Research Article

Effect of *Flacourtia inermis* Peel Extract on Oxidative Stability of Sunflower Oil and Virgin Coconut Oil

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Abstract

Flacourtia inermis (Sinhala name: Lovi) is a popular underutilized fruit grown in Sri Lanka. The present study aimed to determine the effect of lovi peel extract on oxidative stability of selected edible oils. Lovi peel extract was obtained by sonication with 70% ethanol. Its antioxidant activity was measured as IC_{50} (μgmL^{-1}) and total phenol content was measured as gallic acid equivalent (GAE) using 2,2-diphenyl-1-picrylhydrazyl assay and Follin Ciocalteu assay. Sunflower oil and virgin coconut oil were treated with three different levels of lovi peel extract (500, 1000 and 2000 ppm) and positive control: α-tocopherol at 500 ppm. These samples were stored at 65±5°C for 21 days and their free fatty acid contents and peroxide value were measured at 3-day intervals using AOAC methods. Antioxidant activity (IC_{50}) and total phenol content of lovi peel extract were 227.14 ± 4.12 μgmL^{-1} and 4.87 ± 0.01 mg GAE/g respectively. Free fatty acid content of sunflower oil (0.10%) and virgin coconut oil (0.28%) treated with 2000 ppm level of lovi peel extract were significantly lower than their positive controls; sunflower oil (0.22%) and virgin coconut oil (0.29%) after 21 days of storage period. Further, peroxide values of sunflower oil (12.38%) and virgin coconut oil (1.19%) treated with 2000 ppm level of lovi peel extract were significantly lower than their positive controls; sunflower oil (17.94%) and virgin coconut oil (1.39%) after 21 days of storage period. In conclusion, lovi peel extract is effective in controlling the oxidative deterioration of sunflower oil and virgin coconut oil at 2000 ppm level.

Keywords: *Antioxidant, free fatty acid, peroxide value*

1. Introduction

Lovi (*Flacourtia inermis*) is one of the most important seasonal fruit crop grown in Sri Lanka with promising antioxidant properties. Other than Sri Lanka, this fruit crop also found in Indonesia, Malaysia, South India, Philippines and parts of Africa. These fruits consist of sour, astringent and sweet taste. Tree is moderate sized *and* fruits are deep red in colour. Fruits provide essential nutrients for human diet and reduce wide range of diseases (Alakolanga *et al.*, 2014).

Oils play an important role in human diet and widely used in food production. Edible oils consist of high levels of polyunsaturated fatty acids which lead to oxidation process. Oxidation of oil is one of the major concerns in food production sector. It associated with many adverse changes including off-flavour generation, rancid odours, discolouration, and textural changes losses of nutritional quality and shortens the shelf life of the product due to the development of toxic and oxidized compounds such as ketones, aldehydes and various organic acids (Lercker & Rodriguez-Estrada, 2002).

Influx of literature has proven the positive effect of natural antioxidant on oxidative stability of oils mostly after addition of plant extract during accelerated storage. Similarly, antioxidant activity of pomegranate peel extract in stabilization of sunflower oil under accelerated condition (Iqbal *et al.*, 2008), antioxidant efficacy of unripe banana peel extract in sunflower oil during accelerated storage (Ling *et al.*, 2015), oxidative stability ghee as affected by natural antioxidants extracted from food processing wastes (EI-Shourbagy *et al.*, 2014) and oxidative stability of vegetable oils as affected by sesame extracts during accelerated oxidative storage (Abdelazim *et al.*, 2013) have been reported.

Though many studies acknowledged the antioxidant, antifungal and antibiotic activity of *F. inermis*, studies directed to investigate the effect of *lovi* peel extracts on oxidative stability of edible oils are still scanty. Moreover, significant amount of by-products are generated with increasing production of fruit products giving rise to many negative economic and environmental problems. The conversion of unutilized by-products into valuable products could be a good alternative to reduce waste and ensure the environmental safety. *Lovi* peel is the part of plant biomass waste which serves as potential source of natural antioxidants. Thus, in this backdrop the effect of *F. inermis* peel extract as natural antioxidant on oxidative stability of sunflower and virgin coconut oil was determined at accelerated conditions for 21 days.

