ANTIOXIDATIVE EFFECT OF CHAYA LEAF EXTRACT ON REFINED SOYBEAN OIL

BY

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OUTLINES

- **INTRODUCTION**
- **MATERIALS AND METHODS**
- **RESULTS AND DISCUSSION**
- **CONCLUSION AND RECOMMENDATION**
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ABSTRACT

- *The antioxidative effect of methanol and water chaya leaf extracts on Refined Soybean Oil (RSBO) stored for twelve months at room temperature (27^0C-33^0C) was determined by monitoring the colour, refractive index, free fatty acid (FFA), acid value (AV) and peroxide value (PV)*
- *Extracts of chaya leaf were prepared by separately dissolving dried, ground and sieved orange peel into acetone, chloroform, ethylacetate, methanol and water in ratio 1:10 for 72hours*
- *The methanol chaya leaf extract (MCLE) and water chaya leaf extract (WCLE) were separately added at varying concentrations (200 ppm to 1000 ppm) to RSBO*
- *The oil was also dosed with 200 ppm butylatedhydroxytoluene (BHT)*
- *Maximum yield of extracts were obtained with water (14.33±1.10%) and methanol (12.56±0.98%)*
- *The colour and refractive index of RSBO containing the extracts ranged between 10.0-18.5 units and 1.470-1.472 respectively whereas the colour and refractive index of RSBO with no additive (0 ppm) and 200 ppm BHT were 8.0-10.0 units and 1.470 accordingly*
- *The FFA and AV of RSBO containing methanol (expect 200 ppm) and water chaya leaf extracts were lower than RSBO containing no additive and 200 ppm BHT*
- *The PV of RSBO containing chaya leaf extracts were lower than PV of RSBO that contained no additive and 200 ppm BHT*
- *The methanol and water chaya leaf extracts are more effective than butylatedhydroxytoluene in combating both hydrolytic and oxidative rancidity of RSBO.*

INTRODUCTION

- **1.1 Refined Soybean oil**
  - **Rich in polyunsaturated fatty acids**
  - **Containing decreased total cholesterol, low density lipoprotein cholesterol**
  - **Containing beneficial high density lipoprotein cholesterol (Yanishlieva and Marinova, 2001)**
  - **Good sources of essential fatty acids (Linoleic acid, Linolenic acid) (Odoemelam 2005;Salma & Tanveer, 2005; Onyeike *et al*., 2000).**

INTRODUCTION CONT'D

1.2 Major Storage Problems of Refined Soybean oil

- Development of rancidity (hydrolytic and oxidative)
- Unnecessary economic losses
- Off-flavour quality
- Deterioration of taste of foods
- Colour defects
- Health problems e.g atherosclerosis, stroke, hypertension, heart attack, obesity etc.(Ullah *et al*., 2003; Manji *et al*., 2006)

INTRODUCTION CONT'D

1.3 Prevention of Edible Oils Quality Deterioration

- **Use of Synthetic antioxidants e.g BHA, BHT, PG, TBHQ etc**
- **Use of Natural antioxidants e.g beta-carotene, tocopherol, polyphenols, natural colourants, phytochemicals, Vitamin C etc.**

INTRODUCTION CONT'D

1.4 ANTIOXIDANTS

- **Increase shelf life of lipids by reducing process of lipid peroxidation**
- **Inhibit attack by oxygen/ozone,**
- **Reducing agents**
- **Slow or prevent oxidation of other chemicals**

(Aluyor & Ori-Jesu, 2008; Goli *et al.*,2006).

QUALITIES OF AN IDEAL ANTIOXIDANT FOR LIPID

- **Have no harmful physiological effect**
- **Constitute no objctionable flavour, odour or colour**
- **Effective in low concentration**
- **Be lipid soluble**
- **Be ready availabe**
- **Be economical**
- **Be persistently following processing to provide effective protection to food (Fennema, 1997)**

PREVIOUS RESEARCH

- **Rehab 2010 reported the improvement of the stability of fried sunflower oil by using different levels of pomposia (*Syzygium cumini*)**
- **Effectiveness of adding natural antioxidants to sunflower was studied by Carelli *et al.*, 2005**
- **Mian et al., 2003 studied the antioxidative effect of red peper oil in edible oils**
- **The effect of light, red pepper oil (at 0.5 and 0.1%) and BHT (at 0.02%) antioxidants on stability of sunflower and soybean oils was investigated by Ullah et al., 2003**
- **In 2009, Amoo and Arawande reported the stability studies on refined soybean oil stored under light and dark conditions using tin, plastic and glass containers**
- **Goil et al.,2005 reported the antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts.**
- **Armando et al., 1998 studied the antioxidant activities of grapefruit seed extract containing tocopherol, citric acid and ascorbic acid in sunflower and soybean oils**
- **Antioxidant activity of methanolic extract of some species of phlomis and stachys on sunflower oil was investigated by Morteza-Semnani in 2006.**

