International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Storage Stability of Crude Cotton Oil with Respect to Carotenoids and Total Tocopherol by Comparing with Refined Cotton Oil

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Abstract: ***The antioxidant potential of sesame and sunflower meal acetone extracts was investigated in crude cotton oil (CCO) containing natural ocurring antioxidants such as carotenoids and tocopherols and also compared with synthetic antioxidants. Storage stability of CCO was examined by measuring carotenoids and total tocopherols during storage period of 120 days at 50 °C. Carotenoids and total tocopherols were degraded during accelerated storage. Results showed that tertiary butylated hydroxy quinone (TBHQ) was most effective antioxidants. Sesame and sunflower meal extracts could lower the degradation of carotenoids and tocopherols during storage period of 120 days and have high antioxidant efficacy against oil oxidation even higher than propyl gallate (PG). Among both meal extracts, sesame meal extract is more effective against vegetable oil protection than sunflower meal extracts.***

Keywords: Carotenoids; Crude cotton oil; Free radicals; Oxidation; Sesame and sunflower meal extracts; Tocopherols

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1. Introduction

Oxygen is essential to life, but our body generates reactive oxygen species (ROS) by reacting with it, commonly known as free radicals. These compounds are normally formed when body response to stress and can damage healthy cells. These free radicals participate in the etiology of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, aging etc (Harman, 1958; Halliwell and Gutteridge, 1997). Oxidation process is one of the common routes for producing free radicals in food, drugs and living system. Our body produces range of protective antioxidants against these free radicals and ROS. The foods which are rich in antioxidants boost the body own supply. Plants also produce many antioxidants for their protection. The antioxidants which are present in vegetables, fruits, herbs, spices, nuts, whole grains, tea, coffee and extra virgin olive oil are useful for us. For good health, a diet rich in foods which contained high level of antioxidants must be consumed.

Free radicals are any species which have one or more unpaired electrons and capable of independent existence. Free radicals and ROS like superoxide, hydroxyl radical, peroxyl radical as well as non radical species such as hydrogen peroxide (H_2O_2) are highly reactive substances formed in cells as a result of metabolic processes (Niki 1992; 2001). These are highly reactive and destructive molecules and commonly involved in human health and disease. When our body is exposed to free radicals and ROS, many harmful substances are produced that damage cells and tissues and leads to number of disease processes. These may cause reversible or irreversible damage to biological molecules such as DNA, proteins or lipids (Goldberg, 2003). Free radicals can be quenched by molecules having antioxidant activity called antioxidants. Antioxidants are the substances which absorb electrons from molecules and oxidize it (Halliwell and Gutteridge, 1995) and can also delay the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu *et al*., 1998). Antioxidants and radicals can follow free radical addition, hydrogen abstraction and electron transfer mechanism (Britton, 1995; Tsuchihashi *et al*., 1995; Liebler and McClure, 1996; Edge *et al*., 1997)

Kamal-Eldin and Appelqvist (1996) evaluated that vegetable oils have two groups of natural antioxidants such as tocopherols, carotenoid pigments and some sterols. Tocopherols are organic chemical compounds exhibited with vitamin E activity. Name of tocopherol was derived from Greek words *tokos* (birth) and *pherein* (to bear or carry) i.e. to carry a child. Tocopherols are the most important group of natural antioxidants that is present in crude and refined edible oils. Anjani and Khabiruddin (2017) also found that crude and refined groundnut oil has 75.6±0.09 and 34.85±0.05 mg/kg carotenoids, respectively. And total tocopherol content was 670±7.5 and 219±1.9 ppm respectively. In nature, carotenoids are mainly responsible for red, yellow and orange colors. Natural color of fruit and vegetables are mainly due to carotenoids. Food rich in vegetables and fruits pigmented with carotenoids have been of great interest because of their potential health use against chronic diseases.

In present study crude cotton oil was supplemented with sesame and sunflower meal extract against oxidation during accelerated storage in comparison with synthetic antioxidant such as Tertiary Butylated Hydroxy Quinone (TBHQ), Propyl Gallate (PG), with respect to carotenoids and total tocopherol. Results obtained were also compared with refined cotton oil.

2. Materials and Methods

2.1. Materials

The seeds of cotton, sesame and sunflower were collected from farmer's field. These seeds were cleaned manually, to

remove stones, damaged and immature seeds. After cleaning, the seeds were ground into fine powder. The seed oil of cotton was extracted and studied for their chemical parameters. The dried defatted seed meal of sunflower and sesame were extracted with acetone and further used as antioxidants. The commercially available chemicals from qualigens, merk and ranbaxy of highest purity, were used in present investigation.

