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Oxidative Stability of Soybean Oil Supplemented with Pod Coat Extracts of Cluster Bean

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Authors' contributions

This work was carried out in collaboration between both authors. Author SN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SN and SS managed the analyses of the study. Author SN managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The aim of present work was to determine the potency of phenolic extracts from cluster bean pod coats in improving shelf life of soybean oil. Pod coats of cluster bean were extracted with three solvents (acetone, ethylacetate and chloroform). Crude soybean oil samples were stabilised by adding different extracts at 1000 and 2000 ppm concentration and effect of addition of these extracts on oxidative stability of crude soybean oil at 50°C was observed. Rate of oxidative degradation of crude soybean oil was lowest in the sample treated with acetone extract (2000 ppm). The value of various oxidative quality parameters of crude soybean oil supplemented with acetone extract (2000 ppm) after 28 days of incubation are peroxide value (26.77 meq/kg oil), free fatty acids content (2.637%), p-anisidine value (25.18), TOTOX value (78.72), conjugated dienes (28.47%), conjugated trienes (13.97%) and TBA value (31.59 meq malonaldehyde/g). Results of present study indicate that pod coat extracts of cluster bean possess remarkable antioxidant potential and considerably lowered the rate of oxidative deterioration of crude soybean oil.

Keywords: Cluster bean; pod; soybean; oil; stabilization.

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ABBREVIATIONS

ACE: Acetone extract; EAE: Ethylacetate extract; CFE: Chloroform extract; SBO: Soybean oil.

1. INTRODUCTION

Cellular injury or oxidative damage occurring from reactive oxygen species (ROS) or free radicals now seems the primary mechanism underlying a number of human neurodegenerative disorders, inflammation, diabetes, autoimmune pathologies, viral infections, and digestive system disorders. In order to scavenge any oxidant or reactive intermediates which are continuously generated in cells, an array of intra and extracellular antioxidant mechanism is necessary. An antioxidant is a substance which considerably delays or prevents oxidation of substrate, even at very low concentrations in comparison to oxidizable substrate [1].

Fats and oils from various sources have an important place in food industry as well as other industrial sectors. Fats and oils are tested to find out their function in food, to check extent of adulteration or deterioration. From quality perspective, lipid oxidation is the main quality control parameter for the processor and consumers of vegetable oils. Lipid oxidation causes rancidity and usually it is the conclusive factor for determining the shelf life of lipid rich food products. Lipid oxidation is deterioration process which leads to the formation of a number of primary and secondary oxidation products. These degradation products adversely affect the aroma, flavour, taste and overall quality of edible oils or other lipid rich foods. The use of synthetic antioxidants such as propyl gallate (PG), butylated hydroxyl anisole (BHA) and butylated hydroxy-toluene (BHT) in lipid rich food is highly discouraged due to their toxicity and perceived carcinogenicity. However the use of natural antioxidants derived from plants in food as well as in medicines is gaining attention due to their health improvement effects [2,3,4,5]. The phytochemicals that play an important role as antioxidants are polyphenols, caffeic acid, tannins, cinnamic acid, ferulic acid, kaempferol, flavonoids, isoflavonoids, proteins, polypeptides, polysaccharides etc [6].

Legumes are important part of healthy and balanced diet. Besides being a nutritious food, legumes can also help in preventing many diseases [7]. The medicinal properties of legumes are due to presence of pharmaceutical compounds for curing or improving human health [8]. Alkaloids, glycosides, phenols, flavonoids, polysterols, phytic acids, tannins, saponins and protease inhibitors are the active compounds found in legumes. *Cyamopsis tetragonoloba* (L.) Taub commonly known as guar is a cash crop of the family Leguminosae [9]. Guar seed is used in various industries for its galactomannan rich endosperm which acts as viscosity enhancer. Guar gum is used in mining, paper manufacturing, explosives, textile, ore flotation, oil drilling and many other industrial purposes. Fresh or dry forage of guar is also used as cattle feed. It is used as cover crop and green manure in agriculture [10]. Young tender pods of guar are eaten as a vegetable or snacks. The growing interest in the replacement of synthetic food antioxidants by natural ones has fostered research on the screening of agricultural waste for identifying new antioxidants. Present work is an attempt to investigate the ability of pod coat extracts of cluster bean to reduce peroxidation and improve shelf life of soybean oil.

