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Antioxidant Potential of Sericin Extracted from Silkworm (*Bombyx mori*) Cocoon on the Stability of Some Edible Oils.

A. O. Adetuyi *, T. S. Fasoto, K. F. Olupona, O. Ayodele, K. O. Olorunfemi and O. Odediran,
Department of Chemistry
The Federal University of Technology, Akure PMB 704, Nigeria
*Corresponding author's e-mail: aoadetuyi@futa.edu.ng

ABSTRACT

Antioxidant potential of silkworm cocoon extracts (sericin) on soya bean oil and palm kernel oil was investigated for a period of twelve weeks at room temperature by dosing various concentrations (0.2 - 1.0%) of the extracts into refined soya bean oil and palm kernel oil. Extract of sericin from silkworm cocoon was extracted with distilled water, 0.1 M citric acid, sodium carbonate and urea solutions. The antioxidant potential of the extracts were monitored every two weeks by measuring the acid value (AV), peroxide value (PV), free fatty acid (FFA) and the refractive index (RI) of the oil samples for twelve weeks. Physicochemical parameters such as percentage yield, volatile matter, and ash content fell within limits for the extracts and the edible oils with the exception of acid values of the soya bean and palm kernel oils which were higher than recommended values as described by FAO/WHO (2009); AOAC (2005); and Anon (2003). Antioxidant activities of all the extracts were determined using ferric reducing antioxidant power (FRAP) Fe^{2+} chelating activity (%) and DPPH radical scavenging activity (%). All the extracts considered were observed to show significant antioxidant potential with DPPH radical scavenging activity (%) of 14.94 ± 0.73, 13.78 ± 1.19, 13.18 ± 1.06, and 13.04 ± 0.75 for silkworm sericin extracted with citric acid, sodium carbonate, hot water, and urea, respectively.

Keywords: Antioxidant, Cocoon, edible oils, Sericin, Silkworm.

INTRODUCTION

The need for viable substance that will hydrolytically and oxidatively stabilize oils has been on the rise simply because some cardiovascular infections such as high blood pressure (hypertension), stroke, thrombosis and arteriosclerosis are linked to the uncontrolled ingestion of fats and oils as well as their derivative products (Ruger *et al.*, 2002; Shaker, 2006). Antioxidants can be found in various groups of compounds with relation to various reactive oxygen species. Synthetic antioxidants in the form of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely employed in food processes as a result of their ability to prevent deterioration of food and to improve their shelf life (Amir *et al.*, 2005). Synthetic chemicals such as BHA, BHT, tertiary butylated hydroxyquinine (THBQ) and propyl gallate (PG) are found useful in stabilizing edible oils and fats against oxidative rancidity (Ullah *et al.*, 2003). However, the application of synthetic antioxidant could increase the risk of cancer occurrence and damage of liver in human (Namiki, 1990). The unsafe nature (mutagenicity, toxicity, and carcinogenicity) of these synthetic materials have been looked into which has led to the stoppage of these additives in food and other food products internationally (Malecke, 2002; Murkovic, 2003; Enrol *et al.*, 2004). Therefore, there is the need for alternative sources in natural antioxidants, in line with this, there is a growing trend in finding suitable and safer natural alternatives to synthetic antioxidants

that are currently used to prevent lipid peroxidation (Marinova and Yenishlieva, 1997; Abdalla and Roozen, 1999; Rehab, 2010; Arawande and Amoo, 2009). This study is aimed at determining the utilization of silkworm cocoon (*Bombyx mori*) extract sericin- (a natural macromolecular protein of silkworm which constitutes 25-30% of silk protein) to provide effective, cheap and natural antioxidant.

Materials and Methods

The edible oil samples used were refined soya bean oil (RSBO) and refined palm kernel oil (RPKO). The oils were obtained from JOF Ideal Family Farms Limited, Owo, Nigeria before they were fortified with Vitamin A. Silkworm cocoons were obtained from Ondo State Ministry of Agriculture, Akure, Nigeria.

Preparation of the Cocoon Extract

The silkworm cocoons were washed with distilled water and thoroughly rinsed. The cocoons were cut into small sizes, dried in an oven at 105^{o}C for 6 h. 10 g of the dried sample was boiled with 250 mL of distilled water for 2 h at 80^{o}C. The boiling was stopped when the solution volume reached 100 mL. The solution was filtered and the filtrate was further boiled until the solution was reduced to 10 mL which subsequently solidified on cooling. The entire process was repeated using 250 mL of 0.1 mol/dm^3 solutions of sodium carbonate, citric acid and urea respectively for the extraction. Percentage extraction was calculated by difference in the initial 10 g mass used and the resulting solidified extract obtained (Hae *et al.*, 2000).

