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STABILIZATION OF SUNFLOWER OIL USING CORN SILK EXTRACT AND ITS METAL CHELATES

Mariyam Yousaf[1], Muhammad Imran[1*], Zunaira Zulfiqar[1], Shoomaila Latif[2], Tanveer Hussain Bokhari[3], Fatima Izhar[1], Summia Rehman[1], Liviu Mitu[4*], Tabassum Yousaf[5]

[1]Institute of Chemistry, University of the Punjab, Lahore, Pakistan
[2]Department of Chemistry, University of Lahore, Lahore, Pakistan
[3]Department of Chemistry, G.C. University, Faisalabad, Pakistan
[4]Department of Nature Sciences, University of Piteşti, Piteşti, 110040, Romania
[5]Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan
[1*]Corresponding author. E-mail: imran_inorganic@yahoo.com
[4*]Corresponding author. E-mail: ktm7ro@yahoo.com

ABSTRACT

Antioxidants are wisely employed in various food products, physiology and pathology for controlling different oxidative routes. Natural antioxidants should replace synthetic ones since they are destructive for human's health. In this regard, corn silk extract (CSEE) was obtained and subsequently screened for antioxidant potential by different assays (DPPH, β-carotene, FRAP, ABTS, total phenol CSE and flavonoids content). Cu(II) and Zn(II) chelates of this extract were also prepared to compare their antioxidant potential with uncomplexed extract. Stabilization of sunflower oil by CSEE and its respective chelates [CSE-Cu(II) and CSE-Zn(II)] at variable concentrations (250 ppm, 500 ppm, 1000 ppm) was investigated by applying three parameters, like PV, FFA and IV values. The results were compared with synthetic standards such as BHA and BHT. BHT and CSEE 1000 ppm possessed maximum oil stabilizing capacity.

Keywords: corn silk (*Maydis stigma*), antioxidant activity, natural antioxidants, synthetic antioxidants, metal chelates, oil stabilization.

INTRODUCTION

Antioxidants are those compounds that prevent the oxidation of other molecules by hindering the proliferation of oxidizing chain reactions and also possess ability to cope against harmful products such as free radicals, etc. (Velioglu et al., 1998). Free radicals disrupt bio-membranes, cells and tissues inside body and change the structure of DNA. Antioxidants may scavenge these free radicals and convert them into stable compounds.

Moreover, antioxidants have tremendous applications in dietary, cosmetic, pharmaceutical, pathological and nutraceutical industry. Peels and other parts of fresh fruits and vegetables are best source of these natural antioxidants. Some notable examples of such sources in literature include turmeric, mangoes, bitter gourd, kiwi, gooseberry, nuts, grains, meat, fish, carrots, apricot, cherries, hazelnuts and spinach, etc. (Ali et al., 2008).

The percentage of lipid contents in various foods is different and deterioration of lipids by lipid oxidation is a complicated process. This process changes the taste, texture, appearance and shelf life of food products (Yoon et al., 1985; McClements et al., 2000). Synthetic antioxidants such as BHA, BHT and TBHQ, etc. are used to preserve and protect food from such process (Suja et al., 2004). However, these have volatile nature and decompose on heating. Moreover, such synthetic antioxidants have noxious effects on human health, so it's better to replace synthetic with natural antioxidants such as essential oils and plant extracts (Al-Mamary et al., 2002).

Corn silk (*Maydis stigma*) is the part of *Zea mays* which belongs to Poaceae family. *Zea mays* is an annual herb grown in tropical and temperate regions of the world. It is considered to be rich source of carbohydrates, proteins (zein), lipids, salts such as calcium, magnesium, sodium, volatile oils,

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sugars, dietary fibres and Vitamins (A,B,C) (Liu et al., 2011). Phytochemicals components such as steroids, alkaloids, flavonoids, polyphenols, catechin, vanillic acid, anthocyanins and terpenoids that are responsible for antioxidant activity are also present in it (Velazquez et al., 2005).

Accessible literature survey reveals that the antioxidant potential of corn silk extract has been reported by some researchers (El-Ghorab et al., 2007; Ebrahimzadeh et al., 2008; Singh et al., 2009; Dong et al., 2014).

In this work, we collected the waste corn silk from local market of Lahore and investigated its antioxidant potential activity together with its Cu(II) and Zn(II) chelates. Moreover, stabilization studies of sunflower oil using CSE and respective chelates [CSE-Cu(II), CSE-Zn(II)] are also part of this paper.