2. Experimental Section

2.1 Extraction of Natural Antioxidant

Well ripen *lovi* fruits were collected from the Western Province of Sri Lanka. Freeze dried fruit peel was ground to a fine powder with a laboratory grinder. Dried, powdered peel was sieved (mesh size; 1 mm) and stored at -18°C temperature until extraction. Freeze dried powder (20 g) was dissolved in 200 mL of 70% ethanol and extracted using ultrasonic cleaner (model UC -10 A, China, Input 220/50 Hz, frequency 40 kHz, 50 w, Tank size L 150 × W 135× H 100,) at 40 ±5°C for 30 minutes. Extract was filtered through Whatman No.1 filter paper and residues were re-extracted twice to obtain maximum yield. Combined extracts concentrated in *vacuo* (model HS-2005V-N, Input 200-240 V, manufactured by

HAHNSHIN scientific Ltd) at 40±5°C temperature. Crude extract was stored at 4°C until the analysis.

2.2 Analysis of Crude Extract

2.2.1 Determination of Total Phenol Content

Total phenol content was determined according to the method of ISO 14502-1 with slight modifications. First, crude extract was dissolved in 70% methanol to obtain 60 mg/L solution. Tenfold diluted Follin Ciocalteu reagent (1.0 mL) was added to 200 µL plant extract and kept for 8 minutes. Then 800 µL of 7.5% (w/v) Na_2CO_3 (aq) was added and kept for 1 hour. The absorbance was measured at wavelength 765 nm (UV/Visible SP-UV 50 VDB spectrophotometer). Gallic acid was used as a standard and calibration curve was plotted using the absorbance values of gallic acid solution series. The phenolic content of plant extracts was calculated using the calibration curve and express as mg of gallic acid equivalents (GAE)/g extract.

2.2.2 Determination Antioxidant Activity

The crude extracts (150-325 ppm) were tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Ali *et al.,* 2018). Each solution (50 µL) was mixed with 50 µL of 4×10^{-4} M methanolic DPPH Solution and kept in the dark at room temperature for 20 minutes. The absorbance was measured at 517 nm using microplate spectrophtometer (Multiskan Go, Version100.40, Thermo Fisher Scientific) against a reaction blank (50 µL of Methanol and 50 µL of the crude extract). A mixture of 50 µL of 4×10^{-4} M DPPH solution and 50 µL of methanol was used as a reaction control. Percent inhibition was calculated for each concentration and IC50 was calculated for crude extract. Below mentioned equation was used to calculate percent inhibition.

$$\text{Present Inhibition} = \frac{\text{Ac - As}}{\text{Ac}} \times 100 \quad (1)$$

where,

A_c- Absorbance of reaction control

A_s- Absorbance of the test sample

2.3 Storage Study of Edible Oils

2.3.1 Sample Preparation for Storage of Oils

Oil samples with crude extract were prepared with three different concentrations *viz* 500 ppm, 1000 ppm and 2000 ppm along with control (no added peel extract) and α - tocopherol (500 ppm) was used as positive control. First, oil sample was pre heated in a water bath at 50°C for 3 hours. Crude extract of fruit peel were

dissolved in a 150 µL of absolute ethanol to facilitate uniform dispersion of extract in oil. Oil samples with different concentration of extracts were kept in an ultrasonic water bath at 60°C for 30 minutes to obtain a homogenous dispersed sample. Storage study of oils were conducted as per the method describe by Besbes *et al.,* 2004 with slight modifications. Treated oils were completely filled into glass bottles (volume 25 mL) wrapped with aluminium foil, sealed properly and stored in an oven at 65±5°C for 21 days. Oil samples were drawn at three days intervals for analysis. Three replicates were used for the storage study.

2.3.2 Determination of Peroxide Value (PV)

Peroxide values of oil samples drawn at 3-days intervals were determined according to the standard procedures described as AOAC 965.33 (2016). Five grams of oil sample was dissolved in 30 mL of acetic acid: chloroform solution (3:2).Then, 0.5 mL of saturated KI solution was added into it and kept to stand with occasional shaking for one minute and placed in the dark for 5 minutes. Then, 30 mL of distilled water was added and mixture was titrated slowly with 0.01 M sodium thiosulfate. A blank was conducted according to same procedure without addition of oil. According to the following equation peroxide value of all samples was calculated as milliequivalents/kg (mEq/kg).

$$\text{Peroxide value (mEq/kg)} = \frac{S \times M \times 1000}{\text{Sample weight (g)}} \qquad (2)$$

where,

S - Volume of $Na_2S_2O_3$ used (blank corrected)