2.2. Preparation of extracts

Sesame and sunflower meals were dried and ground into a fine powder in an electric grinder. One hundred grams of samples were 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 ºC. Ten grams of above obtained residue was extracted with acetone (150 ml) by Soxhlet method for 8 h. Extracts were filtered, solvent removed (in a rotary evaporator below 40 ºC), weighed and residue was redissolved in acetone (100 ml) to give a solution of known concentration. It was stored in refrigerator untill further use.

2.3. Extraction of oil

Dried and ground seed samples (100 g) of cotton was taken in thimble and placed in soxhlet apparatus. A dry pre-weighed solvent flask ('a', g) containing petroleum ether and condenser were attached for each sample in three replicates. The heating rate was adjusted to give a condensation rate of 2-3 drops/sec and extracted for 8 h. Removed thimble and retained petroleum ether. The excess of petroleum ether was evaporated from the solvent flask on a hot water bath and dried the flask on desiccators and weighed ('b', g).

Oil content in sample (% dry weight basis)=$\frac{(b-a)\times 100}{\text{W of sample (g)}}$

b = W of seed powder before extraction
a = W of seed powder after extraction

2.4. Oil storage studies

Acetone extracts of sesame and sunflower meal at concentrations (500, 1000 and 2000 ppm) were separately added to crude cotton oil. Experiments were also carried out with synthetic antioxidants 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 at 50ºC for storage period of 120 days in an incubator. Samples were analyzed after 20, 40, 60, 80, 100, 120 days to follow the oxidative changes. Required quantity of the sample was taken out periodically and studied for carotenoids and total tocopherol. Carotenoid was determined by the method of Vasconcellous *et al*., (1980) and total tocopherol was determined by the method of Philip *et al*., (1954).

3. Results and Discussion

3.1. Carotenoids

The color in oils is mainly due to the presence of carotenoid pigments and/or chlorophyll pigments. Carotenoids protect cells against the effect of light, air and sensitiser pigments having the ability to quench singlet oxygen and can also serve as antioxidants under conditions other than photosensitisation (Krinsky, 1989). Some crude oils can have unexpectedly high pigmentation caused by field damage, improper storage, or faulty handling during crushing, and extraction. Carotenoids are type of antioxidant, higher value of this indicates higher antoxidant activity.

The loss of carotenoid contents in different samples of crude cotton oil (CCO) stabilized with sesame and sunflower meal extracts, TBHQ, PG and control during 120 days of storage is delineated in table 1. Initial carotenoid value of CCO was 126.09±2.52 mg/kg. The carotenoid value of CCO samples containing control, TBHQ (200 ppm), PG (200 ppm), sesame meal extracts (500, 1000 and 2000 ppm) and sunflower meal extracts (500, 1000 and 2000 ppm) were 17.79±0.46, 43.43±1.05, 24.23±0.53, 29.9±0.65, 31.21±0.68, 33.82±0.84, 25.76±0.59, 27.32±0.62 and 28.88±0.69 mg/kg, respectively, after the end of 120 days.

Carotenoids content of CCO was degraded during the storage stabilized with various concentrations of sesame and sunflower meal extracts. Carotenoids content of control CCO (without the antioxidant) decreased to 17.79 mg/kg after 120 days of storage from initial value of 126.09 mg/kg. A significant difference in value of carotenoids was observed between the control and CCO samples containing synthetic antioxidants, sesame and sunflower meal extracts. These results indicated that sesame and sunflower meal extracts inhibited the degradation process of carotenoid. Antioxidant effect of different extracts was in following order; TBHQ (200 ppm) > sesame meal extract (2000 ppm) > sesame meal extract (1000 ppm) > sesame meal extract (500 ppm) > sunflower meal extract (2000 ppm) > sunflower meal extract (1000 ppm) > sunflower meal extract (500 ppm) > (PG 200 ppm). Results of CCO were compared with refined cotton oil (RCO) and outlined in figure 1. In RCO carotenoids of control sample was declined to 7.14 mg/ kg. There is more degradation of carotenoids in RCO as comparative to CCO. Antioxidant effect of different extracts in RCO was in following order; TBHQ (200 ppm) > sesame meal extract (2000 ppm) > sesame meal extract (1000 ppm) > sesame meal extract (500 ppm) > (PG 200 ppm) > sunflower meal extract (2000 ppm) > sunflower meal extract (1000 ppm) > sunflower meal extract (500 ppm). Results showed that natural extracts are more effective in preservation of CCO than RCO because during chemical refining of crude oil, natural antioxidants are lost. Chemical refining can reduce about 18.47 % of the tocopherols contents (Hassan *et al*., 2011). Jung *et al*., (1989) also found that degumming, alkali refining, bleaching and deodorization removed 99.8% phospholipids, 90.7% iron, 100% chlorophyll, 97.3% free fatty acids and 31.8% tocopherols from crude soybean oil. During incubation TBHQ was most effective and maintained the maximum carotenoid value.

