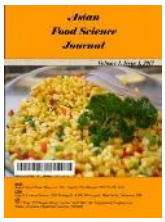

Asian Food Science Journal

1(3): 1-10, 2018; Article no.AFSJ.40040

Optimization of Palm Olein Stability during Storage Using Natural Antioxidants from Cocoa Pods Extract

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GBT and HMW designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors GBT, FTD, HMW, MJK, and MSLK managed the analyses of the study. Authors GBT and FTD managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2018/40040
Editor(s):
(1) Aneta Popova, Chief Assistant Professor, Department of Catering and Tourism, University of Food Technologies, Bulgaria.
Reviewers:
(1) Engr. Jelili Babatunde Hussein, Modibbo Adama University of Technology, Nigeria.
(2) Justyna Płotka-Wasylka, Gdańsk University of Technology, Poland.
(3) Sharif Md. Al-Reza, Islamic University, Bangladesh.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/23629

Original Research Article

Received 18th December 2017
Accepted 26th February 2018
Published 14th March 2018

ABSTRACT

Aims: The objective of this study is an attempt to optimize palm olein stability during storage using natural antioxidants from *Theobroma cacao* pods.
Study Design: Drying of fresh cocoa pods, extraction of natural antioxidants and its application as antioxidants to optimize oxidative stability of palm olein during storage.
Place and Duration of Study: University of Dschang, Cameroon and Council for Scientific and Industrial Research-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, India, from March 2016 to January 2017.
Methodology: The plant material was extracted and its reducing power, metal ion chelating

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activity evaluated. The extract was added in refined palm olein. Peroxide, *p*-Anisidine and total oxidation (TOTOX) values were determined as response variables to evaluate the effect of storage time and extract concentration during storage of oil.
Results: The outcomes showed cocoa pods extract to be efficient in metal ion chelating activity and have the ability to reduce Fe^{3+} to Fe^{2+}. This extract was also found to be efficient in inhibiting peroxide formation and reducing, in the same way, the amount of secondary oxidation products. At 2000 ppm, *Theobroma cacao* pods extract has the ability to extend the shelf life (30 months) of palm olein during storage.
Conclusion: *Theobroma cacao* pods can be exploited as an alternative source of synthetic antioxidants for stabilization of palm olein and other oil systems.

Keywords: Cocoa pods; palm olein; natural antioxidants; oxidation; optimization.

1. INTRODUCTION

Palm olein is the most produced and consumed refined oil in the world and is used for cooking, food formulations and fast food manufacturing [1]. It contains unsaturated fatty acids like oleic acid and linoleic acid, an essential fatty acid [2]. During storage, photosensitized oxidation and autoxidation are mainly responsible for the oxidation of oils. Besides the degradations caused in the quality properties of texture, flavor, color and nutritional value, lipids oxidation leads to the production of several secondary products that have high toxicological potential and mutagenic capacity [3,4]. However, oxidative stability of oils is the main factor that influences their acceptability.

Nowadays, we observed an increase of research works about natural antioxidants plant-based extracts to obtain bioactive compounds that can be used to inhibit the oxidation of foods. These natural compounds have lower toxicity than synthetic antioxidant such as butylated hydroxytoluene and butylated hydroxyanisole [5,6]. Plant-derived products contain a wide range of phenolic compounds such as phenolic acids and flavonoids that mainly contributed to their antioxidant activities [7]. Phenolic compounds can donate hydrogen atoms to free radicals or react with lipid peroxyl radicals and consequently, inhibit the formation of oxidation products. Recently, Womeni et al. [8,9] have proven that soursop flowers and old Cameroonian green tea leaves extract are rich sources of natural antioxidants for stabilization of palm olein during accelerated storage. It is rational to continue the search for new natural sources of antioxidants by screening the infinite plant sources and investigate the possibilities of their application in order to overcome the lipids oxidation during storage of oils.

Cocoa (*Theobroma cacao*) pods are unused plant material of agroindustrial process of cocoa. It contains mainly phenolic acids including caffeic acid, vanillic acid and quercetine [10,11,12]. Many studies suggested that phenolic antioxidants in plants are mainly present in the skin where they play an important role in protecting plants against microorganisms, some insects and others predictors [13]. In order to exploit the agroindustrial wastes as natural sources of antioxidants with application in food industries, Teboukeu et al. [12] have recently optimized the process parameters for the phenolic antioxidants extraction from *Theobroma cacao* pods and their application as an additive to improve the oxidative stability of palm olein during heating at frying temperature. Knowledge concerning the potential effect of this extract on palm olein during storage might encourage future technological applications of this sub-valued by-product.

