Evaluation of Antioxidant Activity of Ethanolic Extract from Irradiated Sunflower (Helianthus Annuus L.) Seeds Hull

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Abstract  
Sunflower seeds hull are by-products of the sunflower industry that has low economic value despite its high content of antioxidants such as phenolic compounds and recently natural antioxidants have gained increased interest because natural food ingredients are safer than synthetic ones. Thus, the aim of investigation to evaluate the antioxidant efficacy of ethanolic extract of irradiated sunflower (Helianthus annuus L.) seeds hull (Sakha-153 variety) at dose levels of 0, 3, 6 and 9 kGy on total phenolic compounds and total antioxidant activity were studied, and the composition of phenolic compounds of ethanolic extract of non-irradiated and irradiated samples was determined by HPLC. The results exhibited that the all extract of samples under investigation possesses high stronger antioxidant activity, especially samples ethanolic extracts of irradiated at dose level of 6 kGy. For further confirmation, the ethanolic extract of irradiated samples at dose level of 6 kGy was investigated on beef minced meat model system. Different concentrations of ethanolic extract of irradiated samples at dose level of 6 kGy and Tert-Butylhydroquinone (TBHQ) were added to beef minced meat and cooked at 100±2ºC for 30 min. after cooling at room temperature, the cooked samples were stored at cold storage 4±1ºC for 7 days. Thiobarbituric acid reactive substances (TBARS) values proved that ethanol extract of Sakha-153 sunflower hulls had a power of inhibiting lipid oxidation comparable to TBHQ and exhibit antioxidant activity superior to TBHQ in the beef minced meat model system. Furthermore, the results showed that the ethanolic extract of irradiated sunflower seeds hull samples at dose level of 6 kGy were effective in inhibiting lipid oxidation of sunflower oil. Thus, these results are very beneficial for using the ethanolic extract of irradiated sunflower seeds hull samples at dose level of 6 kGy as natural stronger antioxidant and cheap price in food industry field.

Keywords: Antioxidant/ Ethanolic extract/ Sunflower seeds hull.

1. INTRODUCTION  
Food lipids undergo a variety of chemical reactions such as accelerated oxidation, thermolysis, and polymerisation under heat exposure (Jinyoung et al. 2008). Oxidation of oils modifies their organoleptic properties and affecting the shelf life of the product. It results in the loss of nutritional value of food as well as changes in color, texture, sensory and other physiological properties. Due to these changes, consumers do not accept oxidised products and industries suffer from economic losses. The oil industry has to pay special attention in this context, as oils, fats and fatty foods suffer stability problems (Iqbal and Bhanger, 2007). Several methods have been reported to measure the oxidative stability of edible oils. The oxidative stability of oils and fats with added antioxidants can be determined during storage under normal ambient conditions and packing. However, in general, oxidation can take a long time to occur, e.g. a few days to a few months, which is impractical for routine analysis. For this reason, accelerated oxidation tests or aging tests are conducted. Tests like Schaal oven test, Sylvester test and Swift test are used for accelerated oxidation test at elevated temperature. Normally Schaal oven test is used for determination of oxidation of oils (Mahuya et al., 2008). The extent of oxidation in oils has been frequently evaluated by measuring the peroxide value (PV). This index is related to the hydroperoxides, the primary oxidation products, which are unstable and readily decompose to form mainly mixtures of volatile aldehyde compounds. The oxidative degradation compounds that derived from degradation of hydroperoxides are generally termed secondary oxidative products which are determined in oils and fats by the p-anisidine (AV) method (Ramadan and Morsel, 2004).

In order to overcome the stability problems of oils and fats, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) have widespread use as food additives in many countries. Recent reports reveal that these compounds may be implicated in many health risks, including cancer and carcinogenesis (Prior, 2004). Hence, there is a tendency towards the use of natural antioxidants of plant origin to replace these synthetic antioxidants. Natural antioxidants such as flavonoids, tannins, coumarins, curcumamnoids, xanthons, phenolics, lignans and terpenoids are found in various plant products (fruits, leaves, seeds and oils) and they are known to protect easily oxidizable constituents of food from oxidation (Jeong et al., 2004). Shui and Leong (2006) found that antioxidants obtained from star fruit residues slowed the rancidification process of oil to a greater extent than did BHT. They stressed the high potential of this residue for preventing oil rancidity. The antioxidant compounds from agri-waste may not only increase the stability of foods, by preventing lipid peroxidation, but in humans or animals may also protect biomolecules and
supramolecular structures (e.g. membranes and ribosomes) from oxidative damage.

