Evaluation of oxidative stability of fried sunflower oil incorporated with clove oil

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Abstract: Clove (*Syzygium aromaticum*) contains phenolic compounds such as eugenol, gallic acid and eugenol acetate. Eugenol exhibits antioxidant activity by mechanism of free radical scavenging and impeding generation of reactive oxygen species. The natural antioxidant potential of clove can be a better choice than synthetic for food applications. In lieu of this, the present investigation was undertaken to evaluate the oxidative stability of sunflower oil incorporated with clove oil (SCO) under frying conditions. The SCO was undergone frying cycles every week and evaluated for oxidative stability by chemically analysing its free fatty acid (FFA), saponification (SV), iodine value (IV), thiobarbituric acid test (TBA) and peroxide value (PV) in comparison to control sunflower oil (without added antioxidant) and added synthetic antioxidant (butylated hydroxytoluene). The results showed that the highest increase in FFA, SV, TBA and PV values in order of control> SCO>BHT after repeated frying cycles. The results support the potential of clove oil in suppressing the oxidative deterioration in fried sunflower oil against the control sample.

Keywords: clove oil, natural antioxidant, sunflower oil, frying, oxidative stability

1. Introduction:

Sunflower oil is one of the most popular vegetable oils and in some countries it is preferred to soybean, cottonseed and palm oils. Sunflower oil is obtained from the seeds of the plant *Helianthus annuus* (Daniel, 1979). The sunflower is the fourth largest oil source in the world, after soybean, palm and canola oil. Traditionally, sunflower oil is excellent for cooking, making salad dressing, margarine but it cannot be used for manufacturing shelf-stable fried foods because of its poor oxidative stability (Gunstone, 2004).

Vegetable oils such as sunflower oil, corn oil, canola oil and olive oil are recommended as best cooking media. They are having many beneficial effects on human body especially in lowering cholesterol because they are high in polyunsaturated fatty acids (Ruxton *et al.*, 2004). However, these fatty acids are susceptible to oxidation and unstable (Alfonso, 2003). Lipid oxidation is the principal cause of food deterioration. It is induced when lipids are exposed to environmental factors such as light, air or high temperature. It produces off-flavour compounds and causes an unpleasant taste of oil which reduces the quality and shelf life of oil. It also decreases the nutritional quality of oil and produces toxic compounds (Rubalya *et al.*, 2012).

There has been an increasing interest in the use of natural antioxidants, such as tocopherols, flavonoids and rosemary (*Rosmarinus officinalis L.*) extracts for the preservation of food materials in recent years. These natural antioxidants avoid the toxicity problems which may arise from the use of synthetic antioxidants, such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and propyl gallate

(PG). Reports reveal that BHA and BHT could be toxic and the higher manufacturing costs, lower efficiency of natural antioxidants such as tocopherols, increasing consciousness of consumers with regard to food additive safety, has created a need for identifying alternative natural and probably safer sources of food antioxidants. Natural antioxidants are more ideal as food additives, not only for their free radical scavenging properties, but also on the belief that natural products are healthier and safer than synthetic ones. Thus, they are more readily acceptable to the modern consumers. There is at present increasing interest both in the industry and in scientific community for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants. Spices and herbs are not just worth in adding flavour to foods, but their antioxidants activity also helps to preserve foods from oxidative deterioration there by increasing their shelf life. As an example ground black pepper has been found to reduce the lipid oxidation of cooked pork (Peter, 2001). Among spices, clove showed the higher content of polyphenols and antioxidant compounds (Perez,2010). The antioxidant activity of clove oil compared with synthetic antioxidants measured as the scavenging of the DPPH radical decreased in the following order: clove oil > BHT > Alfa tocopherol > butylatedhydroxyanisole >Trolox (Gulcin *et al.*, 2012).

Therefore, the present investigation was undertaken to evaluate the effectiveness of clove oil as a source of natural antioxidant and to analyse the oxidative stability of sunflower oil added with clove oil in comparison to synthetic antioxidant (BHT) in frying conditions.

