The Effect of *Coleus Aromaticus* Benth Extracts on the Oxidative Stability of Palm Olein

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ABSTRACT

This study was conducted to determine the thermal oxidative stability of palm oil in the presence of the Coleus aromaticus Benth leaves extracts. The hexane extracted Coleus aromaticus was added to the palm oil as the antioxidant agent. Then followed by several test such as peroxide value (PV), total acid number (TAN), iodine value (IV), and Fourier- Transform Infrared spectroscopy (FTIR) to study the oxidative stability of the palm oil after prolonged exposure to heat at 90°C for 0, 50, 100, 150, 200, 250, and 300 hours. The result of Coleus aromaticus Benth *extract gave the positive effect in improving the oxidative stability of palm oil.*

Keywords: *Coleus aromaticus* Benth, *palm oil, antioxidant, oxidative stability*

Introduction

Palm oil is an important source of edible oil in many tropical countries. Today it is consumed worldwide as a cooking oil, margarine and shortening. It is also used as an ingredient in fat blends and a vast array of food products. Palm oil principal edible use is as an ingredient in prepared foods (primarily baked goods). Palm oil contains about 50% saturated and 50% unsaturated fatty acids (39% monounsaturated and 11% polyunsaturated fatty acids). However, unsaturated oils and fats are susceptible to auto-oxidation process. Polyunsaturated fats and oils are easily oxidized by oxygen by means of a radical chain reaction. The reaction of fatty acids with oxygen causes them to be rancid (Bruice, 2004). Lipid peroxidation can have deleterious effects on the cells and tissues of the human body. Reactive oxygen species can damage cells and other body components (El-Moein *et al.*, 2012).

Antioxidants can delay or prevent the oxidation of lipids by inhibiting the initiation or propagation of an oxidizing chain reaction (Zheng and Wang, 2001). The most widely used synthetic antioxidants in food such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl galate (PG) have been suspected to cause or promote negative health effects (Pokorny, 1991). For this reason there is a growing interest in studies of natural additives as potential antioxidants. Many sources of antioxidants of plant origin have been studied in recent years. These antioxidant properties of many aromatic plants and spices have shown to be effective in retarding the process of lipid peroxidation in oils and fatty foods and have gained the interest of many research groups (Kulisic *et al.*, 2004). Aromatic plants are known for their significant antioxidant content, and have been widely used to preserve foods (Chun *et al.*, 2005).

Coleus aromaticus is a perennial herb, grows to around 50 cm tall, has green heart shaped leaves with scalloped edges. It is the leaves that are used in cooking and as a traditional medicine (Khare *et al.*, 2011). *Coleus aromaticus* has been shown to have antioxidant activity, paticularly due to the high content of phenolic acids and flavonoids (Kumaran and Karunakaran, 2005). The objective of this study is to determine the thermal-oxidative stability of palm oil in the presence of the *Coleus aromaticus* leaves extracts.

Methodology

Plant Material

The plants were obtained in one of the herbs nursery located in Pahang, Malaysia. The dried leaves of *Coleus aromaticus* Benth weighted about 1000g and ground into powder.

Extraction

The powder of *Coleus aromaticus* Benth leaves was extracted with hexane by immersing 96 hour respectively. The mixture was filtered and the hexane was removed using the rotary evaporator. The residue was collected as the extract and stored at 4°C.

Thermal Oxidation Methods

Sample preparation

1% of the extract sample was added into 250 g palm olein by using weight by weight ratio then heated at 90°C. The palm olein without extract sample (PO) was used as references. After adding the extract sample to the oil, the sample was stirred by magnetic stirrer about 20 minutes to ensure homogenous mixture. Each sample was collected in 0, 50, 100, 150, 200, 250, and 300 hours respectively and analytical tests were conducted.

Total Acid Number (TAN)/ acid value test

The acidity test was conducted following AOCS method Cd 3A-63. About 3 ml of sample was weighted in 250 ml Erlenmeyer flask. 25ml of diethyl ether, 25 ml of ethanol 95% and 1 ml of phenolphthalein indicator solution were added into a sample. After that, the sample was shacked gently for 10 minutes until the entire sample was mixed. Then, it was titrated with 0.05M KOH. The amount of KOH was recorded. Blank determination without sample carried out followed the steps mention above. The acid value test is calculated based on the formula as shown below:

$$TAN = [\ (A-B) x\ N\ x\ 56.11\ ]\ /\ wt$$

Where, A is KOH used in titration of mixture (mL), B is KOH used in titration of blank (mL), N is normality of KOH (0.05) and wt is weight of sample (g).

