

Accelerated Storage Stability of Refined Cotton Oil in Term of Free Fatty Acid and Thiobarbituric Acid

ABSTRACT

Aims: To study accelerated storage stability of refined cotton oil in term of free fatty acid and thiobarbituric acid
Place and Duration of Study: Department of Chemistry and Biochemistry, CCS Haryana Agricultural University, Hisar-125004, India, between January 2014 to continuing.
Methodology: The seeds of cotton, sesame and sunflower were collected and 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. Crude oil was chemically refined and stored for 120 days at 50 °C. Free fatty acid (FFA) and thiobarbituric acid (TBA) were studied periodically during accelerated storage.
Results: FFA and TBA values were increased regularly during storage period. All added antioxidants lowered the FFA and TBA. Tertiary butylated hydroxy quinone (TBHQ) was most effective antioxidants. Sesame and sunflower meal extracts appreciably lowered the FFA and TBA values 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.
Conclusion: Sesame and sunflower meals extracts can be used against refined cotton oil oxidation. This is economical also because these are being discarded as by-products.

Keywords: Accelerated storage, free fatty acid, refined cotton oil, thiobarbituric acid

1. INTRODUCTION

Refined cotton oil is edible oil extracted from cotton seed (*Gossypium hirsutum*). Crude cotton oil that is obtained from solvent extraction is further subjected to chemical refining which involves degumming, neutralization, bleaching and deodorization [1].

Oils and fats have wide spectrum of applications. Oils are consumed by human beings in their food since prehistoric times [2]. But the major problem during storage of oil is oxidation. It is responsible for rancid odors and flavors. Oxidation mreaction may be of different types i.e. auto-oxidation, phoyo-oxidation, ketonic oxidation and enzymatic oxidation [3]. Autooxidation is free radical chain mechanism which includes initiation, propagation and termination steps that are repeated again and again. Free radicals are side products of these oxidative reactions. A free radical is defined as any atom or molecule possessing unpaired electrons. Free radicals and ROS like superoxide, hydroxyl radical, peroxy radical as well as non radical species such as hydrogen peroxide (H₂O₂) are highly reactive substances formed in cells as a result of metabolic processes [4-5]. Because of their highly reactive nature, these react rapidly with adjacent molecules via a variety of reactions including hydrogen abstraction (capturing), electron donation and electron sharing [6]. These may cause reversible or irreversible damage to biological molecules such as DNA, proteins or lipids [7]. Free radicals can be quenched by molecules having antioxidant activity called antioxidants. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions [8]. Antioxidants are of two type i.e. preventive antioxidants and chain breaking antioxidants. The Preventive antioxidants deactivate the active species without generation of free radicals, thus minimize the rate of chain initiation in oxidation process. Examples of preventive antioxidants are catalase and glutathione peroxidase. On the other hand chain breaking antioxidants scavenge chain propagating oxygen radicals to form a stable product and reduce lipid oxidation in foods [9].

Numbers of antioxidants are added to oil to prevent oxidation reaction between molecular oxygen and unsaturated fatty acid present in oils. Synthetic antioxidants such as Tertiary Butylated Hydroxy Quinone (TBHQ), Propyl Gallate (PG), Butylated Hydroxy Anisole (BHA) and Butylated Hydroxy Toluene (BHT) are widely used as food additives to improve storage stability [10]. TBHQ is known to be a very effective antioxidant for vegetable oils, and is stable at high temperature [11]. But synthetic chemical antioxidants are discouraged in international market due to serious health issues. Therefore, increasing attention is being directed towards bioactive plant extracts to serve as antioxidants for protection against free radicals. Natural extracts of herbs, vegetables, fruits, cereals and other plant parts have antioxidants such as vitamin E, vitamin C, tannins, phenolics, flavonoids, and proanthocyanidins. In present paper, sesame and sunflower meal acetone extracts are studied as potential antioxidants in storage of refined cotton oil.

2. EXPERIMENTAL DETAILS

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.

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 °C. 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 °C), weighed and residue was redissolved in acetone (100 ml) to give a solution of known concentration.  was stored in refrigerator until 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).

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

Where, b = W of seed powder before extraction

a = W of seed powder after extraction

2.4 Refining of oil

Next, the oil was refined to remove color, odor and bitterness. Refining of oil was done by chemical method [1] in the following steps.

Step 1

Degumming is the first step of refining and involves the removal of easily hydralable phospholipids and metals from the oil. For this, the crude oil was treated with water at room temperatures. The hydrated phospholipids are removed at the end of this step.

Step 2

Small amount of phosphoric acid was added to convert the remaining non-hydralable phospholipids (Ca, Mg salts) into hydralable phospholipids.

Step 3

FFAs are responsible for oil acidity. During neutralization, the oil was treated with excess of sodium hydroxide that reacts with the FFA to form soap stock. The soap stock was separated from the oil by centrifugation.

