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Evaluation of antioxidant efficacy of meal extracts against synthetic antioxidants in crude cotton oil

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Abstract
With the improvement in living conditions, food prepared with addition of chemical preservatives is rejected in market. The antioxidant efficacy of acetone extracts of sesame and sunflower meal was evaluated on stability of crude cotton oil (CCO). Protective effects of sesame and sunflower meal extracts in stabilization of CCO was tested, compared to synthetic antioxidants, by measuring their free fatty acid (FFA), peroxide values (PV), p-anisidine value (AV), thiobarbituric acid (TBA), total oxidation value (TOTOX) and conjugated dienes value (CD) during accelerated storage. Peroxide value is one of the most widely used tests for the measurement of oxidative rancidity in oils and fats. Control sample of CCO found more susceptible to oxidative deterioration as PV of control sample found to increase rapidly after 60 days of storage 50°C. For CCO, sesame meal was found effective to restrict the formation of primary oxidation products. PV, FFA, AV, TBA, TOTOX and CD of CCO stabilized with sesame and sunflower meal extracts were significantly lower. Results indicated that sesame and sunflower meal extracts exhibited stronger antioxidant activity in stabilization of CCO than propyl gallate (PG) while its antioxidant activity was less than that of tert-butyl hydroquinone (TBHQ). Thus acetone extracts of sesame and sunflower meal effectively controlled oxidation process in CCO and it is also environmentally benign.

Keywords: Conjugated dienes value, Crude cotton oil, Peroxide value, PG, Sesame meal extract and TBHQ

1. Introduction
Oils and fats have wide spectrum of applications. Oils are consumed by human beings in their food since prehistoric times. Edible vegetable oils derived from plants are composed of triglycerides, three molecules of fatty acids joined to a glycerol molecule. The minor components include mono and di-glycerides, free fatty acids, phosphatides, sterols, fat-soluble vitamins, phenollic compounds, chlorophyll, carotenoids, tocopherols, pigments, waxes, and fatty alcohols (Salunkhe and Kadam, 1998) [12].

Cotton (*Gossypium hirsutum*) is a fibre crop and is grown in tropical and subtropical regions around world. It is most common crop of India. Cotton is basically grown for its fibre which is the main product of cotton crop. But now a days cotton oil is mostly used for human consumption. Thus, cotton has become a both fibre and oil producing crop. Oil is extracted from seed kernels. The oil is used for cooking, frying, deep-frying, and baking. Because of its neutral taste, cottonseed oil is used to enhance the natural taste of food. Cottonseed oil is highly rich in linoleic acid (omega-6). Cottonseed oil has regenerative properties, acting as an excellent moisturizer and fighting against skin ageing. It is used by the cosmetic industry in skincare products, creams and soaps. Cotton oil is most unsaturated vegetable oil and have high level of tocopherol, thus it is known as Heart Oil.

Lipid oxidation is the decisive factor affecting storage stability of edible oils and fats. It is a natural process that occurs between molecular oxygen and unsaturated fatty acids. Oxidation mechanism of oils is a complex series of reaction and this process occurs through a free radical chain mechanism which results in formation of peroxide free radicals and hydroperoxides. Hydroperoxides are primary oxidation products and rapidly decomposed to secondary reaction products, such as aldehydes and ketones, which cause off-flavours and unpleasant tastes. It also affects the quality of oil. Besides affecting the quality of oil, oxidation process generates toxic compounds through the action of free radicals and reactive oxygen species (ROS) that are harmful for human health. Oxidation reactions start to take place immediately after oil extraction. It cannot be stopped completely; there are some methods that may be taken to slow down this process. The addition of antioxidants to oil is one of the most efficient ways to prevent oxidation reaction between molecular oxygen and unsaturated fatty acids present in oils.

Antioxidants are compounds that inhibit the oxidation process and are widely used to retard the lipid oxidation. Synthetic antioxidants, like Propyl Gallate (PG), Butylated Hydroxy Toluene (BHT), Butylated Hydroxy Anisole (BHA) and Tertiary Butylated Hydroxy Quinone (TBHQ) are used to inhibit oxidation reaction and to improve oxidation stability of vegetable oils during the storage process (Aluyor and Ori-Jesu, 2008) [2].