MATERIAL AND METHODS

Materials and sample collection

Corn silk (*Maydis stigma*) was collected from local market of Lahore, washed with deionised water for removal of dust and finally dried at room temperature for 3 weeks. All chemicals and reagents were of analytical grade and were procured from Fluka/Sigma Aldrich.

Preparation of extract

Dried corn silk was ground to fine powder. Fine powder of corn silk (5 g) was soaked in ethanol (150 mL) for four hours and then stirred well for 48 hours at room temperature. After filtration, the resulting filtrate was evaporated at room temperature to get a semisolid extract. Yield was calculated and the extract was stored at 4°C for further analysis.

Evaluation of antioxidant activity of CSE extract and its chelates

Following different assays were executed to determine the antioxidant activity of corn silk extract.

2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay

DPPH assay was performed by following the reported method of Brand-Williams et al. (1995). Stock solution of DPPH (6×10^{-5} mol/L) was prepared and the operational solution contains 0.1 mL of sample along with 3.9 mL of DPPH. The solution was incubated for 30 minutes in dark at room temperature and then absorbance was checked at 517 nm against ethanol as blank. Results were expressed as percentage inhibition.

Percentage inhibition (%) = $(A_c\text{-}A_t)/A_c \times 100$

where:

A_c = absorbance of control;

A_t = absorbance of sample which reacted with DPPH.

β-carotene Linoleic Acid emulsion system

Antioxidant effect of extract was determined by reported method of Terpinc et al. (2009). Absorbance was recorded at 470 nm after every 15 minutes for 1 hour by maintaining temperature at 50°C against blank till *β*-carotene discharged its colour.

Ferric reducing antioxidant power assay

FRAP assay was performed according to reported method of Benzie et al. (1996). The reaction mixture contained 10 µL extract, 300 µL of freshly prepared FRAP reagent, 30 µL of distilled water and measurement of absorbance was taken at 593 nm. 1 mmol/L of $FeSO_4.7H_2O$ was used to develop the standard curve and the results were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/L of $FeSO_4.7H_2O$.

Determination of Total Flavonoids Content (TFC)

TFC were determined by applying the method of Kim et al. (2003). Absorbance of extract was measured at 510 nm. Catechin at concentrations 0.2 to 1 mg/mL in ethanol was prepared to obtain calibration. The results were reported as catechin equivalent (CE) mg/100 g of crude extract. Analysis was done in three replications.

Determination of Total Phenol CSE Content (TPC)

The TPC of extract was determined by following the method of Iqbal et al. (2008). Absorbance was checked at 765 nm using gallic acid as standard. The results were expressed

as GAE (mg/100 g) of dry weight. Triplicate analysis of extract was done.

Determination of ABTS Radical Cation Scavenging Activity

The ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid) radical cation assay was performed according to testified method of Shalaby et al. (2013). The procedure was conducted by adding 0.9 mL of $ABTS^{\circ+}$ and 0.1 mL of sample solution. Then absorbance was calculated at 734 nm against equal ratio of deionized water and ethanol as blank. The final results were expressed by the formula:

$$ABTS^{\circ+} \text{ radical scavenging } (\%) = (A_c\text{-}A_t)/A_c \times 100$$

where:

A_c = Absorbance of water and $ABTS^{\circ+}$ working solution;

A_t = Absorbance of sample extract which reacted with $ABTS^{\circ+}$ solution.

Preparation of metal chelates

0.1 g of CSE extract was suspended in 20 mL ethanol and stirred. Similarly, 0.1 g of both $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$ were weighed and dissolved separately in distilled water (10 mL). Metal chelates were prepared by mixing 20 mL of extract solution and 10 mL of metal salt solution. The resulting mixture was stirred on hot plate for about 3 hours at 40°C. The resulting precipitates were allowed to settle down overnight at room temperature, filtered, washed with small portion of warm ethanol, dried and finally stored at 4°C for further use. Preparation of metal chelates was proposed comparing the FTIR spectra of [CSE-Cu(II), CSE-Zn(II)] with non-chelated (CSE).