The objective of this study is an attempt to optimize the antioxidative potential of phenolic antioxidants extracts from cocoa pods on the oxidative stability of palm olein during storage.

2. MATERIALS AND METHODS

2.1 Materials

Refined, bleached, and deodorized palm olein, free from additives was obtained from SCS/RAFCA Palm Oil Industry Company Ltd, Bafoussam, West-Cameroon. Fresh cocoa pods were collected at Bafang, Haut Nkam Division, West Cameroon, on April 2016. All the chemicals and reagents used were of analytical reagent grade and obtained from HiMedia Laboratories Pvt. Ltd, Sd Fine Chemicals, Mumbai, India and Sigma-Aldrich, St; Louis, USA.

2.2 Methods

2.2.1 Preparation of extract

Theobroma cacao pods extract was prepared in the optimal extraction condition previously defined by Teboukeu et al. [12]. Fresh *Theobroma cacao* pods were dried in an electric oven at 45°C for 48 h. The dried pods were grounded to pass through a 1 mm sieve. About 25 g of the obtained powder was extracted into 600 ml of methanol for 5.56 h at 47.48°C. The extract was filtered with a Whatman N°1 filter paper. The filtrate was subjected to rotary evaporation at 40°C under reduced pressure for the removal of the solvent. Ferric reducing antioxidant power and metal chelating activity of cocoa pods extract were carried out to complete preliminary antioxidant tests.

2.2.2 Ferric reducing antioxidant power

Method of Oyaizu [14] was performed to evaluate the ability of cocoa pods extract to reduce iron (III) to iron (II). An aliquot of 0.5 mL plant extract (1000 µg/ mL) was mixed with 1 mL phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% aqueous $K_3Fe(CN)_6$ solution, shaken and incubated at 50°C for 30 min. After incubation, 1 mL of 10% trichloroacetic acid solution was added to stop the reaction and the mixture was centrifuged. 1.5 mL of supernatant, 1.5 mL of distilled water and 0.1 mL of 0.1% $FeCl_3$ solution were mixed and incubated for 10 min and absorbance read at 700 nm on a spectrophotometer. A sample blank, containing all the reagents but no extract was prepared under the same conditions. Catechin was used as positive control. A higher absorbance indicates a higher reducing power.

2.2.3 Metal chelating activity

The antioxidant potential of cocoa pods extract was also evaluated by their ferrous ion chelating activity [15]. Into test tubes containing 160 µl of sample solution (1000 µg/ml), 160 µl of aqueous 1, 10-phénanthroline (0,25 %) and 400 µl of methanolic $FeCl_2$ (0.1 %) were added. After 10 min at room temperature, 880 µl of distilled water was added and the absorbance was measured at 510 nm. The metal chelating efficiency of the extract was determined by comparing with the chelating activity of catechin (positive control). The inhibition percentage (IP) of Fe2+-phenanthroline complex formation against blank was calculated by the formula:

$$IP\% = Abs_{sample} \times 100/Abs_{control}$$

2.2.4 Effect of extract concentration and storage time on palm olein stability during storage

2.2.4.1 Experimental design

Central Composite Design (CCD) was used to evaluate the effects of process parameters (concentration of extract and storage time) on the oxidative stability of palm olein. Real and coded levels of the independent variables used are shown in Table 1 and the intervals were determined on the basis of previous work and preliminary test. The design consisted of 10 runs with two replicates at the center point. The peroxide, *p*-Anisidine and total oxidation (TOTOX) values assays expressed as the dependent variables were determined (Table 2).

This experimental design generates a second-degree polynomial model (Y) of the following form:

$$Y = I + ax_1 + bx_2 + cx_1^2 + dx_2^2 + ex_1x_2$$

Where, Y represents the response variables (peroxide value, *p*-Anisidine value, and TOTOX value); x_1 and x_2 are the levels of the independent variables; a and b are the linear terms; e is the interaction term; c and d are the quadratic terms and I is a constant.