Now days, addition of natural antioxidants in edible oil is preferred as comparison to synthetic antioxidants due to health issues. There is a highly demand for effective antioxidants from natural sources as alternatives to replace the synthetic antioxidants. It was reported that the extracts from different plant species possess varying degree of antioxidant activity. Some extracts from plant species have different enhancement effects on the oxidative stability of different plant oils (Bouaziz *et al*., 2008) [5]. Secondary plant metabolites such as phenolic compounds from plant sources are highly valuable for their therapeutic attributes as antioxidants (Sikwese and Duodu, 2007) [14]. Carnosic acid had strong protective effects against lipid oxidation of sunflower oil during accelerated storage (Ying *et al.*, 2010) [17]. Sunflower and soybean meal can be effectively stabilized by potato peels and sugar beet pulp extracts (Mohdaly *et al.*, 2010) [11]. According to Jadhav *et al.*, 1996 antioxidants are classified into primary or chain- breaking and secondary or preventive. Primary antioxidants can react with peroxyl radicals before they combine with unsaturated lipid molecules and convert them into more stable products (such as tocopherol). Secondary antioxidants bind with metal ions, scavenging oxygen, absorbing UV radiation, deactivating singlet oxygen and decomposing hydroperoxides to nonradical products (such as caotenoids, citric acid etc.). The main objective of this work is to evaluate the antioxidant efficacy of natural antioxidants in crude cotton oil during accelerated storage and compared with synthetic antioxidants to justify their potential uses as natural antioxidants in various industries including food industries.

2. Experimental

2.1 Source of materials

The seeds of cotton, sesame meal and sunflower meal were procured from the local market at Hisar (Haryana).

2.2 Sample preparation

A commercial sample of sesame and sunflower meal were dried and then ground into a fine powder. 100 grams of the samples defatted with hexane (3 times × 500 ml) at room temperature. The defatted residue was washed with distill water (3 times × 500 ml) and dried at 50 ^{0}C. 10 grams of the above residue was extracted with 150 ml acetone by Soxhlet method for 8 h. Extracts were filtered, solvent removed (in a rotary evaporator below 40 ^{0}C), weighed and the residue was redissolved in 100 ml of acetone to give an antioxidant solution of known concentration and stored in refrigerator for further use.

2.3 Extraction of cotton oil

The seeds of cotton were ground to powder. 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.

2.4 Accelerated Storage

Acetone extracts of sesame and sunflower meal at concentrations (500, 1000 and 2000 ppm) were separately added to CCO. Experiments were also carried out with TBHQ and PG at 200 ppm, and control set without added antioxidants. Each container was appropriately labelled and samples were stored in uniform glass beaker wrapped with aluminium foil. All the samples were prepared in duplicate. The oil samples were then stored in an incubator at 50^{0}C for 120 days to accelerate the degradation of the oil. Required quantity of the oils were withdrawn at day 20, 40, 60, 80, 100 and 120 and studied for the oxidative quality indices.

2.4.1 Peroxide value

Peroxide value (meq/kg) of oil samples were measured according to AOAC (1990) method.

2.4.2 p-Anisidine value

P-Anisidine assay was carried out according to the procedure in AOCS method (1998).

2.4.3 Total oxidation values

Total oxidation (TOTOX) values of oil samples were determined using the following equation according to Shahidi and Wanasundara, 2008:

TOTOX value = 2×PV+ AV

2.4.4 Free fatty acids

Free fatty acid content (% as oleic acid) of oil samples was determined according to AOAC (1990) method.

2.4.5 Conjugated dienes

Conjugated dienes was assessed based on IUPAC method (1987).

2.4.6 Thiobarbituric acid value

Thiobarbituric acid value (meq/kg) was determined according to the method of Johansson and Marcuse, 1973.