M - Molarity of $Na_2S_2O_3$

2.3.3 Determination of Free Fatty Acid (FFA) Content

Free fatty acid contents of oil samples drawn at 3-days intervals were determined according to the procedure described as AOAC940.28 (2016). Accurately weighted oil sample was dissolved in 20 mL of previously neutralized absolute ethanol. The mixture was titrated with 0.05 M sodium hydroxide in the presence of phenolphthalein as indicator with vigorous shaking until permanent faint pink colour appeared and persisted for more than 1 minute. Percentage of free fatty acids of each oil samples was calculated as oleic acid using the following equation.

$$\%\ \text{FFA (oleic acid)} = \frac{V \times C \times 282.46 \times 100}{M} \qquad (3)$$

where,

V - Volume of sodium hydroxide reacts in titration (mL)

C - Concentration of sodium hydroxide (M)

M - Mass of oil sample (g)

2.4 Statistical Analysis

Mean values of all data were obtained from triplicate determinations. Values were expressed are mean ± SD. Data were subjected to analysis of variance (ANOVA) according to the GLM (general linear model). Data processing was conducted by the Minitab 16.1 version of computer software package.

3. Results and Discussion

3.1 Antioxidant Activity and Total Phenol Content of Crude Extract

Varieties of methods are documented to assess the antioxidant activity of plant materials. In the present study DPPH assay was employed to determine the antioxidant activity of LPE. Results of the present study revealed that antioxidant activity of *lovi* peel extract (LPE) measured as IC_{50} (227.140 ± 4.12 μgmL^{-1}) was significantly ($p<0.05$) higher than that of α-tocopherol (29.795 ± 3.22 μgmL^{-1}). Therefore, antioxidant activity of LPE is significantly lower than that of α-tocopherol. Alakolanga *et al.,* (2015) have observed comparatively high antioxidant activity (66.2 ppm) in ethyl acetate extract of *F. inermis* whole fruit. However, methanolic extract of the reported study exhibited comparatively low antioxidant activity (212.95 ppm). The disparity of the results may be due to the difference of the extraction technique employed and the fruit material used by two studies.

The total phenolic content of a LPE was calculated based on the calibration curve prepared using gallic acid as standard. Total phenol content of LPE was 4.87 ± 0.01 mg GAE per g extract. However, the study of Alakolanga and others (2015) has reported the polyphenol content of *lovi* fruit as 1.28 g gallic acid equivalents per 100 g of fresh fruit. Extraction solvent, extraction temperature and time, particle size and the nature of fruit material may contribute to the disparity of the results.

3.2 Variation of Free Fatty Acid Content (FFA) of Virgin Coconut Oil with Added LPE

FFAs are responsible for undesirable flavour and aroma development in oils, thus FFA content serve as one of the important quality parameters of oils. Various chemical reactions are contributed for the formation of FFA such as hydrolysis,

oxidation due to free radical formation and cleavage of double bonds during frying (Ma *et al.*, 2015). Initial quality of the VCO used for the present study was good as proven by the comparatively low FFA content (0.26±0.04%). The variation of FFA content of VCO samples treated with LPE is shown in Figure 1 along with the control and positive control, α-tocopherol.