Step 4

The purpose of bleaching (or decolorising) is to reduce the levels of pigments such as carotenoids and chlorophyll, but this treatment also further removes residues of phosphatides, soaps, traces of metals, oxidation products, and proteins. Bleaching was done with acid-activated clay minerals to adsorb coloring components and to decompose hydro peroxides.

Step 5

Deodorisation is a vacuum steam distillation process that removes the relatively volatile components, aldehydes and ketones, which give rise to undesirable flavours, colours and odours in oil. This is feasible because of the great differences in volatility between these undesirable substances and the oil. Depending on the residence time in the deodoriser, the process was carried out at low pressure (2 – 6 mbar) and elevated temperatures (180° - 270°C), since the substances responsible for odours and flavours are usually volatile.

2.5 Oil storage studies

Acetone extracts of sesame and sunflower meal at concentrations (500, 1000 and 2000 ppm) were separately added to refined 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 50°C 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 free fatty acid and thiobarbituric acid.

2.6 Free fatty acid

Free fatty acids were determined by AOAC method [12]. Fifty ml of denatured alcohol was added to one g of oil sample in a 250 ml conical flask. The flasks were swirled and few drops of phenolphthalein were added, the content was titrated against 0.1N sodium hydroxide till a permanent light pink color appeared which persisted for at least 1 min. Blank was titrated similarly in the absence of oil.

$$\text{Free fatty acids (in terms of oleic acid)} = \frac{100 \times 18.2 \times V}{W \text{ of oil} \times 10 \times 1000}$$

Where, V = Volume of 0.1N sodium hydroxide used.

W = Weight of oil

2.7 Thiobarbituric acid

The TBA value was determined according to the method of Johansson and Marcuse [13]. One hundred mg of oil sample was dissolved in 25 ml of 1-butanol. The above sample was mixed thoroughly with 5 ml of TBA reagent and incubated at room temperature overnight and absorbance was measured at 530 nm using a spectrophotometer. At the same time, a reagent blank (without TBA reagent) was also run.

$$\text{TBA} = \frac{(A-B) \times 50}{W \text{ of sample (g)}}$$

Where, A = absorbance of test sample

B = absorbance of blank sample

3. RESULTS AND DISCUSSION

3.1 Free fatty acid

FFA is defined as the milligram of potassium hydroxide required to neutralize the free acids in one gram of the oil. It is an important oil quality indicator during each stage of processing. FFA of any lipid is measure of hydrolytic rancidity [4]. Higher value of FFA of any lipid, higher the degree of hydrolytic rancidity that set-in [15]. In crude oils, FFA estimates the amount of oil that will be lost during refining steps designed to remove fatty acids.

Table 1 depicts FFA of refined cotton oil (RCO) stored with TBHQ, PG, sesame and sunflower meal acetone extracts for 120 days. It was observed that FFA of RCO samples increased during storage period. Results showed that RCO samples containing 500 ppm to 2000 ppm sesame meal extracts had lower FFA values than oil samples containing 200 ppm PG in the three month of storage. Sunflower meal extracts at all varying concentrations were not as effective as 200 ppm PG in lowering FFA of RCO. Control sample of RCO reached the maximum FFA up to 10.12 ± 0.27 from the initial value of 0.67 ± 0.01 (% as oleic acid). Here also, RCO treated with TBHQ (200 ppm) was showed minimum increase in FFA up to 7.19 ± 0.17 . RCO treated with PG (200 ppm), sesame meal extracts (500, 1000, 2000 ppm), sunflower meal extracts (500, 1000 and 2000 ppm) were increased up to 8.85 ± 0.23 , 8.84 ± 0.21 , 8.61 ± 0.19 , 8.46 ± 0.17 , 9.12 ± 0.2 , 8.99 ± 0.22 and 8.87 ± 0.23 (% as oleic acid), respectively on the 120th day of storage. As the concentration of extracts increases, the FFA of RCO gradually decreases. The FFA of oil samples containing extracts was lower than FFA of oil which contained no additives (control). The results are in agreement with Anjani et al. [16]. They found that supplementation of crude and refined soybean oil with sesame and sunflower meal extracts strongly inhibited the rise of FFA during storage period.

Table 1 Variation of free fatty acid (% as oleic acid) value of refined cotton oil during storage period of 120 days at 50°C