PROBLEM STATEMENT/JUSTIFICATION

- **There is quality deterioration and economic loss during storage of edible oils and consumption of such oils leads to health risk due to free radicals production.**

- **And the use of synthetic preservatives called synthetic antioxidants post additional health risk because they are toxic and mutagenic hence their usage as preservatives are being discouraged globally in international market.**

- **Plants are considered as good sources of antioxidants. Plant foods (fruits and vegetable) are good for human consumption because they have potential to delay the onset of many age-related diseases, and this is due to their high level of antioxidants and phytonutrient**

CHAYA LEAF

- botanically known as *Cnidoscolus acontifolus*
- an ever green leafy vegetable that is available throughout the year
- a fast growing perennial shrub
- native to the Yucatan Peninsula of Mexico and Central American
- English names of the plant are tree spinach, treadsifly and cabbage star
- its Spanish names are chaya, copapayo and papayeulo
- French names is manioc batard.
- Jatropha aconitifolia is its Latin name (Oboh, 2005; Burkilli, 1995)
- Yoruba names of the plant are Efo Iyanapaya or Efo Jerusalem

- very rich in total phenolic compound and flavonoids (kaempferol-3- O-glycosides and quercetin-3-O-glycosides) (Joseph and Hima, 2004)

Fig.1:Chaya Leaf

Fig.2:Chaya Plant (Tree Spinach)

AIM &OBJECTIVES

The aim of this research work is to examine the antioxidative pontency of Chaya leaf extracts and compare its potency with that of synthetic antioxidant (butylatedhydroxytoluene (BHT)) on preservation of refined soybean oil

The specific objectives of this research are to:

(i) **obtain extracts of Chaya Leaf using different solvent (acetone, chloroform, ethylacetate,methanol and water);**

(ii) investigate the antioxidative potential of two highest solvent yield extracts at varying concentrations (200ppm-1000ppm) on refined soybean oil;

(iii) **determine the effect of the extracts on colour and refractive index; and**

(iv) compare the antioxidant activities of the extracts (natural) with Butylated Hydroxyl Toluene (BHT) (synthetic).

EXPECTED CONTRIBUTION TO KNOWLEDGE

The research found suitable source of antioxidants for refined soybean oils that effectively replaced the costly synthetic antioxidants (BHT) that are toxic, carcinogenic and mutagenic.

MATERIALS AND METHODS

- **MATERIALS**

- **Chaya leaves were obtained in local farm areas in Owo, Ondo-States, Nigeria**

- **Refined Soybean Oil (RSBO) was purchased from JOF Ideal Family Farms Limited, Owo before it was fortified with Vitamin A**

MATERIALS AND METHOD CONT'D

Chaya Leaf Pre-treatment

- **Chaya leaf was rinsed in wate cut into smaller pieces for eas drying, air-dried, ground an finally sieved to give 40mm mes size powder prior to solven extraction and analysis (Goli *et al* 2005).**

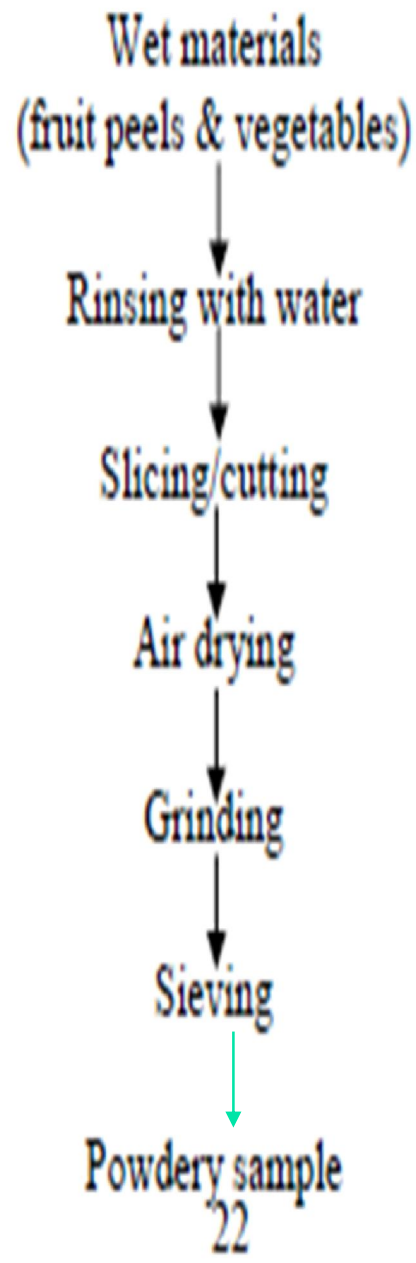

Fig 2.1: Flow chart of Plant Material Pretreatment.