2.2.4.2 Sample preparation

The schaal oven test illustrated by Sultana et al. [16] was used with slight modifications. The

Table 1. Coded and real levels of independent variables used in the RSM design for study effect of extract concentration and storage time on the palm olein stability

Independent variables	Coded levels				
	-α (-1.68)	-1	0	+1	+ α (1.68)
	Real levels				
Extract concentration (ppm), X_1	500	720	1250	1780	2000
storage time (days), X_2	0	7	23	39	46

schaal oven test (storage at 65-70°C) is a good simulation of normal storage conditions. Evans et al. [17] showed that heating of oils for 08 hours at 65°C is equivalent to one month storage at room temperature. The extract was dissolved and separately added to 100 g of preheated RBD palm olein (at 50°C for 3 h) at concentrations indicated by the full factorial design (Table 2). Stabilized oil samples were placed in dark brown glass bottles with narrow necks and subjected to accelerated storage in an electric hot air oven at 70°C (08 hours heating cycle per day) for 46 days. Samples were collected according to the time (days) indicated by the experimental design (Table 2) and stored in the refrigerator for further analysis. The oxidative deterioration level was assessed by measuring oxidation parameters.

2.2.4.3 Measurement of oxidation parameters

The peroxide value (PV) of oil samples was determined following the spectrophotometrical IDF standard method, 74A: 1991 [18]. The *p*-Anisidine value (AV) was assessed according to AOCS Official Method CD 18-90 [19], and the total oxidation (TOTOX) calculated using the following equation: TOTOX= 2 PV + AV [20].

2.3 Statistical analyses

STATGRAPHICS Plus 5.0 was used for the experimental design and statistical analysis of the data. All responses were determined in duplicate and the power of the model was determined by evaluating the coefficient of determination (R^2) obtained from the analysis of variance (ANOVA). Statistical significance of the model variables was determined at 5% probability level. Response surfaces and contour plots were plotted using Sigma Plot v11.0 (c) systat.

3. RESULTS AND DISCUSSION

3.1 Preliminary Antioxidant Tests

3.1.1 Ferric reducing antioxidant power (FRAP)

Frap activity of cocoa pods extract in comparison to catechin (powerful ferric reducer) is presented in Fig. 1. It can be seen from the figure that no significant difference ($P > 0.05$) was registered between the activity of both catechin (2.18±0.17) and the extract (2.01±0.21). This is the proof of the ability of cocoa pods extract to donate its electron and reduce Fe^{3+} to Fe^{2+}. Cotelle [21] have proven that phenolic compounds, mainly flavonoids are powerful metal reducers. These results are in agreement with those of Womeni et al. [9] who demonstrated that green tea leaves extract are powerful ferric reducers.

3.1.2 Metal chelating activity

Metal ion chelating activity of *Theobroma cocoa* pods extract in comparison to catechin is illustrated in Fig. 2. This result shows the capacity of extract to reduce the concentration of the transition metal that catalyses peroxidation. In fact, transition metal ion like iron can stimulate lipid peroxydation by Fenton reaction and can also accelerate lipid peroxydation by decomposing lipid hydroperoxides into alkoxyl and peroxyl radicals that can perpetuate the chain reaction [22]. The same result was obtained by Loizzo et al. [23] who showed higher chelating ability of *annona cherimola* (cherimoya) peel.

3.2 Optimization of Palm Olein Stability during Storage

Table 2 shows the peroxide, *p*-Anisidine and total oxidation values of palm olein supplemented with cocoa pods extract obtained from the 10 experiments. Storage time and extract concentration were taken as independent variables because they can influence oxidative stability of oils during processing and storage [8, 12,24,25]. The initial chemical characteristics of fresh palm olein used in this study are illustrated in experiment N° 10. The fresh palm olein without additives was of good quality, as shown by its low peroxide value (< 10 ppm), low *p*-Anisidine value (≤ 20) and low TOTOX value (<26) as recommended by homologation [26, 27]. These responses variables changes during storage.