Iodine Value Test (IV)

Iodine value test was conducted following AOCS method Cd 1b-87. In determining the iodine value, 0.4 g of sample was weighted into 500 ml conical flask before adding 20 ml cyclohexane and 25 ml Wijs reagent. The mixture was shaken vigorously and continuously for 1 minute before left in the dark for 1 hour. Blank determination was prepared by 20 ml KI and 150 ml distilled water added in both flasks (including blank) and shake slowly until homogenize. The solutions in both flasks were titrated with 0.1N sodium thiosulfate until faint yellow color solution appeared. A few drops of starch solution were added to the solution as indicator until blue color was observed. The titration was continued until the blue color discharged or become clear. The Iodine Value test is calculated based on the formula as shown below:

$$\%\ of\ iodine\ value = [\ (\ B-S)\ x\ N\ x\ 12.96g\ I\ ]\ /\ wt.\ of\ oil$$

Where B is titration of blank (mL), S is titration of test sample (mL) and N is normality of Na_2SO_3.

Peroxide Value Test (PV)

Peroxide value test was conducted according to AOCS official method Cd8-53. About 2.5 g of sample was weighted into 250 ml Erlenmeyer flask and followed by 15ml of 3:2 acetic acid-chloroform solutions. After that, 0.5 ml of saturated KI solution was added. Then, the solution occasional was shaken for 1 minute followed by adding 30 ml of distilled water and titrated with 0.1N sodium thiosulfate until the yellow color almost disappeared. 0.5 ml of starch solution was added as indicator and titration was continued until the blue color disappeared. Blank determination of reagent was conducted daily by using the same steps without sample. The blank titration must not exceed 0.1ml of the 0.1N sodium thiosulfate solutions. The calculation of Peroxide Value is as below:

$$PV = [\ (S-B)\ x\ N\ x\ 1000\ ]\ /\ wt$$

Where S is amount of Na_2SO_3 in mL used to titrate sample, B is Na_2SO_3 used to titrate blank, N is normality of Na_2SO_3 and wt is gram sample used.

Infrared Spectroscopy Analysis

The Fourier transform infrared (FTIR) spectra of the sample were recorded in the mid-IR region 4000 – 400cm^{-1} at 4 cm^{-1} with 16 scans by Perkin Elmer FTIR system spectrum GX spectrophotometer.

Result and Discussion

Total Acid Number (TAN)

The acid number was used to indicate the relative changes of total acid number (TAN) for palm oil (PO) and palm oil with 1% of *Coleus aromaticus* extract (PO+1% Extract) during thermal oxidizing condition. TAN is due to the oxidation of all the oil which produces peroxides and then undergoes further reaction to form alcohol, ketones, aldehydes, and carboxylic acid.

According to the TAN value of palm oil and palm oil with extract sample, in the early stage of heating process, there were not many differences in TAN as it increased slowly after heating at the temperature of 90°C. The result showed that *Coleus aromaticus* extract has proved slightly effective as antioxidant which can reduce oxidation of palm oil. Table 1 showed the TAN of original palm oil increased slowly from 1.08mg KOH/g to 1.50mg KOH/g.

Table 1: Summary of Total Acid Number with hours for original palm oil and palm oil with *Coleus aromaticus* extract.

	Total Acid Number (TAN) (mg KOH/g)	
Hour	Palm Oil	Palm Oil + 1% Extract
0	1.08	1.06
50	1.16	1.15
100	1.20	1.16
150	1.26	1.26
200	1.36	1.36
250	1.47	1.36
300	1.50	1.46

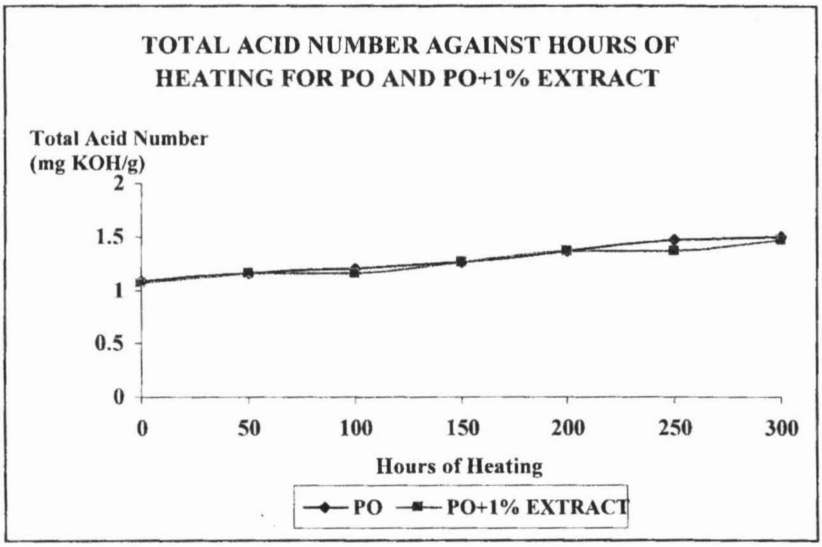

Figure 1: Total Acid Number against Hours of Heating for Palm Oil and Palm Oil + 1% Extract

