OpenAccessPub

Journal of Antioxidant Activity

ISSN: 2471-2140

DOI: 10.14302/issn.2471-2140.jaa-21-3960

Research Article

Freely Available Online

Antioxidant Activity of Pod Coat Extracts of Pigeon Pea (*Cajanus Cajan* L.) and Their Efficacy in Stabilization of Soybean Oil

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Running Title:

Stabilization of SBO with pigeon pea

Keywords:

Antioxidant, peroxide, phenol, Thiobarbituric acid

Received: Sep 15, 2021

Accepted: Sep 24, 2021

Published: Oct 04, 2021

Editor:

Ana Carolina Carolina, University of SÃ£o Paulo Faculty of Veterinary Medicine and Animal Science

Abstract

When lipids are exposed to heat, light and oxygen, it leads to oxidation. The addition of antioxidants is required to preserve colour, flavour and vitamin destruction. Present study was, therefore, planned to investigate pod coat of pigeon pea as possible sources of natural antioxidants and to assess their efficacy in stabilization of crude soybean oil during normal storage (28 days at 50°C). Study revealed that acetone pod coat extract of pigeon pea showed richness in total phenolics (17.72 mg/g), flavonoids (9.00 mg/g) and tannins (2.21 mg/g) while the extract of ethyl acetate was found enriched in tocopherols content (9.56 mg/g).

The IC_{50} value of acetone extract was found to be lowest, exhibited potent antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric thiocyanate (FTC) methods. After adding synthetic and natural antioxidants in oil, Peroxide, p-Anisidine, Thiobarbituric acid value, Conjugated dienes, trienes and free fatty acids content were measured every 4 days. Acetone pod coat extract (2000ppm) of pigeon pea gave strong antioxidant efficacy in stabilization of crude soybean oil and hence could be recommended as natural antioxidants for food applications. The research

explored the possibility of using pod coat of pigeon pea as imminent sources of green antioxidants and to evaluate their efficacy in stabilization of crude soybean oil.

Introduction

The use of natural extracts for medicinal purpose has taken prominent dimensions in the past few decades, largely due to the discovery that these contain not only primary metabolites and minerals but also a great variety of secondary metabolites having antioxidant properties [1] [2]. Purified constituents of many plants have shown unique therapeutic potential. In edible oil processing industry the main reason of lipid deterioration is oxidation. When lipids are exposed to various environmental factors like high temperature, light and oxygen, it leads to oxidative reactions which produce undesirable flavour and odour thus brings down the quality of lipids and lipid-rich foods. Inclusion of antioxidants is, therefore, required to conserve colour and flavour along with prevention of vitamin destruction. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ) and propyl gallate (PG) are generally used to preserve fatty foods. Investigations have demonstrated, however, that uncontrolled and overdosing of these synthetic antioxidants could be toxic and carcinogenic to humans [3]. Although natural antioxidants such as tocopherols are also used as antioxidants in preservation of food but have met with little commercial success due to higher manufacturing costs and lower efficiency. Nevertheless, keeping in mind the economic feasibility and increasing awareness of consumers regarding safe food additive, a need of identifying alternative safer sources has been created.

Various plant extracts are being investigated throughout the world with rapidly rising awareness about the disease-preventive and health promoting properties of polyphenols and other antioxidants found in plants [4]. Pigeon pea (*Cajanus cajan* L.) commonly known as *arhar* or red gram is a perennial legume of Fabaceae family. It is an important legume of semi-arid, tropical and sub tropical regions. It contains ample amount of crude fibres, starch, fat, protein, manganese, potassium, calcium, minerals and trace elements. Seeds of pigeon pea are traditionally recommended to treat piles and biliousness. The leaves of this pulse crop are used in the treatment of abdominal tumours, diabetes, wounds and sores [5]. In China, an infusion of leaves is used for curing yellow fever, anaemia, hepatitis, ulcer and urinary infection [6]. Young leaves are chewed for treating cough and diarrhoea [7]. Leaves have been found to possess anti-oxidant properties and are reported to have copious amount of stilbenes, flavonoids and isoflavonoids [8]. The pods and seeds of this plant are rich source of minerals which are consumed essentially as vegetable at early stage of development. The extract from roots is capable to act as sedative, alexeritic, vulnerary, expectorant, and anthelminthic [9]. The foliage and other parts of plant because of their high nutritional value act as excellent fodder for livestock [10]. This study is an attempt to investigate pod coat of pigeon pea as effective sources of green antioxidants and to investigate their efficacy in stabilization of soybean oil.