3.2. Total tocopherol

Tocopherols are natural antioxidants, which are present in all vegetable oils in different amounts that play a key role in

preserving oil from rancidity during storage thus prolonging its shelf-life (Ruiz-Lopez *et al*., 1995). Tocopherols act as biological kidnappers of free radicals and could prevent diseases, besides possessing an important nutritional function for human beings as a source of Vitamin E (Monahan *et al.*, 1993; Brigelius- Flohe *et al*., 2002). The tocopherol content of foods is important to protect food lipids against autoxidation and, thereby to increase their storage life and their value as wholesome foods.

The total tocopherol content of CCO samples stabilized with sesame and sunflower meal extracts, TBHQ, PG and control during 120 days of storage is delineated in table 2. Total tocopherol of control CCO sample declined to 10±0.23 after 120 days from an initial value of 934±22.4 mg/kg, while total tocopherol contents of samples stabilized with TBHQ (200 ppm), PG (200 ppm), sesame meal extracts (500, 1000 and 2000 ppm) and sunflower meal extracts (500, 1000 and 2000 ppm) were 174±3.6, 45±1.1, 79±1.7, 93±1.6, 105±2.1, 57±1.3, 60±1.3 and 85±1.9 mg/kg, respectively, after 120 days of storage.

The presence of tocopherols contributes to oxidative stability of edible oils, which is considered to be one of the most important functional properties of oils. This property is especially important in fried or processed foods using vegetable oils. The experiments showed that the stability of different tocopherols and tocotrienols present in the refined vegetable oils depend on the kind of tocopherol derivatives and the fatty acid composition of the oil, in particular on polyunsaturated fatty acid content (Rossi *et al.*, 2007). In present study, of total tocopherol present in crude cotton oil was 934 while in refined cotton oil it was 616 mg/kg. About 34% tocopherol lost during refining process. Refining results was almost similar to Tasan and Demirci, 2005. They observed that the average losses of total tocopherols content in sunflower oil during the chemical and physical refining processes reached up to 30.2% and 35.5% respectively. Degradation of total tocopherol in crude and refined cotton oil at 50°C is shown in fig. 2. Total tocopherol was destroyed faster in RCO than CCO. Total tocopherol was decreased to 10 and 0.9 mg/kg in control sample of CCO and RCO at 120 day of storage. C&RCO stabilized with natural as well as synthetic antioxidants has low degradation rates of total tocopherol. Stability order of different antioxidants was similar to carotenoids degradation.

4. Conclusions

There is a rapid loss in carotenoids and tocopherol in cotton oil with increase in storage period and their degradation take place at lower rate in samples stabilized with antioxidants than control sample. Sesame and sunflower meals have high antioxidant potential against cotton oil oxidation. Present study could be an effective introduction to the antioxidant potential of seed meals that are being discarded as by-products.

5. Acknowledgments

The author is grateful to University Grants Commission, New Delhi, India for awarding junior research fellowship. The authors thank Dr. M. Khabiruddin for support and constant encouragement.

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Tables

Table 1: Variation of carotenoid (mg/kg) value of crude cotton oil during storage period of 120 days at 50°C