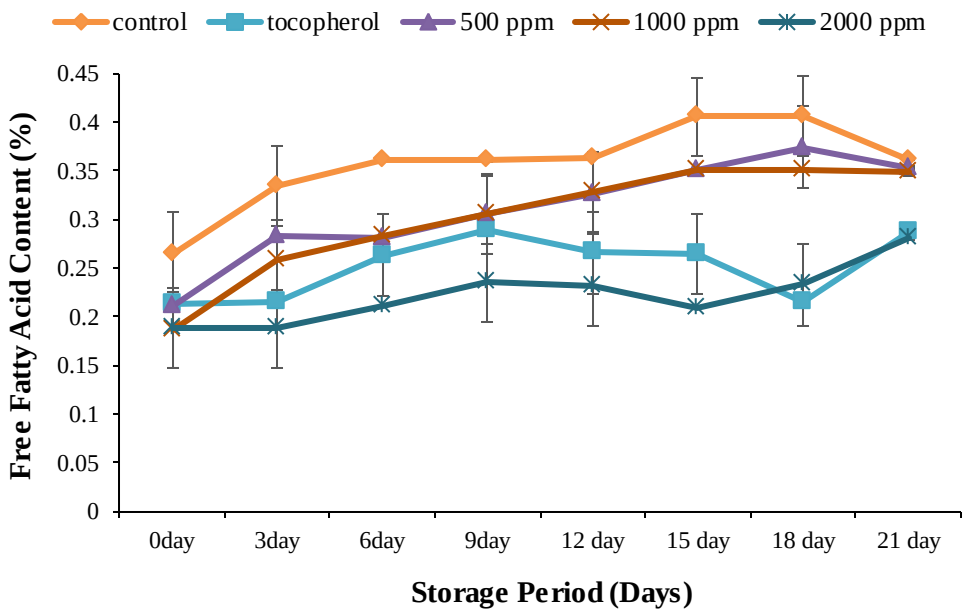

Figure 1: Variation of free fatty acid content of virgin coconut oil with added *lovi* peel extracts

Initially FFA content of treated and nontreated VCO samples were not significantly different from each other. With the time FFA contents of all the samples increased as shown in Figure 1. After 21 days of storage VCO (control) sample exhibited the highest FFA content (0.36 ±0.00%) while VCO sample treated with 2000 ppm level of LPE had the lowest FFA value (0.28±0.00%). Further, after 21 days of storage period FFA content of oil sample treated with 2000 ppm level of LPE was significantly lower than that of positive control the α-tocopherol added sample (0.29±0.00 %). Thus, LPE at 2000 ppm concentration level is more effective in delaying the deteriorative oxidation reactions compared to that of positive control at 500 ppm.

The variation of FFA content of SO sample against the positive control α-tocopherol is illustrated in Figure 2. FFA content of SO sample ranged from 0.10±0.04% to 0.29±0.00% after 21 days of accelerated storage period. FFA content of all samples were not significantly different in initial stages but FFA content of all SO samples increased with the storage time. FFA content of the

control sample was not significantly different from that of 500 ppm and 1000 ppm LPE added samples. FFA content of 2000 ppm LPE added SO sample (0.10±0.04%) was significantly ($p<0.05$) lower than that of the positive control (0.22±0.00%) after 21 days of storage period. Research results have proven that 2000 ppm level of LPE added oil has positive impact on oxidative stability of SO under study conditions.

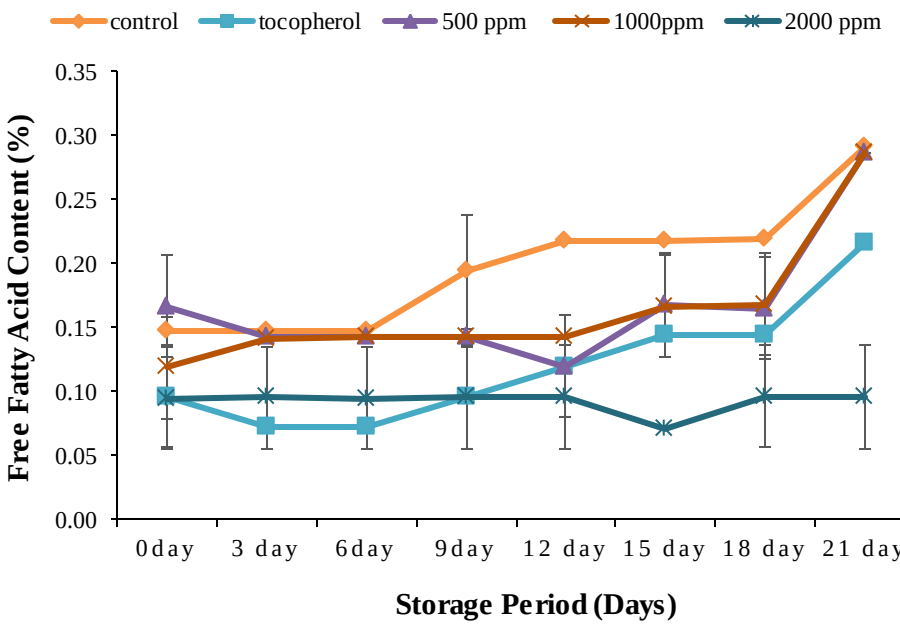

Figure 2: Variation of free fatty acid content of sunflower oil with added *lovi* peel extracts

3.3 Variation of Peroxide Value (PV) of Virgin Coconut Oil with Added LPE

Peroxide value use to measure the progression of lipid oxidation as it measures the peroxide formation in early stages of lipid oxidation. The peroxide values of VCO samples treated with LPE are shown in Figure 3 along with the control and positive control α-tocopherol. The peroxide value of the treated samples showed no significant difference ($p>0.05$) for first 3 days. There was no significant difference between control and 500 ppm, 1000 ppm levels during the time period between day 6 and days 18. However, PV of all oil samples increased during storage and thereby indicated the progression of lipid oxidation. Several studies have proven that PV of VCO increased during storage at 63°C (Rohman *et al.*, 2011). Conversely, initial PV of reported study was lower (0.42±0.009 meq/kg oil) than that of the present study (1.20±0.00 meq/kg oil).