Sample	Storage period (days)						
	0	20	40	60	80	100	120
Control	0.67 ± 0.01	2.9 ± 0.07	5.06 ± 0.09	6.48 ± 0.14	7.79 ± 0.21	9.23 ± 0.23	10.12 ± 0.27
Tbhq (200 ppm)	0.67 ± 0.01	1.47 ± 0.03	2.81 ± 0.05	3.69 ± 0.07	4.64 ± 0.09	5.82 ± 0.12	7.19 ± 0.17
Pg (200 ppm)	0.67 ± 0.01	2.53 ± 0.04	4.37 ± 0.09	6.01 ± 0.13	6.96 ± 0.16	7.3 ± 0.15	8.85 ± 0.23
Sesame meal extract (500 ppm)	0.67 ± 0.01	2.2 ± 0.04	4.32 ± 0.08	5.7 ± 0.11	6.32 ± 0.12	7.21 ± 0.15	8.84 ± 0.21
Sesame meal extract (1000 ppm)	0.67 ± 0.01	2.14 ± 0.04	4.26 ± 0.08	5.43 ± 0.1	6.14 ± 0.15	7.05 ± 0.17	8.61 ± 0.19
Sesame meal extract (2000 ppm)	0.67 ± 0.01	2.05 ± 0.03	4.15 ± 0.07	5.31 ± 0.13	5.92 ± 0.12	6.99 ± 0.14	8.46 ± 0.17
Sunflower meal extract (500 ppm)	0.67 ± 0.01	2.45 ± 0.06	4.65 ± 0.09	6 ± 0.15	6.77 ± 0.14	7.83 ± 0.19	9.12 ± 0.2
Sunflower meal extract (1000 ppm)	0.67 ± 0.01	2.32 ± 0.07	4.58 ± 0.06	5.86 ± 0.12	6.48 ± 0.12	7.64 ± 0.18	8.99 ± 0.22
Sunflower meal extract (2000 ppm)	0.67 ± 0.01	2.29 ± 0.05	4.45 ± 0.07	5.74 ± 0.14	6.25 ± 0.16	7.39 ± 0.15	8.87 ± 0.23

*Values are mean $\pm$ standard error

3.2 Thiobarbituric acid

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 per-oxidation, was determined by spectroscopic analysis [17]. The effects of sesame and sunflower meal extracts on the stability of RCO in term of TBA are outlined in Table 2. TBA value of RCO sample without antioxidant (control) was increased from 2.73 ± 0.07 to 97.26 ± 2.52 meq/kg after 120 days of storage, while TBA values for samples containing TBHQ (200 ppm), PG (200 ppm), sesame meal extracts (500, 1000 and 2000 ppm) and sunflower meal extracts (500, 1000 and 2000 ppm) were 66.87 ± 1.67 , 75.14 ± 1.81 , 76.81 ± 2.07 , 74.29 ± 1.85 , 71.78 ± 1.72 , 86.64 ± 2.25 , 82.21 ± 2.05 and 79.64 ± 1.91 , respectively. All the additives slightly lowered TBA value of RCO. Sesame meal extracts at all varying concentrations were more effective than 200 ppm PG in controlling TBA of refined cotton oil. Sunflower meal extracts was less effective than oil other antioxidant during storage of RCO. However, TBA of RCO gradually decreased as the concentration of sesame and sunflower meal extracts increased in the oil sample for three month of storage. The result is in agreement with Anjani and Singh [18], they investigated the effect of TBHQ, PG, sesame and sunflower meal extracts on the stability of crude cotton oil (CCO) for three months and found that increased TBA in oils with the addition of sesame and sunflower meal extracts was lower than those under the addition of PG and control but higher than TBHQ.

Table 2 Variation of thiobarbituric acid (meq/kg) value of refined cotton oil during storage period of 120 days at 50°C

Sample	Storage period (days)						
	0	20	40	60	80	100	120
Control	2.73±0.07	9.28±0.19	25.84±0.64	49.37±1.18	61.46±1.47	75.43±1.88	97.26±2.52
Tbhq (200 ppm)	2.73±0.07	5.24±0.12	14.63±0.33	30.96±0.80	44.27±1.06	56.26±1.41	66.87±1.67
Pg (200 ppm)	2.73±0.07	8.72±0.18	17.99±0.48	37.85±0.94	52.12±1.3	63.54±1.71	75.14±1.81
Sesame meal extract (500 ppm)	2.73±0.07	8.56±0.21	17.35±0.45	38.63±0.99	51.38±1.28	63.43±1.64	76.81±2.07
Sesame meal extract (1000 ppm)	2.73±0.07	7.95±0.22	16.22±0.4	36.58±0.87	50.5±1.31	61.85±1.54	74.29±1.85
Sesame meal extract (2000 ppm)	2.73±0.07	7.9±0.18	16.14±0.37	34.34±0.89	49.68±1.24	61.13±1.52	71.78±1.72
Sunflower meal extract (500 ppm)	2.73±0.07	9.06±0.23	20.69±0.49	42.76±1.11	56.06±1.34	70.16±1.75	86.64±2.25
Sunflower meal extract (1000 ppm)	2.73±0.07	9±0.19	21.34±0.53	40.88±1.04	55.22±1.32	67.78±1.69	82.21±2.05
Sunflower meal extract (2000 ppm)	2.73±0.07	8.89±0.2	18.45±0.47	39.24±0.98	52.34±1.25	65.61±1.57	79.64±1.91

*Values are mean ± standard error

4. CONCLUSION

Concluding the results, we can say that acetone extracts of sesame and sunflower meal have been proven to be alternative sources of natural antioxidants and more effective than synthetic antioxidants i.e. PG. These antioxidants are preferred economically also.

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