Preparation and Extraction of Chaya Leaf

- **Ten grams of the powdery sample was weighed into five cleaned and dried reagent bottles**
- **100ml of each solvent (methanol, ethhylacetate, acetone, water and chloroform) was separately added to each bottle and left for 72hours during which it was intermittently shaken on a shaking orbit machine**
- **The mixture was filtered through a 0.45µm Nylon membrane filter**
- **The extracts were evaporated to dryness under reduced pressure at 40^0C by a rotary evaporator**
- **Weight of extract obtained was used to calculate the percent yield of extract in each solvent**

(Arawande and Komolafe, 2010; Amir *et al.*, 2005).

ADDITION OF ADDITIVES TO REFINEDSOYBEAN OIL

- **Vary concentrations (200ppm to 1000ppm i.e 0.02g/100g to 0.10g/100g Oil sample) of methanol and water extracts were separately added to oil samples (RSBO)**

- **Oil sample was mixed with synthetic antioxidant of BHT(200ppm i.e 0.02g/100g Oil)**

- **Oil sample without any additive (antioxidant) was used as a control.**

- **Plastic containers of equal capacity were used.**

- **Stored at room temperature ranging from 27^0C to 33^0C.**

MATERIALS AND METHODS CONTD

Determination Of Antioxidative Performance Of Water And Methanol Bioactive Extracts Of Chaya leaf on Refined Soybean Oil

- Refractive Index using Abbey Refractometer
- Colour using Lovibond Tintometer
- Free Fatty Acid (FFA) (determined monthly for 12months)
- Acid Value (AV) (determined monthly for 12months)
- Peroxide Value (PV)(determined monthly for 12months)

(AOCS, 2004)

RESULTS AND DISCUSSION

Table1: Extractive Value (% Yield) of Chaya Leaf

Solvent	*Extractive Value (% Yield)
Acetone	6.17 ^{b} ±0.21
Chloroform	4.98 ^{a} ±0.13
Ethylacetate	6.54 ^{b} ±0.18
Methanol	12.56 ^{c} ±0.98
Water	14.33 ^{c} ±1.10

NOTE: Within column, mean values followed by the same superscript are not significantly different at P ‹ 0.05 level according to Duncan Multiple Range Test (DMRT).;

***Mean Value of triplicate determination ± Standard Deviation**

Table2: Changes in Colour and Refractive Index of Refined Soybean Oil stored with varying concentration of Methanol and Water Chaya Leaf Extract and 200ppm BHT.

Concentration of Additive	Colour(Units) in 1 inch cell	Refractive Index at 40^0C
0ppm(No additive)	1R+3Y=8.0	1.470
200ppm MCLE	1R+7Y=12.0	1.470
400ppm MCLE	1R+7Y=12.0	1.471
600ppm MCLE	1.2R+9Y=15.0	1.471
800ppm MCLE	1.5R+10Y=17.5	1.472
1000ppm MCLE	1.5R+11Y=18.5	1.472
200ppm WCLE	1R+5Y=10.0	1.470
400ppm WCLE	1R+6Y=11.0	1.470
600ppm WCLE	1R+6Y=11.0	1.470
800ppm WCLE	1R+6Y=11.0	1.470
1000ppm WCLE	1R+7Y=12.0	1.471
200ppm BHT	1R+5Y=10.0	1.470

MCLE= Methanol Chaya Leaf Extract; WCLE= Water Chaya Leaf Extract, BHT= Butylated hydroxyl toluene ,