Determination of Antioxidant Potential of Secirin

The antioxidant potential of the extract was investigated by dosing varying concentrations (0.2 – 1.0 %) of the extracts into soya bean oil and palm kernel oil. Also, 0.2 % butylated hydroxyl toluene was added to another oil sample which served as reference sample while an oil sample without any additive served as control. All the oil samples were adequately exposed to the sun and monitored every two weeks for a period of twelve weeks by measuring the free fatty acid (FFA), acid value (AV), peroxide value (PV), and refractive index (RI) (Arawande and Amoo, 2009).

Physicochemical Analysis

Determination of Ash Content

Three clean porcelain crucibles were placed in a muffle furnace for 15 min at 800^{o}C. The crucibles were removed and cooled in a desiccator. The weight of each crucible was taken (W_1). 2 g of each solid sample was added into each crucible and weighed (W_2). The crucibles were placed in a muffle furnace and the temperature was slowly increased from 200 – 550^{o}C to avoid incomplete ashing. The calcination was stopped when the ash became grey in appearance. The crucibles and their contents were reweighed (W_3) (AOAC, 2005). The percentage ash was calculated as follows:

$$\%Ash = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \qquad (1)$$

Determination of Specific Gravity

A clean and dry specific gravity bottle was weighed accurately (M_1) and was filled with water at 30^{o}C. The stopper was inserted to exclude extra water and the bottle with its stopper was wiped dry. The bottle with the water content was weighed (M_2). The bottle was emptied and dried perfectly before being filled with the oil sample at 30^{o}C and the stopper was inserted in order to exclude surplus oil. The top of the stopper and the bottle was wiped clean and was finally weighed (M_3) (AOAC, 1993).

$$Specific\ gravity\ at\ 30^{o}C = \frac{weight\ of\ oil\ (M_3 - M_1)}{weight\ of\ water\ (M_2 - M_1)} \qquad (2)$$

Determination of Insoluble Impurity, Moisture and Volatile Matter in Oil and Secirin

A clean and dried Petri-dish was weighed (M_1) and 100 g of oil sample was weighed into the petri-dish. The weight (M_2) of both oil sample and the dish was obtained before drying. The dish

with its content was kept in an oven within the temperature range of 105-110^{o}C for 3 h until a constant weight was obtained. The dish was covered before being removed from the oven and kept in a desiccator, the weight (M_3) was obtained after cooling. Thus, % moisture and volatile matter is calculated as follows:

$$\%Moisture\ and\ volatile\ matter = \frac{loss\ in\ weight}{weight\ of\ sample} = \frac{M_2 - M_3}{M_2 - M_1} \times 100 \quad (3)$$

Each oil sample (20 g) was weighed accurately into 500 mL flask. 200 mL of petroleum ether was added and the flask was closed and shaken vigorously. Thereafter, it was allowed to stand for 30 min at room temperature. A filter paper (Whatman No. 4) was dried at 100^{o}C and cooled. This was weighed and used to filter the mixture (micelle) in the flask. The residue in the filter paper was washed with fresh petroleum ether separately. The filter paper was then dried in an oven at 100^{o}C to a constant weight. The filter paper was cooled in a desiccator and weighed again (AOAC, 1993).

The percentage insoluble impurities was calculated as follows:

$$\%\ Insoluble\ impurity = \frac{gain\ in\ the\ weight\ of\ filter\ paper}{weight\ of\ oil\ sample} * 100 \quad (4)$$

Determination of Free Fatty Acid and Acid Value

Accurately measured 25 mL of 95% absolute ethanol was poured into 150 mL conical flask. Two drops of 1% phenolphthalein indicator was added into it. The ethanol was neutralised to faint pink colour with 0.1 M KOH from the burette. 5 g of oil sample was added into the neutralised ethanol in the conical flask and heated till it started boiling, it was shaken and cooled, three drops of phenolphthalein indicator was added into the mixture, and titrated with 0.1 M KOH solution until a faint pink colour was observed which lasted for about a minute (AOAC, 1993). The free fatty acid and acid value were calculated as follows:

$$\%\ Free\ fatty\ acid = \frac{titre\ value * concentration\ of\ KOH * Z}{weight\ of\ oil\ sample} \quad (5)$$

$$Acid\ value\ (mg\ KOH/g\ oil) = \frac{titre\ value * concentration\ of\ KOH * 56.11}{weight\ of\ oil\ sample} \quad (6)$$

Where Z is a constant and its value depends on the type of oil. For palm kernel oil, Z value is 20.0 (as % lauric acid) while for soybean oil, Z value is 28.2 (as % oleic acid).