The palm oil with 1% extract in Figure 1 showed the increasing of acid value from 1.06mg KOH/g to 1.46mg KOH/g proportionally to the heating temperature and the expected time. Although there was only a slight difference of acid value between palm oil and palm oil with extract, the palm oil with additives added still showed lower acid value than palm oil without additive-added.

Iodine Value

Iodine value (*IV*) test was conducted to determine the degree of unsaturation in the palm oil. The decreased iodine values can be attributed to the destruction of double bond by oxidation. The heating oil samples provide energy to excite the molecule of oil, the double bond at the chain will break down after molecules absorbed enough energy. The tendencies of the allylic hydrogen to oxidize enable the formation of peroxide and decrease the double bonds of the samples (Chan, 1987).

The Table 2 showed that *IV* decreased slowly from 0 hour to 300 hours proportionally to the heating temperature and the exposed time. The *IV* of original palm oil at 0 hour is 62.9439 and decreased to 44.1721 after 300 hours of heating. The decreasing of *IV* means that the heating treatment will cause the unsaturated part becomes less while the saturated part increases. This indicated that thermal able to destruct the double bonds of the oil samples (O'Brien, 2004).

Table 2: Summary of Iodine Value ($gI_2/100g$) with hours for original palm oil and palm oil with 1 % *Coleus aromaticus* extract.

	Iodine Value (*IV*) ($gI_2/100g$)	
Hour	Palm Oil	Palm Oil + 1% Extract
0	62.9439	63.2759
50	54.8859	60.7600
100	52.0729	58.3993
150	48.9048	56.5052
200	47.6664	55.2288
250	47.3389	52.3364
300	44.1721	51.1388

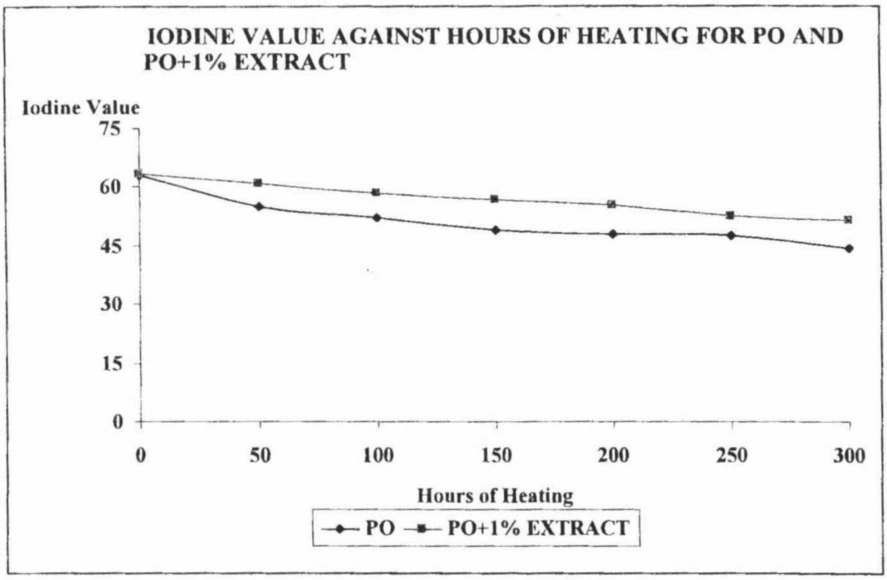

Figure 2: Iodine Value against Hour of Heating for Palm Oil and Palm Oil + 1% Extract.

The iodine value for palm oil with *Coleus aromaticus* extract has less decreased compared to palm oil without extract as shown in Figure 2. It shows that it has better thermal-oxidative stability which it can reduce the saturation of double bond in palm oil.

Peroxide Value

The peroxide value (PV) was used to indicate the level of peroxides that produced from the oxidation process. Table 3 shows that the peroxide value of original palm oil is fluctuated. In the beginning of 100 hours, the peroxide value of original palm oil increased correlates to hour of heating. This due to formation of hydro peroxide of unsaturated fatty acid that were obtained as a result of lipid oxidation (Chan, 1987).