Sunflower (Helianthus annuus) is an important oil seed crop of the world and it ranks third in production next to groundnut and soyabean (Byrareddy, 2008). Current usage of sunflower seed or its byproducts as human food is so low that it might be considered as underutilized. Its use, however as an animal feed is considerable and growing. For humans, sunflower seeds are used mainly as a snack, the seeds being roasted and eaten as peanuts and chestnuts. Decorticated sunflower seeds feature much more in vegetarian diets and are sold primarily in health food stores as an effective alternative protein source (Nwokolo, 1996). To prepare refined products from sunflower such as flour, concentrates, isolates, textured vegetable protein etc., thus sunflower seeds have to be decorticated. Hence, effective utilization of the hulls seems to be essential. Sunflower seeds are covered with highly fibrous hulls which comprise about 15-25% of the seed. These hulls are mostly used for livestock bedding. A small amount of sunflower hulls can be added to animal feed as a source of fiber because of its needle like nature which can damage the gastrointestinal tract. It was experimented on the use of sunflower hulls as supplementary fuel to coal-fired power plants (Crum et al. 1992). Sunflower hull is a byproduct of industrial oil extraction of dehulled seed. However, it also has a high insoluble fiber content which reduces digestible energy (Silva et al., 2002).

Therefore, the objectives of this study were to evaluate the antioxidant effectiveness of ethanolic extract from irradiated sunflower (Helianthus annuus L.) seeds hull of Sakha-153 variety at dose levels of 0, 3, 6 and 9 kGy. The aim of was also to evaluate the antioxidant effectiveness of ethanolic extract from irradiated sunflower seeds hull during oxidation of sunflower oil under accelerated conditions and to compare its antioxidant activity with commercially available antioxidants Tert-Butylhydroquinone TBHQ. The study also attempted to identify the antioxidant compounds present in the extracts using chromatographic and spectroscopic techniques. In addition to the best ethanolic extract from irradiated and non-irradiated of sunflower seeds hull was selected to test in a beef minced meat model system.

2. MATERIALS & METHODS

2.1 Sunflower seeds hull samples:
Shelled sunflower seeds of Sakha-153 variety were purchased from Agriculture Research Center, Giza, Egypt. They were dried at 45°C for 3 days. Then the sunflower seeds hulls were hand-shelled. Sunflower seeds hull were ground using a blender (Toshiba El Araby MX-5100/5200 Sample Mill, Egypt). The milled sunflower seeds hull were packed in plastic bags and sealed by heat and stored at 4±1°C until used.

2.2 Irradiation Treatments:
For irradiation treatments, the samples of sunflower seeds hull samples exposed to gamma irradiation at dose levels of 0, 3, 6 and 9 kGy using an experimental 60Co Russian gamma chamber, in Cyclotron Project, Nuclear Research Center, Atomic Energy Authority, Abou-Zaabal, Egypt.

2.3 Preparation of ethanolic extract from non-irradiated and irradiated sunflower seeds hull:
To obtain the ethanolic extracts, the non-irradiated and irradiated of sunflower seeds hull samples were defatted by two extractions with n-hexane (50 mL each 10 g sunflower seeds hull) for 12 hours each one at room temperature. The dry defatted sunflower seeds hull (20g) was extracted with 300mL ethanol for 24 hours by maceration in darkness at room temperature. The extract was filtered and the residue was extracted again under the same conditions. The combined filtrate was evaporated to dryness in a rotary evaporator (Buchi R 110, Frawil, Switzerland) at 40°C to a final volume of 5 mL.

2.4 Proximate composition
Moisture, lipid, protein, ash, caloric carbohydrates contents, vitamin C and total phenol as Gallic acid were determined according to A.O.A.C. (2000) official method.

2.5 Determination of polyphenols
The total phenolic compounds present in the ethanolic extract of no-irradiated and irradiated sunflower seeds hull at dose levels of 3, 6 and 9 kGy samples were determined spectrophotometrically by using the Folin-Denis reagent described in A.O.A.C (2000). The concentration of total phenolic compounds in the peanut skins and hulls samples were determined by comparison with the absorbance of the standard, catechin at different concentrations.