2. MATERIALS AND METHODS

2.1 Preparation of Extracts and Stabilization of Oil Samples

The threshed pods of cluster bean were taken from the experimental field of CCS HAU Hisar, Haryana. Pod coats were separated from grass residues and grinded to powder. The powdered samples were extracted by petroleum ether (60-80°C). 100 g dried defatted powdered samples were then extracted separately by the soxhlet method using acetone, ethylacetate and chloroform for 8 h. Oil was extracted by Soxhlet method using petroleum ether (60-80°C) for 8 h. Solvent extraction processes include basically three steps: preparation, extraction, and desolventizing. Butylated hydroxytoluene (Synthetic antioxidant) was added with in legal limits (100 and 200 ppm) while extracts were added at 1000 and 2000 ppm concentration in crude soybean oil. Control sample was also prepared. Samples were prepared in triplicate and immediately stored in dark at 50°C for a period of 28 days. Required amount of the samples was removed after every 4 days and analysed in triplicate for the following oxidative quality parameters.

2.2 Determination of Peroxide Value of Oil Samples

Peroxide value of stabilised and control sample was determined according to AOAC method [11]. 5 g oil was dissolved in 5 mL of acetic acid/ chloroform (3:2 v/v) mixture. To the mixture added 0.5 mL freshly prepared saturated aqueous solution of potassium iodide (KI) solution and kept it undisturbed for 1 minute. 30 mL distilled water was added and the solution was titrated against sodium thiosulphate solution (0.01N) using starch as indicator. Titration was done till blue colour just disappeared. Peroxide value (meq O_2/kg oil) of sample was calculated using the formula:

$$\text{Peroxide value}\ \left({}^{\text{meq}}/_{\text{kg}}\right) = \frac{1000 \times \text{S} \times \text{N}}{\text{W}}$$

Where S = volume of sodium thiosulphate used in mL, N = normality of sodium thiosulphate used and W = weight of oil sample (g).

2.3 Determination of Free Fatty Acid Content of Oil Samples

Free fatty acids were determined as described by the method of Rao et al. [12]. To one gram of oil sample added 50 mL denatured alcohol and mixed thoroughly. A few drops of phenolphthalein were added and the mixture was titrated against 0.01N sodium hydroxide (NaOH) solution till a permanent light pink colour appears which persist at least for one minute. The percentage of free fatty acids was calculated by using the following formula:

$$\text{Free fatty acids (in terms of oleic acid)} = \frac{100 \times 282 \times \text{V}}{\text{weight of oil} \times 10 \times 1000}$$

V = volume of 0.01N sodium hydroxide (NaOH) solution used.

2.4 Determination of p-anisidine Value of Oil Samples

Formation of secondary oxidation products was measured by p-anisidine value according to the American Oil Chemists' Society (AOCS) method [13]. 0.5 g oil sample was dissolved in isooctane and the volume was made up to 50 mL with isooctane. 5 mL of this solution was mixed with 1 mL of 0.25% of p-anisidine reagent and kept in the dark for 10 minutes. Absorbance was measured at 350 nm using the spectrophotometer. A blank test (without p-anisidine reagent) was also prepared.

$$\text{p} - \text{anisidine value} = \frac{(\text{A}_2 - \text{A}_1) \times 1.2 \times 50}{\text{W(g)}}$$

Where A_2 = absorbance of oil sample, A_1 = absorbance of blank and W = weight of oil sample in grams.

2.5 Determination of Total Oxidation (TOTOX) Values of Oil Samples

Total oxidation (TOTOX) values were calculated according to the method of Shahidi and Wanasundara [14]. Anisidine value is used in conjunction with peroxide value to calculate total oxidation.

Total oxidation (TOTOX) value = 2PV + p-AV

2.6 Estimation of Conjugated Dienes (CD) and Conjugated Trienes (CT) in Oil Samples

Frankel method [15] was used for estimation of conjugated dienes and conjugated trienes in oil samples. To 100 mg oil sample taken in a flask added 75 mL of isooctane. Mixture was warmed with continuous shaking for complete dissolution. Resulting solution was cooled to room temperature and further isooctane was added to make a final concentration of about 0.5 g/ L to the oil sample. The absorbance of the solution was measured at 234 and 268 nm for conjugated dienes and conjugated trienes, respectively. The conjugated dienes and trienes are represented as the percentage of conjugated dienoic and trienoic acid and were calculated from the formula:

$$\text{Conjugated dienoic or trienoic acid (\%)} = 0.84 \times \frac{\text{A}_\text{s}}{\text{bc} - \text{K}_0}$$

Where K_0 = absorptivity by acid or ester groups, A_s = absorbance at 234 nm (CD) or 268 nm (CT), b = cell length and c = concentration in gram per litre.