R = Red Slide; Y = Yellow Slide

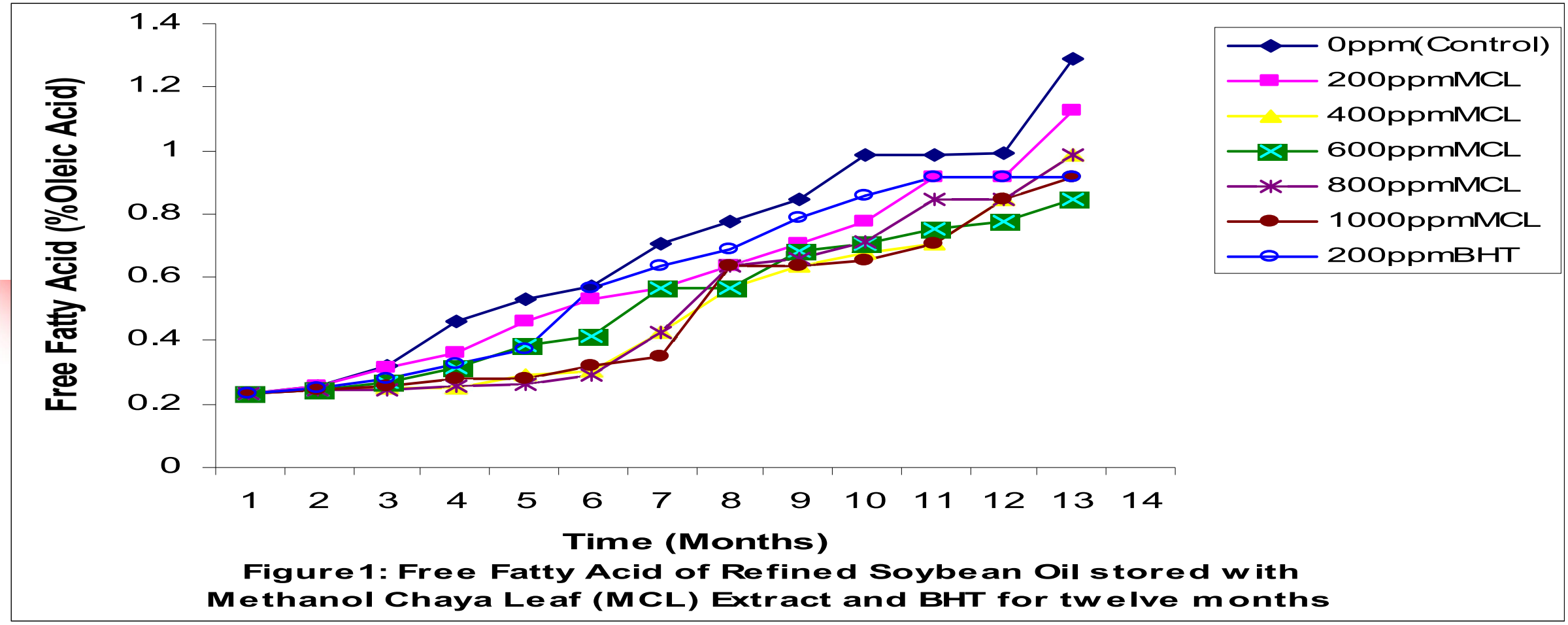

Figure1: Free Fatty Acid of Refined Soybean Oil stored with Methanol Chaya Leaf (MCL) Extract and BHT for twelve months

