Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2018; 7(2): 292-296
Received: 03-01-2018
Accepted: 04-02-2018

**Anjani**
Department of Chemistry and Biochemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

**Rajvir Singh**
Department of Chemistry and Biochemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

**Correspondence**
**Anjani**
Department of Chemistry and Biochemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

# Antioxidant efficacy of meal extracts in stabilization of crude soybean oil

**Anjani and Rajvir Singh**

**Abstract**
Fats and oils are important food constituents of human diet but major disadvantage of these products are oxidative deterioration. In present study, oxidative deterioration of crude soybean oil (CSO) was determined in term of conjugated dienes (CD), total oxidation value (TOTOX), thiobarbituric acid value (TBA), total tocopherol and carotenoids. To slow down oxidation process sesame and sunflower meal extracts are used as potential antioxidants and their antioxidant efficacy is also compared with synthetic antioxidants such as tertiary butylated hydroxy quinone (TBHQ) and propyl gallate (PG).The stabilized CSO samples were subjected to accelerated storage for 120 days at 50 °C and analysis was done periodically after every 20 days. The overall order of antioxidant potential of different antioxidants measured by various oxidative parameters was; TBHQ > sesame meal extracts > sunflower meal extracts > PG. Results revealed that supplementation of CSO with meal extracts greatly improved its shelf-life and can be alternatively used in place of synthetic antioxidants.

**Keywords:** soybean oil, crude, stabilization, efficacy

## Introduction
Earth is a green planet due to plants which are considered the first living organisms born on it. Life cannot exit on earth without plants and their presence is inevitable for clean air, food, water, clothing, flavours, cosmetic, ornamental, fumigants, insect deterrents and fragrance, plants have also served humanity in the treatment of ailments (Akerele, 1993) [1]. From ancient time people are using many plant parts to cure diseases. Side effects of synthetic drugs have also drawn attention on the use of plant materials as a source of medicines for human ailments. Recently, WHO (World Health Organization) found that 80 per cent of world population depend upon herbal medicines for primary health treatment (Mukherjee, 2002 and Bodeker *et al.*, 2005) [16, 4]. Treatment with medicinal plants is very safe without any side effects. The biggest benefit of these remedies is synchronization with nature. There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, aging etc. Oxidation process is one of the routes for producing free radicals in food, drugs and living system. Environmental pollutants, radiations, chemicals, toxins, and physical stress produce free radicals which cause depletion of the immune system antioxidants, change gene expression and induce abnormal proteins (Halliwell, 1996; 2002) [9, 10].
Soybean (*Glycine max*) is an annual crop originated in Asia. Temperate climate is suitable for its cultivation. It is a legume species, widely grown for its high quality protein, edible bean and oil. It is classified as an oil seed rather than a pulse, like most legumes. Soybean is a good source of organic compounds, antioxidants, vitamins and minerals. Its oil has high amount of polyunsaturated fatty acids which are very essential for human beings. It is extensively used oil in world because of its low cost. Defatted soybean meal is a significant and economic source of protein for animal feeds.
Consumption of polyunsaturated vegetable oils has substantially increased day by day. But higher rate of lipid oxidation in these oils leads to development of rancidity and decreases the quality of oil (Kamal-Eldin, 2003; Arawande *et al.*, 2010) [13, 3]. Oxidation of oils is a series of chemical reactions and is a major cause of oil deterioration. This process not only affects the nutritional quality but also generate potential toxic compounds by reacting with reactive oxygen species (ROS) and free radicals. These compounds have adverse effects on human health and are major cause of degenerative diseases such as early ageing and cancer (Krishnaiah *et al.*, 2010) [14].
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 (Gunstone and Norris, 1983) [8]. TBHQ is known to be a very effective antioxidant for vegetable oils, and is stable at high temperature (Coppen, 1989) [6]. 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. Phenolic compounds have been known to possess antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilatory activities (Breinholt 1993; Shahidi and Naczk 1995; Duthie *et al.*, 2000) [5, 19, 7]. These compounds are reported to have pronounced chemopreventive effect through modulation of molecular events that damage DNA and other biomolecules (Tahara *et al.*, 2005) [22]. However, the antioxidant activity of these extracts depends on the isolation procedures and type of active components of extracts (Pokorny and Trojakova, 2001) [18]. The effectiveness of antioxidants varies with temperature, processing conditions and storage (Coppen, 1989) [6].

