IJFANS

International Journal of Food And Nutritional Sciences

Volume 2, Issue 3, Jul-Sep-2013, www.ijfans.com e-ISSN: 2320-7876

Official Journal of IIFANS

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

e-ISSN 2320 –7876 *www.ijfans.com*
Vol.2, Iss.3, Jul-Sep 2013

Research Paper *Open Access*

A PRELIMINARY STUDY ON EVALUATION OF ANTIOXIDANT ACTIVITY AND OXIDATIVE STABILITY OF WHEAT GERM OIL IN POULTRY AND MUTTON MEAT SYSTEMS

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ABSTRACT

The present preliminary study investigates the lipid oxidation inhibition activity and oxidative stability of wheat germ oil processed with poultry and mutton meat in comparison with sunflower oil. The antioxidant activity and oxidative stability of wheat germ oil was evaluated in meat model systems in which the mutton and poultry meats were processed in wheat germ oil (WGO) in a conventional oven at 180^0C for 35 min. Wheat germ oil significantly reduced the thiobarbituric acid reactive substances (TBARS) values during 21 days refrigerated storage (4 ± 1^0C) and showed better oxidative stability against increase of peroxide value and free fatty acid content during 21 days of refrigerated (5 ± 1^0C) and room temperature (28 ± 5^0C) storage conditions. The results showed that wheat germ oil inhibit the lipid oxidation process in both the meat systems. In case of PV and free fatty acid content both oils were showing a significant increase during storage at room temperature than refrigerated storage condition. However, wheat germ oil showed lesser peroxides and free fatty acid contents compared to sunflower oil and also showed better oxidative stability during the storage period.

Key words: Wheat germ oil, Thiobarbituric acid reactive substances, peroxide value and free fatty acids.
Abbreviations: WGO-Wheat Germ Oil: WGOC- Wheat Germ Oil Processed with Chicken: WGOM-Wheat Germ Oil processed with Mutton: SFO- Sunflower Oil: SFOC-Sunflower Oil Processed with Chicken: SFOM- Sunflower Oil Processed with Mutton].

INTRODUCTION

Meat foods and vegetable oils are among the most important sources of dietary fat which play an important role in human nutrition by providing bulk calories, essential fatty acids, fat soluble vitamins (A & E) and natural antioxidants including polyphenols, flavanoids etc in the diet (Papadima and Bloukas 1999, Vural *et al.*, 2004). Fats and oils will undergo variety of chemical reactions during heat processing and storage such as accelerated oxidation, thermolysis and polymerization (Jinyoung *et al.*, 2008, Adel *et al.*, 2010). During these periods the nutritional and sensorial quality of the food lipids may decrease due to the formation of primary (peroxides, free fatty acids) and secondary oxidation products (TBARS) (Iqbal and Bhanger 2007). Consumption of these oxidative products from dietary oils and fats are reported as unsafe to the humans because as they are free radical in nature which will cause cancer, atherosclerosis and other possible diseases in humans. Antioxidants will play an important role in the protection of fats and oils from oxidation process during heat processing and storage conditions by quenching free radical formations. Moreover the antioxidants that are present in vegetable oils proved to be a better source for dietary antioxidants to the humans. The major antioxidants present in the vegetable oils are polyphenols -olive oil and tocopherols- sunflower oil, soya bean oil, wheat germ oil (Silva *et al.*, 2010). Tocopherols are the fat soluble vitamins having greater antioxidant activity in retarding oxidation and quenching the free radical formation in the vegetable oils during processing and storage periods. The extent of oxidation process in oils and fats greatly depends on their fatty acid composition .The fats and oils that are rich in unsaturated fatty acids especially polyunsaturated fatty acids are proved to be more susceptible to oxidation process due to the presence of liable double bonds (Frankel 1989; Yoshida *et al*.,1992; Kamal-Eldin and Appelqvist 1996; Kamal-Eldin and Andersson 1997; Kamal-Eldin 2006; Sakai *et al.*,2010). However studies have proved that consumption of oils that are rich in unsaturated fatty acids may help to decrease the life style diseases. On the other hand many health organizations and health professional are recommending the consumption of particular ratio of the fatty acids in the diet where the polyunsaturated fatty acids are the predominant ones. Thus in present scenario food scientists and nutritionists have been trying to put their effort in order to develop healthier foods by using these oils in a better way. It is therefore important to evaluate the oxidative stability of oils as affected by processing and

storage conditions (Adel *et al.,* 2010) before being used in functional food formulations.