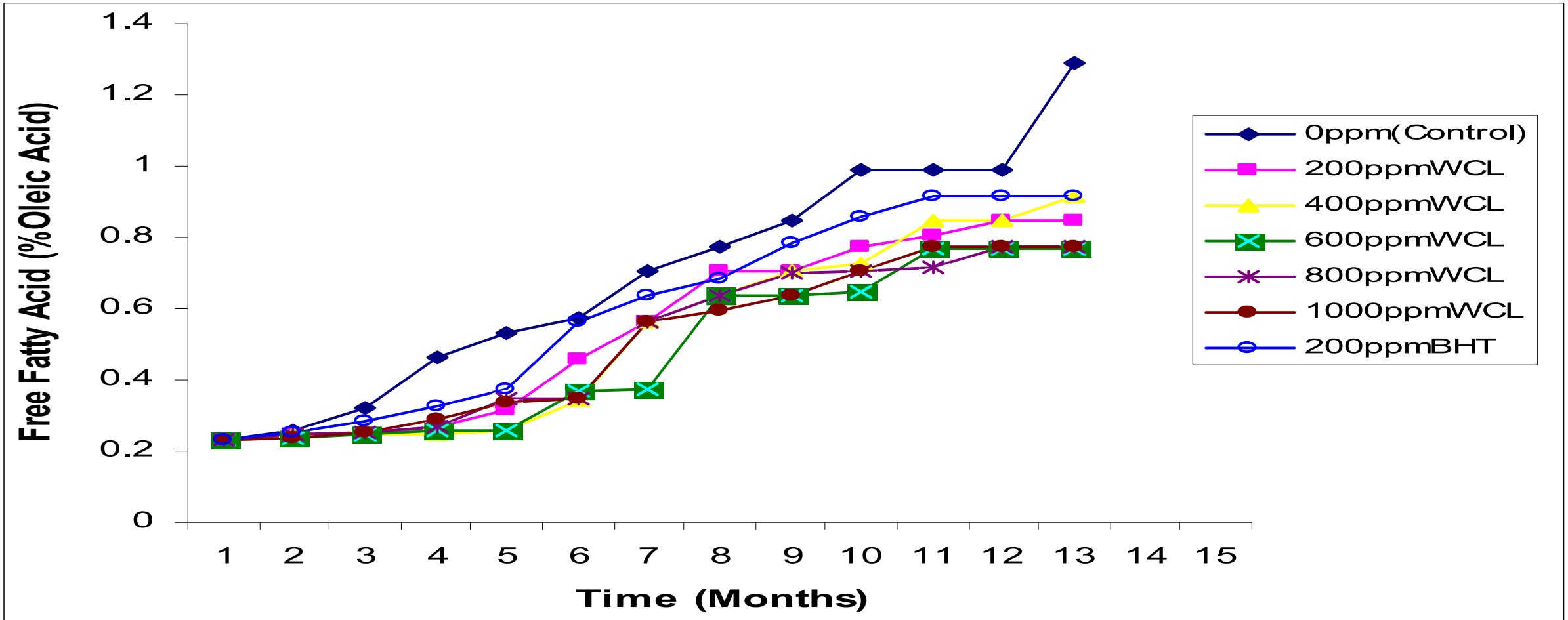

Figure2: Free Fatty Acid of Refined Soybean Oil stored with Water Chaya Leaf (WCL) Extract and BHT for twelve months

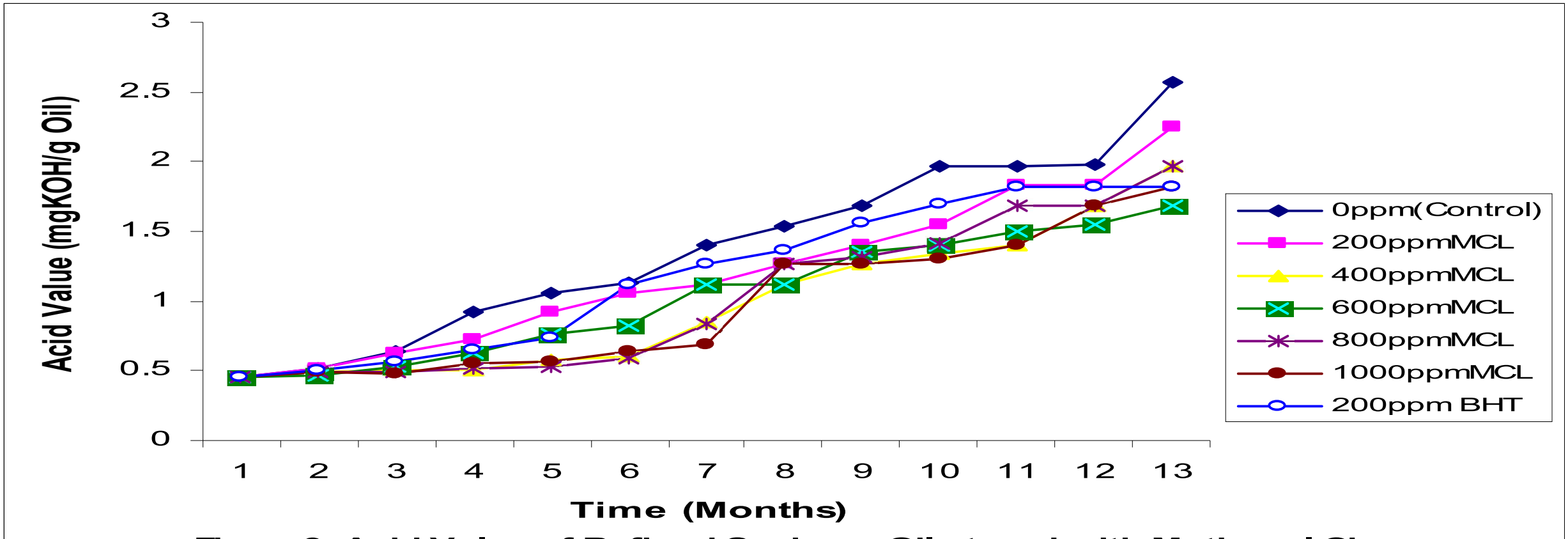

Figure3: Acid Value of Refined Soybean Oil stored with Methanol Chaya (MCL) Extract and BHT for twelve months