Table 3: Summary of Peroxide Value with hours for original palm oil and palm oil with *Coleus aromaticus* extract.

	Peroxide Value (PV) (mg KOH/g)	
Hour	Palm Oil	Palm Oil + 1% Extract
0	3.5987	2.3989
50	5.5949	3.5969
100	7.5939	3.5970
150	9.5996	7.5909
200	15.5975	13.5809
250	11.5579	9.5847
300	23.5209	21.5225

The Figure 3 shows the palm oil with 1% extracts decreasing in peroxide value against hours of heating. From the graph plotted, shows *Coleus aromaticus* extract started to prevent the oil sample from the formation of hydro peroxide in the early stage. It indicates that *Coleus aromaticus* extract can function very well as antioxidant to maintain the lower PV in the heating process.

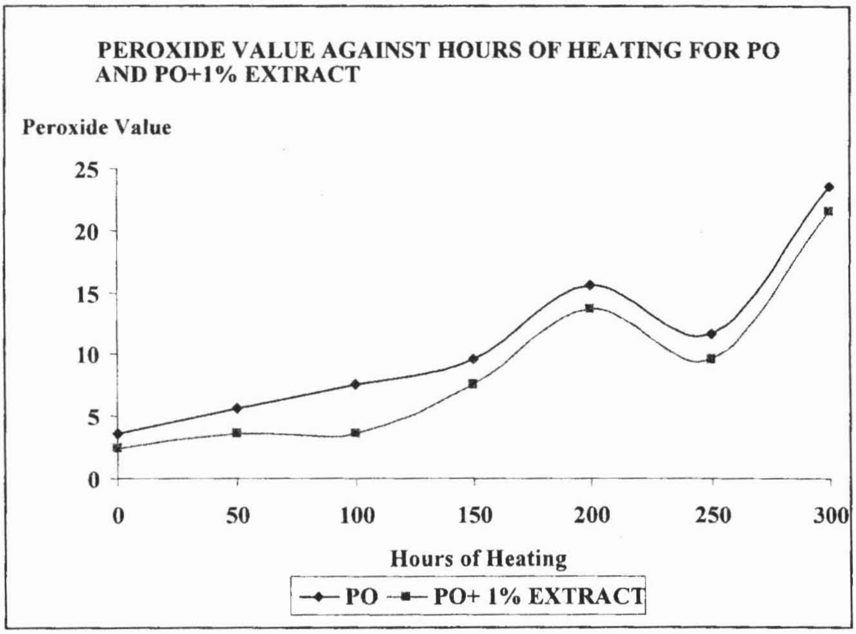

Figure 3: Peroxide Value against Hour of Heating for Palm Oil and Palm Oil + 1% Extract.

Infrared Spectroscopy Analysis

The IR spectrum of palm oil and palm oil with 1% extract at 300 hours of heating is shown in Figure 4 (PO) and Figure 5 (PO+1% Extract), The changes of the structures of main functional group of palm oil without extract and palm oil with *Coleus aromaticus* extract after heating was observed. The CH stretching from the alkane at 2925 $cm^{-1}$and 2855 cm^{-1} , while at absorption band of 3005 cm^{-1} also gives the functional group of CH stretching but in alkene structure of C-C-H. The structure of C=O stretch of triglyceride ester and fatty acid can be identified from absorption band at 1746 cm^{-1}.