Oxidation is a major problem in meat and meat products which will deteriorate the quality and decrease the shelf life of the products (Nunez de Gonzalez *et al.,* 2008, Vural, Javidipour & Ozbas 2004). Lipid oxidation in meat products may badly affects major quality attributes of meat products such as color, flavor, texture, overall acceptability and nutrition value of the products (Lee *et al.,* 2011). This oxidation process will leads to formation of primary and secondary oxidative products during cooking and processing which are carcinogenic in nature and decrease the acceptability of the meat products for human consumption (Kanner 1994; Gray *et al.,* 1996, Morrissey *et al.,* 1998; Yanishlieva *et al.,* 2006; Devatkal and Naveena 2010; Einafshar *et al.,* 2012)

Research studies have proved that dietary supplementation of vitamin E in the form of tocopherlos, tocopherol acetate etc, increased the oxidative stability of raw and cooked chicken, pork and lamb meat during refrigerated and frozen storage condition (Ana Rivas-Cañedo *et al.,* 2013). Moreover studies have been indicated that addition of tocopherol into the meat and meat products may increase the shelf life of the products by inhibiting the oxidation process (Tang *et al.,* 2006; Castro *et al.,* 2011).

In recent years vegetable oils have got greater importance in the development of healthier meat products. These vegetable oils have been used as animal fat replacers to increase the unsaturated fatty acids and to decrease the cholesterol content of the final product. Addition of these vegetable oils as fat replacers may increase the nutritional value whilst it may also help in lower lipid oxidation in meat and meat products due to presence of natural antioxidants such as polyphenols and tocopherlos etc (Delgado-Pando *et al.,* 2011, 2012; Salcedo-Sandoval *et al.,* 2013).

Among the vegetable oils wheat germ oil is one of the known richest sources of vitamin E. The total unsaturated and polyunsaturated fatty acid of wheat germ oil is nearly about 81 and 64% respectively. Vitamin E in wheat germ oil is available in the forms of α, β and γ tochopherols. Among these alpha tocopherol constitutes about 60% in total tocopherlos. Clinical studies have proved that dietary supplementation of wheat germ oil improves immune system function, reduced obesity and cardiovascular risks. Some other clinical studies have reported that it may enhance the physical activity during endurance exercise periods (Michael Eisenmenger Nurhan Turgut Dunford 2008).

With our knowledge till to date there are no research studies have been conducted on the antioxidant and oxidative stability of wheat germ oil in model meat systems. With this the main objective of this present preliminary work is to evaluate the lipid oxidation inhibition activity and oxidative stability of wheat germ oil processed with mutton and poultry meat to find its suitability for the development of functional meat products.

MATERIALS AND METHODS

Wheat germ oil was purchased from Falcon oils Pvt. Ltd, Banglore. Fresh lean Poultry and mutton meat were obtained from a local abattoir Mysore. All other chemicals and reagents used for the present study were thiobarbituric acid reactive substances, glacial acetic acid, neutral alcohol, sodium thiosulphate, chloroform, potassium iodide procured from SD fine chemicals (India).

PREPARATION OF POULTRY AND MUTTON MEAT SYSTEMS WITH VEGETABLE OILS

Chicken or mutton meat (70 gm uniform pieces 2×2cm) were processed in 30gm of each oil(wheat germ oil & sunflower oil) poured in 10cm diameter moisture dishes in a conventional oven at 180^{o}C for 35min. The meat samples were cooked until the core temperature of the meat reached to 72^{o}C measured with a digital thermometer. For control samples the oils were processed without meat samples in same conditions. After processing the oil and meat pieces were separated and the oil was weighed and poured in air tight glass containers (Borosil) and meat samples were packed in low density poly propylene (LDPE,75micron) pouches and stored at -20^{o}C till to usage (1day). On the next day the oil and meat samples were taken off for the real experiment. The oil samples were stored at both refrigerated and room temperature storage conditions (light) whereas the meat samples were stored at refrigerated condition only. The total study was conducted for 21 days in which the oil samples were analyzed for their peroxide value, free fatty acids and the meat samples were analyzed for TBARS values during storage period. The analysis was carried out for every three days of the total storage period. The outline of the experimental procedure is shown in Figure 1.