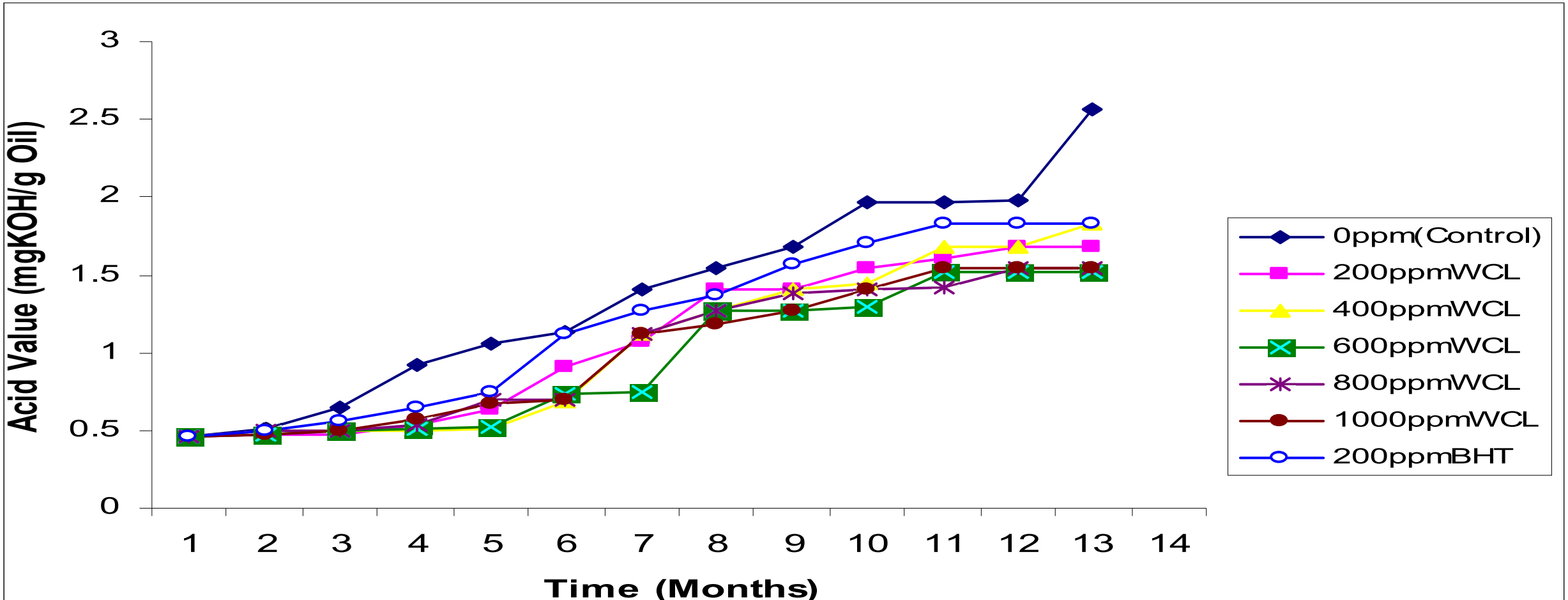

Figure4: Acid Value of Refined Soybean Oil stored with Water Chaya Leaf (WCL) Extract and BHT for twelve months