2. MATERIALS AND METHODS

2.1 Raw materials: Refined sunflower oil without added antioxidant was procured from the Bassava oil mill of Basavakalyan, Dist: Bidar Karnataka state and dried clove buds were purchased from the local market of Loni Kalbhor, Pune.

2.2 Chemicals: Analytical grade chemicals were made available from Food Process and Product Technology laboratory, MIT College of food technology, Loni, Kalbhor.

2.3 Packaging material: Packaging material such as white colour 250 and 500 ml PET bottles with cap were sourced from local market of Pune.

2.4 Processing equipment: The analytical instruments or equipment's viz. thermometer, Lovibond Tintometer, dryer, grinder, weighing balance, shaker, water bath, spectrophotometer, incubation chamber with thermostat was made available from MIT College of Food Technology.

2.5 Methodology:

2.5.1 Sample preparation: The control sunflower oil sample was without added antioxidants. Sample two was prepared by adding clove oil in sunflower oil (SCO) and sample three was sunflower oil added with 0.02% BHT.

2.5.2 Process standardization for extraction of clove oil by hydro-distillation

Hydro-distillation is one of the most commonly used methods for extraction of essential oils Jeyaratnam *et al*., (2016). During hydro-distillation method, powered samples (100 g dried and ground clove buds) was soaked into distilled water. 100 g dried clove sample was taken into 500ml round bottom flask and subjected to hydro-distillation for 4-6 hours. and dehydrated by using anhydrous sodium sulphate.

2.5.3 Free fatty acid (FFA) value

Free fatty acid was estimated by method given by A.O.A.C. (2000). Weighed sample (5g) was well mixed with 50 ml of neutral ethyl alcohol into 250 ml conical flask. Few drops of phenolphthalein indicator were added. Titrated against 0.1N potassium hydroxide (KOH). Shaking was done until a pink colour, which persists for 15 seconds. FFA has expressed as % oleic acid.

$$\text{Free fatty acid value (mg KOH/g)} = \frac{\text{Titer value x Normality of KOH x 56.1}}{\text{Weight of sample (g)}}$$

The FFA calculated as oleic acid using the equation: 1ml 0.1N KOH = 0.028g oleic acid

2.5.4 Peroxide value (PV)

Peroxide value was estimated by using method given by A.O.A.C. (2000). Weigh out 5g of sample into a 500 ml conical flask, add 30ml acetic acid chloroform mixture and dissolve it. Add 0.5 ml of saturated potassium iodide solution mix well and allow to stand for 1 min. Then add 430 ml of water, 3-4 drops of starch indicator and mix well. Titrate against standard 0.01 N sodium thiosulphate with vigorous shaking to liberate all from chloroform layer until the blue color just disappears. Treat the blank similarly in the absence of Sample.

$$\text{Peroxide value (meq } O_2\text{/kg of sample)} = \frac{\text{A x N x 1000}}{\text{Weight of sample}}$$

2.5.5 Thiobarbituric acid (TBA) test

Dissolve an aliquot of melted fat sample in 10 ml of carbon tetrachloride. Add 10ml of TBA reagent. Shake thoroughly for 4 min. Transfer the contents to a separating funnel and withdraw the aqueous layer into a test tube. Immerse the tube in a boiling water bath for 30 min. and prepare the blank in absence of sample, cool it, read the optical density of the sample against the blank at a wavelength of 538 nm Tarladgia *et al.,* (1960).