2.6 Antioxidant activity study (DPPH free radical-scavenging assay)
The scavenging effect on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by modifying methods of Brand-Williams, et al.(1995) and Gamez, et al. (1998). The ethanolic extract of no-irradiated and irradiated sunflower seeds hull at dose levels of 3, 6 and 9 kGy samples were separately mixed with ethanol to prepare test solution of 1 mg/ml sample. DPPH was dissolved in ethanol and mixed with the ethanolic extracts of sunflower seeds hull samples. The solution was adjusted to a final DPPH concentration of 100 µM. The mixture was shaken vigorously and left to stand for 5 - 60 min in the darkness at room temperature. The amount of DPPH remaining in each period of stand was determined spectrophotometrically at 540 nm, using a microtitr
plate reader (Biorad 680, USA). The extracts of RGS test solution was diluted to different concentrations (0.1-1.0 mg/ml). After vigorous shake, the mixtures were left to stand for 30 min. Tert-Butylhydroquinone (TBHQ) was used to compare the scavenging activity. The radical scavenging activity was calculated as % inhibition from the following equation:

% inhibition as OD = \left( \frac{OD_{\text{sample}} - OD_{\text{blank}}}{OD_{\text{blank}}} \right) \times 100.

2.7 β-Carotene/linoleic acid bleaching

The ability of extracts and synthetic antioxidants to prevent the bleaching of β-carotene was assessed as described by Keyvan et al. (2007). In brief, 0.2 mg of β-carotene in 1 mL of chloroform, 20 mg of linoleic acid and 200 mg of Tween 20 were placed in a round-bottom flask. After removal of the chloroform, 50 mL of distilled water were added and the resulting mixture was stirred vigorously. Aliquots (3 mL) of the emulsion were transferred to tubes containing extract or synthetic antioxidant. Immediately after mixing 0.5 mL of extract solution (10 mg extract/10 mL solvent), an aliquot from each tube was transferred to a cuvette and the absorbance at 470 nm was recorded (Abs0). The remaining samples were placed in a water bath at 50°C for 2 h, then the absorbance at 470 nm was recorded (Abs120). A control with no added extract was also analysed. Antioxidant activity was calculated as follows:

Antioxidant activity (%) = \left[ 1 - \frac{(Abs_{\text{sample}}^0 - Abs_{\text{sample}}^{120})}{(Abs_{\text{control}}^0 - Abs_{\text{control}}^{120})} \right] \times 100

Where Abs0 sample is the absorbance of sample at 0-time, Abs120 sample is the absorbance after 120 min, Abs0 control is the absorbance of control at 0-time and Abs120 control is the absorbance of control after 120 min.

2.8 Preparation of cooked beef minced meat as a model system:

Ethanol extract of irradiated sunflower seeds hull at dose level of 6 kGy was chosen and its antioxidant activity was examined on a beef minced meat model system according to Shahidi and Pegg (1990). The hull extract was dissolved in 2 ml of 99% ethanol (absolute) and added to the beef minced meat (20% by weight of water) at the following concentrations (250, 500 and 1000 ppm). These concentrations were used to test their antioxidant effectiveness (Onyeneho and Hettiarachchy 1991). A sample with Tert-Butylhydroquinone (TBHQ) (200 ppm) and a control sample containing only 2 ml of 99% ethanol without antioxidant were also prepared. Meat systems were thoroughly homogenized and cooked at 100±2°C (He and Shahidi, 1997) in a thermostated water bath (Julabo Labortetechnik GMBH Seelbach / Germany) for 60 min., samples were cooled to room temperature, transferred into plastic bags and then stored for 7 days at 4±1°C. Samples were analysed for lipid oxidation (TBARS) on days 0, 1, 3, 5 and 7.

2.9 Thiobarbituric acid value (TBARs)

Lipid oxidation was determined as a 2-thiobarbituric acid reactive substances (TBARs) value by using the method described by Ahn et al. (1999) with some modifications. Cooked beef minced meat (5 g) and 15ml of deionized distilled water were homogenized with 50 µl butylated hydroxyanisol (7.2%) for 15 s. One milliliter of the meat homogenate was transferred to a disposable test tube and then 1ml of thiobarbituric acid/trichloroacetic acid (20mM TBA in 15% trichloroacetic acid) solution was added. The mixture was vortexed and incubated in a boiling water bath for 15 min, then cooled in cold water for 10min, and centrifuged for 15min at 2500g at 4°C. Absorbance was measured at 532nm and lipid oxidation was reported as mg malondialdehyde/kg meat.