The highest peroxide value (1.93±0.12 meq/kg oil) was observed in control sample after 21 days of storage. Meanwhile, the lowest peroxide value (1.19±0.01 meq/kg oil) was reported in the VCO sample treated with 2000 ppm level of LPE after 21 days of storage (Figure 3). There was no significant difference between the VCO treated with α- tocopherol (1.39±0.00 meq/kg oil) and the oil treated with 2000 ppm level of LPE after 21 days storage. Thus, indicates the effectiveness of LPE at higher concentration in attenuating the lipid oxidation.

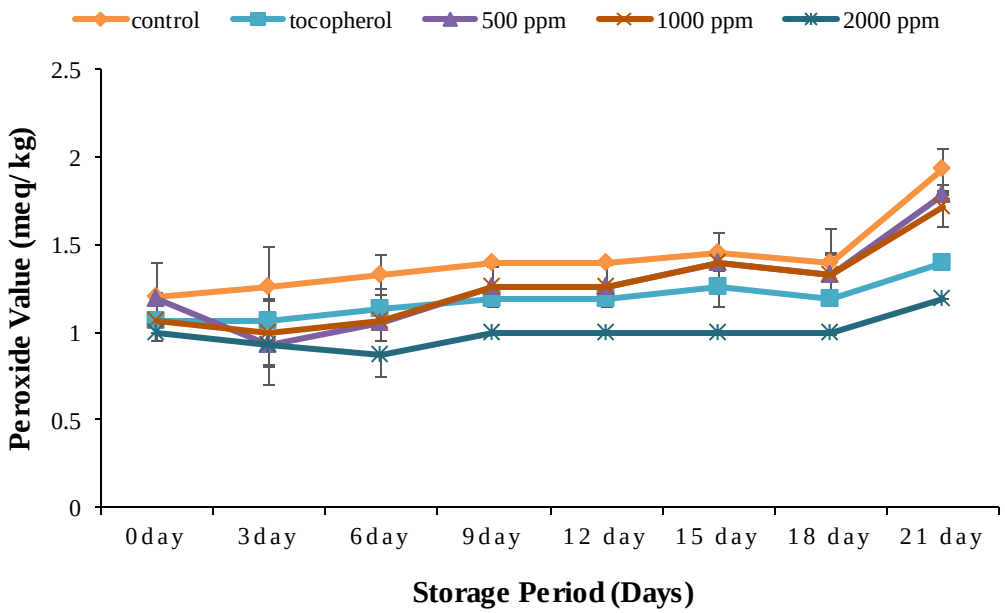

Figure 3: Variation of peroxide value of virgin coconut oil with added *lovi* peel extracts

Peroxide values of SO samples treated with LPE are shown in Figure 4. There was no significant difference between control and treatments until day 6. However, PVs of all tested oil samples increased with time which indicates the generation of hydroperoxides in SO (Szydłowska-Czerniak & Rabiej, 2018). Moreover, PVs of sunflower oil samples were higer than that of VCO owing to high amount of unsaturated fatty acids present in SO which are more prone to oxidation (Wójcickiwt *et al.*, 2015). From day 9 onwards PV of SO treated with 2000 ppm level of LPE was significantly ($p<0.05$) lower than that of positive control, α- tocopherol. The PV of oil sample treated with 2000 ppm level of LPE was 12.38±0.46 meq /kg after 21 days of storage period. Meanwhile, PVs of control and α- tocopherol treated samples were recorded as 28.30±0.58 meq/kg and 17.94±0.35 meq/ kg, respectively.