In this study, sesame (*Sesamum indicum* L.) and sunflower (*Helianthus annus* L.) seed meals were studied as potential antioxidant agents to improve the shelf-life of oils. The objectives of present investigation were:

- To prepare extracts of sesame and sunflower seed meals
- To extract the crude oil of soybean by solvent extraction
- To study the oxidative stability of crude soybean oil supplemented with meal extracts

## Experimental

### Materials

The seeds of soybean, sesame and sunflower were collected from the 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 soybean was extracted and studied for their various chemical parameters. The dried defatted seed meal of sunflower and sesame were extracted with acetone and further used as antioxidants.

### Extracts Preparation

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 $^0$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 $^0$C), weighed and residue was redissolved in acetone (100 ml) to give a solution of known concentration. It was stored in refrigerator untill further use.

### Oil extraction

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.

### Storage of oil samples

CSO samples supplemented with TBHQ 200 ppm, PG 200 ppm, sesame and sunflower meal at concentrations (500, 1000 and 2000 ppm) were incubated at 50 °C for 120 days to study oxidative stability. Control sample also incubated that contained no additives. Samples were stored in uniform glass beaker wrapped with aluminium foil and each container was appropriately labelled. Required quantity of the oils were withdrawn at day 20, 40, 60, 80, 100 and 120 and studied for the oxidative quality indices.

### Analytical Procedures

#### Conjugated dienes (CD)

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

#### Total oxidation (TOTOX) values

Total oxidation (TOTOX) values of oil samples were determined using the following equation according to Shahidi and Wanasundara, 2008 [20]:

Total oxidation (TOTOX) values = 2×PV+ AV

#### Thiobarbituric Acid Value (TBA)

Thiobarbituric Acid Value was determined according to the method of Johansson and Marcuse, 1973 [12].

#### Total tocopherol

Total tocopherol was determined by the method of Philip *et al.* (1954) [17].

#### Carotenoids

Carotenoids content was evaluated by the method of Vasconcellous *et al.* (1980) [23].

## Results and Discussion

### Conjugated dienes (CD)

Increase in hydroperoxides due to oxidation is accompanied by increase in UV absorption of oxidized fats and oils (Shahidi and Zhong, 2005) [21]. One of the first steps in the oxidation of linoleate ($C_{18:2}$) or higher PUFA in an oil is a shift in the position of the double bond. The shift occurs as one hydrogen atom is lost from the active methylene group positioned between two double bonds existing in the original 1, 4-pentadiene configuration (White, 1995) [24]. A pentadienyl radical is formed and reacted with oxygen to form conjugated hydoperoxides. The resulting conjugated dienes exhibit an intense absorption at 234 nm. The data depicted in table 1 revealed that CD values of CSO samples were increased gradually with storage period of 120 days at 50 °C. Highest increased CD was observed in control sample i.e. 30.87±0.77 from initial value of 3±0.07 % as dienoic acid. Results indicated that there may be rapid deterioration in control sample during storage period. The presence of TBHQ gave the lowest percentage change in CD value of CSO followed by sesame meal extract, sunflower meal extracts while PG gave lowest CD value when compared with CSO in the absence of the antioxidant during the storage period. Although sesame meal extract is superior to sunflower meal extract at all concentrations. Formation of high contents of CD may be related to the presence of higher contents of PUFA (Liu and White, 1992) [15] in soybean oil.

**Table 1**: Variation of Conjugated dienes value (% as dienoic acid) of crude soybean oil during storage period of 120 days at 50°C

| Sample | Storage period (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 20 | 40 | 60 | 80 | 100 | 120 |
| Control | 3±0.07 | 5.6±0.12 | 9.27±0.22 | 11.9±0.33 | 17.19±0.41 | 23.62±0.54 | 30.87±0.77 |
| TBHQ (200 ppm) | 3±0.07 | 4.1±0.09 | 4.9±0.12 | 7.1±0.15 | 9.4±0.21 | 12.36±0.28 | 17.74±0.39 |
| PG (200 ppm) | 3±0.07 | 5.4±0.12 | 8.42±0.21 | 10±0.21 | 13.29±0.29 | 21.07±0.46 | 25.64±0.56 |
| Sesame meal extract (500 ppm) | 3±0.07 | 5±0.12 | 6.03±0.15 | 9.7±0.25 | 10.84±0.27 | 19.36±0.41 | 21.36±0.49 |
| Sesame meal extract (1000 ppm) | 3±0.07 | 5±0.11 | 5.92±0.15 | 9.7±0.26 | 10.1±0.22 | 15.28±0.36 | 20.76±0.47 |
| Sesame meal extract (2000 ppm) | 3±0.07 | 5±0.1 | 5.41±0.12 | 9.55±0.25 | 10.14±0.22 | 14.77±0.35 | 19.48±0.44 |
| Sunflower meal extract (500 ppm) | 3±0.07 | 5.1±0.11 | 6.29±0.16 | 10.1±0.24 | 14.29±0.35 | 16.73±0.4 | 22.46±0.53 |
| Sunflower meal extract (1000 ppm) | 3±0.07 | 5.16±0.11 | 6.69±0.18 | 9.8±0.19 | 13.84±0.29 | 15.42±0.32 | 20.11±0.42 |
| Sunflower meal extract (2000 ppm) | 3±0.07 | 5.2±0.13 | 6.63±0.18 | 9.6±0.18 | 13.89±0.29 | 15.23±0.33 | 21.58±0.43 |