2.7 Determination of Thiobarbituric Acid Value (TBA) of Oil Samples

Determination of Thiobarbituric acid value (TBA) of oil sample was carried out following the

method given by Marcuse and Johansson [16] with slight modification. Oil sample (100 mg) was dissolved in 25 mL 1-butanol. To a 25 mL aliquot of the above solution added 5 mL (0.2%) TBA reagent and mixed vigorously. Resulting solution was incubated at room temperature. Absorbance was noted at 530 nm with spectrophotometer Spectronic 20 (Milton Roy Company). Simultaneously, a reagent blank was also prepared. Thiobarbituric acid value (mequiv of malonaldehyde/g) was calculated as:

$$\text{Thiobarbituric acid value} = \frac{50 \times (\text{A} - \text{B})}{\text{W}}$$

Where A = absorbance of test sample, B = absorbance of blank sample and W = weight of sample in mg.

3. RESULTS AND DISCUSSION

Oxidative quality of control as well as stabilised oil samples were measured by various parameters after every 4 days for a period of 28 days. Peroxide value represents the peroxide and hydroperoxides formed at early stage of lipid peroxidation [17]. A typical pattern in the rise of peroxide value was observed in almost all the oil samples (Table 1). Control showed highest peroxide value among all the samples which indicates highest extent of oxidation. The peroxide value (meq O_2/kg) of control varied from 3.40 to 41.62. Oil samples stabilised by BHA had exhibited lowest peroxide value however among the different pod coat extracts of cluster bean acetone extract was most efficient in retarding the formation of primary oxidation products. The high peroxide value is due to the formation of unstable hydroperoxides that ultimately converts to short chain acids, aldehydes, ketones, and alcohols and thus causes off flavour and odour [18].

The peroxide values of various samples at the end of incubation period were found in the following order:

> Control > CFE (1000 ppm) > EAE (1000 ppm) > CFE (2000 ppm) > ACE (1000 ppm) > EAE (2000 ppm) > ACE (2000 ppm) > BHA (100 ppm) > BHA (200 ppm)

Assessment of free fatty acid content of oil samples was done after stabilising with extracts of pod coat of cluster bean in different solvent systems. The enlargement in free fatty acid content with incubation time in control as well as stabilised oil samples is displayed in Table 2. It was observed that free fatty acid content of all the samples increased regularly and continuously during incubation. In contrast to other extracts acetone extract at 2000 ppm concentration manifested highest antioxidant activity after BHA in relation to free fatty acid content. In control free fatty acids content increased from 0.567 to 4.223 which are significantly higher than other samples stabilised with various extracts and BHA. Results of present investigation are comparable to the finding of Anwar et al. [19] regarding oxidative deterioration of soybean oil at ambient and sunlight storage. Free fatty acids content of various samples at the end of storage period were in following order:

> Control > CFE (1000 ppm) > EAE (1000 ppm) > CFE (2000 ppm) > ACE (1000 ppm) > EAE (2000 ppm) > ACE (2000 ppm) > BHA (100 ppm) > BHA (200 ppm)

Literature evaluation shows that for the degradation of oil as well as formation of free fatty acids, reaction of oxygen in presence of light is the primary step followed by formation of various compounds based on organic nature as well as free fatty acid formation which are responsible for spoilage of lipid rich food products [20]. During lipid oxidation, hydroperoxides decompose to give secondary oxidation products (aliphatic aldehydes, ketones, acids, alcohols and hydrocarbons) which are quite stable at higher temperatures and accountable for off-odours and off-flavours of oil. As a high peroxide value means poor quality but a low peroxide value is not always indicative of good quality of oil. Peroxide value alone can give misleading results. In order to have a better monitoring of lipid oxidation process, simultaneous detection of primary and secondary lipid oxidation products is necessary. p-anisidine value represents the extent of aldehydic secondary oxidation products in oil (McGinley, 1991). Maximum enhancement in p-anisidine value was observed in control. p-Anisidine value of control varied from 2.49 to 48.41 during incubation period. Oil samples treated with BHA showed least increase in p-anisidine value (Table 3). The higher increase of p-anisidine value of samples treated with extracts in comparison to those treated with BHA indicates the reduced capability of extracts in chelating metal ions. BHA is more effective in retarding the formation of secondary oxidation products [21]. The p-

anisidine values of various samples at the end of incubation period were found in the following order:

> Control > CFE (1000 ppm) > EAE (1000 ppm) > ACE (1000 ppm) > CFE (2000 ppm) > EAE (2000 ppm) > ACE (2000 ppm) > BHA (100 ppm) > BHA (200 ppm)

Peroxide value and p-anisidine value collectively provides a broad overview of the oxidation process in oils and fats. This is a mathematical prediction of oxidative stability and the value is calculated as TOTOX value. TOTOX value of all the samples conspicuously increased during incubation period (Table 4). TOTOX value of control taster increased from 9.29 (at zero time) to 131.65 on 28th day on storage. Soybean oil samples containing BHA (100 and 200 ppm) had low TOTOX value (51.70 and 42.84 respectively) at the end of incubation period. All extracts lowered rate of oxidative deterioration of oil sample although they were more effective at 2000 ppm concentration. The TOTOX values of different samples at the end of incubation period were found in the following order:

> Control > CFE (1000 ppm) > EAE (1000 ppm) > CFE (2000 ppm) > ACE (1000 ppm) > EAE (2000 ppm) > ACE (2000 ppm) > BHA (100 ppm) > BHA (200 ppm)

Hydroperoxides of polyunsaturated fatty acids present in oil undergoes rearrangements and forms conjugated dienes and trienes which absorbs at 234 nm and 268 nm respectively [22,23]. Change in absorbance at 234 and 268 nm is determined with absorptivity coefficient (K_o) is used as a measure of oxidative deterioration of oil. Conjugated diene (CD) and conjugated triene (CT) contents of soybean oil samples supplemented with different pod coat extracts of cluster bean, BHA and control are shown in Table 5 and 6 respectively. Conjugated dienes and trienes content increased with increase in incubation period. The oil samples stabilized with pod coat extracts of cluster bean exhibited lower concentration of conjugated dienes and trienes relative to the control oil sample, indicating the antioxidant properties of the extracts. Conjugated dienes (%) and conjugated triene (%) of control increased from 0.36 and 0.16 to 36.44 and 17.36 respectively during incubation. The higher the increase in conjugated dienes and conjugated trienes, higher will be the magnitude of oxidative deterioration and lower will be oxidative stability of the oil [19].

Conjugated dienes (%) in different samples at the end of storage was in following order:

> Control > CFE (1000 ppm) > CFE (2000 ppm) > EAE (1000 ppm) > EAE (2000 ppm) > ACE (1000 ppm) > ACE (2000 ppm) > BHA (100 ppm) > BHA (200 ppm)

Conjugated trienes (%) in different samples at the end of incubation period was in following order:

> Control > CFE (1000 ppm) > CFE (2000 ppm) > EAE (1000 ppm) > EAE (2000 ppm) > ACE (1000 ppm) > ACE (2000 ppm) > BHA (100 ppm) > BHA (200 ppm)

During oxidative degradation of oil, peroxide formed at initial stage break down to lower molecular weight non-volatile compounds like malonaldehyde. TBA value measures the oxidative deterioration of oil with respect to formation of malonaldehyde [24]. TBA value of control as well as stabilised oil samples increased at the beginning and reached to a maximum value, then decreased towards the end (Table 7). It might be due to further oxidation of secondary oxidation products (aldehydes) to carboxylic acids [25]. TBA value (meq of malonaldehyde/g) of control oil sample enhanced from 4.47 (initially) to 49.77 (16th day) and then decreased to 41.67 (28th day). TBA value of samples treated with BHA (100 and 200ppm) as well as control showed an increasing trend till 16th day of incubation however TBA value of the samples stabilized with clusterbean pod coat extract showed longer increasing trend (till 24th day of storage). This could be explained by slow release of phenolic compounds from extracts which results in more development of oxidation products such as malonaldehyde. TBA value of different samples at the end of storage period was in following order:

> Control > CFE (1000 ppm) > EAE (1000 ppm) > ACE (1000 ppm) > CFE (2000 ppm) > EAE (2000 ppm) > ACE (2000 ppm) > BHA (100 ppm) > BHA (200 ppm)

Table 1. Peroxide values (meq/kg) of crude soybean oil samples stabilised with different concentrations of BHA and pod coat extracts of cluster bean

Sample ↓ Incubation days→	0	4	8	12	16	20	24	28
SBO (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
SBO+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
SBO+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
SBO+ACE (1000ppm)	3.40±0.01	4.67±0.02	7.57±0.04	9.94±0.02	13.07±0.03	18.49±0.02	24.39±0.02	29.81±0.04
SBO+ACE (2000ppm)	3.40±0.01	4.43±0.02	6.02±0.02	8.87±0.02	11.77±0.03	15.80±0.02	20.95±0.02	26.77±0.04
SBO+EAE (1000ppm)	3.40±0.01	4.96±0.02	7.98±0.02	10.52±0.02	15.63±0.02	20.56±0.04	26.16±0.01	31.19±0.05
SBO+EAE (2000ppm)	3.40±0.01	4.79±0.02	6.58±0.01	9.41±0.02	12.46±0.03	16.66±0.02	22.63±0.04	28.69±0.06
SBO+CFE (1000ppm)	3.40±0.01	5.13±0.02	8.19±0.01	11.07±0.02	16.91±0.03	22.72±0.03	27.90±0.01	32.84±0.05
SBO+CFE (2000ppm)	3.40±0.01	5.01±0.01	6.99±0.02	10.54±0.02	13.38±0.02	17.69±0.03	24.11±0.07	30.84±0.05

Table 2. Free fatty acids content (%) of crude soybean oil samples stabilised with different concentrations of BHA and pod coat extracts of cluster bean

Sample ↓ Incubation days→	0	4	8	12	16	20	24	28
SBO (Control)	0.567±0.002	1.163±0.005	1.651±0.008	2.187±0.010	2.693±0.013	3.186±0.016	3.712±0.018	4.223±0.023
SBO+BHA (100 ppm)	0.567±0.002	0.862±0.004	1.141±0.006	1.431±0.007	1.681±0.009	1.936±0.010	2.279±0.012	2.519±0.013
SBO+BHA (200 ppm)	0.567±0.002	0.719±0.030	0.989±0.005	1.196±0.006	1.342±0.080	1.588±0.008	1.879±0.009	2.009±0.011
SBO+ACE (1000ppm)	0.567±0.002	0.933±0.005	1.245±0.006	1.548±0.010	1.866±0.009	2.154±0.014	2.531±0.016	2.902±0.023
SBO+ACE (2000ppm)	0.567±0.002	0.794±0.006	1.128±0.008	1.309±0.009	1.593±0.005	1.939±0.012	2.297±0.013	2.637±0.015
SBO+EAE (1000ppm)	0.567±0.002	1.053±0.005	1.373±0.008	1.696±0.009	1.973±0.011	2.281±0.013	2.637±0.015	3.003±0.017
SBO+EAE (2000ppm)	0.567±0.002	0.842±0.006	1.193±0.005	1.429±0.007	1.679±0.008	2.068±0.010	2.371±0.011	2.828±0.014
SBO+CFE (1000ppm)	0.567±0.002	1.128±0.003	1.474±0.005	1.768±0.008	2.049±0.010	2.352±0.013	2.714±0.014	3.117±0.017
SBO+CFE (2000ppm)	0.567±0.002	0.953±0.003	1.238±0.006	1.512±0.008	1.776±0.010	2.182±0.012	2.489±0.015	2.993±0.018

Table 3. p-anisidine values of crude soybean oil samples stabilised with different concentrations of BHA and pod coat extracts of cluster bean