Stabilization of sunflower oil

Samples preparation

Refined bleached deodorized (RBD) sunflower oil (SFO) was obtained from local refinery. Different concentrations (250, 500, and 1000 ppm) of ethanolic extract of CSE, CSE-Zn(II) and CSE-Cu(II) were prepared and added into RBD sunflower oil separately. Similarly, 200 ppm concentrations of BHT and BHA were prepared and also added into the oil in parallel fashion for relative analysis (Iqbal et al., 2007).

A control sample of only oil without any antioxidants was also prepared. All samples were stored at room temperature for 45 days and readings were calculated at 15 days interval followed by zero day reading.

Determination of Iodine value (IV), Free fatty acid value (FFA) and Peroxide value (PV)

The stabilization of sunflower oil was determined by measuring three parameters like iodine value (IV), peroxide value (PV) and FFA value by an already reported AOAC standard method (Pomeranz et al., 1994).

Statistical Analysis

All data were set in triplicate and results were reported as mean ± standard deviation. Significance of differences for all data ($p < 0.05$) was calculated with the help of one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The percentage yield of corn silk extract in ethanol was found to be 4.13 ± 0.12%. The synthesized Cu(II) and Zn(II) chelates were stable solids towards air and moisture. FTIR spectrum of CSE exhibited stretching frequencies at 3440, 2930, 1618, 1215, 1056 (cm^{-1}) which could be assigned to (–OH) (str.), –CH (str.), –NH (ben.), C–O (str.) and S=O (str.) vibrations, respectively (Figure 1). Upon comparison of FTIR spectrum of CSE extract with CSE-Zn(II), CSE-Cu(II), a shift in IR stretching frequencies was found. These shifts reveals the coordination of CSE with Zn(II) and Cu(II) ions through various functional groups as (Figure 1).

Ethanolic corn silk extract (CSE) as well as CSE-Cu(II) and CSE-Zn(II) were examined by different methods to assess their antioxidant potential. Phenols and flavonoids are main phytochemicals that impart antioxidative ability to extracts.

Table 1 showed that maximum amount of phenols and flavonoids was present in CSE extract compared to the respective Cu(II) and Zn(II) chelates. This might be due to

involvement of OH groups in chelation with Cu(II) and Zn(II) ions. In FRAP assay, extract showed the reduction ability of Fe^{3+} to $Fe2^{+}$, which was maximum for ethanolic CSE extract and comparatively less for Zn(II) and Cu(II) chelates. This reduction ability of ethanolic CSE extract proved its better antioxidative potential (Table 1).

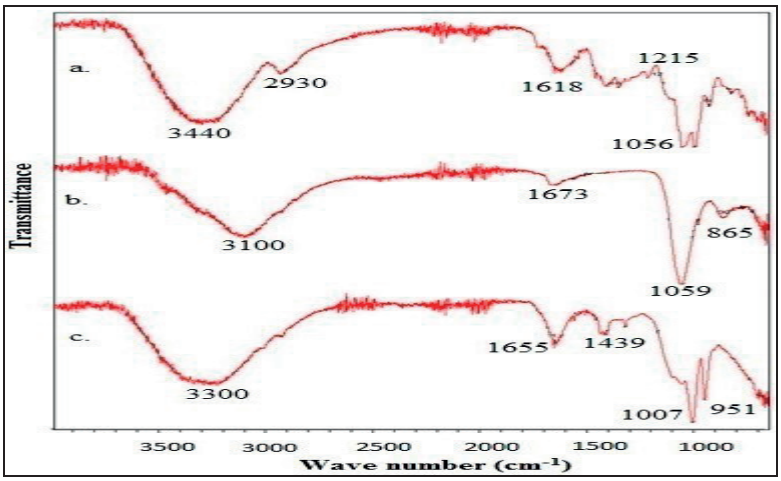

Figure 1. FTIR spectra; (a) CSE extract (b) CSE-Cu(II) (c) CSE-Zn(II)

Table 1. Total phenolic, flavonoid content and FRAP assay of corn silk extract and its chelates

Sample	Total phenolic contents (mg/100 g)	Total flavonoid contents (mg/100 g)	FRAP assay (mmol/L of $FeSO_4$)
CSE	1.19 ± 0.01	1.71 ± 0.01	0.621 ± 0.01
CSE – Zn(II)	0.96 ± 0.00	1.3 ± 0.01	0.466 ± 0.01
CSE – Cu(II)	0.047 ± 0.00	1.19 ± 0.00	0.320 ± 0.02