Both the FFA and AV of oil samples were fortnightly monitored for a period of twelve weeks.

Determination of Peroxide Value

About 5 g of oil sample was weighed into 250 mL conical flask and 30 mL of glacial acetic acid-chloroform mixture (3:2) was added into the flask. 0.5 mL of saturated KI was added and was agitated for 60 sec, 30 mL of distilled water was added. The resulting mixture was titrated with 0.01 M sodium thiosulphate with constant stirring until the yellow colour precipitate was about disappearing. Thereafter, 0.5 mL of 1% starch solution was added and the titration was continued until the blue-black colour disappeared. The blank determination was also carried out (AOAC, 1993).

$$peroxide\ value\ (mEq\ O_2/kg\ oil) = \frac{sample\ titre - blank\ titre * conc.of\ Na_2S_2O_3 * 1000}{weight\ of\ oil\ sample} \quad (7)$$

This parameter was also monitored fortnightly for a period of twelve weeks. But the concentration of sodium thiosulphate was changed from 0.01 M to 0.02 M as the PV value was increased.

Determination of Refractive Index

Abbe's refractometer was used for refractive index determination. Warm water was circulated into the cavity around the prism and a thermometer was fitted in a hole in the cavity for reading the temperature at which the refractive index reading was taken (30^{o}C). The prism was opened and cleaned with acetone. Then a drop of oil sample was put on one part of the prism and it was closed. The mirror below the eyepiece was adjusted and the reading was taken up to three decimal places where the sharp demarcation of light and shade appeared and the temperature on the thermometer was noted. The refractive index was reported as η (AOAC, 2000).

Determination of Reducing Power

About 2.5 mL of sericin extract was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1 g/100 mL potassium ferrocyanide. The mixture was incubated at 50^{o}C for 20 min. Thereafter 2.5 mL of 10 g/100 mL ferric chloride. The absorbance was later measured at 700 nm. The higher the absorbance, the higher the reducing power (Pulido *et al.*, 2000; Kuda *et al.*, 2005; Iqbal *et al.*, 2005).

Determination of Ferrous Ion Chelating Activity

Ferrous ion chelating activity of each sample was measured according to the method of Decker and Hultin (1992). 0.1 mL of distilled water was added to 0.1 mL of the extract and thereafter 0.025 mL of 0.5 mM ferrozine (mixture of 0.25 mL of 1 mM $FeSO_4$ and 1 mL of HCl buffer (pH 7.4) was added to the mixture and allowed to stand for 20 min at room temperature and the absorbance (Abs 2) was measured (Igba *et al.*, 2005).

$$Ferrous\ ion\ chelating\ activity\ (\%) = \frac{1-(sample\ Abs\ 2 - Sample\ Abs\ 1)}{control\ Abs\ 2 - control\ Abs\ 1} * 100 \quad (8)$$

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

One millilitre of sample extract was pipetted into 50 mL conical flask, 2 mL of freshly prepared methanolic solution of DPPH (5.9 mg/100 mL methanol) was added and homogenized properly, after 30 min, absorbance of the mixture was taken at 517 nm on a digital spectrophotometer (Igbal *et al.*, 2005; Heinomen *et al.*, 1998). DPPH radical scavenging activity was calculated from a standard calibration curve using the formula:

$$\%DPPH = \frac{absorbance\ of\ sample\ extract \times dillution\ factor}{10000 \times weight\ of\ sample} \quad (9)$$

RESULTS AND DISCUSSION

The results of percentage yield, volatile matter, and ash content using different solvents are presented in Table 1. The percentage yield across all solvents ranged between 21.43 to 22.00% which fell within the values of 17 to 32% reported by Rui (1998). The nature of dissolving solvent may alter the percentage yield and properties of sericin from silkworm cocoon (Teramoto *et al.*, 2004).