*Values are mean ± standard error*

**Total oxidation (TOTOX) values**

The data delineated in figure 1 revealed that TOTOX values of CSO samples were increased gradually with storage period of 120 days at 50 °C. Initial TOTOX value was 16.94±0.4. The maximum increase of TOTOX was observed in control sample with no additives (763.31±15.6). TBHQ has maximum stabilization effect with minimum increase in TOTOX i.e. 347.64±7.69. Under accelerated storage of 120 days, the increase of TOTOX value was in following sequence in ascending order: TBHQ 200 ppm (347.64±7.69) < sesame meals extract 1000 ppm (459.69±9.19) < sesame meals extract 2000 ppm (462.36±9.68) < sunflower meals extract 2000ppm (500.29±11.4) < sesame meals extract 500 ppm (500.41±10.5) < sunflower meals extract 500 ppm (545.73±10.8) < sunflower meals extract 1000 ppm (545.99±10.3) < PG 200 ppm (604.77±12.3) < control (763.31±15.6), respectively, after 120 days.

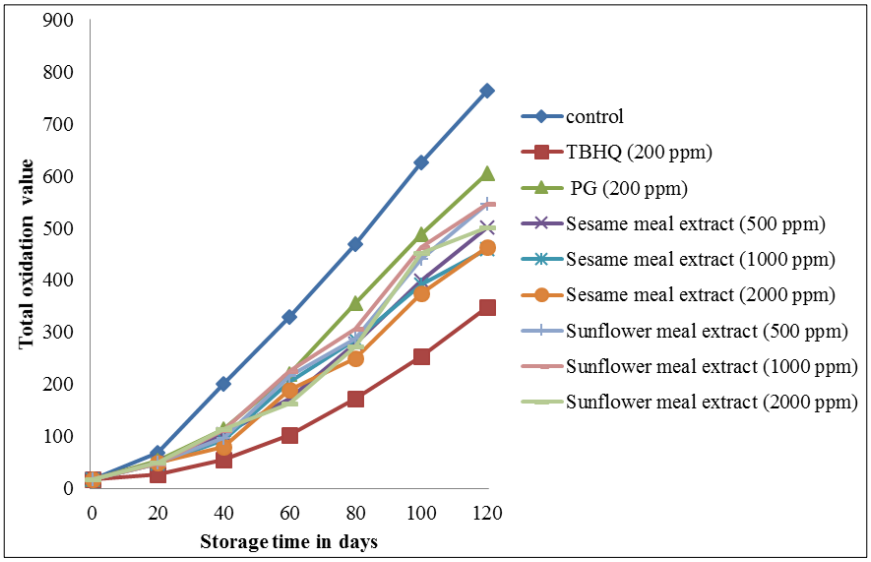


**Fig 1:** Relative increases in total oxidation value of crude soybean oil samples supplemented with extracts for 120 days at 50°C

**Thiobarbituric acid value (TBA)**

As outlined in table 2, TBA value of CSO samples was increased gradually with storage period of 120 days at 50 °C. Highest increased TBA was observed in control sample i.e. 132.39±2.79 from initial value of 15±0.39 meq/kg which is significantly higher than those of the other samples containing sesame meal extracts (500, 1000, 2000 ppm); sunflower meal extracts (500, 1000, 2000 ppm); PG (200 ppm) and TBHQ (200 ppm). Under accelerated storage of 120 days, the increase of TBA value was in following sequence in ascending order: TBHQ 200ppm < sesame meals extract 500ppm < sunflower meals extract 500ppm < sesame meals extract 1000ppm < sesame meals extract 2000ppm < sunflower meals extract 2000ppm < PG 200ppm < sunflower meals extract 1000ppm < control, respectively, after 120 days. Results found were almost similar to Anjani and Singh, 2018.