DPPH method is widely used in measuring of radical quenching ability of plant extracts and is a fast, easy and cheap method for assessment of antioxidant potential. Initially DPPH radical was purple in colour and, on reacting with antioxidants, colour changed from purple to yellow. This colour transformation shows the radical scavenging ability of extract (Molyneux, 2004). The percentage inhibition values were in range BHT (65.25-83.19), BHA (62.33-76.44), CSE (17.27-55.22), CSE-Zn(II) (10.44-37.10) and CSE-Cu(II) (9.8-35.82). These are graphically represented in Figure 2. This figure shows that % inhibition was increased by increasing concentration (0.2-1 mg/mL) of CSE extract and its chelates. The trend of % inhibition was as follows: BHT > BHA > CSE > CSE-Zn(II) > CSE-Cu(II).

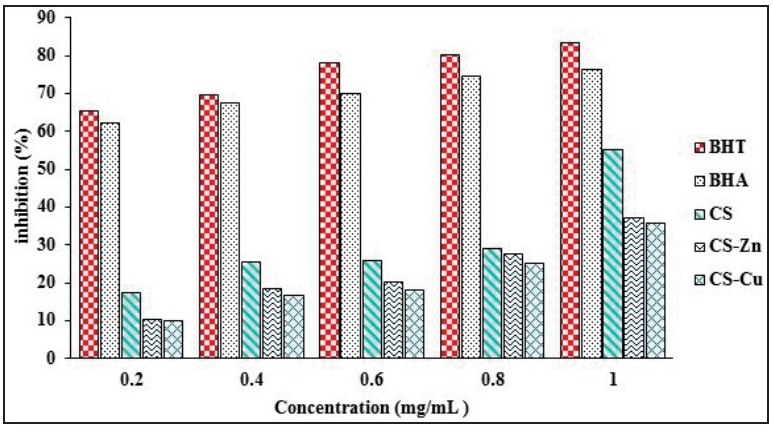

Figure 2. Percentage inhibition of BHT, BHA, CSE, CSE-Cu(II), CSE-Zn(II) extracts at different concentrations

ABTS method depends on the ABTS radical, which is produced by using strong oxidizing agent ($KMnO_4$ or $K_2S_2O_8$) with the commercially available ABTS salt. Phenols present in extract donates hydrogen to $ABTS^{\circ+}$ and stabilize it with blanching of its blue colour (Awika et al., 2003). The obtained results by this method are represented in Figure 3, which revealed scavenging activity as follows: BHT > BHA > CSE > CSE-Zn(II) > CSE-Cu(II).

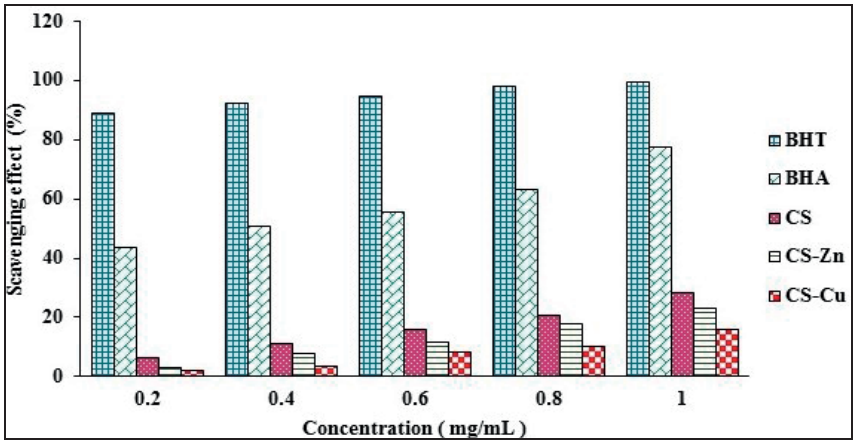

Figure 3. Comparison of scavenging effect (%) of BHT, BHA, CSE, CSE-Cu(II), CSE-Zn(II) extracts

β-carotene method depends upon reduction of emulsions yellow colour formed by mixing linoleic acid and Tween 20 that act as emulsifier lipids (Koleva et al., 2002). Figure 4 shows graph between absorbance at 470 nm and time required by the extracts to bleach *β*-carotene. It can be concluded from graph that with the passage of time (0-60 min), CSE extract showed maximum antioxidant potential (0.071-0.038) followed by CSE-Zn(II) (0.091-0.070) and CSE-Cu(II) (0.098-0.075). Similar for BHA and BHT it was (0.081-0.046) and (0.077-0.042) respectively. Least reduction was observed in control (0.1-0.079). This trend is in agreement with related studies reported by us recently while studying antioxidant potential of turnip's peel (Rehman et al., 2017).