2.10 Identification of phenolic compounds of ethanolic extract of non-irradiated and irradiated sunflower seeds hull using HPLC analysis

HPLC analysis was carried out in a Shimadzu make binary system with LC-10 AD model pump, a 7125 model Rheodyne injector fitted with a 20 ml sample loop, a SPD-10 A UV Visible detector, and with a CR7Ae plus integrator for data acquisition, analysis and display. The analysis was carried out using a Waters mbondapak C18 column (4.6 mm ID × 25 cm) in the reversed phase with a guard column of C18 (Supelco). The mobile phase used was methanol: water (70:30) with a flow rate of 1 ml/min. The UV-Visible detector was set at 290 nm, with a detector sensitivity of 0.005 AUFS (Bailey’s Industrial Oil and fat Products, 1996).

3. RESULTS & DISCUSSIONS

3.1 Chemical composition

The chemical composition (protein, fat, ash, crude fiber and total carbohydrates contents) of non-irradiated and irradiated sunflower seeds hull samples of Sakha-153 variety at dose levels of 3, 6 and 9 kGy on dry weight basis are presented in Table (1). The results showed no significant differences between non-irradiated and irradiated sunflower seeds hull samples. Meanwhile, the data revealed that the guava seed samples had high percentage of crude fiber in all samples under investigation.
Defatted sunflower (Helianthus annuus L.) seeds hull.

<table>
<thead>
<tr>
<th>Treatments (kGy)</th>
<th>Chemical composition (%)</th>
<th>Moisture</th>
<th>Protein</th>
<th>Ash</th>
<th>Oil</th>
<th>Crude fiber</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>7.98±0.23</td>
<td>7.25±0.17</td>
<td>1.92±0.24</td>
<td>1.85±0.18</td>
<td>65.33±0.27</td>
<td>15.67±0.21</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>7.92±0.31</td>
<td>7.22±0.25</td>
<td>1.94±0.08</td>
<td>1.83±0.24</td>
<td>65.26±0.16</td>
<td>15.83±0.32</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>7.89±0.09</td>
<td>7.21±0.35</td>
<td>1.93±0.12</td>
<td>1.86±0.32</td>
<td>65.44±0.05</td>
<td>15.67±0.18</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>7.95±0.16</td>
<td>7.24±0.17</td>
<td>1.97±0.31</td>
<td>1.84±0.15</td>
<td>65.42±0.09</td>
<td>15.85±0.07</td>
</tr>
</tbody>
</table>

Means with the same letters within the columns are not significantly different (p>0.05).

* By difference

3.2 Total phenolic compounds

Phenolic compounds are widely distributed in the plant kingdom. These compounds serve as important antioxidants because of their ability to donate a hydrogen atom or an electron in order to form stable radical intermediates. Hence, they prevent the oxidation of various biological molecules (Cuvelier et al. 1992). Total phenolic compounds (mg/100g as gallic acid) in non-irradiated and irradiated sunflower seeds hull samples are tabulated in Table (2). The results showed that the ethanolic extract of non-irradiated and irradiated samples possess significant total phenolic compounds. In addition to the data revealed that the ethanolic extract of non-irradiated and irradiated samples possesses higher significant values of total phenolic compounds compared with the ethanolic extract of non-irradiated samples. Moreover, the results illustrated that the ethanolic extract of irradiated samples at dose level of 6 kGy possesses higher significant values of total phenolic compounds than the non-irradiated samples. These phenomena may be due to that the higher irradiation doses facilitate certain polyphenolic compounds to be more extractable (Gil et al. 2002). Pedrosa et al. (2000) found that the total phenolic compounds (g/kg) of the hull in different geno-types of sunflower were 0.0780 for Tesoro, 0.376 for Marks, 0.232 for Clip, 0.732 for Vyp and 0.1810 for Nanta. They added that the total phenolic compounds of kernels of different genotypes range from 1.6867 to 1.2867 g/kg kernel. Harrison and Were (2007) observed that increase in phenolic contents and antioxidant activity was observed in almond skin extract (ethanol extract) irradiated at doses greater than 4 or 12.7 kGy. Similarly Kim et al. (2008) showed that electron-beam irradiation of citrus pomaces could increase polyphenolic compounds and scavenging activity of citrus pomaces extracts in methanol, ethanol and water at the absorbed doses of 3.6–37.9 kGy.