3. Results and discussion

3.1 Peroxide value

The effects of TBHQ (200 ppm), PG (200 ppm), sesame meal extracts (SME) 500, 1000, 2000 ppm and sunflower meal extracts (SuME) 500, 1000, 2000 ppm added into cotton oils, respectively were shown in Figure 1. There were regular increases of PVs for all samples and increased in acceleration after 60 days. The total increased in PV was followed in descending order by control > PG (200 ppm) > SuME (500 ppm) > SuME (1000 ppm) > SuME (2000 ppm) > SME (500 ppm) > SME (1000 ppm) > SME (2000 ppm) > TBHQ (200 ppm) which achieved a maximum PV of 178.45±0.7, 144.62±0.5, 121.19±0.2, 112.67±0.4, 107.36±0.1, 104.55±0.5, 100.9±0.3, 91.28±0.08 and 61.32±0.08 meq/kg of oil, respectively at day 120. CCO containing sesame and sunflower extracts had lower PV values than oil sample containing PG during storage. As the concentration of the extracts increases, the PV of CCO gradually decreases. The maximum range of PV contents of sesame and sunflower acetone extracts were in fully corroboration Tavasalkar *et al.*, 2012. Results delineated that SME and SuME controlled hydroperoxide formation effectively and proved the superiority of sesame and sunflower meal acetone extracts as natural antioxidants.

3.2 p-Anisidine value

There were regular increases of AV for all samples stabilized with the extracts, TBHQ, PG and control (Figure 2). Under

the accelerated storage for 120 days, the total increased of AVs was in the following sequence: Control > PG (200 ppm) > SuME (500 ppm) > SuME (1000 ppm) > SuME (2000 ppm) > SME (500 ppm) > SME (1000 ppm) > SME (2000 ppm) > TBHQ (200 ppm). Maximum AV was increased in control sample (164.7±0.8) while TBHQ had minimum increase (76.39±0.06). It was clearly observed that all the varying concentrations of sesame and sunflower extracts were effective in lowering the AV of CCO. This is in accordance with Abdelazim *et al.*, (2013) who reported that supplementation of sunflower and soybean oil with sesame cake extract strongly inhibited the rise of the anisidine value during the storage period. And also sesame cake extract was more active than BHA and BHT, but less active than TBHQ. During storage period of 120 days TBHQ was most effective in preservation of CCO. Difference in antioxidant activity of different antioxidants may be due to chemical structures. The stability of phenoxy radicals reduces the rate of propagation and further reactions and increases the oxidative stability of lipids (Ying *et al.*, 2010) [17].

3.3 Total oxidation value

The TOTOX values of all the samples were increased over storage period illustrated in Figure 3. Rate of increase of TOTOX values were in following ascending order: TBHQ (200 ppm) < SME (2000 ppm) < SME (1000 ppm) < SME (500 ppm) < SuME (2000 ppm) < SuME (1000 ppm) < SuME (500 ppm) < PG (200 ppm) < Control, with maximum values of 199.03±1.7, 302.73±2.5, 325.43±2.5, 337.65±2.0, 341.7±2.2, 357.79±2.1, 377.71±2.9, 430.85±2.4 and 521.6±3.1, respectively at day 120. Results revealed that the antioxidant activity of PG in stabilizing CCO was not as good as that of the extracts used in this study. The low antioxidant activity of PG in stabilizing CCO was due to fewer efficacies in reducing hydroperoxides formation because there is high increase in PV.