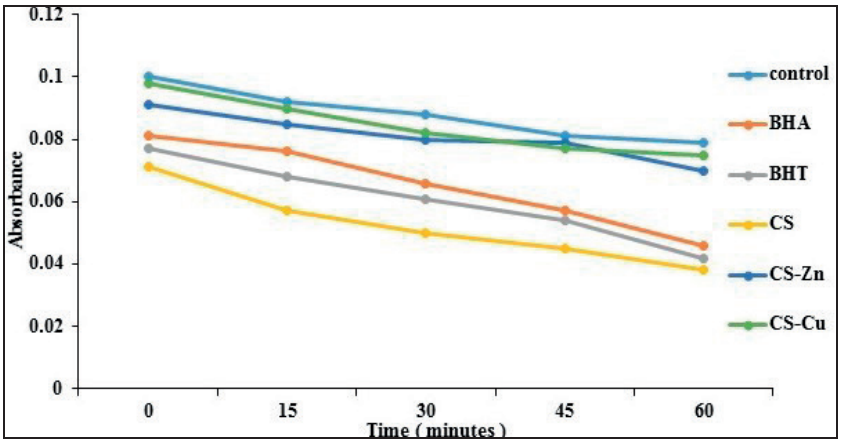

Figure 4. Graph of samples absorbance at different time intervals (minutes)

Stabilization of sunflower oil

Peroxide value (PV)

The peroxide value is useful method for the determination of extent of oxidation of lipids, fats or oils by measuring the peroxides formed during the storage (Shantha et al., 1994; Rasheed et al., 2017). Figure 5 depicts the PV of all samples during the storage periods of 45 days at room temperature. On zero day, PV of control, BHA, BHT, CSE 1000 ppm, CSE 500 ppm, CSE 250 ppm, CSE-Zn(II) 1000 ppm, CSE-Zn(II) 500 ppm, CSE-Zn(II) 250 ppm, CSE-Cu(II) 1000 ppm, CSE-Cu(II)

500 ppm and CSE-Cu(II) 250 ppm, was 8.7 ± 0.24, 6.9 ± 0.28, 6.7 ± 0.15, 7.4 ± 0.12, 7.5 ± 0.14, 7.8 ± 0.12, 7.1 ± 0.2, 7.3 ± 0.21, 7.3 ± 0.22, 7 ± 0.27, 7.3 ± 0.21 and 7.6 ± 0.25 ($meqO_2/kg$), respectively. Increase in PV showed the rancidity of oils happened during said storage period of oil, while on 45th day, the PV of control, BHA, BHT, CSE 1000 ppm, CSE 500 ppm, CSE 250 ppm, CSE-Zn(II) 1000 ppm, CSE-Zn(II) 500 ppm, CSE-Zn(II) 250 ppm, CSE-Cu(II) 1000 ppm, CSE-Cu(II) 500 ppm and CSE-Cu(II) 250 ppm, was 55.8 ± 0.42, 19.8 ± 0.28,16.8 ± 0.18, 18.4 ± 0.29, 20.9 ± 0.24, 23.8 ± 0.21, 24.1 ± 0.21, 38.6 ± 0.24, 40.1 ± 0.21, 45.9 ± 0.41, 50.7 ± 0.44 and 51.4 ± 0.41 ($meqO_2/kg$), respectively. These values showed a trend for stabilization of SFO as follows: BHT > CSE 1000 > BHA > CSE 500 > CSE 250 = CSE-Zn(II) 1000 > CSE-Zn(II) 500 > CSE-Zn(II) 250 > CSE-Cu(II) 1000 > CSE-Cu(II) 500 > CSE-Cu(II) 250 > control. Maximum rancidity was observed in control, while minimum rancidity was exhibited by CSE 1000.