Materials and Methods

Pigeon pea was grown in experimental field of CCS Haryana Agricultural University Hisar, Haryana (India) with a temperature range from 26°C to 30°C in rainy season (June to October). The threshed pod coats of pigeon pea were dried and ground into powder, using an electric grinder. The powdered sample was extracted by petroleum ether (60-80°C). Hundred grams of dried, defatted and powdered sample was then extracted separately by the Soxhlet method using acetone, ethyl acetate and chloroform for 8h. The extracts were evaporated under a vacuum at 40°C to the required dryness using a rotary evaporator. The dried extracts of acetone (AE), ethyl acetate (EAE) and chloroform (CE) were kept in a refrigerator until they were analyzed.

Chemical and Phytochemical Analysis

Total tocopherols were determined by the method of Philip [11]. Tannin content was determined by Pearson method using tannic acid as standard [12]. Total phenolics were estimated spectrophotometrically by

Folin-Ciocalteu method keeping gallic acid as a standard [13]. The antioxidant activity of different extracts was determined by percent inhibition of DPPH method [14] and by Ferric thiocyanate (FTC) method [15] while aluminum chloride colorimetric method [16] was used for the determination of flavonoid content.

Oil Extraction

Oil was extracted by Soxhlet method using petroleum ether (60-80°C) as a solvent for 8 h. Oil extraction is a three step process which includes: preparation, extraction, and desolventization.

Addition of Various Extracts in Soybean Oil

Each of the dried pod extracts was added into soybean oil at 1000 and 2000 ppm concentrations. Experiments were also carried out with synthetic antioxidant, Butylated hydroxy anisole (BHA), and control set without added antioxidants. All the samples were homogenized thoroughly and were incubated, in triplicate, at 50°C in thermostat. Required quantity of the samples was removed periodically and studied for their oxidative quality indices.

Determination of Peroxide Value in Oil Samples

Peroxide value (meq/kg) of oil samples were estimated by AOAC method [17].

Determination of p-Anisidine Value in Oil Samples

p-Anisidine assay was carried out according to the procedure described in AOCS method [18]. The absorbance was measured at 350 nm.

Determination of Thiobarbituric Acid in Oil Samples

Thiobarbituric acid (meq/kg) value (TBA) was determined according to the method given by Marcuse and Johansson with slight modification [19]. Absorbance was measured at 530 nm. At the same time, a reagent blank (without TBA reagent) was also done.

Estimation of Conjugated Dienes and Trienes in Oil Samples

Conjugated diene and conjugated triene of oil samples were assessed according to method described by Frankel [20]. The absorbance of the solution was measured at 234 nm and 268 nm for conjugated dienes and conjugated trienes, respectively.

Estimation of Free Fatty Acid in Oil Samples

Free fatty acids were determined as described by Rao method [21].

Statistical Analysis

The data obtained were analyzed using the analysis of variance (ANOVA) in Online Statistical Analysis (OPSTAT) available at http://www.hau.ac.in. Correlation analyses of polyphenolic composition and their antioxidant activities were carried out using Pearson correlation programme in Online Statistical Analysis.

Results and Discussion

Phytochemical Constituents and DPPH Free Radical Scavenging Activity of Pod Coat Extracts of Pigeon Pea

Among three solvents, acetone gave highest yield (4.88g/100g) followed by ethyl acetate (4.35g/100g) and chloroform extract (3.68g/100g). The disparity in yield of different extracts is a result of differences in polarities of compounds present in pod coat [22]. Acetone extract showed highest amount of total phenolics ((17.72 mg Gallic Acid Equivalent per gram (GAE)/g)) followed by ethyl acetate extract (15.51 mg GAE/g) and chloroform (11.46 mg GAE/g) extract. Flavonoid content was also found in decreasing order in the extracts of acetone ((9.00 mg Catechin Equivalent per gram (CAE)/g)), ethyl acetate (7.19 mg CAE/g) and chloroform (6.01 mg CAE/g). Similarly, pod coats of Pigeon pea yielded highest amount of tannins, 2.21 mg Tannic Acid Equivalent per gram (TAE/g) followed by2.04 mg TAE/g and 1.80 mg TAE/g, when extracted with acetone, ethyl acetate and chloroform, respectively. Tocopherols content, on the other hand, was maximum in the extract retrieved using ethyl acetate (9.56 mg tocopherol/g) followed by acetone (7.01 mg tocopherol/g) and chloroform (4.31 mg tocopherol/g).