3.4 Free fatty acids

Results for CCO showed that FFA increased with storage time (figure 4) and CCO sample without the antioxidant (control) reached a maximum of 8.5±0.05 (% oleic acid) after 120 days of storage. CCO sample with TBHQ showed minimum increase in FFA during storage. When comparing different samples, the total increased of FFA value during 120 days storage were in the order of Control > PG (200 ppm) > SuME (500 ppm) > SuME (1000 ppm) > SuME (2000 ppm) > SME (500 ppm) > SME (1000 ppm) > SME (2000 ppm) > TBHQ (200 ppm). The FFA of CCO samples which contained TBHQ, PG, sesame and sunflower acetone extracts, was lower than FFA of oil sample that contained no additive. The FFA of CCO containing sesame and sunflower extracts was lower than the FFA of oil containing PG. The FFA of the oil sample containing extracts slightly decreased as the concentration of extracts increased in the oil. Hence increase in the extract concentration in the oil reduces the rate at which the oil undergoes oxidative rancidity. The result is in agreement with Chung *et al.*, (2012), they investagated the effect of PG, BHA and garlic extract on the stability of crude Jatropha oil (CJO) for 12 months and found that increased values FFA in the oils with the addition of garlic extract was lower than those under the addition of PG and BHA conditions as well as control.

3.5 Conjugated dienes

Measurement of CD is a good parameter for the determination of oxidative stability of the oils. Lipids containing methylene interrupted dienes or polyenes show a shift in their double bond position during oxidation. The resulting conjugated dienes exhibit intense absorption at 232 nm. The increase in CD contents is proportional to the uptake of oxygen. Greater the levels of CD lower will be the oxidative stability of the oils (Bushra *et al.*, 2007). CD of CCO samples, treated with sesame and sunflower meal extracts, PG and TBHQ, stored at 50 °C were measured during a period of 120 days as presented in Figure 5. The initial CD was 4.43±0.02 and increased up to 37.62±0.02 during period of 120 days. By adding sesame and sunflower meal extracts to CCO, the rate of CD increase was reduced; however, the effect depends on the dosage of extracts. Results of the CD indicated that the antioxidant activities of sesame and sunflower meal extracts was stronger than PG but weaker than that of TBHQ.

3.6 Thiobarbituric acid value

During the oxidation process, peroxides are generally decomposed to lower molecular weight compounds. One such compound is malonaldehyde, which is measured by TBA method. Malonaldehyde, a compound which is used as index of lipid peroxidation, was determined by spectroscopic analysis during course of study. The TBA for crude cotton oil samples stabilized with the extract, TBHQ, PG and control (Figure 6). It was clearly observed that all the varying concentrations of sesame and sunflower extracts were effective in lowering the TBA of CCO. The capability of these extracts to reduce TBA of CCO slightly increased as the concentration of the extract increased. It was revealed sesame meal extract is more stable than sunflower meal extract. Throughout the 120 days of storage the TBA of CCO that contained no additive was higher than oil samples that contained additives (extracts, PG and TBHQ). As the concentration of sesame and sunflower extracts increased in the oil sample, the TBA of the oil decreased remarkably. During storage TBHQ was most effective in preservation of CCO. Difference in antioxidant activity of different antioxidants may be due to chemical structures. Results found were in accordance with Singh *et al.*, (2004) [15]. They found that volatile oil and acetone extract of ajwain had significantly ($P < 0.05$) lower TBA than BHA and BHT in stabilization of linseed oil at 80 °C.

4. Conclusions

From present study, it could be suggested that the oxidative parameters in crude cotton oil increased during storage time and stability of oil decreased relatively. The changes of these values in the oils with addition of TBHQ, PG, sesame and sunflower extracts may be inhibited as compared to control. It could be concluded that the antioxidant activity of sesame and sunflower meal acetone extracts were higher than the synthetic antioxidant propyl gallate. Among the extracts, sesame meal extract had higher antioxidant activity than sunflower meal extract. Thus, sesame and sunflower meal extracts could be used to maintain the oxidative stability of crude cotton oil during long-term storage.