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

**Table 3:** Variation of thiobarbituric acid (meq/kg) value of crude soybean oil during storage period of 120 days at 50°C

| Sample | Storage period (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 20 | 40 | 60 | 80 | 100 | 120 |
| Control | 15±0.39 | 26.9±0.64 | 42.85±1.15 | 77.31±0.1.85 | 92.79±2.08 | 105.67±2.14 | 132.39±2.79 |
| TBHQ (200 ppm) | 15±0.39 | 17.48±0.41 | 22.22±0.57 | 37.16±0.85 | 48.11±1.12 | 72.26±1.44 | 98.49±2.13 |
| PG (200 ppm) | 15±0.39 | 20.6±0.45 | 30±0.71 | 50.56±1.05 | 61.06±1.28 | 91.78±1.85 | 119.46±2..19 |
| Sesame meal extract (500 ppm) | 15±0.39 | 19.3±0.42 | 30.62±0.72 | 40.26±0.89 | 63.72±1.34 | 85.07±1.69 | 106.17±2.24 |
| Sesame meal extract (1000 ppm) | 15±0.39 | 18.9±0.45 | 31.72±0.78 | 41.29±0.85 | 62.72±1.46 | 85.16±1.73 | 111.12±2.46 |
| Sesame meal extract (2000 ppm) | 15±0.39 | 18.9±0.48 | 31.44±0.8 | 41.78±0.87 | 62.55±1.37 | 82.76±1.95 | 112.54±2.59 |
| Sunflower meal extract (500 ppm) | 15±0.39 | 19.71±0.51 | 32.7±0.85 | 41.65±0.85 | 64.18±1.45 | 86.12±2.15 | 108.68±2.35 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Sunflower meal extract (1000 ppm) | 15±0.39 | 19.7±0.53 | 30.08±0.69 | 43.46±0.92 | 62.88±1.56 | 83.24±2.09 | 123.34±2.79 |
| Sunflower meal extract (2000 ppm) | 15±0.39 | 19.43±0.49 | 30.1±0.65 | 44.1±1.04 | 63.03±1.48 | 83.26±2.12 | 118.19±2.63 |

Values are mean ± standard error

**Total tocopherol**

Concerning the tocopherol of stored CSO samples, the results obtained in figure 2 revealed that total tocopherol contents decreased gradually in all oil samples during storage. The lowest decrease in tocopherol was found in oil sample stabilized with TBHQ. The oil sample treated with meal extracts showed a lower deterioration of tocopherol content as compare to control and PG. Results also revealed that meal extracts treatment at 2000 ppm is more effective than 1000 ppm, which is more effective than 500 ppm. Under accelerated storage of 120 days, decline in total tocopherol was in following sequence in descending order: TBHQ 200 ppm (346±9.3) > sesame meals extract 2000 ppm (218±5.6) > sesame meals extract 1000 ppm (218±5.6) > sunflower meals extract 2000 ppm (174±3.4) > sesame meals extract 500 ppm (165±4.4) > sunflower meals extract 1000 ppm (160±3.6) > sunflower meals extract 500 ppm (151±4.1) > PG 200 ppm (148±3.8) > control (90±1.7), respectively, after 120 days.