DETERMINATION OF TBARS VALUE

Oxidative stability of meat samples was determined by estimating the changes in thiobarbituric acid reactive substances content by using the method of Yun-Sang Choi et al (2010) with some minor modifications. Briefly the procedure is as follows: approximately 10g of each meat sample was homogenized with 90ml of distilled water and 7.5ml of 0.1NHCl by using Ultraturrax blender (Ika-Werke, GmbH & Co, Staufen, Germany). The resulted homogenate was then allowed for distillation, the first 50ml of distillate was collected. From this 20ml of distillate was taken and mixed with 2ml of 0. 20M TBARS solution (90% glacial acetic acid) and the resulted mixture were heated in boiling water bath for 35min and after tubes were cooled to room temperature. The pink color chromogen formed in the test solution was measured spectrophotometrically at 538nm by using UV spectrophotometer (Lambda 15 UV/VIS spectrophotometer, Perkin-Elmer, USA). A blank sample was also prepared by mixing 2ml of TBARS solution and 20ml of distilled water. The TBARS determinations of each sample were done in triplicate and the results were expressed as mg malondialdehyde (MDA/kg) of each meat sample.

DETERMINATION OF PEROXIDE VALUE

Peroxide value of the oil samples were analyzed during both refrigerated and room temperature storage period for 21 days. Estimation of peroxide value in fat samples had a great importance in order to identify their rancidity during storage conditions. This is an iodometric based method in which it measures the iodine produced from the potassium iodide by the peroxides present in the fat/oils respectively. PV of the samples was analyzed according to the method of AOCS Official Method Cd 8-53 (2003). Briefly the procedure is as follows: approximately 4-5g of each fat sample was taken in 250ml Erlenmeyer flask. To this 10ml of chloroform, 15ml of acetic acid and 1ml of freshly prepared saturated potassium iodide solution were added and kept in the dark for 5min with occasional shaking. Further, to this 10ml of distilled water was added and mixed thoroughly with the solution and titrated against 0.1N sodium thio sulphate solution until yellow color of the solution disappeared. Finally about 0.5% of freshly prepared starch solution was added as an indicator and titration was continued until the blue color just disappears. One blank reagent was prepared without the fat sample added. The peroxide value of the oil samples was determined by using the following formula;

$$\text{Peroxide value (meq/kg)} = \frac{\text{ml of sodium thiosulphate} \times \text{N of sodium thiosulphate}}{\text{Weight of the sample}}$$

DETERMINATION OF FREE FATTY ACID CONTENT IN PROCESSED OILS

Changes in the free fatty acid content of each oil sample were analyzed during both room and refrigerated storage condition for 21 days. The free fatty acid content of the samples was measured according to the method of Sahoo, Sharma and Chatli (2011). Briefly the procedure used is as follows: approximately 1-2g of fat sample was taken in to 150ml conical flask, to this 30ml of neutral alcohol (60^{o}C) was added and the contents were mixed properly until the complete dissolution of fat occurs. In the second step the resulted solution was titrated against 0.01N potassium hydroxide by using phenolphthalein as an indicator. The change in the color of solution to light pink determines the end point of titration. The test was carried out in triplicate and the percentage of free fatty acids was calculated as equivalents of oleic acid. A blank was prepared without the oil sample. Percentage of free fatty acids was estimated by using the following formula;

$$\text{\% free fatty acids (as oleic acid)} = \frac{\text{volume 0.01 KOH used} \times 28.2 \times 0.1}{\text{weight of the sample}}$$

$$\frac{\text{volume 0.01 KOH used} \times 28.2 \times 0.1}{\text{weight of the sample}}$$

STATISTICAL ANALYSIS

Data were analyzed by using COSTAT software. One- way ANOVA was used to find the significant difference between the experimental samples in terms of their TBARS content, peroxide value and free fatty acid content in both the meat and oil samples respectively.

RESULTS AND DISCUSSION

From the present study results showed better antioxidant and oxidative stability of wheat germ oil compared with sunflower oil in terms of lower TBARS value, peroxide value and free fatty acid content during 21 days of room and refrigerated storage conditions. Briefly the results and discussion are as follows.