5. Acknowledgments

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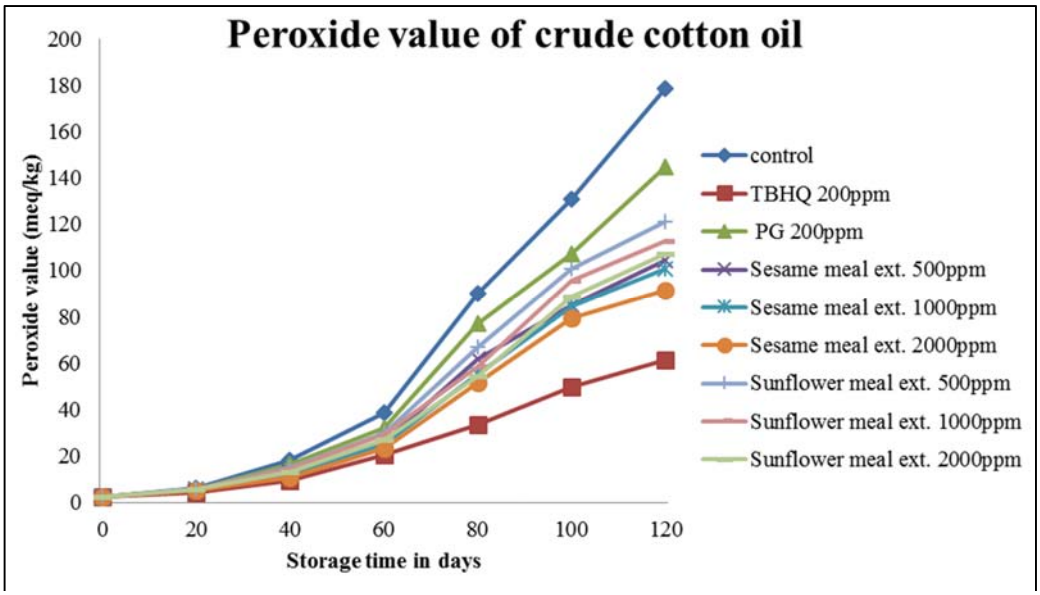

Fig 1: Relative increases in peroxide value of crude cotton oil samples treated with sesame and sunflower meal extracts at 50°C

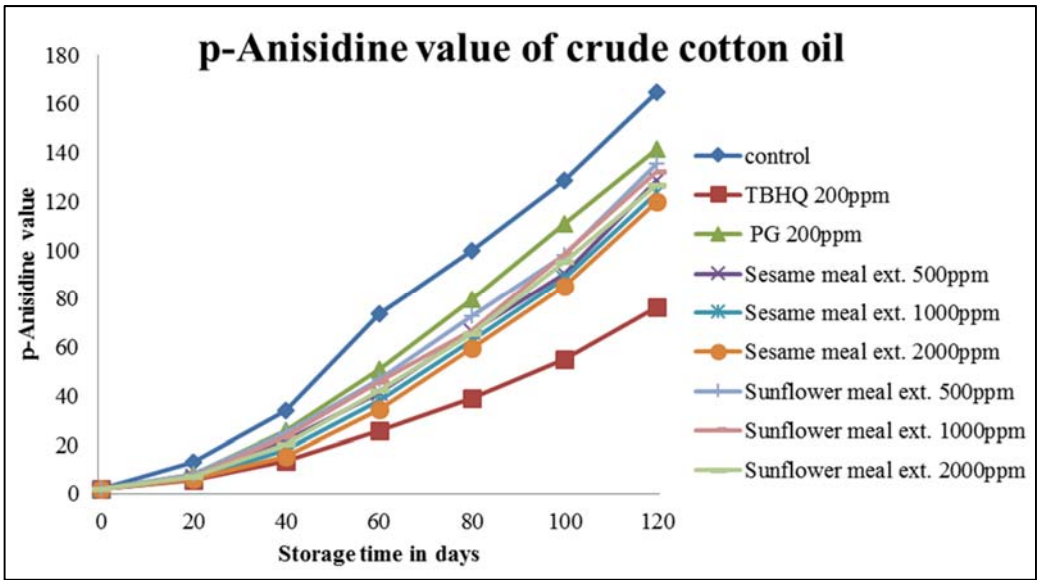

Fig 2: Relative increases in p-Anisidine value of crude cotton oil samples treated with sesame and sunflower meal extracts at 50°C