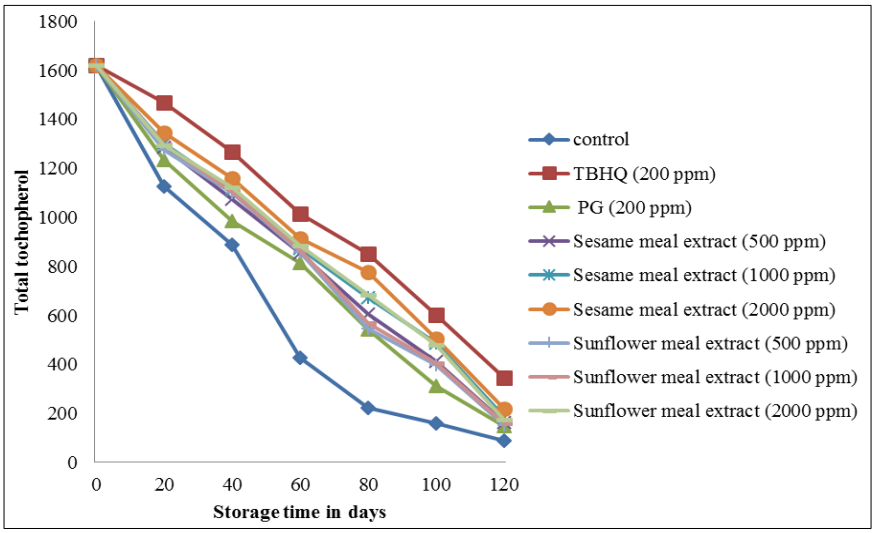


**Fig 2:** Relative deterioration of total tocopherol of crude soybean oil samples supplemented with extracts for 120 days at 50°C

**Carotenoids**

The changes in carotenoid contents of CSO during storage at 50 °C for 120 days are shown in figure 3. Generally it can be observed from results that carotenoid contents decreased gradually in all oil samples as storage period increased. The highest degradation was observed in control sample. This may due to absence of antioxidants in control sample. The sample stabilized with TBHQ has lowest degradation of carotenoids. Also the oil sample treated with meal extracts showed a lesser deterioration of carotenoids contents as compare to control. Under accelerated storage of 120 days, decline in carotenoids was in following sequence in descending order: TBHQ 200 ppm (18.56±0.49) > sesame meals extract 2000 ppm (15.86±0.33) > sunflower meals extract 1000 ppm (14.42±0.36) > sunflower meals extract 2000 ppm (13.02±0.31) > sesame meals extract 1000 ppm (11.11±0.24) > PG 200 ppm (10.68±0.24) > sesame meals extract 500 ppm (9.06±0.19) > sunflower meals extract 500 ppm (7.35±0.16) > control (3.52±0.08), respectively, after 120 days. Sesame and sunflower meal extracts at 2000 ppm and 1000 ppm concentrations are more effective than PG 200 ppm but less effective at 500 ppm concentration.

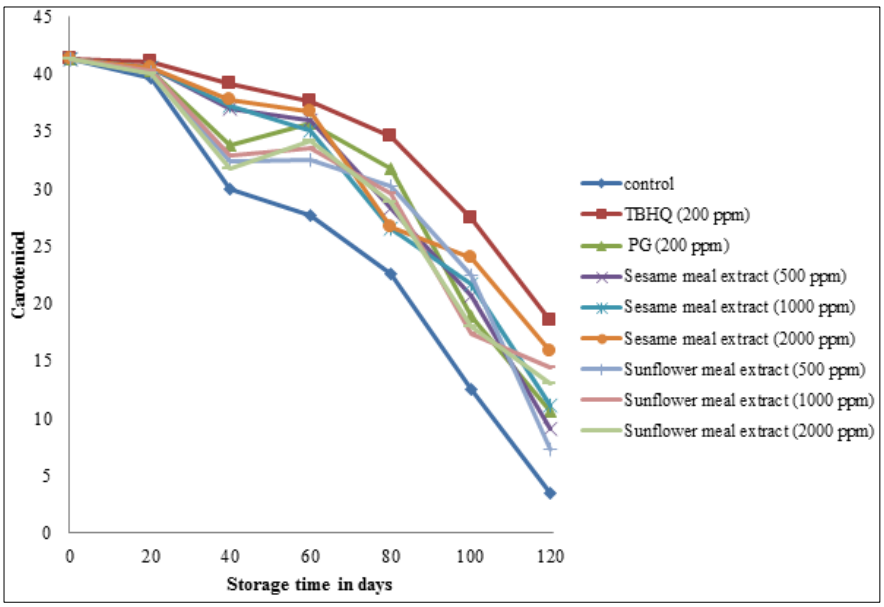


**Fig 3:** Relative deterioration of carotenoids content of crude soybean oil samples supplemented with extracts for 120 days at 50°C

## Conclusion

Storage of crude soybean oil for longer time resulted in undesirable changes in their chemical properties and deteriorates the nutritional quality of oil. This problem can be minimized by treating oil samples with synthetic as well as natural antioxidants. So, sesame and sunflower meal can be used as potential antioxidants and also more effective than propyl gallate.

## Acknowledgement

The author is grateful to University Grants Commission, New Delhi, India for awarding junior research fellowship.