Antioxidant activities were evaluated by

2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) method. Maximum DPPH free radical scavenging activity exhibited by BHA (91.14%) at 0.9 mg/ml concentration and IC_{50} value was 0.28 mg/ml (Fig.1). Maximum antioxidant activity was exhibited by the extracts of acetone (78.21%), ethyl acetate (66.08%) and chloroform (60.18%) at 1.0 mg/ml concentration. Acetone extract per ml depicted lowest IC_{50} value equals to 0.52 mg followed by 0.60 mg and 0.66 mg, in case of ethyl acetate and chloroform, respectively. Acetone extract exhibited highest free radical scavenging activity. The results of correlation analysis in acetone extract showed that total phenols show positive and significant correlation with flavonoids (r = 0.968*) and tocopherols (r = 0.985*). Flavonoids were significantly correlated with tocopherols (r = 0.996**) and IC_{50} value by DPPH method (r = 0.985*). Tocopherols show positive and significant correlation with IC_{50} value of DPPH methods (r = 0.967*).

The FTC method was used to evaluate the level of lipid peroxidation by measuring the absorbance of hydroperoxide of linoleic acid. In this method, the concentration of peroxide decreases as the antioxidant activity increases. The highest per cent inhibition was shown by extracts of acetone (68.22) followed by ethyl acetate (62.09) and chloroform (57.01). Higher free radical scavenging activity and strong antioxidant activity in FTC method of acetone extract is clearly due to higher amount of phenolics, flavonoids and tocopherol content as compared to other extracts.

Peroxide Value (PV) of Oil Samples

Concentration of peroxides and hydroperoxides formed during the initial stages of lipid oxidation determines the peroxide value of any sample. Hydroperoxides are produced as primary oxidation product during lipid oxidation [23]. Further breakdown of oil into nonvolatile and volatile secondary products may deteriorate its quality [24]. Therefore the level of peroxides gave a clear indication of prevention in lipid oxidation by chemical or natural antioxidants. A continuous increase in peroxide value was observed in all the oil samples with increasing incubation period (Table 1). Initially rate was very slow became fast after 12th day of incubation following which it continued to increase further, and reached to a maximum value after 28 days of incubation. The peroxide value (meq O_2/kg) of control sample reached a maximum of 41.62±0.05 after 28 days of incubation. A significant ($p < 0.05$) difference in peroxide value was perceived between the control and soybean oil containing chemical (BHA) and natural antioxidants which slowed down the formation of peroxides edifying good antioxidant efficacy of different pod coat extracts in stabilizing oil.

The antioxidants under study can be arranged in following descending order depending upon their competence in preserving the standards of the studied oil: BHA (200ppm), BHA (100ppm), acetone extract (2000ppm), ethyl acetate extract (2000ppm), acetone extract (1000ppm), chloroform extract (2000ppm), ethyl acetate extract (1000ppm) and chloroform extract (1000ppm). Among all pod coat extracts, the acetone extract of pigeon pea revealed low peroxide values and better antioxidant activity because of the existence of extortionate phenolics quantity. However, among all the samples, BHA (200ppm) was found to be the most effective in reducing the peroxide value of oil. Similar kind of results showing the antioxidant potential of agro wastes extracts and their application in stabilization of corn oil [25]. They also observed a significant ($p < 0.05$) stabilization in peroxide value of corn oil in the presence of BHT (200ppm) and methanolic extracts of different plant materials (600ppm).

p-Anisidine Value of Oil Samples

p-Anisidine value represents the extent of secondary oxidation products *i.e.* aldehyde in fats and oils [26]. The p-Anisidine value of control sample increased from 2.49±0.04 to 48.41±0.13 after 28 days of incubation (Table 2). Differences observed between the p-Anisidine values of control and experimental samples were statistically significant. However inhibitory effect