3.2.1 Analysis of variance

The coefficient of determination (R^2), the coefficients of the second-order polynomial equation and significant effect of independent variables were established on the basis of experimental data (Table 3). Results shows that only the storage time in linear terms significantly affects ($P < 0.05$) the peroxide, anisidine and TOTOX values of oil samples. The mathematical models of relationship for peroxide value (Y_1), anisidine value (Y_2) and TOTOX value (Y_3) with extract concentration (X_1) and storage time (X_2) is given by the equations:

$Y_1 = -1.66 + 0.007X_1 + 0.16X_2 - 0.000003X_1^2 - 0.00006X_1X_2 + 0.01X_2^2$

$Y_2 = 4.52 - 0.003X_1 + 0.002X_2 + 0.000001X_1^2 + 0.000004X_1X_2 + 0.0005X_2^2$

$Y_3 = 1.19 + 0.01X_1 + 0.34X_2 - 0.000005X_1^2 - 0.0001X_1X_2 + 0.02X_2^2$

The coefficient of determinations (R^2) indicates that the observed mathematical models can be valid (R^2 greater than 75 %) [28] and is able to explain 96.24 %, 88.62 %, and 96.42 % of the results in the case of peroxide value, *p*-Anisidine value and TOTOX value, respectively.

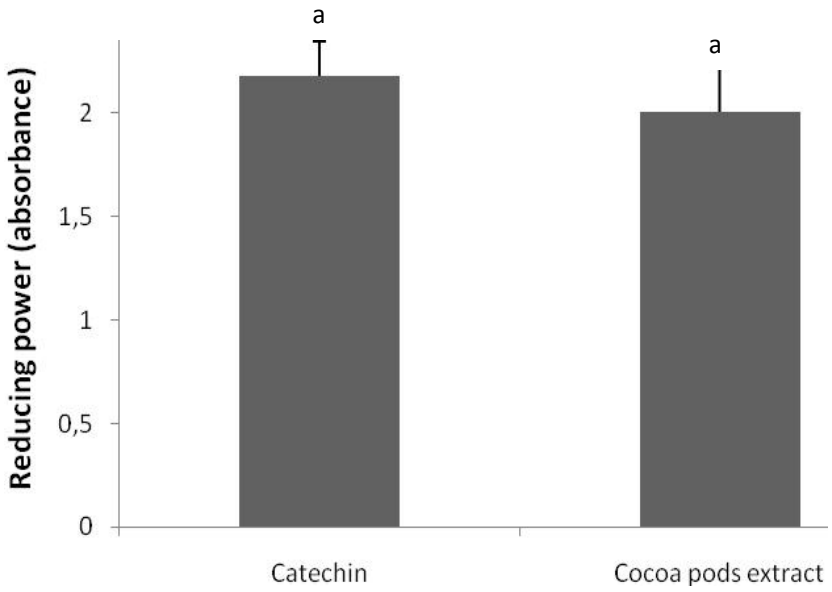

Fig. 1. Ferric reducing antioxidant power of cocoa pods extract

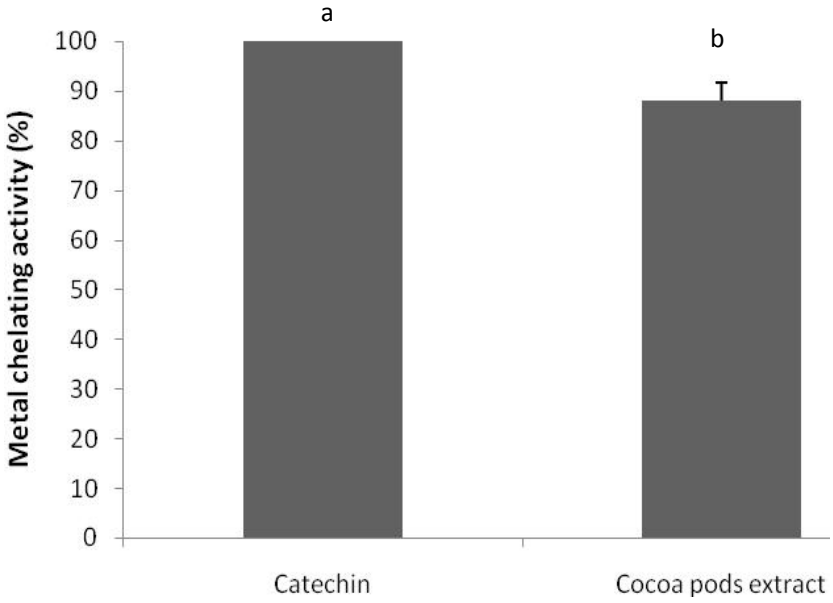

Fig. 2. Metal chelating activity of cocoa pods extract

Table 2. Experimental design observed and predicted values of parameter effect on the oxidative stability of palm olein during storage