Table 1: Physicochemical Parameters of Silkworm Sericin extracted using Different Solvents

Extracting solvent	Yield (%)	Appearance	Volatile matter at 105^{o}C	Ash content at 550^{o}C
Water	22.00 ± 0.34	Light yellow solid	20.31 ± 0.01	0.74 ± 0.00
Urea	22.97 ± 0.16	Light yellow solid	21.72 ± 0.02	0.74 ± 0.01
Na_2CO_3	21.44 ± 0.10	Light yellow solid	20.42 – 0.04	0.81 ± 0.02
Citric acid	21.43 ± 0.13	Light yellow solid	20.43 ± 0.02	0.76 ± 0.02

Values represent means of triplicate determination ± standard deviation

Though the highest percentage yield was obtained for urea extract (22.97%) which may be due to the molecular similarity of urea with the serine in the extract, the lowest value was obtained for citric acid treatment (21.43 ± 0.13%) due to protein denaturing and dissolution of the sericin. Acid causes an increase in density clustering which denatures protein (Haque *et al.,* 2005). A sericin solution turns into a gel after a period of time and can return to a solution upon heating. During the gelation of sericin solution, when the moisture content is very low, it forms a solid matter which also returns to solution upon heating. This sol-gel state transition is one characteristic of sericin that is different to fibroin (Kim, 2007).

The high volatile matter of sericin using different treatment methods ranged between 20.31 and 21.72% with lowest volatile matter obtained for water extract and highest obtained for urea extract. Low ash content is a factor of high purity of the obtained sericin and an approval of a degumming process (Rui, 1998). The ash contents obtained (0.74 to 0.81%) across different treatments were lower than the values reported by Rui (1998).The result indicated silkworm cocoon from local sericulture was of high purity.

Physicochemical Properties of Edible Oils

The physicochemical properties of raw soya bean oil and raw palm kernel oil are presented in Table 2. The specific gravity of oil is the ratio of the weight of the oil to the weight of equal volume of water. The specific gravity for refined soya bean oil and palm kernel oil are 0.921 ± 0.005 and 0.912 ± 0.003, respectively. The values are within the range (0.9 to 1.16) stipulated by FAO/WHO (2009) for edible oil.

The refractive index of oil is a physical parameter that measures the angle through which a beam of light is bent when passing through a thin film of oil. The refractive index values for refined soybean and palm kernel oils were 1.470 ± 0.0001 and 1.452 ± 0.0001, respectively. The presence of double bond (unsaturation) increases the refractive index of organic compound, therefore the higher the unsaturation, the greater is the effect on refractive index (Oderinde *et al.,* 2008; Adewuyi and Oderinde, 2009). This suggested that refined palm kernel oil was more saturated than refined soybean oil. The values obtained for these oils were within the standards stipulated by Standard Organisation of Nigeria (SON, 2000).

Table 2: Physicochemical Properties of Refined Soybean Oil and Refined Palm Kernel Oil

Parameter	RSBO	RPKO	Standard
Specific gravity	0.921 ± 0.005	0.912 ±0.003	0.9– 1.16 (FAO/WHO, 2009)
Acid value (mgKOH/g oil)	1.234 ± 0.001	1.568 ± 0.001	< 3.0 (Fakhri and Qadir, 2011)
Peroxide value (Meq O_2/kg oil)	2.399 ± 0.003	2.798 ± 0.003	≤10 (Anon, 2003)
Free fatty acid (%)	0.620 ± 0.0005	0.601 ± 0.0005	≤ 1.5 (Anon, 2003)
Moisture (MIV%)	0.04 ± 0.01	0.11 ± 0.03	≤ 0.05% (Anon, 2003)
Refractive index	1.470 ± 0.0001	1.452 ± 0.0001	1.4 – 1.7 (AOAC, 2005)
Insoluble impurity (%)	0.01 ± 0.00	0.04 ± 0.00	≤ 0.05% (Anon, 2003)

Values represent means of triplicate determinations ± standard deviation: RSBO – refined soybean oil; RPKO – refined palm kernel oil

Acid value (AV) is an important index of physicochemical property of oil which is used to indicate the quality, age, edibility and suitability of oil for use in industries such as paint and food (Akubugwo *et al.,* 2008). AV is expressed as the amount of KOH in milligram necessary to neutralize free fatty acids contained in 1.0 g of oil. According to Demian (1990), acid values are used to measure the extent to which glycerides in the oil have been decomposed by lipase and other

physical factors such as light and heat. Thus, the acid values of the soya bean and palm kernel oil (1.234 ± 0.001 and 1.568 ± 0.001 respectively) were less than 1.371 ± 0.651 reported for soya bean oil by Arawande *et al.* (2012). This suggests that the refined soya bean and palm kernel oils are susceptible to lipase action