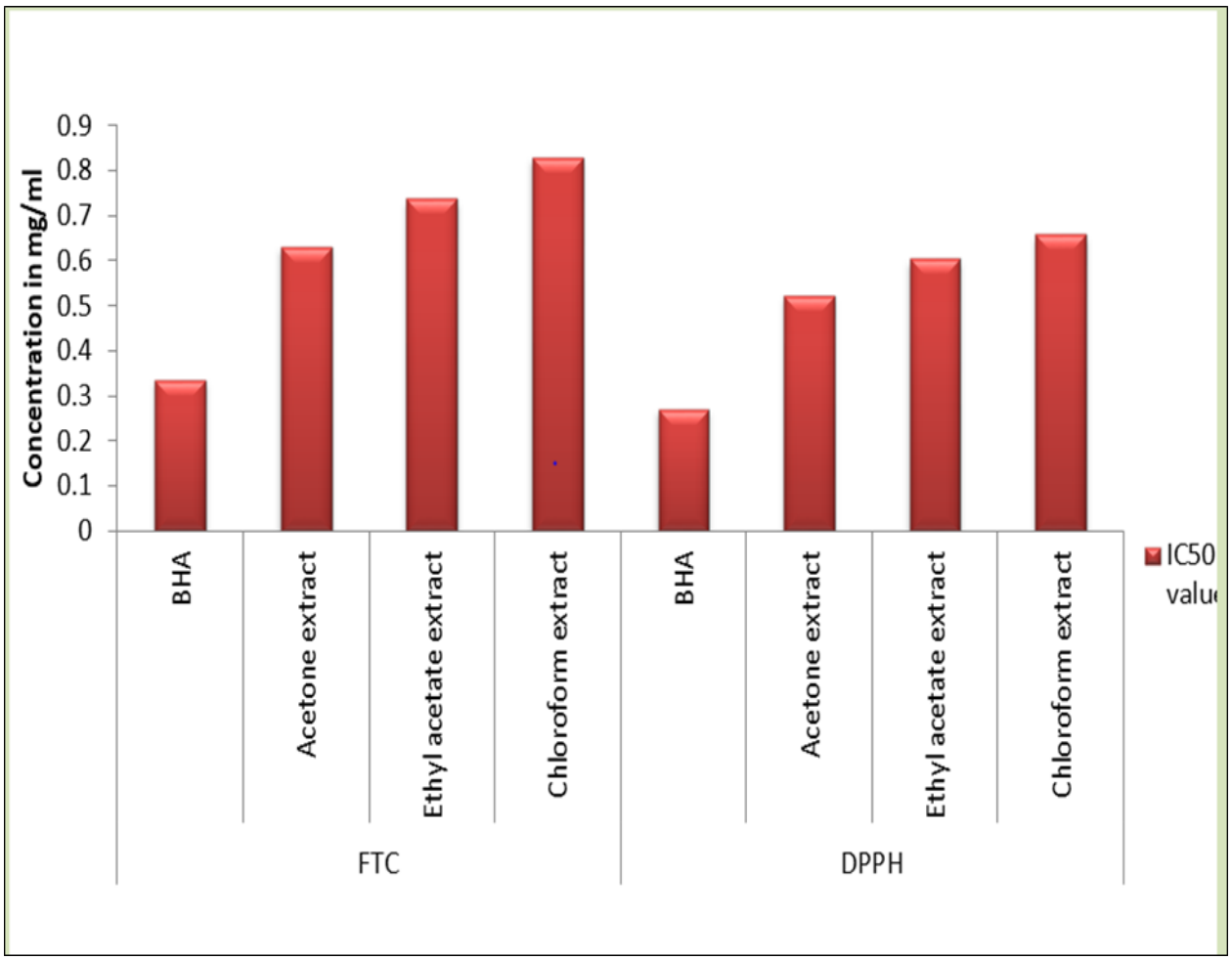

Figure 1. IC_{50} values of various pod coat extracts of pigeon pea by DPPH and FTC method

Table 1. Peroxide values of crude soybean oil samples supplemented with synthetic and natural antioxidants