N°	Extract (ppm)	Storage time (days)	Experimental peroxide value (ppm)	Predicted peroxide value (ppm)	Experimental *p*-anisidine value	Predicted *p*-anisidine value	Experimental TOTOX value	Predicted TOTOX value
	X_1	X_2	EY_1	PY_1	EY_2	PY_2	EY_3	PY_3
01	0 (1250)	0(23)	10.03±0.58	10.03	2.97±0.15	2.97	23.05±1.33	23.05
02	0(1250)	0(23)	10.03±0.58	10.03	2.97±0.15	2.97	23.05±1.33	23.05
03	1(1780)	1(39)	17.73±0.72	17.07	3.97±0.21	3.96	39.44±1.65	39.01
04	1(1780)	-1(7)	2.30±0.39	2.35	3.08±0.26	2.78	7.69±1.04	7.98
05	-1(720)	1(39)	20.52±0.10	2.96	4.04±0.06	4.42	45.08±0.26	44.44
06	-1(720)	-1(7)	2.94±0.45	2.88	3.29±0.41	3.45	9.19±1.31	9.45
07	α(2000)	0(23)	7.61±0.56	7.89	3.36±0.12	3.26	18.59±1.24	18.96
08	-α(500)	0(23)	12.22±1.14	12.05	3.54±0.08	3.78	27.98±1.38	27.08
09	0(1250)	α(46)	32.92±1.18	32.05	4.16±0.21	4.05	70.01±2.57	70.71
10	0(1250)	-α(0)	2.032±0.21	2.034	2.08±0.11	2.01	6.14±0.53	6.45

Table 3. Regression coefficients (RC), *p* values and coefficient of multiple determinations (R^2) for peroxide value, *p*-Anisidine value and TOTOX value following CCD

	Peroxide value		*p*-Anisidine value		TOTOX value	
	CR	***P value***	**CR**	***P value***	**CR**	***P value***
X1 : Extract (ppm)	0.007	0.279	-0.0030	0.590	0.01	0.269
X2 : Storage time (days)	0.169	0.0006*	0.0028	0.0069*	0.34	0.0006*
X1 X1	-0.000003	0 .509	0.000001	0.0975	0.000005	0.580
X1 X2	-0.00006	0.722	0.000004	0.832	-0.0001	0.731
X2 X2	0.010	0.098	0.0005	0.354	-0.02	0.092
Constant	-1.665		4.523		1.19	
R^2 (%)	96.24		88.62		96.42	
R^2 (adjusted) (%)	91.55		76.89		91.95	

* *Independent variable that significantly ($p < 0.05$) affect the response*

3.2.2 Analysis of main effect plots and contour plots

3.2.2.1 Analysis of main effects plots

Effect of extract concentration and storage time on the oxidative stability of palm olein during storage is presented in Fig. 3. We observed that peroxide, anisidine and total oxidation values of oils samples enriched with extract increases with storage time. However, the addition of *Theobroma cacao* pods extract at high concentrations decreases mainly peroxide and TOTOX values. Peroxide value measures hydroperoxides [29], anisidine value assess secondary oxidation products like 2-alkenal, 2,4-alkadienal [30] and TOTOX value measures both primary and secondary oxidation products, and provides a better determination of the progressive oxidative deterioration of oils [9]. The general increase in all responses variables might be attributed to the formation of hydroperoxides, a thermolabile species whose breakdown leads to the formation of secondary products. Cocoa pods extract inhibited peroxide formation and reduced by the same way the amount of secondary oxidation products in oil. Many studies showed the presence of phnolics compounds in *Theobroma cocoa* pods extract [10,11,12]. These compounds are able to provide a hydrogen atom to stabilize the free radicals present in oil and consequently increase its oxidative stability [31]. Similar results were obtained by Teboukeu et al. [12] in the same lipid matrix, with the same extract but during heating at frying temperature. Also, these results are in accordance with those reported in previous work [8,25,32] who have proven that natural plant extract can limit total oxidation of vegetable oils.

