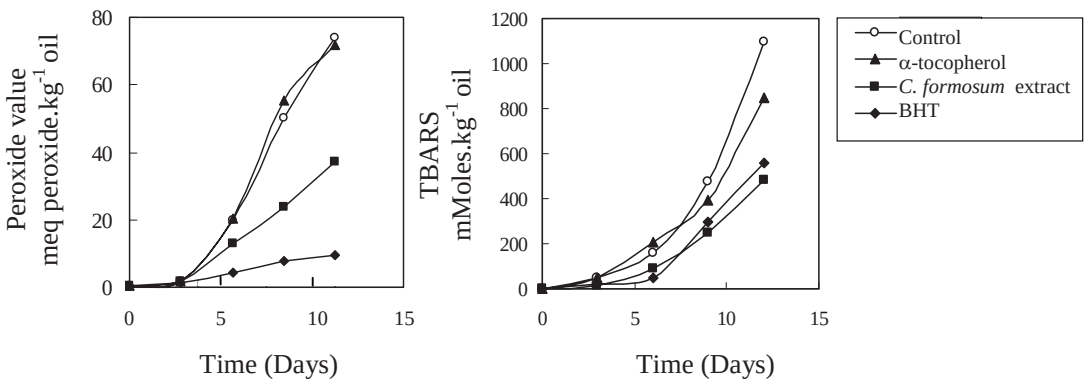


**Figure 1** Effect of antioxidants on the oxidative stability of non-stripped soybean oil at 60°C, assessed by the determination of (a) PV and (b) TBARS. Data points represent mean (n=3) ± standard deviation.

lipid oxidation in vegetable oils. Since *C. formosum* extract contained 60% chlorogenic acid, it could act as hydrogen donator and metal chelator. The greater effectiveness of *C. formosum* extract in decreasing secondary oxidation products is due to the lower extent of metal catalyzed hydroperoxide decomposition, as substantiated by the lowest value of TBARS (Figure 2b).

**Effect of stripping on soybean oil stability**

In order to understand the role of natural antioxidants further, the time (days) taken by the natural oil (i.e. non-stripped) to reach a Peroxide Value of 50 meq. $kg^{-1}$ ($PV_{50}$) was determined and compared with stripped oil (Figure 3). It is evident that the $PV_{50}$ values of stripped soybean oils are lower than those of non-stripped oils indicating lower antioxidant activity in the former. In stripped oil, the formation of hydroperoxide (Figure 2a) was the greatest in the control sample in comparison with oil containing α-tocopherol, *C. formosum* extract and BHT. Moreover, this parameter decreased after 9 days of storage. This can be explained by the breakdown of hydroperoxide to secondary oxidation products, which is also measured by the TBARS assay. The TBARS of control confirmed this aspect (Figure 2b).

The antioxidant activity of non-stripped soybean oil containing an extra of 100 mg $kg^{-1}$ of α-tocopherol was not different to that of the non stripped oil itself. The $PV_{50}$ value of non-stripped oil containing α-tocopherol was similar to that of the control (Figure 3). On the other hand, the $PV_{50}$ of stripped soybean oil containing α-tocopherol was greater than that of the control. This data confirms the benefits imparted by natural tocopherols present in soybean oil in terms of antioxidant activity.