Sample ↓ Incubation days→	0	4	8	12	16	20	24	28
SBO (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
SBO+BHA (100 ppm)	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
SBO+BHA (200 ppm)	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
SBO+ACE (1000 ppm)	2.49±0.04	6.51±0.02	9.92±0.04	13.61±0.06	18.93±0.05	21.60±0.08	25.13±0.09	29.71±0.13
SBO+ACE (2000 ppm)	2.49±0.04	4.81±0.02	8.13±0.03	11.28±0.05	14.67±0.06	17.53±0.08	20.08±0.05	25.18±0.09
SBO+EAE (1000 ppm)	2.49±0.04	6.96±0.04	11.68±0.06	15.23±0.08	20.63±0.05	23.78±0.06	27.18±0.08	31.61±0.11
SBO+EAE (2000 ppm)	2.49±0.04	5.92±0.03	10.27±0.04	13.06±0.05	16.25±0.09	19.27±0.08	22.72±0.11	26.86±0.13
SBO+CFE (1000 ppm)	2.49±0.04	7.32±0.05	12.35±0.07	16.07±0.08	21.76±0.12	25.69±0.10	28.91±0.14	33.77±0.16
SBO+CFE (2000 ppm)	2.49±0.04	6.54±0.05	12.19±0.06	14.83±0.04	17.63±0.08	21.18±0.11	24.64±0.09	28.37±0.15

Table 4. Total oxidation values of crude soybean oil samples stabilised with different concentrations of BHA and pod coat extracts of cluster bean

Sample ↓ Incubation days→	0	4	8	12	16	20	24	28
SBO (Control)	9.29±0.09	23.61±0.04	34.16±0.08	47.04±0.05	64.97±0.06	87.67±0.13	107.60±0.10	131.65±0.20
SBO+BHA (100 ppm)	9.29±0.09	15.46±0.05	22.03±0.06	25.54±0.09	32.34±0.11	38.92±0.16	45.47±0.14	51.70±0.17
SBO+BHA (200 ppm)	9.29±0.09	11.10±0.07	16.89±0.10	21.67±0.11	26.99±0.12	30.01±0.14	36.77±0.17	42.84±0.19
SBO+ACE (1000 ppm)	9.29±0.09	15.85±0.08	25.06±0.10	33.49±0.12	45.07±0.24	58.58±0.32	73.91±0.19	89.33±0.18
SBO+ACE (2000 ppm)	9.29±0.09	13.67±0.20	20.17±0.14	29.02±0.17	38.21±0.14	49.10±0.24	61.98±0.31	78.72±0.19
SBO+EAE (1000 ppm)	9.29±0.09	16.88±0.09	27.64±0.14	36.27±0.16	51.89±0.19	64.90±0.27	79.50±0.31	93.99±0.45
SBO+EAE (2000 ppm)	9.29±0.09	15.50±0.13	23.43±0.11	31.88±0.20	41.17±0.24	52.59±0.20	67.98±0.15	84.24±0.20
SBO+CFE (1000 ppm)	9.29±0.09	17.58±0.09	28.73±0.21	38.21±0.32	55.58±0.30	71.13±0.25	84.71±0.20	99.39±0.43
SBO+CFE (2000 ppm)	9.29±0.09	16.56±0.12	26.17±0.11	35.91±0.09	44.39±0.10	56.56±0.14	72.86±0.16	90.05±0.24

Table 5. Conjugated dienes (%) of crude soybean oil samples stabilised with different concentrations of BHA and pod coat extracts of cluster bean

Sample ↓ Incubation days→	0	4	8	12	16	20	24	28
SBO (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
SBO+BHA (100 ppm)	0.36±0.02	1.58±0.03	4.59±0.08	8.29±0.06	12.34±0.08	16.26±0.13	20.63±0.10	24.57±0.16
SBO+BHA (200 ppm)	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
SBO+ACE (1000 ppm)	0.36±0.02	1.95±0.05	5.29±0.06	9.49±0.08	13.51±0.10	18.06±0.12	23.47±0.14	29.20±0.16
SBO+ACE (2000 ppm)	0.36±0.02	1.59±0.05	4.93±0.08	8.68±0.02	12.93±0.11	17.43±0.15	22.77±0.13	28.47±0.21
SBO+EAE (1000 ppm)	0.36±0.02	2.16±0.08	5.86±0.09	10.13±0.12	14.96±0.10	19.56±0.12	24.87±0.14	30.58±0.18
SBO+EAE (2000 ppm)	0.36±0.02	1.77±0.04	5.42±0.06	9.02±0.08	14.01±0.07	18.88±0.09	24.04±0.10	29.91±0.13
SBO+CFE (1000 ppm)	0.36±0.02	2.29±0.06	6.14±0.10	10.46±0.13	15.52±0.14	20.16±0.17	25.49±0.20	31.09±0.23
SBO+CFE (2000 ppm)	0.36±0.02	2.05±0.04	5.68±0.08	9.89±0.12	14.81±0.11	19.68±0.13	24.84±0.14	30.62±0.19