### Table (2): Total phenolic compounds (mg/100g as gallic acid) in extract of non-irradiated and irradiated defatted sunflower (Helianthus annuus L.) seeds hull.

<table>
<thead>
<tr>
<th>Total phenolic compounds</th>
<th>Control</th>
<th>Gamma irradiation doses (kGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defatted sunflower seeds hull</td>
<td>35.29±0.28</td>
<td>38.25±0.16</td>
</tr>
</tbody>
</table>

Capital letters were used for comparing between means in the rows and columns, respectively. Means with the same letters are not significantly different (p>0.05).

3.3 Radical-scavenging activity

The scavenging activity (%) on DPPH radical of the ethanolic extracts of non-irradiated and irradiated sunflower seeds hull samples on the DPPH radical are tabulated in Table (3). The results showed that the ethanolic extract of non-irradiated sunflower seeds hull possesses marked scavenging ability from 79.85% to 94.18% with increasing extract concentration from 50 to 200µg/ml on DPPH radical. Meanwhile, the results indicated that the ethanolic extract of irradiated sunflower seeds hull samples at dose levels of 3, 6 and 9 kGy had higher marked scavenging activity of DPPH radical compared with non-irradiated samples. In addition to the data exhibited that the ethanolic extract of irradiated sunflower seeds hull samples at dose level of 6 kGy possess marked scavenging activity from 86.45 to 99.13% with increasing extract concentration from 50 to 200µg/ml on DPPH radical in comparison with non-irradiated and irradiated samples at dose levels of 3 and 9 kGy. Thus, the higher scavenging effect on DPPH radical of the ethanolic extract of irradiated samples, especially irradiated samples at dose level of 6 kGy could be attributed either to the higher phenolic content or to the change in percentage or configuration of some components of the irradiated sunflower hull samples. Yen and Duh (1995) found radical-scavenging activity of 89.3% inhibition in 1500 mg/ml methanolic extract from peanut hulls on the DPPH radical. Yen and Duh (1995) found radical-scavenging activity of 89.3% inhibition in 1500 mg/ml methanolic extract from peanut hulls on the DPPH radical.
3.4 β-Carotene/linoleic acid bleaching assay

In this assay, oxidation of linoleic acid produces hydroperoxide-derived free radicals that attack the chromophore of β-carotene, resulting in bleaching of the reaction emulsion. An extract capable of retarding/inhibiting the oxidation of β-carotene may be described as a free radical scavenger and primary antioxidant (Liyana-Pathirana and Shahidi, 2006). As can be seen in Table (4) all extracts were capable of inhibiting the bleaching of β-carotene by scavenging linoleate derived free radicals. Moreover, the results revealed that, ethanol extracts had comparable scavenging ability to the synthetic antioxidants TBHQ.

Furthermore, the results exhibited that the ethanolic extract samples of irradiated sunflower seeds hull at dose of level of 6 kGy had the highest antioxidant activity was observed with respective value 99.21% at concentration 200 µg/ml. thus, the ethanolic extracts of irradiated samples especially, the ethanolic extract of irradiated samples at dose level of 6 kGy had stronger antioxidant activity than no-irradiated samples under investigating.

Table (3): Scavenging activity (%) on DPPH radical of the ethanolic extracts of non-irradiated and irradiated sunflower (Helianthus annuus L.) seeds hull.

<table>
<thead>
<tr>
<th>Concentration of samples (µg/ml)</th>
<th>TBHQ</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>88.36 ±0.76</td>
<td>79.85 ±0.41</td>
<td>83.59 ±0.63</td>
<td>86.45 ±0.27</td>
<td>79.68 ±0.87</td>
</tr>
<tr>
<td>100</td>
<td>96.03 ±0.34</td>
<td>89.54 ±0.16</td>
<td>93.77 ±0.95</td>
<td>95.67 ±0.19</td>
<td>90.73 ±0.38</td>
</tr>
<tr>
<td>150</td>
<td>99.15 ±0.26</td>
<td>91.61 ±0.57</td>
<td>95.82 ±0.81</td>
<td>98.52 ±0.68</td>
<td>93.48 ±0.45</td>
</tr>
<tr>
<td>200</td>
<td>99.44 ±0.65</td>
<td>94.18 ±0.37</td>
<td>96.67 ±0.46</td>
<td>99.63 ±0.29</td>
<td>95.69 ±0.94</td>
</tr>
</tbody>
</table>

Capital letters were used for comparison between means in the rows. Means with the same letters are not significantly different (p>0.05).