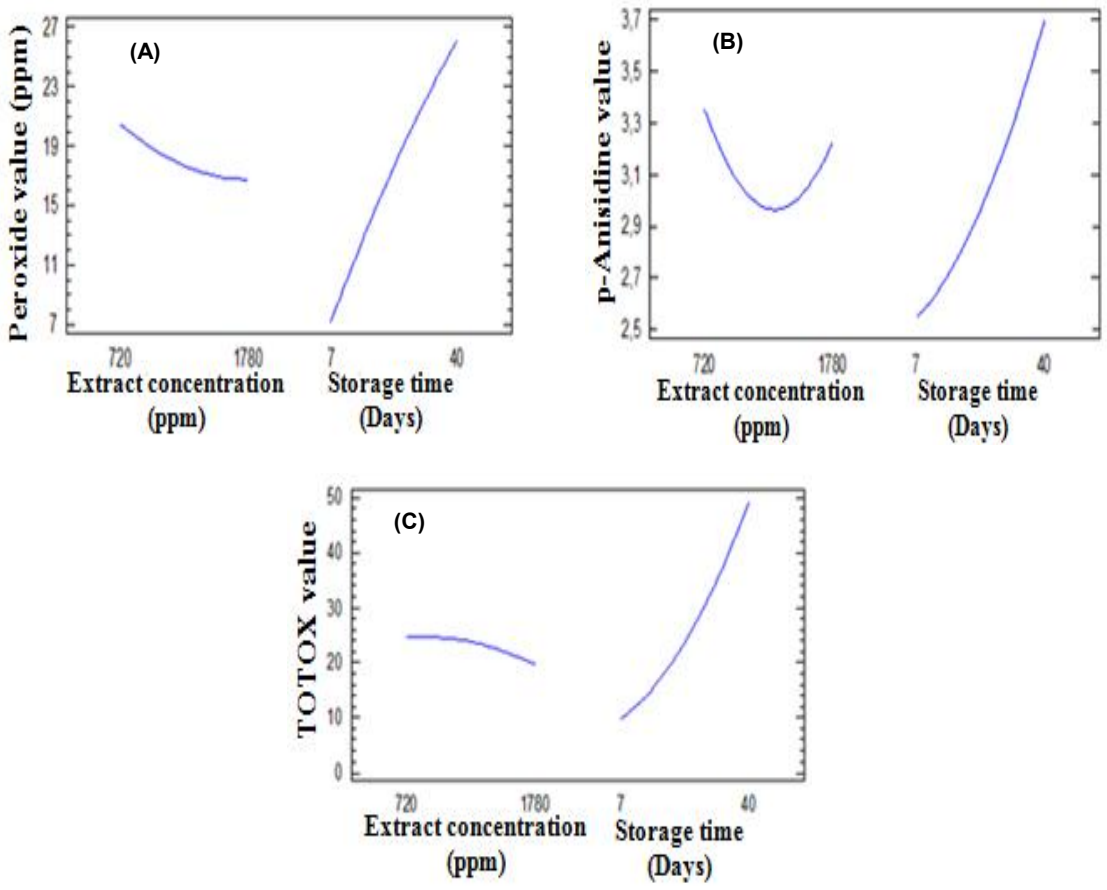

Fig. 3. Main effects plots showing effect of extract concentration and storage time on the peroxide value (A), anisidine value (B) and TOTOX value (C) of oil during storage

3.2.2.2 Analysis of contour plots

Fig. 4. is a contour plot showing the effect of the process parameters on the peroxide value (A), anisidine value (B) and TOTOX value (C) during storage of sample oils enriched with cocoa pods extract at different concentrations. On the basis of the quality standards for oils with respect to peroxide value (<10 ppm), *p*-Anisidine value (≤ 20) and TOTOX value (<26), the areas of interest have been delineated. Considering TOTOX value that measures both hydroperoxides and their breakdown products, and provides a better estimation of the progressive oxidative deterioration of fats and oils, it would be advisable to store palm olein enriched with cocoa pods extracts (2000 ppm) at 70°C for 1 to 30 days in order to preserve its quality.

Considering assertion of Evans et al. [17] who showed that heating of oils for 08 hours at 65°C is equivalent to one month storage at room temperature, we can conclude that the supplementation of palm olein with 2000 ppm of cocoa pods extract can extend its preservation to 30 months at room temperature.