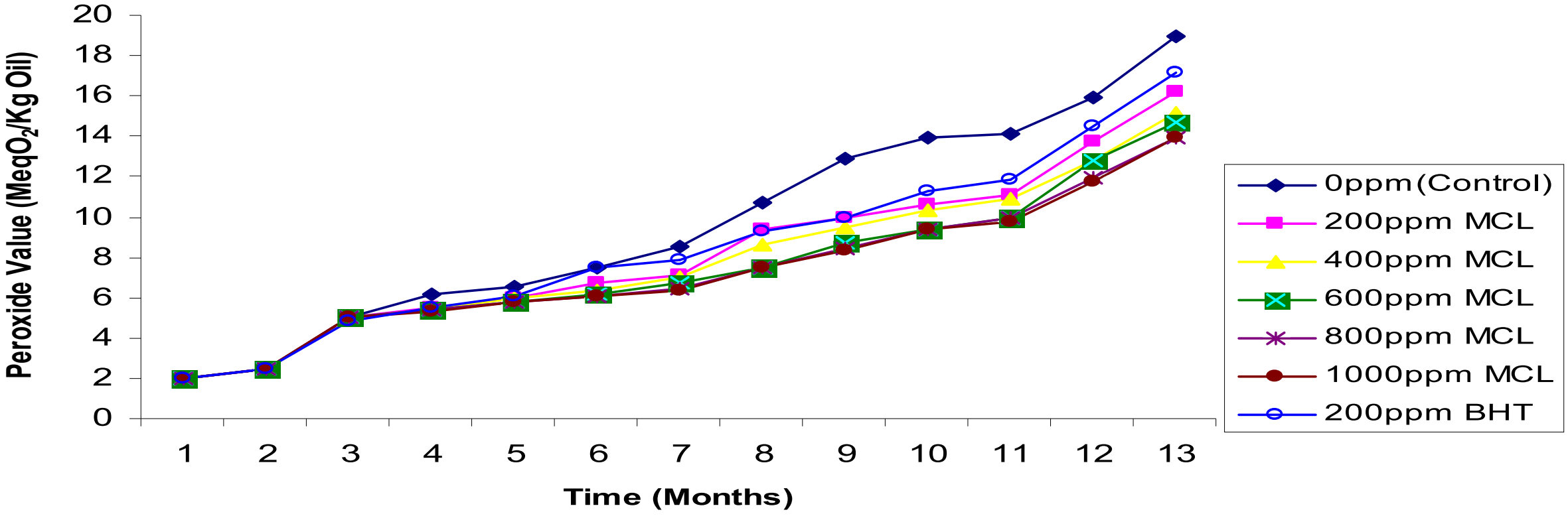

Figure5: Peroxide Value of Refined Soybean Oil stored with Methanol Chaya Leaf (MCL) Extract and BHT for twelve months

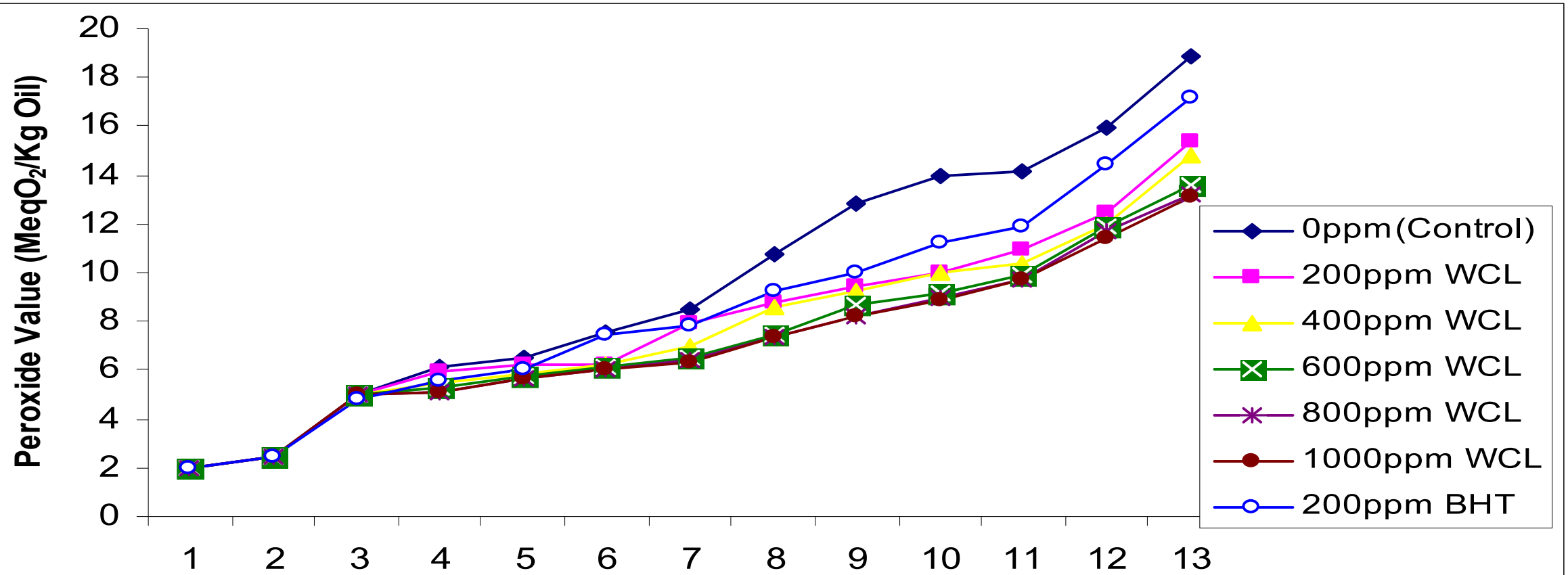

Figure6: Peroxide Value of Refined Soybean Oil stored with Water Chaya Leaf (WCL) Extract and BHT for twelve months