Table 6. Conjugated trienes (%) of crude soybean oil samples stabilised with different concentrations of BHA and pod coat extracts of cluster bean

Sample ↓ Incubation days→	0	4	8	12	16	20	24	28
SBO (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
SBO+BHA (100 ppm)	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
SBO+BHA (200 ppm)	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
SBO+ACE (1000 ppm)	0.16±0.02	1.17±0.02	2.35±0.04	4.09±0.06	6.40±0.05	9.02±0.08	11.12±0.09	14.62±0.10
SBO+ACE (2000 ppm)	0.16±0.02	1.03±0.04	2.55±0.04	4.05±0.06	6.11±0.05	8.95±0.06	10.96±0.11	13.97±0.08
SBO+EAE (1000 ppm)	0.16±0.02	1.24±0.01	2.61±0.09	4.31±0.04	7.02±0.05	9.69±0.06	11.79±0.09	15.22±0.10
SBO+EAE (2000 ppm)	0.16±0.02	1.10±0.01	2.63±0.05	4.31±0.06	6.59±0.05	9.62±0.08	11.63±0.08	14.81±0.09
SBO+CFE (1000 ppm)	0.16±0.02	1.30±0.02	2.64±0.03	4.49±0.04	7.33±0.07	9.96±0.05	12.09±0.13	15.66±0.07
SBO+CFE (2000 ppm)	0.16±0.02	1.19±0.03	2.79±0.05	4.41±0.06	6.95±0.07	9.93±0.09	11.86±0.10	15.24±0.11

Table 7. Thiobarbituric Acid Value (meq Malonaldehyde/g) of crude soybean oil samples stabilised with different concentrations of BHA and pod coat extracts of cluster bean

Sample ↓ Incubation days→	0	4	8	12	16	20	24	28
SBO (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
SBO+BHA (100 ppm)	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
SBO+BHA (200 ppm)	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
SBO+ACE (1000 ppm)	4.47±0.02	9.89±0.05	14.13±0.09	21.19±0.10	28.11±0.12	32.29±0.13	35.47±0.16	33.49±0.17
SBO+ACE (2000 ppm)	4.47±0.02	8.26±0.04	11.54±0.08	19.03±0.09	25.05±0.12	28.62±0.14	32.38±0.14	31.59±0.16
SBO+EAE (1000 ppm)	4.47±0.02	10.16±0.06	15.22±0.08	22.31±0.10	29.24±0.11	33.58±0.13	36.68±0.14	34.39±0.18
SBO+EAE (2000 ppm)	4.47±0.02	8.91±0.05	12.86±0.06	20.11±0.09	25.65±0.10	29.72±0.14	33.69±0.16	32.77±0.19
SBO+CFE (1000 ppm)	4.47±0.02	10.32±0.08	16.29±0.10	23.66±0.11	30.32±0.13	34.11±0.15	37.22±0.17	35.44±0.20
SBO+CFE (2000 ppm)	4.47±0.02	9.52±0.03	13.29±0.05	20.56±0.08	26.23±0.10	30.28±0.12	34.42±0.14	33.25±0.17

4. CONCLUSION

The analysis of oxidative degradation of soybean oil, supplemented with various pod coat extracts and BHA under accelerated condition in terms of standard chemical indices, seems to be an important tool to determine the ability of these extracts to inhibit lipid oxidation. Incubation of oil samples under accelerated condition lead to the formation of hydroperoxides and secondary oxidation products. Supplementation of soybean oil with pod coat extracts and BHA improved the oxidative stability of oil. All the extracts significantly lowered the rate of oxidative deterioration of crude soybean oil but acetone extract (2000 ppm) was most effective, this can be attributed to higher concentration of antioxidants in acetone extract. These antioxidant compounds might be polar in nature hence more soluble in polar solvent. Although, supplementary investigations are desirable to identify active constituents that are involved in antioxidative system and develop their approaches for food industries as well as pharmaceutical industries.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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