DETERMINATION OF TBARS VALUES

Determination of extent of lipid oxidation by quantifying the thiobarbituric acid reactive substances content (malonaldehyde-MDA) in meat and meat products is an appropriate method to assure the quality of meat products for human consumption. Figure 2 and 3 shows the trends of TBARS formation in poultry and mutton meat processed with wheat germ oil and sunflower oil during storage at refrigerated condition for 21 days. There were no significant differences found in values of TBARS in meat systems at 0 day processed with either SFO/ WGO. However there were great results showed significant increase in the thiobarbituric acid reactive substances in poultry and mutton meat processed with wheat germ oil compared with sunflower oil. During storage period the TBARS values increased linearly along with storage period. The TBARS values of chicken meat processed in wheat germ oil and sunflower oil were increased from 0.15 on day 0 to 0.78 mg MDA/kg of meat sample on day 21and it was in the range of 0.15 to 1.02mg MDA/kg in sunflower oil processed samples. Whereas in mutton meat it was increased from 0.17on day 0 to 0.65 mg MDA/kg of meat sample (21days- SFO) and it was 0.17 (0d) to 0.55 mg MDA/kg (21days-WGO).The use of vegetable oil as fat replacers to modify the fatty acid composition was well studied (Delgado-Pando *et al.,* 2011, 2012; Salcedo-Sandoval *et al.,* 2013) whereas their effect on lipid oxidation process was scarcely reported. It is well reported that controlling lipid oxidation is a major challenge to the meat processers because lipid oxidation is the process involves the development of free radicals, off flavors which are all together can decrease the acceptability and nutritional quality of the meat and meat products (Morrissey *et al.,* 1988). Wheat germ oil successively delayed the lipid oxidation process in both the meat systems during refrigerated storage period. The reason for this may be the presence of high content of tocopherols in wheat germ oil which can have the ability to delay/ control the lipid oxidation process resulted in lower TBARS values. Previous studies also reported that addition of alpha tocopherol into the meat products significantly reduced the lipid oxidation process during 300 days refrigerated storage condition (Muhammet Irfan Aksu and Mukerrem Kaya 2005). Moreover other researchers reported that during refrigerated storage of pork (Pfalzgraf *et al.,*1995) and turkey meat addition of vitamin E significantly reduced the TBARS values (Sante

and Lacourt 1994). In respect with the present results Rodríguez-Carpena *et al.,* (2012) reported that burger patties prepared with avocado, sunflower and olive oils controlled lipid oxidation process compared with the control samples containing pork back fat. The authors reported presence of natural tocopherols in these oils helped in lessen the oxidation process in meat products. In line with the present study the previous studies also reported that tochopherols are the major components that can help in controlling lipid oxidation process in meat and meat products. In the present study, at the end of the storage period, greater lipid oxidation inhibition effect was observed in chicken meat samples when compared with the mutton meat samples processed with both the oils. Therefore, the use of wheat germ oil exhibited an important antioxidant activity towards controlling of lipid oxidation in poultry and mutton meat during 21 days stor

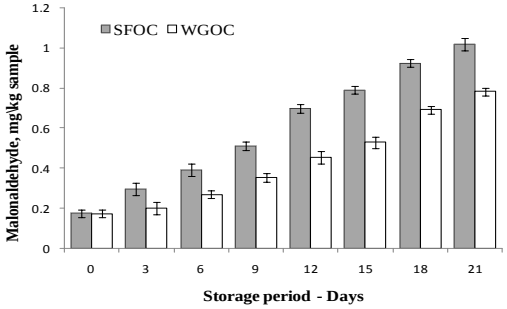

Figure- 1 TBARS values of chicken meat processed with Sunflower oil and wheat germ oil stored at refrigerated condition for 21 days

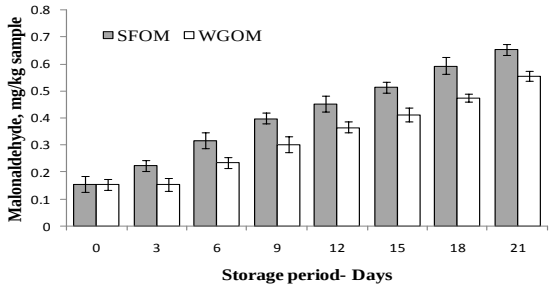

Figure- 2 TBARS values of mutton meat processed with Sunflower oil and wheat germ oil stored at refrigerated condition for 21 days