Table3: Mean Value of Some Selected Quality Properties of Refined Soybean Oil stored with varying concentration of Methanol and Water Chaya Leaf Extract and 200ppm BHT over a period of twelve months

Concentration of Additive	*Free Fatty Acid (FFA) (% Oleic acid)	*Acid Value (AV) (mgKOH/gOil)	*Peroxide Value (PV) ($meqO_2$/KgOil)
0ppm(No additive)	$0.689^{c} \pm 0.327$	$1.372^{c} \pm 0.653$	$9.6597^{c} \pm 5.275$
200ppm MCLE	$0.600^{b} \pm 0.800$	$1.195^{b} \pm 0.557$	$8.138^{b} \pm 4.173$
400ppm MCLE	$0.493^{a} \pm 0.258$	$0.982^{a} \pm 0.514$	$7.808^{a} \pm 3.869$
600ppm MCLE	$0.517^{ab} \pm 0.222$	$1.032^{ab} \pm 0.441$	$7.443^{a} \pm 3.684$
800ppm MCLE	$0.511^{ab} \pm 0.278$	$1.01^{ab} \pm 0.553$	$7.265^{a} \pm 2.452$
1000ppm MCLE	$0.489^{a} \pm 0.248$	$0.972^{a} \pm 0.496$	$7.205^{a} \pm 3.422$
200ppm WCLE	$0.540^{ab} \pm 0.252$	$1.067^{ab} \pm 0.506$	$7.924^{a} \pm 3.826$
400ppm WCLE	$0.525^{ab} \pm 0.268$	$1.044^{ab} \pm 0.534$	$7.618^{a} \pm 3.669$
600ppm WCLE	$0.476^{a} \pm 0.227$	$0.948^{a} \pm 0.452$	$7.209^{a} \pm 3.380$
800ppm WCLE	$0.505^{a} \pm 0.223$	$1.005^{a} \pm 0.444$	$7.073^{a} \pm 3..278$
1000ppm WCLE	$0.502^{a} \pm 0.224$	$0.998^{a} \pm 0.445$	$7.011^{a} \pm 3.225$
200ppm BHT	$0.596^{b} \pm 0.273$	$1.185^{b} \pm 0.543$	$8.477^{bc} \pm 4.476$

NOTE: Within each column, mean values followed by the same superscript are not significantly different at P ‹ 0.05 level according to Duncan Multiple Range Test (DMRT);
*Mean Value of Quality Properties ± Standard Deviation.
MCLE= Methanol Chaya Leaf Extract; WCLE= Water Chaya Leaf Extract, BHT= Butylated hydroxyl toluene

CONCLUSION & RECOMMENDATION

- Water and methanol had the highest extract values among other solvents used
- Addition of varying concentrations (200ppm-1000ppm) of both water and methanol chaya leaf extracts to RSBO had increased effects on the colour units of the oil.
- Chaya leaf extract didnot have insignificant effect on the refractive index of RSBO
- The optimal concentration of water chaya leaf extract in decreasing the hydrolytic rancidity of RSBO was 600ppm.
- Both extracts were able to reduce hydrolytic rancidity of RSBO than 200ppm BHT
- There was decrease in peroxide value of RSBO as the concentration of water and methanol extracts of chaya leaf increases
- All the varying concentrations of methanol and water chaya leaf extracts were superior to 200ppm BHT in combating oxidative rancidity of RSBO.
- Water chaya leaf extract proved superior to methanol chaya leaf extract in improving oxidative stability of refined soybean oil stored in plastic bottles.
- Water and methanol extracts of chaya leaf are potential antioxidants which can replace the synthetic antioxidant (BHT) which is costly, toxic and carcinogenic
- Higher concentrations (above 1000ppm) of these extracts can further be examined on this oil and other edible oils as well as comparing their antioxidant activities with other synthetic antioxidants such as BHA, PG etc

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THANKS FOR LISTENING

WHAT WILL FOLLOW?

(SUGGESTIONS, COMMENTS & QUESTIONS)