Sample	Incubation days							
	0	4	8	12	16	20	24	28
Control	3.40±0.01	5.89±0.04	8.86±0.06	12.31±0.05	19.15±0.04	26.98±0.07	33.71±0.04	41.62±0.05
BHA (100ppm)	3.40±0.01	4.51±0.01	6.61±0.03	7.76±0.03	9.51±0.03	10.87±0.04	12.13±0.02	13.38±0.03
BHA (200ppm)	3.40±0.01	3.85±0.02	5.48±0.02	6.72±0.02	7.86±0.02	8.45±0.03	9.72±0.03	10.86±0.02
AE (1000ppm)	3.40±0.01	4.82±0.02	7.92±0.04	10.68±0.02	14.67±0.03	19.61±0.04	25.67±0.06	31.12±0.07
AE (2000ppm)	3.40±0.01	4.66±0.01	6.13±0.02	9.33±0.01	12.21±0.02	16.93±0.03	21.55±0.02	27.77±0.03
EAE (1000ppm)	3.40±0.01	5.01±0.02	8.13±0.02	11.40±0.04	16.97±0.02	21.08±0.03	27.31±0.02	31.61±0.05
EAE (2000ppm)	3.40±0.01	5.04±0.01	6.93±0.02	10.49±0.01	13.46±0.03	17.78±0.02	23.12±0.03	29.19±0.05
CE (1000ppm)	3.40±0.01	5.32±0.01	8.46±0.02	11.86±0.02	17.31±0.03	23.68±0.02	28.08±0.04	33.12±0.14
CE (2000ppm)	3.40±0.01	5.46±0.02	7.12±0.01	11.09±0.04	14.34±0.02	19.43±0.03	24.51±0.02	31.60±0.04

Notes. The values are expressed as mean ± SD;BHT- butylated hydroxyl toluene; AE-acetone pod coat extract of pigeon pea; EAE-ethyl acetate pod coat extract of pigeon pea ; CE-chloroform pod coat extract of pigeon pea .

Table 2. p-Anisidine values of crude soybean oil samples supplemented with synthetic and natural antioxidants

Sample	Incubation days							
	0	4	8	12	16	20	24	28
Control	2.49±0.04	11.83±0.06	16.44±0.08	22.42±0.06	26.67±0.09	33.71±0.05	40.18±0.09	48.41±0.13
BHA (100ppm)	2.49±0.04	6.44±0.02	8.81±0.05	10.02±0.06	13.32±0.08	17.18±0.10	21.21±0.07	24.94±0.10
BHA (200ppm)	2.49±0.04	3.45±0.04	5.93±0.05	8.23±0.03	11.27±0.06	13.11±0.07	17.33±0.06	21.12±0.09
AE (1000ppm)	2.49±0.01	6.70±0.03	10.21±0.05	14.34±0.06	19.81±0.08	25.42±0.11	28.82±0.14	33.61±0.16
AE (2000ppm)	2.49±0.04	5.79±0.04	9.01±0.06	12.92±0.05	15.62±0.08	18.75±0.06	22.38±0.10	26.08±0.10
EAE (1000ppm)	2.49±0.04	7.14±0.04	11.89±0.06	16.37±0.07	22.48±0.09	26.84±0.12	30.30±0.13	32.24±0.17
EAE (2000ppm)	2.49±0.04	6.37±0.05	11.41±0.06	14.37±0.07	17.38±0.08	20.39±0.09	24.43±0.11	27.61±0.14
CE (1000ppm)	2.49±0.04	8.58±0.05	12.94±0.06	18.68±0.09	24.67±0.16	28.49±0.10	32.08±0.14	35.19±0.19
CE (2000ppm)	2.49±0.04	7.29±0.05	13.07±0.06	15.79±0.08	19.43±0.10	22.09±0.11	26.51±0.14	29.98±0.16

Notes. The values are expressed as mean ± SD;BHT- butylated hydroxyl toluene; AE-acetone pod coat extract of pigeon pea ; EAE-ethyl acetate pod coat extract of pigeon pea ; CE-chloroform pod coat extract of pigeon pea

of pod coat extracts of pigeon pea was lower than BHA. p-Anisidine values of various crude soybean oil samples on 28th day of incubation were arranged in descending order as follows: control, CE (1000ppm), AE (1000ppm), EAE (1000ppm), CE (2000ppm), EAE (2000ppm), AE (2000ppm, BHA (100ppm), BHA (200ppm). Among all extracts, the acetone extract (2000ppm) had higher antioxidant activity than ethyl acetate and chloroform

Thiobarbituric Acid (TBA) Value of Oil Samples

During oxidation process, peroxides decompose to lower molecular weight compounds such as malonaldehyde. TBA value measures the rate of oxidative rancidity in terms of formation of a non-volatile compound, malonaldehyde [27]. TBA value of all the samples initially increased and reached to a maximum value followed by a slight decrease towards the end (Table 3). It could be due to oxidation of secondary oxidation products and formation of carboxylic acids [28]. TBA value (meq of malonaldehyde/g) of control increased from 4.47±0.02 (at zero time) to 49.77±0.09 (16th day) and then decreased to 41.67 ±0.17 (28th day). All the varying concentrations of pod coat extracts were effective in lowering the TBA value of different samples. The capability of these extracts to reduce TBA value of different samples slightly increased as the concentration of the extract increased. Maximum TBA value, was observed on 16th day of incubation, in case of control and soybean oil sample supplemented with BHA (100 and 200ppm), while in case of soybean oil supplemented with all the other extracts the maxima was observed on 24th day of incubation.