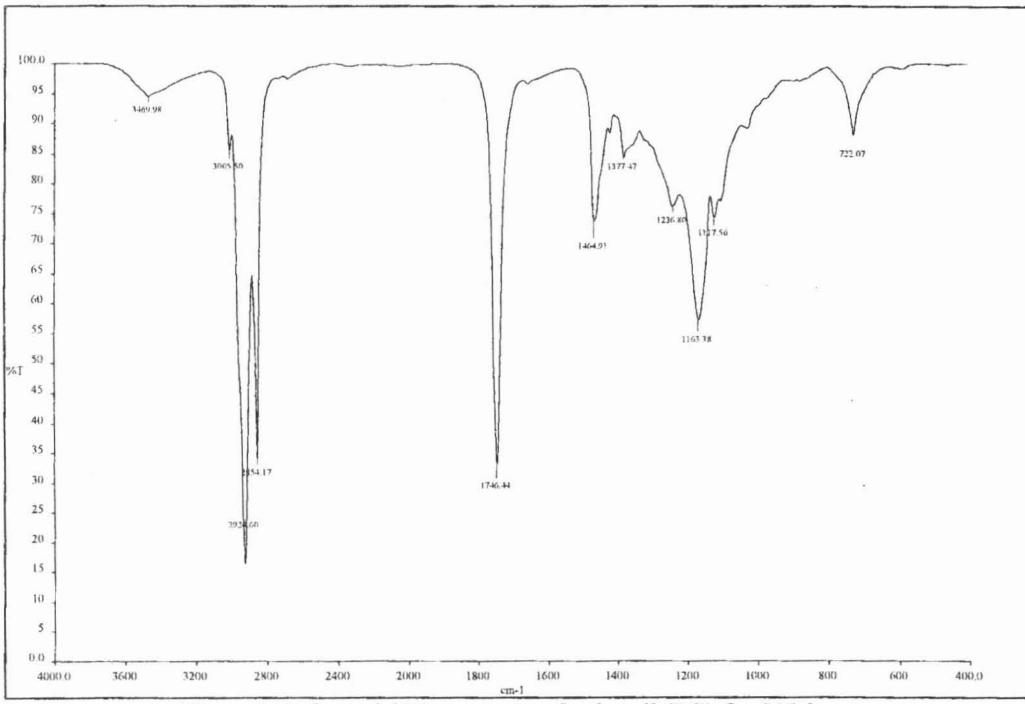

Figure 4: Infra-red (IR) spectrum of palm oil (PO) for 300 hours

Figure 4 shows the oxidation occurred causes the O-H region band near 3470 cm^{-1} become wider compared with palm oil with extract in figure 5. It might be due to the increased hydroperoxide group that were obtained as a result of lipid oxidation (Frankel, 1984). Therefore, the palm oil with *Coleus aromaticus* extract is more stable which decreased the formation of the hydroperoxide in the palm oil toward thermal oxidative stability.

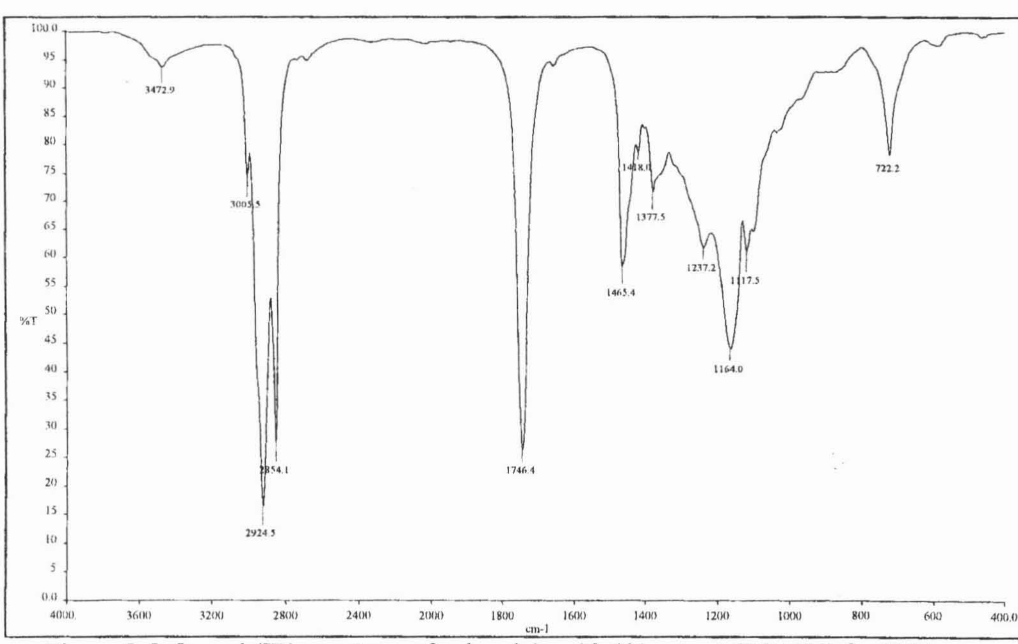

Figure 5: Infra-red (IR) spectrum of palm olein with 1% extract (PO+1%) for 300 hours.

Conclusion

The heating of palm oil with *Coleus aromaticus* extract increases the oxidative stability of palm oil. It can be shown by the lower Peroxide Value (PV), lower Total Acid Number (TAN) value and relatively higher Iodine Value (IV) for all heating time. Therefore, it could be concluded that the *Coleus aromaticus* leaves extract can act as a natural antioxidant which improves the oxidative stability of palm oil.

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