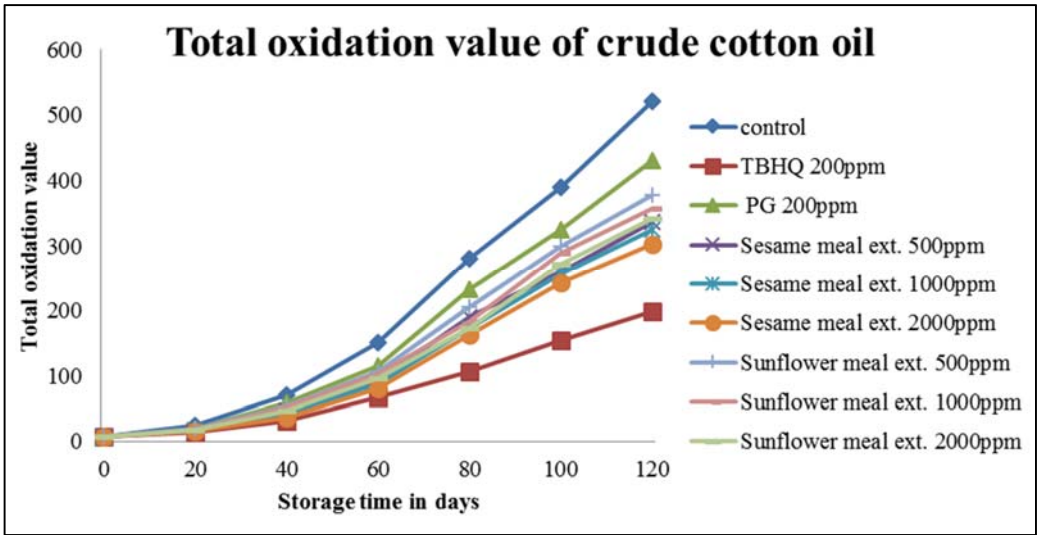

Fig3: Relative increases in total oxidation value of crude cotton oil samples treated with sesame and sunflower meal extracts at 50°C

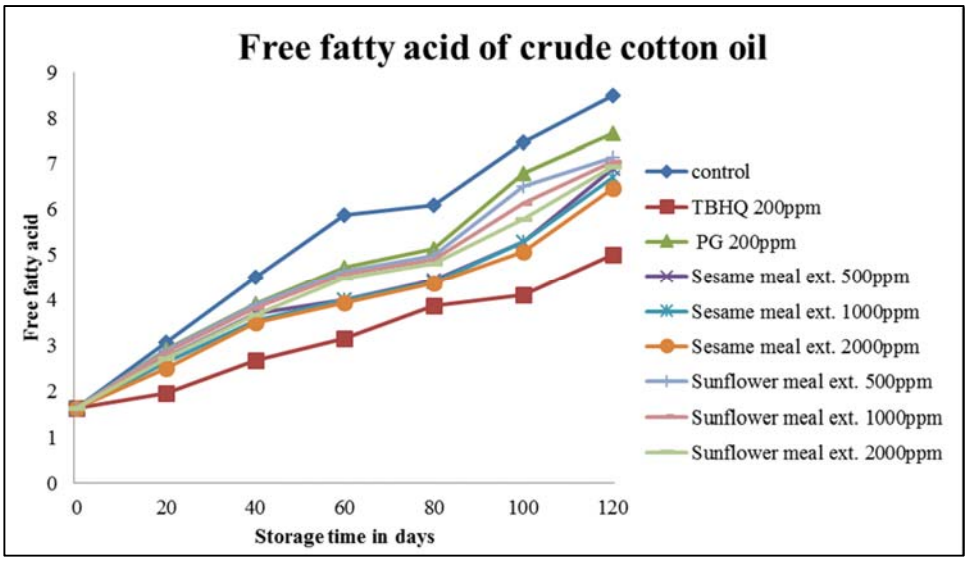

Fig4: Relative increases in free fatty acid of crude cotton oil samples treated with sesame and sunflower meal extracts at 50°C