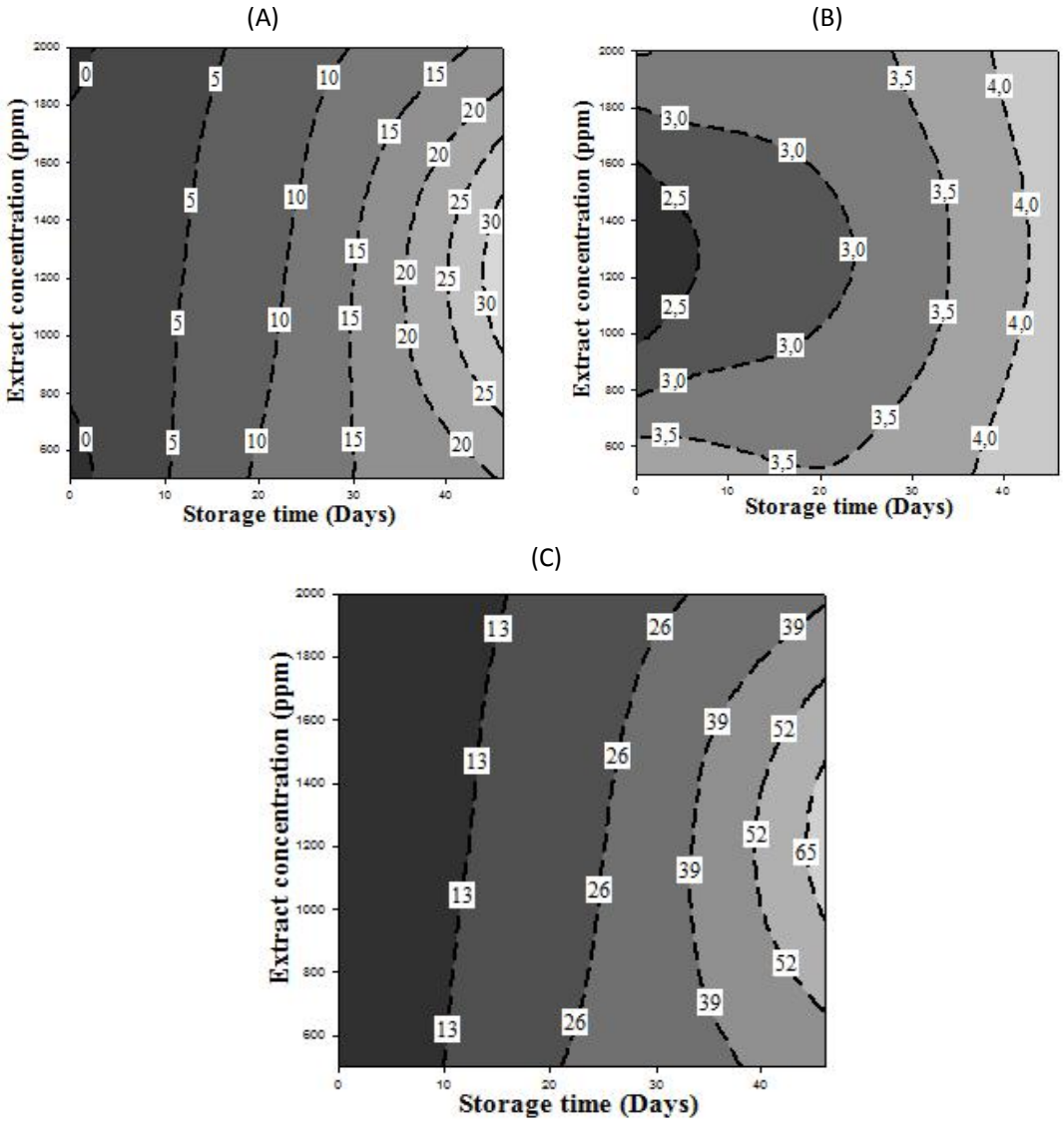

Fig. 4. Contour plots showing the effect of storage time and extract concentration on the peroxide value (A), anisidine value (B) and TOTOX value (C) of palm olein during storage

4. CONCLUSION

Results indicate that cocoa pods extract has a high reducing power and metal ion chelating activity. This extract was also found to be efficient in inhibiting peroxide formation and reducing in the same way the amount of secondary oxidation products. Oxidation of palm olein increase with storage time and cocoa pods extract have the capacity to extend the shelf life of oil at room temperature. *Theobroma cacao* pods can be exploited industrially as an alternative source of synthetic antioxidants for stabilization of palm olein and other oil systems.

ACKNOWLEDGEMENTS

The authors would like to thank the TWAS-CSIR, and the Department of Science and Technology, Government of India, for the financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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