TBA value (as mg malonaldehyde per 1000 g sample) = 7.8XA

(A= absorbance of sample against blank)

2.5.6 Saponification value

Weigh accurately 1 to 2 g of oil sample into a 250 ml of conical flask, 25ml of alcoholic KOH was added to dissolve the oil completely. The mixture was boiled for 30 min. on a boiling water bath and allowed to cool to room temperature. 2-3 drops of phenolphthalein indicator were added and mixed. The mixture was titrated against 0.5N HCl until the pink colour disappears. Treat the blank similar in absence of oil Ranganna, (2015).

$$\text{Saponification Value of an oil (mg KOH)} = \frac{(Blank - Titre) \times 28.06}{\text{Weight of oil(g)}}$$

2.5.7 Iodine value:

Weight 0.2-0.3g of oil into 500ml conical flask. 20ml of chloroform was added and to dissolve the oil completely. Then 25ml of hanus iodine solution was added and mixed well.the stoppered flask was kept in dark for 30 min. and 20ml of KI solution was added and mixed well. The mixture was titrated against standard 0.1N sodium thiosulphate using starch as an indicator with vigorous shaking to extract the iodine from the chloroform layer. The blank was conducted similarly in the absence of oil Ranganna, (2015).

$$\text{Iodine number of oil } (gI_2/100g \text{ oil}) = \frac{A \text{ X } N \text{ X } 0.1269 \text{ X } 100}{\text{Weight of oil (g)}}$$

Where,

A=ml of $NA_2S_2O_3$

N=Normality of $NA_2S_2O_3$ solution

2.5.8 Oil frying study

During frying 500g of sunflower oil sample viz, control sunflower oil sunflower oil incorporated with clove oil and BHT were fried whose temperature was set to be 180ºC. Four wet cotton balls each weighing 2 g were immersed one after another and cotton balls were fried exactly for 7 min. followed by cooling the sunflower oil at room temperature. The entire cycle was repeated for 4 times Shende, (2014).

3. RESULT AND DISCUSSION

3.1 Oxidative stability of fried SCO

3.1.1 Effect of frying on free fatty acid value

Oil is exposed to air and moisture during frying at elevated temperature which results in the hydrolysis of triacylglycerol's, further this results in the release of free fatty acid. This released FFA are more susceptible to thermal oxidation and they cause odour and off flavours in the fried foods and frying media Bensmira *et al.*, (2007). Therefore, FFA value is one of the indicators used to assay the oil quality. In the present study, FFA (% oleic acid) value showed increase in both control, SCO and BHT added sunflower oil. Before frying the FFA value for all the three samples was 0.08%, during the frying period increasing FFA value was recorded in control sample (0.21%), SCO1 (0.12%) and BHT added sample (0.11%). The increase in FFA during frying period was less in SCO1 and BHT oil sample as compared to control. The values for both natural and synthetic antioxidants added oil samples ranged between 0.082-0.12% and 0.083-0.11% respectively, from the first to eighth week of analysis. This increase in FFA was more pounced in case of control sample which showed that the natural antioxidant (clove oil) was efficient to slow down the rate of formation of FFA. . The results for increased FFA in control sunflower oil were in accordance with, (Man and Jaswir ,2000) comparative reduction in FFA by addition of rosemary and saga extract in palm oil the same trend was observed by, Bensmira *et al*.,(2007) by addition of thyme and lavender oil in sunflower seed oil.