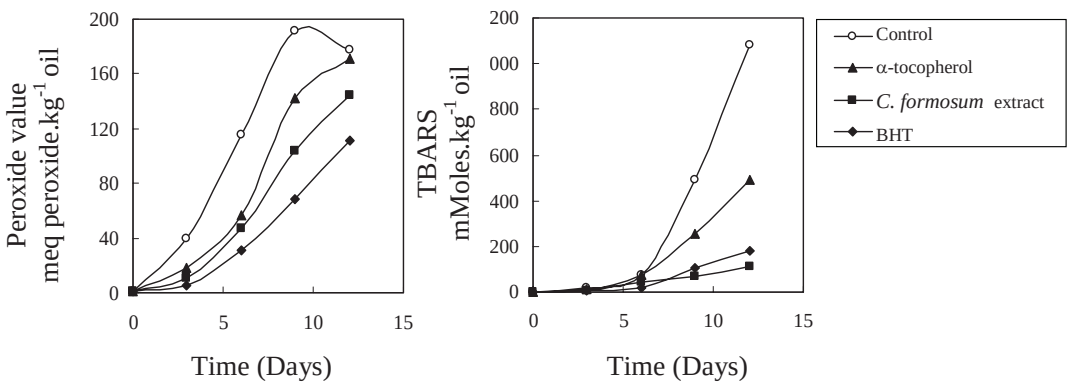


**Figure 2** Effect of antioxidants on oxidative stability of stripped soybean oil at 60°C, assessed by (a) PV and (b) TBARS. Data points represent mean (n=3) ± standard deviation.

**Table 2** Oxidative stability index (OSI) value of stripped soybean oil.

| Antioxidants | OSI value (h) |
|---|---|
| No antioxidant | 1.82 ± 0.01 [a] |
| α-tocopherol 100 mg.$kg^{-1}$ | 14.53 ± 0.01 [b] |
| *C. formosum* extract 100 mg.$kg^{-1}$ | 15.64 ± 0.01 [c] |
| BHT 100 mg.$kg^{-1}$ | 18.94 ± 0.03 [d] |

Note : OSI data followed by different letters within each column are significantly different according to Duncan's multiple range test at $p < 0.05$. The data represent means ± standard deviation derived from triplicate measurement.

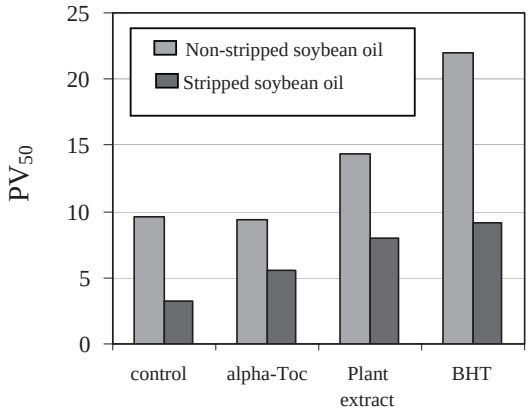


**Figure 3** Time (days) for non-stripped and stripped soybean oil to reach PV of 50 Meq.Kg$^{-1}$ at 60°C. α-tocopherol, *C. formosum* extract and BHT are added at a concentration of 100 mg.kg$^{-1}$.

The $PV_{50}$ values observed in BHT containing stripped and non-stripped soybean oils were greater than those observed in *C. formosum* extract containing oil, under otherwise the same conditions. This is due to synergistic interactions between BHT and natural tocopherols in soybean oil. It is known that BHT and tocopherols can act as primary antioxidants. Combinations of several free radical scavengers (FRS) often result in synergistic inhibition of lipid oxidation. This occurs when one FRS reacts more rapidly with free radicals than the other as a result of differences in bond disassociation energies and/ or stearic hindrance of FRS/FR interactions (Decker, 1997). The present study has clearly shown that naturally present minor components in soybean oil, including α, γ, δ-tocopherols, strongly influence antioxidant activity. Most of the earlier studies in this area used natural non-stripped oils to draw such conclusion. A comparison between natural oils and oil stripped of antioxidants must be considered to elucidate antioxidant power.

## CONCLUSIONS

The present study showed that the natural tocopherols of soybean oil affected the antioxidant activity of the additives in this study due to the synergistic effect and concentration effectiveness of antioxidants. The antioxidant activity of *C. formosum* extract was more ef fective than that of α-tocopherol but less than that of BHT in stripped and non-stripped oils. Given that *C. formosum* is a natural extract, we believe it to be a more suitable antioxidant.

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