Conjugated Dienes (CD) and Trienes (CT) of Oil Samples

After the formation of hydroperoxides from polyunsaturated fatty acids present in oil, non-conjugated double bonds undergo rearrangement and forms conjugated dienes which absorbs at 234 nm. Conjugated trienes are formed as a result of oxidation and rearrangement of polyunsaturated fatty acids having three or more double bonds which can be estimated as absorption at 268 nm [29] [30]. The change in absorbance at 234 and 268 nm, quantified with absorbance coefficient (K_0) is used as a measure of oxidative degradation of oil.

The increase in conjugated dienes and trienes signifies the increased oxidative degradation and decreased stability of the oil [31]. There was a consistent increase in conjugated dienes and conjugated trienes of all the samples over the storage period (Table 4). Per cent conjugated dienes and trienes of control sample increased from 0.36±0.02 and 0.16±0.02 to 36.44±0.16 and 17.36±0.06, respectively during incubation period of 28 days. Highest percentage contents of CD and CT were observed for control after 28 days of incubation ,indicating greater intensity of oxidation, followed by chloroform extract (1000ppm), chloroform extract (2000ppm), ethyl acetate extract (1000ppm), acetone extract (1000ppm), ethyl acetate extract (2000ppm), acetone extract (2000ppm), BHA (100ppm) and BHA (200ppm). The superior antioxidant activity has been indicated by reduced CD and CT of all the stabilized oil samples as compared to control.

Free Fatty Acid (FFA) Content of Oil Samples

The free fatty acid content is considered as a sign of oil hydrolysis. It usually increases with oxidative degradation of lipids [26]. Free fatty acids measured in terms of percentage oleic acid content of all samples had shown a consistent increase along the storage period (Figure 2). In control sample, the free fatty acids content increased from 0.567±0.002 to 4.22±0.023 % after 28 days of incubation which are significantly higher than other samplesstabilised with various extracts and BHA. The free fatty acid content of soybean oils containing different extracts of pod coat of pigeon pea were lower than the control having no stabilizer. In contrast to other extracts, acetone (2000ppm) pod coat extract manifested superior antioxidant activity in terms of free fatty acid but lower than BHA.

Conclusions

Addition of pod coat extracts of pigeon pea in crude soybean oil gave strong antioxidative efficiency and hence could be used as alternative natural antioxidants

Table 3. TBA values of crude soybean oil samples supplemented with synthetic and natural antioxidants

Sample	Incubation days							
	0	4	8	12	16	20	24	28
Control	4.47±0.02	12.16±0.03	22.78±0.05	37.82±0.07	49.77±0.09	48.52±0.08	44.23±0.12	41.67±0.17
BHA (100ppm)	4.47±0.02	8.57±0.04	12.79±0.06	20.64±0.06	27.44±0.08	26.16±0.10	25.62±0.12	25.18±0.13
BHA (200ppm)	4.47±0.02	7.31±0.03	10.48±0.05	17.26±0.07	24.61±0.08	23.77±0.11	23.05±0.12	22.75±0.13
AE (1000ppm)	4.47±0.02	9.95±0.10	15.58±0.05	21.67±0.08	28.25±0.09	33.38±0.12	36.43±0.14	34.42±0.16
AE (2000ppm)	4.47±0.02	8.68±0.04	12.59±0.06	19.32±0.09	25.28±0.08	29.22±0.13	33.41±0.11	32.38±0.14
EAE (1000ppm)	4.47±0.02	10.22±0.08	16.28±0.11	22.49±0.14	29.12±0.07	33.89±0.10	36.94±0.05	34.99±0.16
EAE (2000ppm)	4.47±0.02	9.07±0.05	13.05±0.04	20.16±0.07	25.87±0.06	29.96±0.08	33.98±0.10	32.93±0.14
CE (1000ppm)	4.47±0.02	10.66±0.05	17.65±0.06	23.75±0.08	30.25±0.10	35.19±0.11	38.13±0.12	36.46±0.14
CE (2000ppm)	4.47±0.02	9.76±0.05	14.31±0.07	20.86±0.05	27.12±0.06	31.89±0.08	35.26±0.10	35.05±0.12

Notes. The values are expressed as mean ± SD;BHT- butylated hydroxyl toluene; AE-acetone pod coat extract of pigeon pea ; EAE-ethyl acetate pod coat extract of pigeon pea ; CE-chloroform pod coat extract of pigeon pea .