Sample	Storage period (days)						
	0	20	40	60	80	100	120
Control	126.09±2.52	102.59±2.15	84.19±1.59	70.68±1.83	53.55±1.01	35.45±0.81	17.79±0.46
TBHQ (200 ppm)	126.09±2.52	116.62±2.56	105.23±2.13	91.94±2.48	82.75±2.23	65.14±1.69	43.43±1.08
PG (200 ppm)	126.09±2.52	104.89±2.21	92.56±1.49	76.26±1.88	62.35±1.62	45.55±1.13	24.23±0.53
Sesame meal extract (500 ppm)	126.09±2.52	112.21±2.36	100.05±2.11	80.99±2.26	72.34±1.95	50.96±1.27	29.9±0.65
Sesame meal extract (1000 ppm)	126.09±2.52	113.64±2.41	100.59±2.11	83.38±1.58	75.41±1.88	52.29±1.31	31.21±0.68
Sesame meal extract (2000 ppm)	126.09±2.52	114.74±2.52	101.62±2.23	85.46±1.62	75.98±2.12	54.66±1.47	33.82±0.84
Sunflower meal extract (500 ppm)	126.09±2.52	108.69±2.28	95.53±1.67	75.39±1.88	67.27±1.88	42.38±1.01	25.76±0.59
Sunflower meal extract (1000 ppm)	126.09±2.52	110.54±2.32	96.17±1.82	76.89±2.07	69.66±1.95	46.61±1.11	27.32±0.62
Sunflower meal extract (2000 ppm)	126.09±2.52	110.56±2.25	98.58±1.56	80.54±2.25	70.19±1.33	49.46±1.28	28.88±0.69

Triplicates of each sample were used for statistical analysis and resulting values are expressed as mean ± S.E.

Table 2: Variation of total tocopherol (mg/kg) value of crude cotton oil during storage period of 120 days at 50°C

Sample	Storage period (days)						
	0	20	40	60	80	100	120
Control	934±22.4	775±19.3	644±16.7	435±9.3	187±4.6	22±0.57	10±0.23
TBHQ (200 ppm)	934±22.4	881±17.6	746±15.6	623±11.1	552±11.1	327±8.8	174±3.6
PG (200 ppm)	934±22.4	800±15.2	670±14.6	470±9.2	256±6.6	150±3.7	45±1.1
Sesame meal extract (500 ppm)	934±22.4	824±21.3	699±16.5	543±11.2	337±8.7	223±5.7	79±1.7
Sesame meal extract (1000 ppm)	934±22.4	836±19.6	701±18.2	576±12.2	358±7.1	240±5.6	93±1.6
Sesame meal extract (2000 ppm)	934±22.4	850±21.7	710±16.3	590±12.9	390±7.5	250±6.2	105±2.1
Sunflower meal extract (500 ppm)	934±22.4	803±18.7	670±16.1	480±11.4	260±4.9	175±2.6	57±1.3
Sunflower meal extract (1000 ppm)	934±22.4	818±18.4	684±16.9	505±10.8	284±5.6	192±3.9	60±1.3
Sunflower meal extract (2000 ppm)	934±22.4	830±18.5	690±15.7	520±10.9	316±4.1	216±4.1	85±1.9

Triplicates of each sample were used for statistical analysis and resulting values are expressed as mean ± S.E.