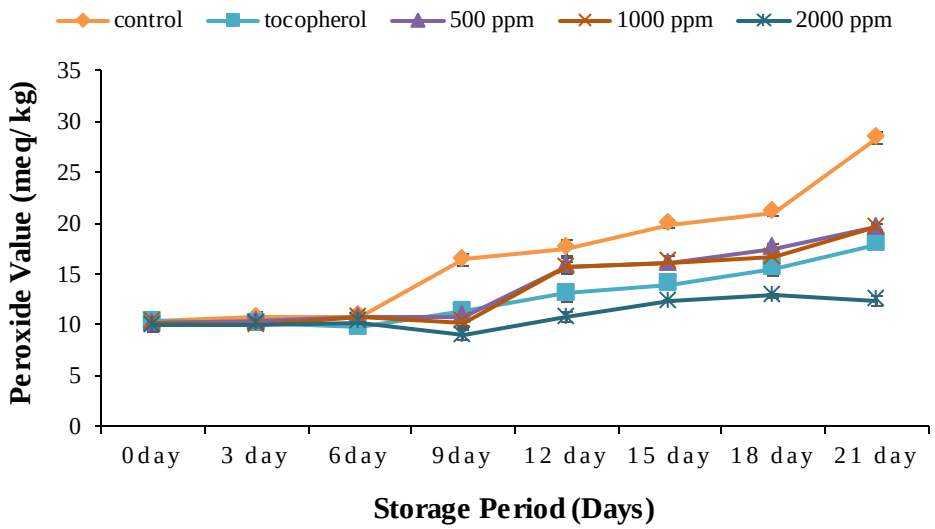

Figure 4: Variation of peroxide value of sunflower oil with added *lovi* peel extracts

FFA content and PV of treated and non-treated VCO samples and SO samples after 21 days of storage period are shown in Figure 5. As illustrated in the Figure 5, FFA content of VCO samples were higher than that of SO. Contrary, SO samples have recorded comparatively higher PVs than VCO samples. However, in both oil samples LPE has demonstrated positive effect on controlling deteriorative oxidation reactions.

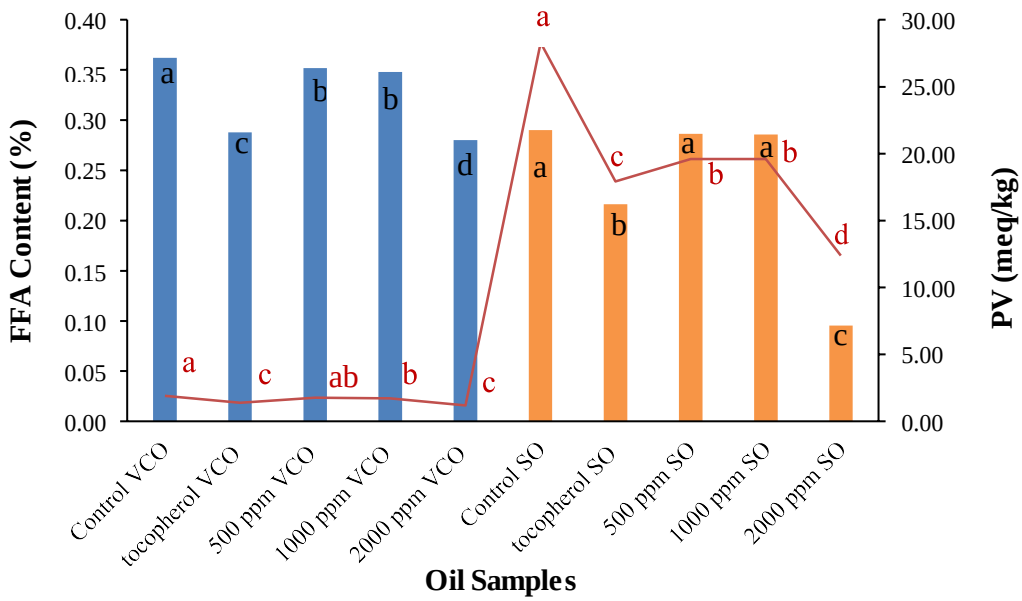

Figure 5: Variation of FFA content and PV of treated and non-treated VCO and SO samples a,b,c,d different letters in the graph indicate significant difference at $p< 0.05$

4. Conclusion

Results of the present study acknowledge that peel of *Flacourtia inermis* as potent source of natural antioxidant with comparatively high total phenolic content. Further, the study has revealed the effectiveness of *F. inermis* peel extract in controlling the lipid oxidation of virgin coconut oil and sunflower oil at 2000 ppm concentration level. Moreover, the findings of storage study have confirmed that the *F. inermis* peel extract could be used as alternative to α-tocopherol to improve the oxidative stability of virgin coconut oil and sunflower oil at higher concentration levels.

5. Acknowledgements

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6. Referencess

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