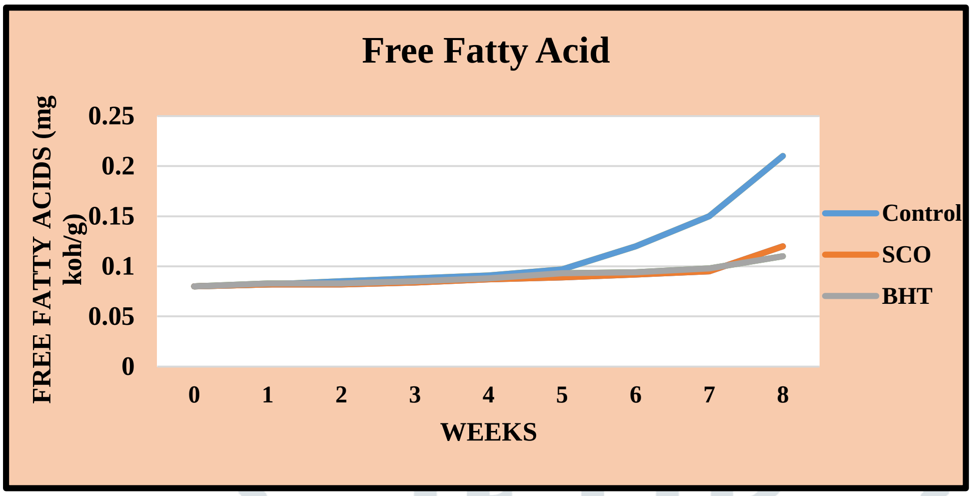

Fig. 1: Variation of iodine value in frying condition against time

3.1.2 Effect on peroxide value

Peroxide formed during oxidation of oil leads to the formation of complex mixture of volatile compounds, such as aldehyde, hydrocarbon, ketones, esters and alcohols which are responsible for deterioration of organoleptic properties (Matthaus *et al*., 2010). Hence their formation impacts shelf life and consumer's acceptance of the oil. There are two factors like high temperature and light, which generally promotes peroxides formation and degradation (Choe *et al.*, 2006). The most evident difference in the sunflower oil after frying was the increase in the peroxide value (PV). It is clear from fig. 2 that the PV on zero day was 0.92 however, after frying the PV of control oil sample increased from 3rd week onwards whereas the increase in SCO and BHT oil samples was from 6th week. This means that the clove oil and BHT were able to retard the oxidative damage for few weeks which could be attributed to the strong antioxidant activity of clove oil and BHT. The obtained results are in close agreement with Choe *et al.*, (2006), Judde (2004).

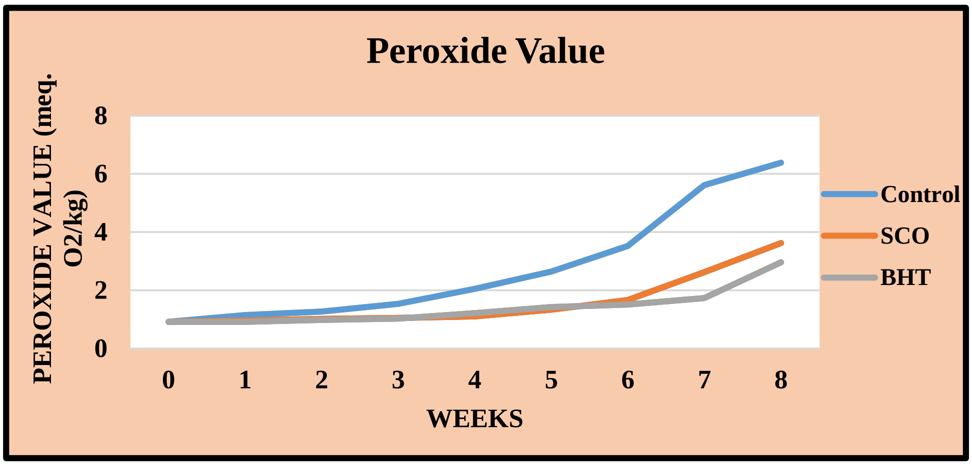

Fig. 2: Variation of peroxide value in frying condition against time

3.1.3 Effect on iodine value

The iodine value (IV) shows degree of unsaturation in oil or fat. A decrease in IV was observed in control, SCO and BHT sample during 8 weeks of frying. Changes in iodine value represents the ability of oil to slow down the occurrence of saturation. During frying the differences in the iodine values are also good indicators of deterioration in oils (Man and Jaswir, 2000). Fig 3 shows that at the end of frying cycle of 8 weeks the maximum variation in IV was recorded in control oil sample (90.63) followed by SCO (117.69) and BHT (123.47). The faster decrease in IV indicates increased rate of saturation in oil which is main reason for higher polaymeration and oxidation in oil (Goburdhun *et al*., 2000). The BHT added sample showed improved hammering in the level of unsaturation followed by the clove oil added oil sample this indicated the capability of clove oil to act as a natural antioxidant. The result in present study are in accordance with Awatif and Mohamed, (1997) a comparable increase in iodine value by addition of sesamol oil in sunflower oil. The same trend was observed by Suja *et al.*, (2004) by addition of sesamol oil in soybean and sunflower oil .