Figure Captions

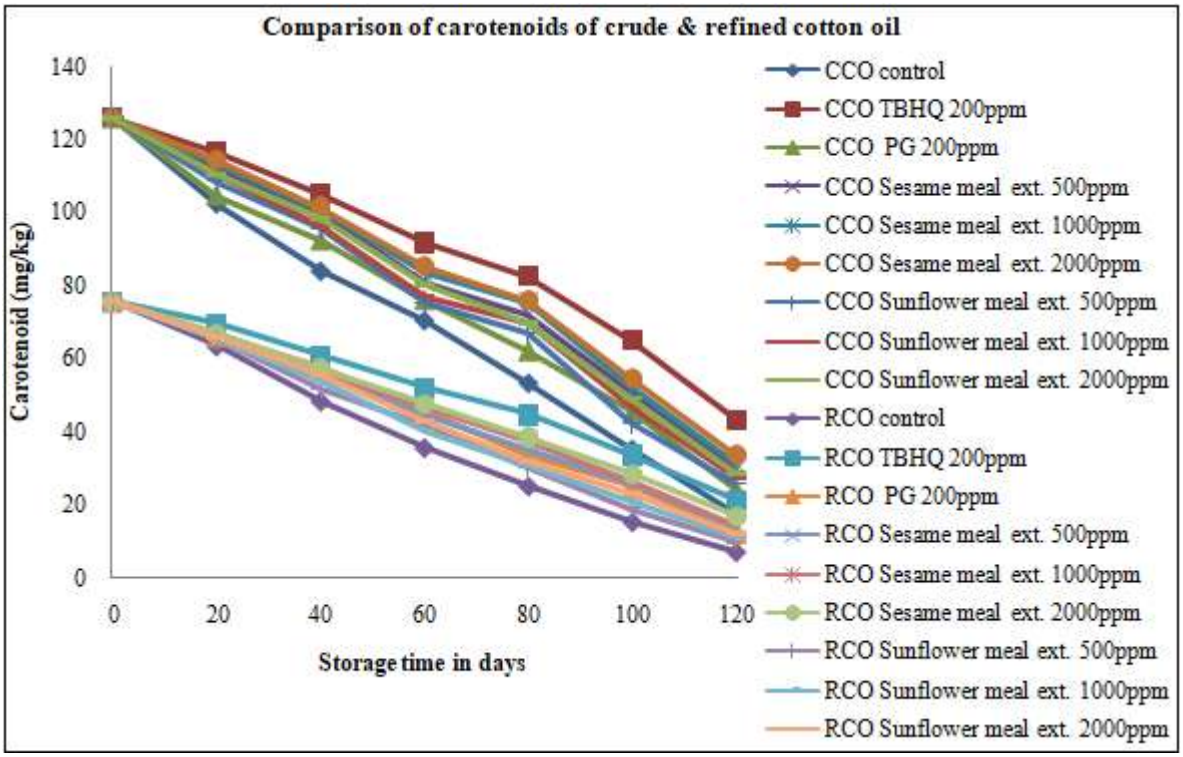

Figure 1: Comparative evaluation of carotenoid (mg/kg) of crude and refined cotton oil supplemented with extracts for 120 days

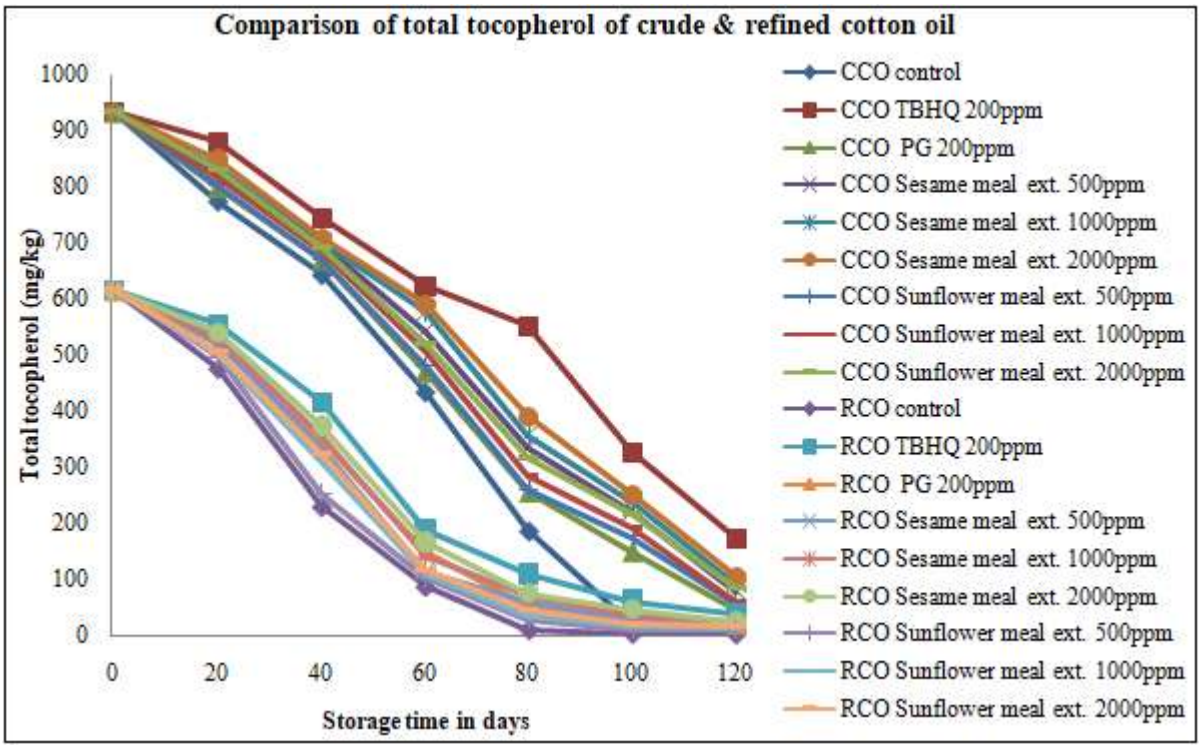

Figure 2: Comparative evaluation of total tocopherol (mg/kg) of crude and refined cotton oil supplemented with extracts for 120 days