Percent FFA is a conventional expression of the percentage mass-fraction of total fat. According to the nature of the fat, it is expressed as lauric acid for coconut, palm kernel, and similar oils, as palmitic acid for palm oil, and oleic acid for soya bean oil (Gunstone, 2004). Oil varied in their %FFA and this variation could be attributed to inadequate refining and decolourization of edible oils (Perkins, 1992). %FFA of 0.620 ± 0.0005 and 0.601 ± 0.005 were obtained for refined soya bean oil and refined palm kernel oil, respectively, and are higher than the recommended value for soya bean oil (0.176) and lower than 2.540 for coconut oil by FAO/WHO (Anon, 2003) but less than 0.689 ± 0.327 obtained for refined soya bean oil reported by Arawande *et al.,* (2012).

The PV of an oil or fat is used as a measurement of the extent to which rancidity reactions have occurred during storage and the best test for oxidative rancidity is the determination of the peroxide value (Fakhri and Qalid, 2011). The PV was determined as an expression of oxidation or rancidity in zero week of oil storage. The results indicate that the refined soya bean oil (more unsaturated) has less PV (2.399 ± 0.003) than refined palm kernel oil (2.798 ± 0.003) which may be related to oxidation or rancidity. The soya bean oil considered in this work has lower PV compared to corn oil reported by (Fakhri and Qalid, 2011). The PV for both oils fit into the recommended value (≤ 10) by WHO/FAO (Anon, 2003).

Low moisture, impurity and volatility (%), and insoluble impurity (%) are clear determinants of high qualities of both refined soya bean and palm kernel oils considered in this study. The recommended matter volatility at 105^{o}C (0.2%) and insoluble impurity (0.05%) by FAO/WHO (Anon, 2003) were higher than values obtained for both oils considered. The low moisture content was indicative that the oils might be able to withstand long period of storage and transportation.

Antioxidant Properties of Silkworm Cocoon Sericin

The results of antioxidant properties of sericin as presented in Table 3 showed that the reducing power (ferric reducing power) ranged between 1.72 ± 0.09 (for citric acid extract) to 1.78 ± 0.00 (for urea extract). It has been reported that the reducing power of bioactive compound is associated with antioxidant activity and that the higher the value, the higher the antioxidant activity (Yildirim *et al.,* 2001; Siddhuraju *et al.,* 2002). The results indicated that the urea extract had highest ability to reduce Fe(III) to Fe(II), while citric acid extract had the least reducing power.

Table 3: Antioxidant Properties of Silkworm Cocoon Sericin

	Reducing power	Fe^{2+} chelating activity (%)	DPPH radical scavenging activity
Water extract	1.76 ± 0.09	6.13 ± 0.35	13.18 ± 1.06
Na_2CO_3 extract	1.75 ± 0.31	6.15 ± 0.15	13.78 ± 1.19
Urea extract	1.78 ± 0.00	6.18 ± 0.29	13.04 ± 0.75
Citric acid extract	1.72 ± 0.12	6.18 ± 0.37	14.49 ± 0.73

Values represent means of triplicate determination ± standard deviation

Ferrous (Fe^{2+}) chelating activity was lowest in water extract (6.13 ± 0.35%) and highest in both urea and citric acid extracts (6.18 ± 0.29). Ferrous chelating is an important factor, in that iron is essential to life because it is required for oxygen transport, respiration and the activities of many enzymes. However, iron is an extremely reactive metal and will catalyse changes in lipids, proteins

and other cellular components (Igba *et al.*, 2005; Decker and Hultin, 1992). Hence, the ability of any compound to chelate ion (II) will enhance its antioxidant activity by binding iron (II), thereby reducing its catalysed oxidative activity that leads to deterioration of lipid and lipid containing foods. High value of ferrous chelating activity promotes high antioxidant activity.

DPPH is often used to evaluate the free radical scavenging ability of any sample (Ebrahimzadeh *et al.*, 2008). The higher the value of DPPH, the higher the antioxidant activity of the substance evaluated (Cotelle *et al.*, 1996). Substances which are able to scavenge free radicals are considered antioxidants (Dehpour *et al.*, 2009). DPPH radical scavenging activity of urea extract was the lowest with the value of 13.04±0.75% while 14.49 ± 0.73% was the highest for citric acid extract. The results implied that all the extracts possessed antioxidant activities, though in varying amounts.