DETERMINATION OF PEROXIDE VALUE

The estimation of peroxide value is an important test for the oils and fats to estimate the oxidative rancidity during storage periods. Peroxides are the primary oxidative products which are formed at initial stages of oxidation process. This peroxide value test involves the measurement of peroxides and hydroperoxides formed during the initial stages of lipid oxidation (Teets & Were 2008). Many research studies have been reported that consumption of oxidized products from fats and oils may lead to several degenerative diseases. Thus there is a lot of importance for the evaluation of oxidative stability oils/ fats to be used in particular healthier food formulations.The separated oil samples of wheat germ oil and sunflower oil processed with meat samples and control were analyzed for their peroxide value during room and refrigerated storage condition for 21 days (Figure 4,5,6). There were significant differences observed in the increase in POV values between all the oil samples along with storage period. As expected the POV of WGO, WGOC, WGOM were found to be 3.15 to 5.66, 3.28 to 8.79 and 3.19 to 9.55meq kg^{-1} (room temperature (RT) storage/0-21days) and it was 3.12 to 3.85, 3.21 to 4.28 and 3.18 to 4.85meq kg^{-1}after 0- 21 days of refrigerated storage condition. Whilst in SFO, SFOC and SFOM the POV were found that 4.28 to 8.11, 4.35 to 14.95 and 4.22 to 13.22 meq kg^{-1} during room temperature storage for 0-21days and it was 4.21 to 5.98, 4.32 to 7.33 and 4.21 to 7.45meq kg^{-1}after 0-21 days of refrigerated storage condition. From the obtained results it was clearly observed that wheat germ oil showed lower peroxide value compared with the sunflower oil during storage period in all the model systems. Furthermore the results obtained during storage at room temperature showed considerably higher peroxide value in all the samples compared with refrigerated storage. This may be due to exposure of oils to light and room temperature where the temperature is in the range of 25-32^{o}C responsible for the more oxidation than refrigeration temperature. Previous studies have also been reported that oxidation of the fats and lipids are more dependent on intensity of light and temperatures and they show more oxidation along withincrease in temperature and light (Abramovi and Abram 2005). From the present study it was observed that after processing the WGO and SFO oils were not shown much increase in their peroxide during refrigerated storage condition. However there was a significant increase in their peroxide value during room temperature storage condition compared with refrigerated storage. The results from the present study indicate that wheat germ oil showed a better oxidative stability with or without food processing compared with sunflower oil in both the storage conditions. It seems that the presence of higher content tocopherlos in wheat germ oil play an important role in their oxidative stability.

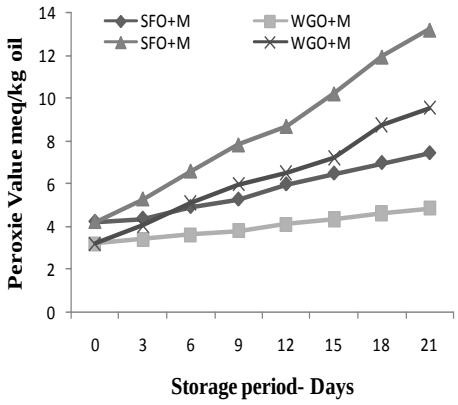

Figure- 4 Peroxide value of sunflower oil and wheat germ oil processed without meat samples and stored for 21 days at room and refrigerated condition

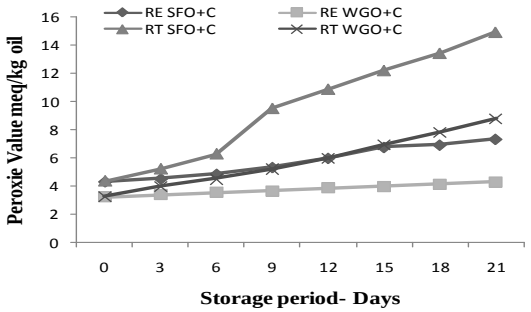

Figure- 5 Peroxide value of sunflower oil and wheat germ oil processed with chicken meat samples and stored for 21 daysat room and refrigerated condition

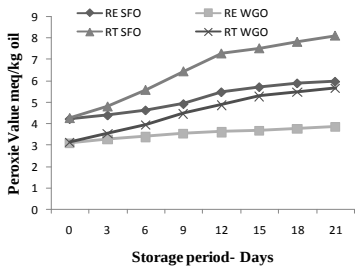

Figure- 6 free fatty acid content of sunflower oil and wheat germ oil processed with mutton meat samples and stored for 21 days at room and refrigerated condition