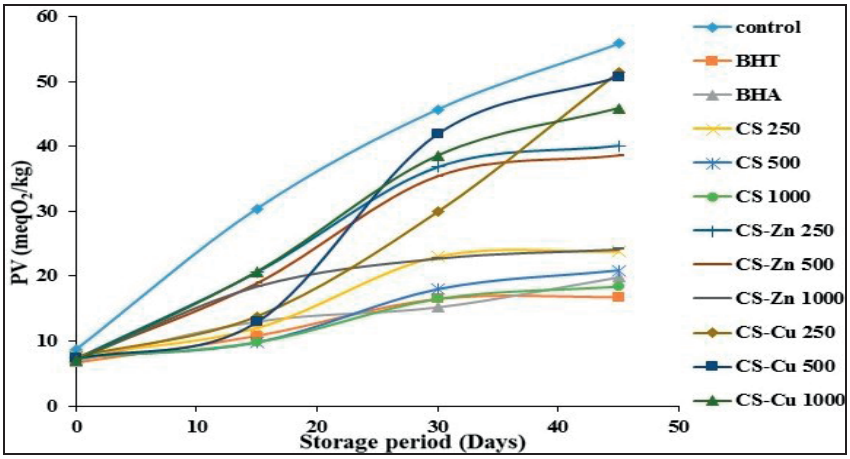

Figure 5. PV value ($meqO_2/kg$) of sunflower oil with BHT, BHA, CSE (250-1000 ppm), CSE-Cu(II) (250-1000 ppm), CSE-Zn(II) (250-1000 ppm) and control

Free fatty acid value (FFA)

FFA value is another parameter of measuring the rancidity of oils, which is obtained due to the hydrolysis of triglycerides in the presence of moisture. However, addition of certain antioxidant containing substances cause reduction in FFA formation in oils (Akhtar et al., 2012). The FFA (%) value during the storage period of 45 days of control, BHA, BHT, CSE 1000 ppm, CSE 500 ppm, CSE 250 ppm, CSE-Zn(II) 1000 ppm, CSE-Zn(II) 500 ppm, CSE-Zn(II) 250 ppm, CSE-Cu(II) 1000 ppm, CSE-Cu(II) 500 ppm and CSE-Cu(II) 250 ppm, was 0.823 ± 0.021, 3.692 ± 0.022, 0.705 ± 0.021, 2.456 ± 0.023, 0.705 ± 0.026, 2.314 ± 0.021, 0.705 ± 0.022, 2.441 ± 0.034, 0.705 ± 0.025, 2.678 ± 0.031, 0.705 ± 0.025, 2.871 ± 0.032, 0.846 ± 0.026, 2.451 ± 0.032, 0.709 ± 0.022, 2.68 ± 0.031, 0.705 ± 0.032, 2.881 ± 0.031, 0.646 ± 0.022, 2.89 ± 0.032, 0.605 ± 0.022, 3.012 ± 0.032 and 0.564 ± 0.027, 3.245 ± 0.34 (%), respectively.

Figure 6 shows the increase in FFA values for all treated samples with the passage of days. The trend of oils stabilization with extracts and control was in the following order: BHT > CSE 1000 > BHA = CSE-Zn(II) 1000 > CSE 500 > CSE-Zn(II) 500 > CSE 250 > CSE-Zn(II) 250 > CSE-Cu(II) 1000 > CSE-Cu(II) 500 > CSE-Cu(II) 250.

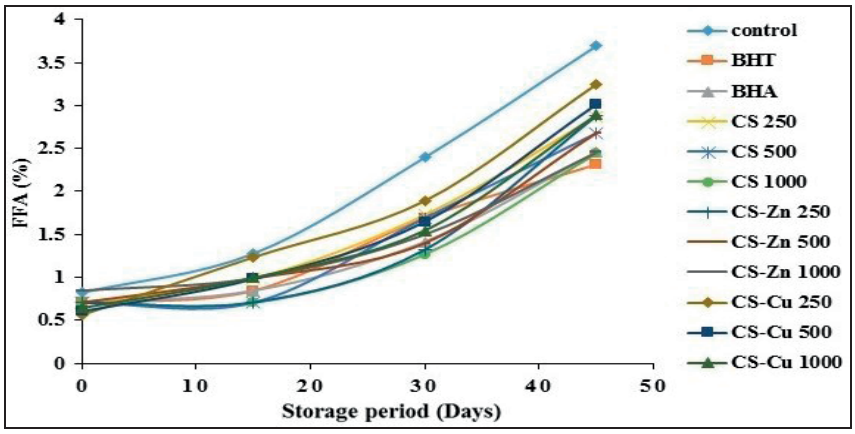

Figure 6. A graph showing FFA (%) value of sunflower oil with BHT, BHA, CSE (250-1000 ppm), CSE-Cu(II) (250-1000 ppm), CSE-Zn(II) (250-1000 ppm) and control