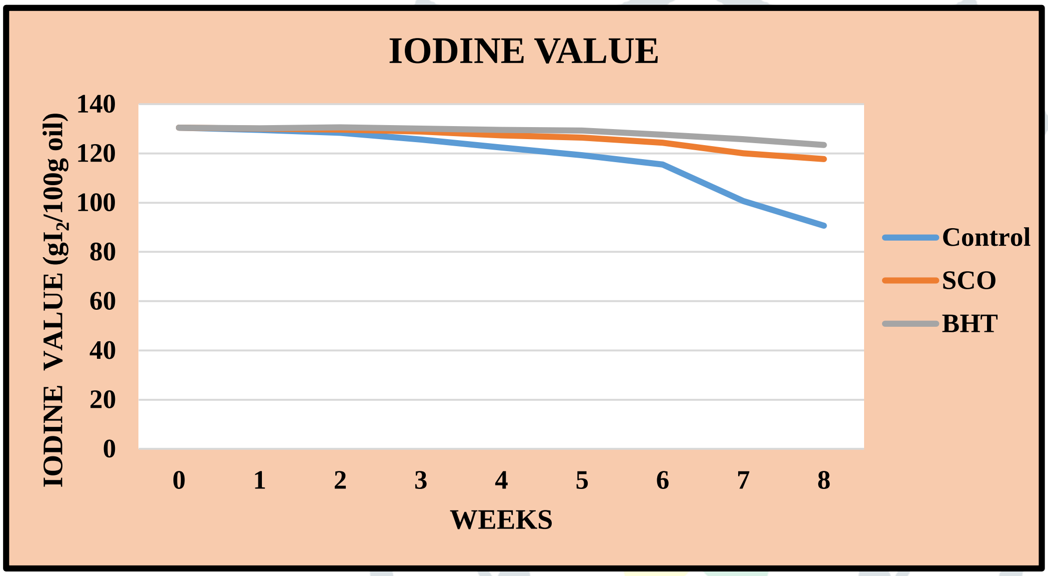

Fig. 3: Variation of iodine value in frying condition against time

3.1.4 Effect on saponification value

Saponification value (SV) assesses the degree of saturation or unsaturation in oil. It gives an idea about the molecular mass of fatty acids in oil Ali *et al.*, (2016). Higher SV values indicates presence of more saturated fatty acids where as a low value denotes higher degree of unsaturation of oil. As shown in fig. 4, the zero day SV was 20.54 mg of KOH/g oil which increased to 30.94, 25.35, and 26.56 mg of KOH/g of oil in control, SCO and BHT added sunflower oil samples respectively. The SCO sample was found to be effective in maintaining the oil saturation during the frying cycle compared to other samples of oil. The result in present study are in accordance with Ali *et al.*, (2016) observed a comparable increase SV by addition of Eucalyptus citriodora extract in canola, rapeseed and sunflower oils.