DETERMINATION OF FREE FATTY ACID CONTENT

The effect of storage period and the meat processing on the formation of free fatty acid content in the oil samples was determined during storage period by using titration method. The control samples WGO and SFO showed significant differences in their free fatty acid content compared with WGOC, WGOM, SFOC and SFOM respectively. Also significant difference was observed between the WGO and SFO (Figure7) in their free fatty acid content during both refrigerated and room storage condition for 21 days. Figure 8 and Figure 9 illustrates the free fatty acid content of wheat germ oil processed with chicken and mutton stored for 21 days at both refrigerated and room temperature showed significant difference in their FFA content. Compared with the SFO, SFOC and SFOM wheat germ oil exhibited lower free fatty acid content. Initially the free fatty acid content of all wheat germ oil systems including WGO, WGOC and WGOM was in the range of 0.1-0.2 (refrigerated), 0.1-0.2 (RT) and it was increased up to 0.2-0.3 (refrigerated) and 0.2-0.5(RT) after 21 days storage period, where as in sunflower oils systems it was 0.3(initial)-0.5(final) (refrigerated) and it was rose up to 0.3(initial)-0.9 (final) (RT) after storage period(21days). This may be due to the presence of high amounts of tochopherols in wheat germ oil inhibited the formation of free fatty acids. From the present results it can assumed that wheat germ oil has greater oxidative stability against storage conditions and processing effects.

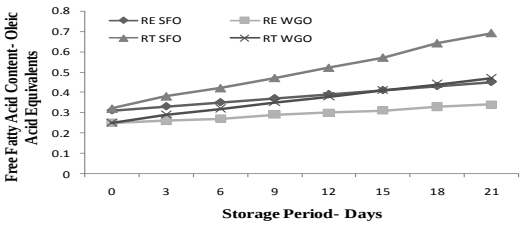

Figure- 7 Free fatty acid content of sunflower oil and wheat germ oil processed without meat samples and stored for 21 days at room and refrigerated condition

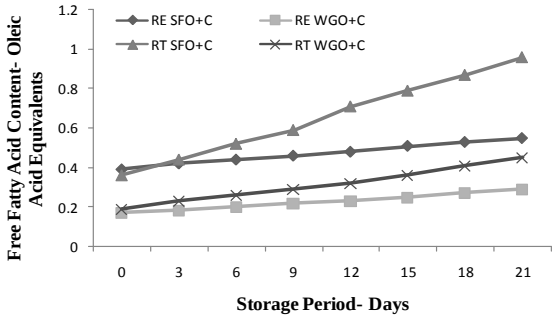

Figure- 3. Free fatty acid content of sunflower oil and wheat germ oil processed with chicken meat samples and stored for 21 days at room and refrigerated condition

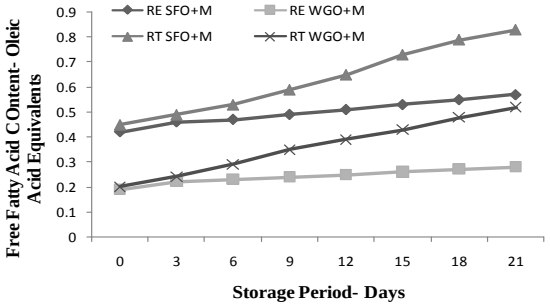

Figure- 4 Free fatty acid content of sunflower oil and wheat germ oil processed with mutton meat samples and stored for 21 days at room and refrigerated condition

CONCLUSIONS

Wheat germ oil showed better inhibitory activity on lipid oxidation in both poultry and mutton meat. The inhibitory activity showed the antioxidant activity of wheat germ oil in controlling oxidation process in meat foods. The presence of highest amounts of tocopherols in wheat germ oil may inhibit the radical chain formation occur during oxidation process. Furthermore in the present study wheat germ oil exhibited better oxidative stability in food processing treatments and during storage period. Wheat germ oil resulted in lower amounts of primary oxidative products such as peroxides and free fatty acids compared with sunflower oil. From this present study it can be suggested that wheat germ oil can inhibit the formation of both primary and secondary oxidation products in meat foods and it can be used as functional fat replacer/ cooking medium for the development of low fat- antioxidant rich novel meat and meat products.

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