## Reference

1. Akerele O. Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram. 1993; 28:13-19.
2. Anjani, Singh R. Evaluation of antioxidant efficacy of meal extracts against synthetic antioxidants in crude cotton oil. Journal of Pharmacognosy and Phytochemistry. 2018; 7(1):1220-1225.
3. Arawande JO, Amoo IA, Lajide L. Effects of Citric Acid and Methanol Extracts of Banana and Plantain Peels on Stability of Refined Soybean Oil. Ethnobotanical Leaflets. 2010; 14:706-714.
4. Bodeker C, Bodeker G, Ong CK, Grundy CK, Burford G, Shein K. WHO Global Atlas of Traditional, Complementary and Alternative Medicine. Geneva, Switzerland: World Health Organization, 2005.
5. Breinholt V. Desirable versus harmful levels of intake of flavonoids and phenolic acids. In J. Kumpulainen & JE. Salonen (Eds.), Natural antioxidants and anticarcinogens in nutrition, health and disease. Cambridge: The Royal Society of Chemistry. 1993; 37:93-105.
6. Coppen PP. Rancidity in Foods (2nd edn.), ed Allen JC & Hamilton R J. Elsevier Applied Science, London, UK, 1989, 83.
7. Duthie GG, Duthie SJ, Kyle JAM. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutrition Research Reviews. 2000; 39:79-106.
8. Gunstone FD, Norris FA. Lipid in Food: Chemistry, Biochemistry and Technology, Pergamon Press New York, 1983, 58-63.
9. Halliwell B. Antioxidants in human health and disease. Annual Review of Nutrition. 1996; 16:33-50.
10. Halliwell B. Effect of diet on cancer development: is oxidative DNA damage a biomarker? Free Radical Biology and Medicine. 2002; 32:968-974.
11. International Union of Pure and Applied Chemistry Standard methods for the analysis of oils, fat and derivatives. 7th Edition, London: Blackwell Scientific, 1987.
12. Johansson L, Marcuse R. Studies on the TBA test for rancidity grading: II. TBA reactivity of different aldehyde classes. Journal of American Oil Chemist's Society. 1973; 50:387-391.
13. Kamal-Eldin A, Makinen M, Lampi AM. In A. Kamal-Eldin, ed., Lipid Oxidation Pathways, AOCS Press, Champaign, Illinois, 2003, 1-36.
14. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Food and Bioproducts Processing. 2010; 157:1-17.
15. Liu H, White PJ. Oxidative stability of soybean oils with altered fatty acid compositions. Journal of American Oil Chemist's Society. 1992; 69:528-532.
16. Mukherjee PW. Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. New Delhi, India: Business Horizons Publishers, 2002.
17. Philip B, Bernard L, William H. Vitamins and Deficiency Diseases, In: Practical Physiological Chemistry, McGraw-Hill company, INC. New York, Toronto, London, 1954, 1272-1274.
18. Pokorny C, Trojakova L. In eds. Yanishlieva N, Gordon M. The Use of Natural Antioxidants in Food Products of Plant Origin. Antioxidants in Food, Woodhead Publishing Ltd. (England), 2001, 355-372.
19. Shahidi F, Naczk M. Methods of analysis and quantification of phenolic compounds. Food phenolic: Sources, Chemistry, Effects and Applications. Technomic Publishing Company, Inc. Lancaster, PA, 1995, 287-293.
20. Shahidi F, Wanasundara UN. Methods for measuring oxidative stability in edible oils, in Akoh CC. and Min D.B. (Ed.) Food Lipids: Chemistry, Nutrition and Biotechnology. New York: CRC Press, 2008, 387-388.
21. Shahidi F, Zhong Y. Lipid oxidation: measurement methods in: Bailey's Industrial Oil and Fat Products, Sixth Edition, Edited by Fereidoon Shahidi, John Wiley & Sons, Inc., 2005, 357-385.
22. Tahara S, Baba N, Matsuo M, Kaneko T. Protective effect of epigallocatechin gallate and esculetin on oxidative DNA damage induced by psoralen plus ultraviolet-a therapy. Bioscience Biotechnology and Biochemistry. 2005; 69:620-622.
23. Vasconcellous JA, Berry JW, Weber CW. The properties of Cucurbita foetidissima seed oil. Journal of American Oil Chemist's Society. 1980; 57:310-313.
24. White PJ. Chapter 9: Conjugated diene, anisidine value, and carbonyl value analyses. In: methods to assess quality and stability of oils and fat-containing foods edited by Warner and Eskin. AOCS Press, Champaign, Illinois, USA, 1995, 159-178.