Free Fatty Acid of Soya Bean Oil Stored with Silkworm Cocoon Extracts of Varying Concentrations

Percent free fatty acid (% oleic acid) values were obtained in the soya bean oil stored with varying amount of silkworm sericin, 0.2 g butylated hydroxyl toluene (BHT) and palm kernel without additive are presented in Table 4.

Table 4: Free Fatty Acid (%Oleic Acid) of Soya Bean Oil Dosed with Varying Concentrations of Silkworm Cocoon Extracts; BHT and Control Oil for 12 Weeks

Extract	Amount (%)	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Control	0	0.620	0.789	0.902	1.013	1.015	1.128	1.184
BHT	0.2	0.675	0.789	0.845	0.902	0.959	1.014	1.128
Water extract	0.2	0.620	0.677	0.733	0.788	0.788	0.902	1.015
Water extract	0.4	0.620	0.789	0.845	0.901	0.899	0.958	1.015
Water extract	0.6	0.620	0.732	0.789	0.846	0.902	0.957	1.013
Water extract	0.8	0.620	0.789	0.789	0.786	0.901	0.958	0.959
Water extract	1.0	0.620	0.732	0.732	0.789	0.901	0.959	0.959
Na_2CO_3 extract	0.2	0.619	0.677	0.733	0.789	0.788	0.845	1.015
Na_2CO_3 extract	0.4	0.732	0.789	0.846	0.902	0.901	0.959	0.958
Na_2CO_3 extract	0.6	0.677	0.731	0.788	0.844	0.902	0.958	1.013
Na_2CO_3 extract	0.8	0.617	0.676	0.789	0.789	0.898	0.958	0.958
Na_2CO_3 extract	1.0	0.563	0.620	0.733	0.789	0.902	0.958	0.959
Urea extract	0.2	0.620	0.675	0.733	0.787	0.787	0.845	0.956
Urea extract	0.4	0.619	0.619	0.789	0.788	0.844	0.902	0.957
Urea extract	0.6	0.563	0.677	0.732	0.789	0.845	0.900	0.902
Urea extract	0.8	0.564	0.676	0.677	0.733	0.732	0.789	0.846
Urea extract	1.0	0.507	0.620	0.733	0.733	0.789	0.846	0.900
Citric acid extract	0.2	0.733	0.787	0.845	0.902	0.959	0.956	1.015
Citric acid extract	0.4	0.789	0.844	0.900	0.958	0.958	1.014	1.015

Citric acid extract	0.6	0.788	0.845	0.845	0.901	0.957	0.902	1.013
Citric acid extract	0.8	0.846	0.901	0.959	1.015	1.015	1.067	1.015
Citric acid extract	1.0	0.959	1.015	1.015	1.071	1.015	1.071	1.128

BHT: Butylated hydroxyl toluene

Table 5: Free Fatty Acid (%Lauric Acid) of Palm Kernel Oil Dosed with Varying Concentrations of Silkworm Cocoon Extracts, BHT and Control Oil for 12 Weeks

Extract	Amount (%)	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Control	0	0.559	0.719	0.880	0.958	0.959	1.079	1.079
BHT	0.2	0.601	0.718	0.838	0.878	0.918	0.997	1.038
Water extract	0.2	0.560	0.640	0.759	0.798	0.799	0.920	0.960
Water extract	0.4	0.559	0.719	0.839	0.879	0.876	0.959	0.959
Water extract	0.6	0.560	0.679	0.780	0.840	0.880	0.958	0.958
Water extract	0.8	0.599	0.719	0.799	0.796	0.879	0.960	0.920
Water extract	1.0	0.559	0.679	0.759	0.799	0.879	0.960	0.920
Na_2CO_3 extract	0.2	0.560	0.640	0.758	0.800	0.798	0.879	0.960
Na_2CO_3 extract	0.4	0.640	0.719	0.839	0.880	0.879	0.960	0.919
Na_2CO_3 extract	0.6	0.599	0.678	0.800	0.839	0.880	0.960	0.958
Na_2CO_3 extract	0.8	0.560	0.639	0.796	0.800	0.876	0.959	0.920
Na_2CO_3 extract	1.0	0.520	0.600	0.759	0.800	0.879	0.960	0.920
Urea extract	0.2	0.560	0.638	0.760	0.798	0.798	0.879	0.917
Urea extract	0.4	0.560	0.559	0.780	0.799	0.838	0.913	0.919
Urea extract	0.6	0.519	0.640	0.759	0.800	0.839	0.918	0.880
Urea extract	0.8	0.520	0.640	0.720	0.760	0.759	0.840	0.840
Urea extract	1.0	0.480	0.600	0.760	0.760	0.800	0.880	0.880
Citric acid extract	0.2	0.640	0.718	0.839	0.880	0.920	0.958	0.959
Citric acid extract	0.4	0.678	0.758	0.878	0.919	0.919	0.998	0.959
Citric acid extract	0.6	0.678	0.799	0.838	0.878	0.913	0.920	0.958
Citric acid extract	0.8	0.720	0.799	0.920	0.960	0.960	1.035	0.960
Citric acid extract	1.0	0.800	0.880	0.959	1.000	0.960	1.039	1.040