Iodine value (IV)

The IV is a degree unsaturation of any fats or oils. Greater the iodine value, more will be the stabilization of oils (Rehman et al., 2017). Figure 7 shows the decrease in IV of all samples. The range of IV for all treated oil samples i.e. control, BHA, BHT, CSE 1000 ppm, CSE 500 ppm, CSE 250 ppm, CSE-Zn(II) 1000 ppm, CSE-Zn(II) 500 ppm, CSE-Zn(II) 250 ppm, CSE-Cu(II) 1000 ppm, CSE-Cu(II) 500 ppm and CSE-Cu(II) 250 ppm was 144.31 ± 0.34 to 39.33 ± 0.22, 144.32 ± 0.32 to 83.35 ± 0.12, 144.32 ± 0.22 to 93.3 ± 0.24, 144.32 ± 0.31 to 76.21 ± 0.26, 144.32 ± 0.30 to 70.34 ± 0.18, 144.32 ± 0.24 to 64.27 ± 0.13, 144.24 ± 0.33 to 83.34 ± 0.22, 144.24 ± 0.31 to 68.90 ± 0.16, 144.32 ± 0.35 to 60.7 ± 0.26, 144.32 ± 0.36 to 55.91 ± 0.12, 144.31 ± 0.35 to 54.11 ± 0.22 and 144.34 ± 0.32 to 49.99 ± 0.20 (gI_2/100 g) during the storage period of 45 days at room temperature. Minimum IV was reported in control while maximum value was in BHT.

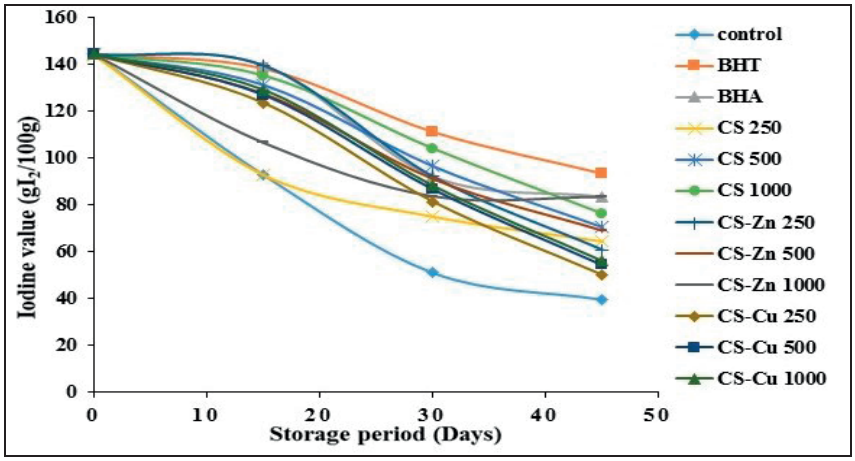

Figure 7. A graph showing IV value (gI_2/100g) of sunflower oil with BHT, BHA, CSE (250-1000 ppm), CSE-Cu(II) (250-1000 ppm), CSE-Zn(II) (250-1000 ppm) and control

Generally, it was observed that CSE exhibited better stabilization compared to CSE-Zn(II) and CSE-Cu(II) and this can be explained due to process of chelation of CSE with metal ions through various coordinating functional groups particularly OH after deprotonation (Afanas'ev et al., 1989; Bravo et al., 1994; Karamac et al., 2007).

CONCLUSIONS

From the study it can be concluded that corn silk extract showed meaningful antioxidative potential measured by different assays. Therefore corn silk extract can be used as an alternative of synthetic antioxidants (BHT, BHA) Corn silk is a

waste of corn production, but can be modified into valuable products for stabilizing oils, etc.

Corn silk extract exhibited better stabilization compared to its chelates with metal ions: CSE-Zn(II) and CSE-Cu(II).

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