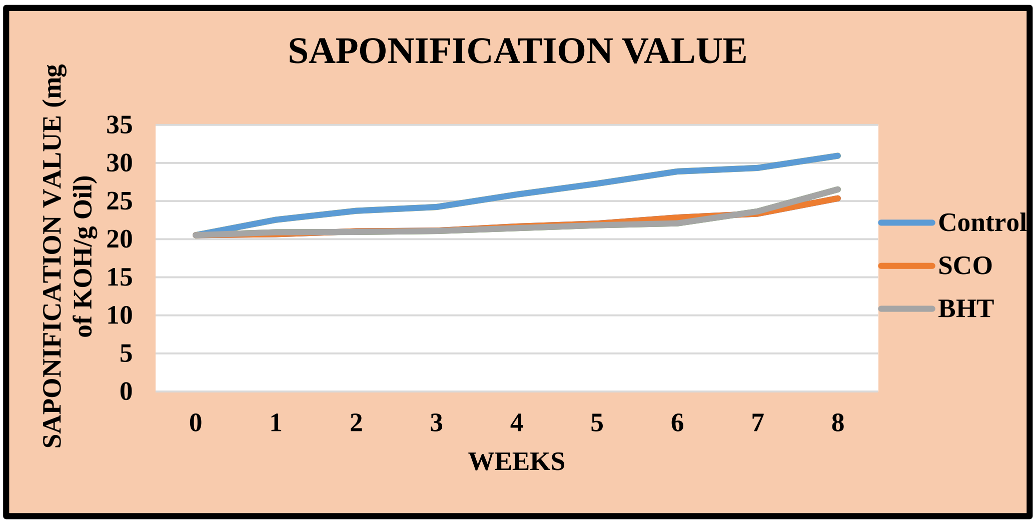

Fig. 4: Variation of saponification value in frying condition against time

3.1.5 Effect on TBA value

Oil oxidation is in two stages, first is formation of hydro peroxide and second is its decomposition leading to the formation of secondary oxidation product, which may react with TBA reagents to form colored compounds whose observation is measured at 530nm Orthoefer and Cooper, 1996. The results of TBA test fig. 5 indicates that sunflower oil without added antioxidants had high TBA value (0.98). The addition of natural (SCO) and synthetic (BHT) antioxidants lowered TBA absorbance values (0.37 and 0.38 respectively). The results indicated ability of clove oil to lower the formation of TBA reacting substances and the antioxidant effect is comparable with synthetic antioxidant during frying period of 8 weeks. The results in present study are in accordance with (Ali, 2010) who observed comparative reduction in TBA by addition of pomposia extract in sunflower oil. The same trend was also observed by (Shaker, 2006) by addition of red grape seed and peel extract in sunflower oil.

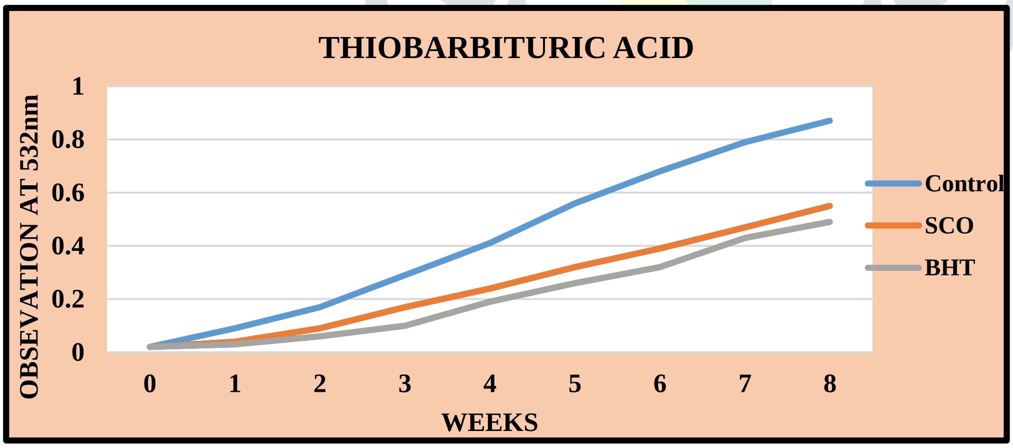

Fig. 5: Variation of thiobarbituric acid value in frying condition against time

4. Conclusion

It can be concluded from the results that clove oil has potential in delaying the oxidative damage in fried sunflower oil. Both the natural and synthetic antioxidants were found to delay the oxidative deterioration of sunflower in given frying conditions compared to control sample. The synthetic antioxidant (BHT) was found to be effective in reducing oxidative deterioration but at lower concentration than clove oil. However, the non-toxicity of natural antioxidant such as clove oil is of importance for edible oils. The activity of natural antioxidant is not comparable with synthetic however the natural antioxidant synergism can be of interest in future.

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