BHT: Butylated hydroxyl toluene

Tables 4 and 5 show the values of free fatty acid of refined soya bean oil and refined palm kernel oil, respectively, stored with varying concentrations of silkworm cocoon water, urea, sodium carbonate, and citric acid extracts, BHT and control oil sample for a period of 12 weeks.

There was an increase in the free fatty acid values with increase in the number of weeks, it was also observed that, increase in dosage favoured free fatty acid.

The free fatty acid values obtained in the refined soya bean oil control sample and 0.2 g BHT stored soya bean oil (Table 4) were higher than corresponding samples of refined palm kernel oil samples (Table 5). The FFA values of both refined soya bean oil and palm kernel oil stored with citric acid extracts were higher than the free fatty acid values for all the silkworm sericin extracts under investigation. The free fatty acid of edible oil is a measure of hydrolytic rancidity index caused by moisture, lipase enzymes and heat (Rossel, 1994). The amount of FFA present in oil is of importance because it indicates hydrolytic activity. According to Codex Alimentarius Commission (CAC) (2001), the recommended % FFA (as oleic acid) limits are 0.3% for refined plant oils; 2.0% for cold pressed and crude oil; and 5% for crude palm oil. The highest values obtained in this study were observed in soya bean oil control sample after twelve week storage period with 1.184%. Generally, the citric acid extracts seemed to have the highest values of FFA, however, the values were below 2% (for cold pressed oil) according to the standard stipulated by CAC (2001); the values were also lower than 5% (for crude palm oil) as stipulated by CAC (2001).

Refractive Index of Soya Bean Oil and Palm Kernel Oil Stored with Silkworm Sericin of Varying Concentrations

The results (Tables 6 and 7) of the refractive index of soya bean oil and palm kernel oil stored with varying concentrations of silkworm cocoon water, urea, sodium carbonate, and citric acid extracts, BHT and the control sample.

Table 6: Refractive Index of Soya Bean Oil Dosed with Varying Concentrations of Silkworm Cocoon Extracts, BHT and Control Oil for 12 Weeks

Extract	Amount (%)	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Control	0	1.470	1.473	1.474	1.475	1.475	1.476	1.477
BHT	0.2	1.473	1.473	1.473	1.472	1.471	1.469	1.469
Water extract	0.2	1.465	1.473	1.471	1.4700	1.470	1.471	1.469
Water extract	0.4	1.465	1.473	1.471	1.470	1.470	1.472	1.469
Water extract	0.6	1.465	1.473	1.471	1.470	1.470	1.470	1.469
Water extract	0.8	1.469	1.472	1.471	1.470	1.470	1.470	1.468
Water extract	1.0	1.464	1.473	1.470	1.470	1.469	1.469	1.468
Na_2CO_3 extract	0.2	1.475	1.470	1.471	1.470	1.469	1.471	1.490
Na_2CO_3 extract	0.4	1.472	1.472	1.472	1.471	1.469	1.470	1.471
Na_2CO_3 extract	0.6	1.475	1.473	1.471	1.472	1.470	1.468	1.470
Na_2CO_3 extract	0.8	1.475	1.472	1.472	1.473	1.470	1.470	1.470
Na_2CO_3 extract	1.0	1.478	1.473	1.471	1.473	1.471	1.470	1.470
Urea extract	0.2	1.470	1.730	1.472	1.471	1.468	1.469	1.469
Urea extract	0.4	1.463	1.473	1.474	1.472	1.471	1.470	1.469

Urea extract	0.6	1.471	1.472	1.474	1.423	1.472	1.471	1.469
Urea extract	0.8	1.470	1.472	1.474	1.472	1.474	1.473	1.470
Urea extract	1.0	1.470	1.473	1.473	1.473	1.473	1.473	1.470
Citric acid extract	0.2	1.462	1.472	1.471	1.471	1.470	1.469	1.469
Citric acid extract	0.4	1.468	1.473	1.471	1.472	1.470	1.470	1.469
Citric acid extract	0.6	1.473	1.472	1.471	1.471	1.470	1.468	1.468
Citric acid extract	0.8	1.468	1.473	1.471	1.471	1.470	1.470	1.470
Citric acid extract	1.0	1.470	1.472	1.470	1.470	1.470	1.470	1.470

Refractive index is a physical attribute of oils and fats, measured by and through which a beam of light is bent when passing through a film of melted fat. This makes it an excellent spot test for uniformity of compositions of oils and fats (O'Brien, 2009).