Table 4. Percent conjugated dienes and trienes in crude soybean oil samples supplemented with synthetic and natural antioxidants

Conjugated dienes								
Sample	Incubation days							
	0	4	8	12	16	20	24	28
Control	0.36±0.02	3.42±0.04	8.43±0.07	13.62±0.10	18.42±0.13	24.02±0.14	30.49±0.13	36.44±0.16
BHA (100ppm)	0.36±0.02	1.58±0.03	4.56±0.08	8.29±0.06	12.34±0.08	16.26±0.13	20.63±0.10	24.57±0.16
BHA (200ppm)	0.36±0.02	1.07±0.02	3.97±0.04	6.93±0.06	10.28±0.09	13.75±0.11	17.38±0.13	21.27±0.16
AE (1000ppm)	0.36±0.02	2.09±0.02	5.69±0.05	9.88±0.06	13.86±0.09	18.36±0.12	23.83±0.15	29.51±0.18
AE (2000ppm)	0.36±0.02	1.73±0.05	5.19±0.06	9.22±0.08	13.29±0.08	17.85±0.10	23.13±0.12	28.97±0.13
EAE (1000ppm)	0.36±0.02	2.36±0.03	6.08±0.06	10.43±0.05	15.04±0.10	19.79±0.12	25.11±0.10	30.79±0.15
EAE (2000ppm)	0.36±0.02	1.94±0.05	5.55±0.10	9.59±0.07	13.89±0.13	18.33±0.10	23.78±0.11	29.44±0.09
CE (1000ppm)	0.36±0.02	2.49±0.04	6.38±0.05	10.78±0.06	15.83±0.08	20.51±0.13	25.81±0.11	31.42±0.16
CE (2000ppm)	0.36±0.02	2.17±0.04	5.95±0.05	10.19±0.07	15.15±0.06	20.03±0.08	25.18±0.08	30.98±0.10
Conjugated trienes								
Control	0.16±0.02	1.79±0.02	3.48±0.04	5.89±0.04	8.89±0.02	11.58±0.05	13.99±0.06	17.36±0.06
BHA (100ppm)	0.16±0.02	1.09±0.03	2.19±0.02	3.44±0.04	4.96±0.05	7.28±0.06	9.77±0.08	12.44±0.05
BHA (200ppm)	0.16±0.02	0.89±0.02	2.05±0.03	3.25±0.02	4.81±0.03	6.49±0.06	8.42±0.04	10.56±0.04
AE (1000ppm)	0.16±0.02	1.22±0.02	2.65±0.02	4.45±0.03	6.72±0.04	9.56±0.06	11.61±0.05	15.03±0.05
AE (2000ppm)	0.16±0.02	1.17±0.02	2.71±0.04	4.31±0.06	6.53±0.05	9.28±0.08	11.42±0.05	14.39±0.08
EAE (1000ppm)	0.16±0.02	1.31±0.03	2.79±0.05	4.61±0.05	7.05±0.06	9.78±0.08	11.88±0.06	15.32±0.09
EAE (2000ppm)	0.16±0.02	1.22±0.02	2.78±0.05	4.42±0.06	6.82±0.08	9.59±0.09	11.72±0.08	14.77±0.09
CE (1000ppm)	0.16±0.02	1.39±0.02	2.91±0.06	4.82±0.05	7.62±0.05	10.23±0.06	12.39±0.07	15.96±0.09
CE (2000ppm)	0.16±0.02	1.27±0.02	2.84±0.04	4.54±0.05	7.27±0.06	10.24±0.05	12.19±0.06	15.57±0.09

Notes. The values are expressed as mean ± SD;BHT- butylated hydroxyl toluene; AE-acetone pod coat extract of pigeon pea ; EAE-ethyl acetate pod coat extract of pigeon pea ; CE-chloroform pod coat extract of pigeon pea.