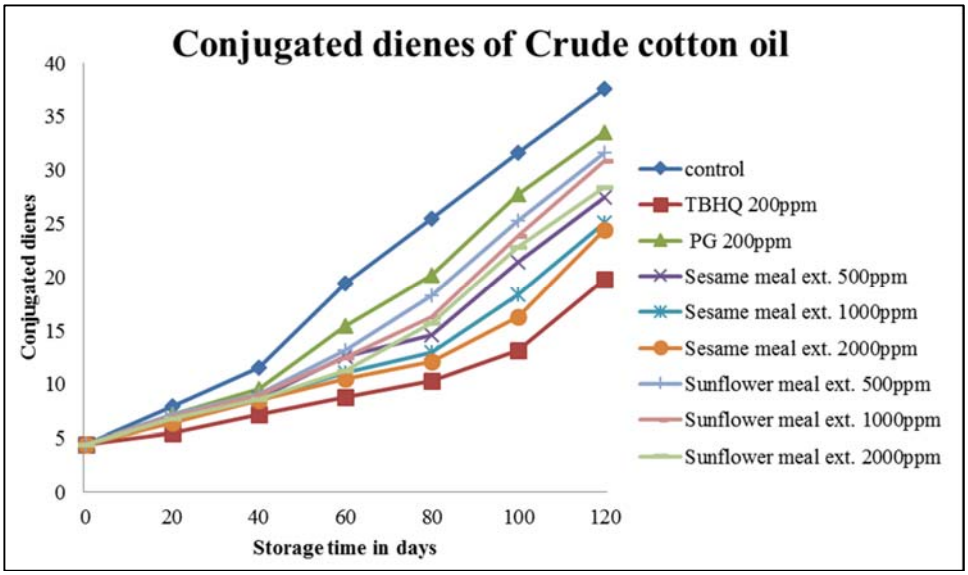

Fig 5: Relative increases in conjugated dienes of crude cotton oil samples treated with sesame and sunflower meal extracts at 50°C

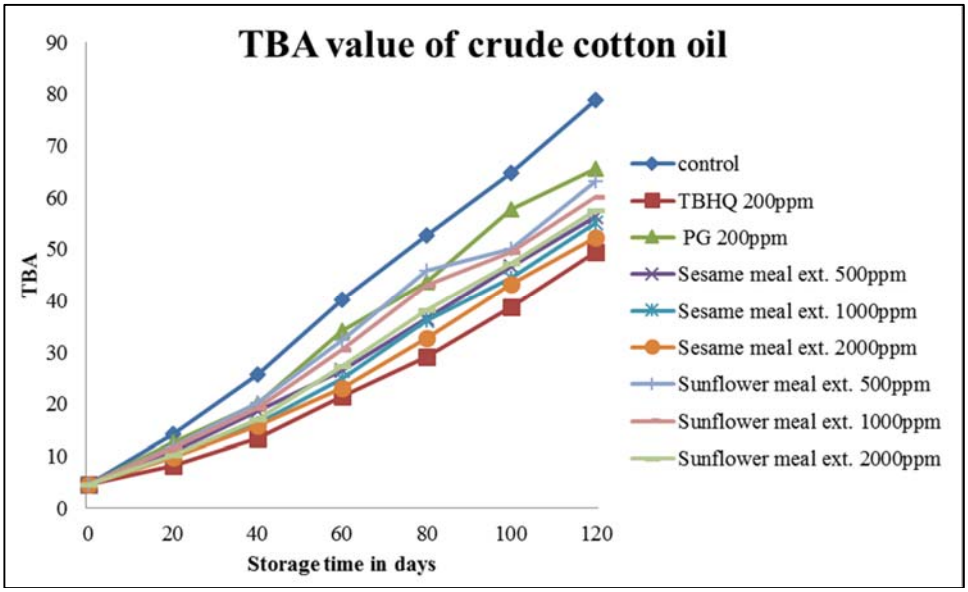

Fig 6: Relative increases in thiobarbituric acid value of crude cotton oil samples treated with sesame and sunflower meal extracts at 50°C

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