Table 7: Refractive Index of Palm Kernel Oil Dosed With Varying Concentrations of Silkworm Cocoon Extracts, BHT and Control Oil for 12 Weeks

Extract	Amount (%)	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Control	0	1.452	1.452	1.453	1.454	1.454	1.455	1.456
BHT	0.2	1.452	1.453	1.452	1.452	1.453	1.453	1.452
Water extract	0.2	1.452	1.453	1.452	1.452	1.452	1.452	1.452
Water extract	0.4	1.452	1.453	1.142	1.452	1.452	1.452	1.452
Water extract	0.6	1.453	1.453	1.142	1.452	1.452	1.452	1.452
Water extract	0.8	1.452	1.453	1.142	1.452	1.452	1.452	1.452
Water extract	1.0	1.452	1.452	1.142	1.452	1.452	1.452	1.412
Na_2CO_3 extract	0.2	1.456	1.454	1.453	1.452	1.452	1.452	1.452
Na_2CO_3 extract	0.4	1.454	1.454	1.453	1.452	1.452	1.452	1.452
Na_2CO_3 extract	0.6	1.453	1.454	1.453	1.452	1.452	1.452	1.452
Na_2CO_3 extract	0.8	1.452	1.454	1.453	1.452	1.452	1.452	1.452
Na_2CO_3 extract	1.0	1.452	1.454	1.453	1.452	1.452	1.452	1.452
Urea extract	0.2	1.452	1.453	1.453	1.452	1.452	1.452	1.452
Urea extract	0.4	1.452	1.453	1.453	1.452	1.452	1.452	1.452
Urea extract	0.6	1.452	1.453	1.453	1.452	1.452	1.452	1.452
Urea extract	0.8	1.452	1.453	1.453	1.452	1.452	1.452	1.452

Urea extract	1.0	1.452	1.453	1.453	1.452	1.452	1.452	1.451
Citric acid extract	0.2	1.452	1.453	1.452	1.452	1.452	1.452	1.451
Citric acid extract	0.4	1.452	1.453	1.452	1.452	1.452	1.452	1.451
Citric acid extract	0.6	1.452	1.452	1.452	1.452	1.452	1.452	1.451
Citric acid extract	0.8	1.452	1.452	1.452	1.452	1.452	1.452	1.451
Citric acid extract	1.0	1.452	1.452	1.452	1.452	1.452	1.452	1.451

BHT: Butylated hydroxyl toluene

The refractive index increases with the unsaturation, and decreases with the mean molecular weight of fatty acids. Hence, the presence of free fatty acid appreciably lowers the refractive index of fats/oils (Cock and Reds, 1966). Refractive index of oil is a physical property that is used to assess oil acceptability and adulteration or purity. The refractive index values were adequately within the range of 1.40 – 1.70 (AOAC, 2000), and this is an indication that the additive could not cause any major adulteration in the oil samples under study.

CONCLUSION

High percentage yield of silkworm sericin, and the ease of extractions coupled with the availability of the cocoon are all indications that the beneficiation of these extracts for both industrial and domestic purposes will be economical. Low ash content (1<) of the local silkworm sericin is an attestation to high and pure quality of the material. Water which is recommended for extracting sericin to be used in foods did give a relatively high percentage yield. The composition of the extracting solvents has effects on the behaviour of silkworm cocoon extracts since all the extracts did not behave in exactly the same manner. Refractive index data revealed that the values obtained for the oil samples were within limits set by standard body, and that the introduction of the extracts into edible oils may not adulterate the oils. The specific gravity, peroxide value, refractive index, moisture and volatile matter results for the two oil samples were within the recommended values but the values obtained for acid values and consequently, the percent free fatty acid were higher than the recommended values which may be due to modification during industrial production processes of oils. Reducing power, Fe^{2+} chelating activity (%) and DPPH radical scavenging activity (%) all revealed the good antioxidant activities of the silkworm sericin.

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