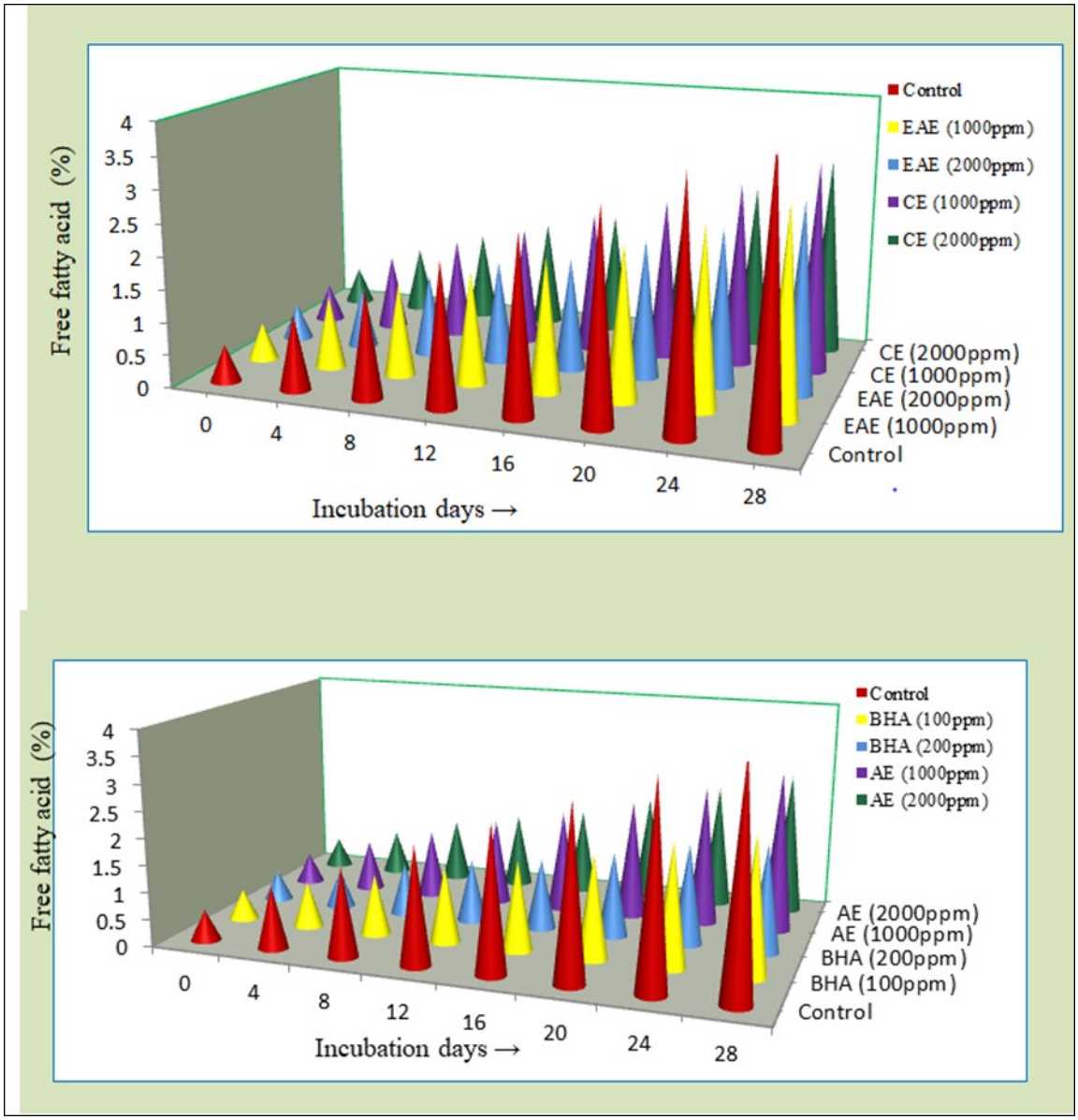

Figure 2. Free fatty acid content of crude soybean oil samples stabilized with BHA and pod coat extracts of pigeon pea retrieved using acetone (A), ethyl acetate and chloroform (B)

for food applications. It was quite clearly depicted that all the extracts considerably reduced the rate of oxidative deterioration of crude soybean oil but acetone extract (2000ppm) was most effective. As future prospects, an understanding of mechanisms involved and the factors affecting the antioxidant activity of these compounds would be of significant importance in exploitation of such natural antioxidants to control lipids oxidation in food.

Conflict of Interest

Authors declare that there is no conflict of interest.

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