TBHQ= Tetr-Butylhydroquinone

3.5 Antioxidant activity of sunflower hull in cooked beef minced meat as a model system

The previous results showed that the ethanolic extract of irradiated sunflower seeds hull samples at dose level of 6 kGy possess higher antioxidant activity than the other treatments under investigation. So, the samples of the ethanolic extract of irradiated sunflower seeds hull were selected to carry out to evaluate its antioxidant activity in cooked beef minced meat as a model system. Thus, Table (5) shows the effect of ethanol extract of irradiated sunflower seeds hull at dose level of 6 kGy on the thiobarbituric acid-reactive substances (TBARS mg malonaldehyde / kg sample) values of cooked beef minced meat stored at 4±1°C in model system at three levels (250, 500 and 1000 ppm) along with TBHQ (200 ppm) and control (with no addition) for comparison. The results obvious that the addition of ethanolic extract of sunflower seeds hull at levels of 250, 500 and 1000 ppm delayed the lipid oxidation of cooked beef minced meat in comparison of control samples at zero time and till end cold storage at (4±1°C). Meanwhile, the data illustrated that the level of 250 ppm of ethanolic extract of sunflower seeds hull less effective than TBHQ (200 ppm) in cooked beef minced meat model system. On the other hand, the results illustrated that the level of 500 ppm of ethanolic extract of sunflower seeds hull had the same effective of TBHQ (200 ppm), but the level of 1000 ppm of ethanolic extract of sunflower seeds hull exhibit more effective than TBHQ (200 ppm) to delay lipid oxidation in cooked beef minced meat model system.

Jayasingh et al. (2002) mentioned that for secondary oxidation products, such as, TBA, no legal threshold exists, but a limit of 1 mg malonaldehyde/kg meat has been suggested for sensory perceived rancidity. Ferial et al. (2011) reported that green tea and thyme leaves extracts contained high level of total phenolic compounds content. The addition of green tea extract, thyme oil extracts either individually or combination (as natural antioxidant extracts) to luncheon roll meat was found to be effective towards reducing biogenic amines (Bas) formation, thiobarbituric acid reactive substances (TBARS) levels, volatile basic nitrogen % (VBN) and acidity %; hence improvement of the stored and refrigerated luncheon meat samples characters can occur. The best anti-oxidative effect was obtained by the combination of green tea and thyme oil extracts. DeJong and Lanari (2009) found that crude polyphenol extracts (50 or 100 mg gallic acid equivalents (GAE)/kg meat) from...
the waste waters of olive oil pomace reduced the formation of 2-thiobarbituric reactive substances (TBARs) in pre-cooked beef (63–83%) and pork (47–66%). Yu et al. (2002) reported that from lipid oxidation. Sanchez-Escalente et al. (2003) found that lipid oxidation was found to be dramatically reduced (P<0.05) in beef patties containing the peppers, either hot or sweet. The TBA values were below 1 in both types of patties. Inhibition was even greater by the effect of cayenne hot peppers, which did not result in TBA elevation.

Table (5): Effect of ethanol extract of irradiated sunflower seeds hull at dose level of 6 kGy on the thiobarbituric acid-reactive substances (TBARs mg malonaldehyde / kg sample) values of cooked beef minced meat stored at 4°C±1C in model system.

<table>
<thead>
<tr>
<th>Cooked beef minced meat</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0.53±0.07</td>
</tr>
<tr>
<td>TBHQ</td>
<td>0.39±0.21</td>
</tr>
<tr>
<td>Ethanolic extract of sunflower seeds hull</td>
<td>0.42±0.13</td>
</tr>
<tr>
<td>200</td>
<td>0.38±0.18</td>
</tr>
<tr>
<td>500</td>
<td>0.26±0.25</td>
</tr>
<tr>
<td>1000</td>
<td></td>
</tr>
</tbody>
</table>

TBHQ = Tert-Butylhydroquinone

Capital letters were used for comparing between means in the columns.

Means with the same letters are not significantly different (p>0.05).

3.6 Identification of phenolic compounds using HPLC

Some authors have reported that the total phenolic content did not correspond well with the antioxidant activity of the extracts. Thus, individual phenolic compounds may provide a better indication of the antioxidant activity of the extracts in vegetable oils (Bin et al. 2008). Thus, Table (6) obvious that the phenolic compounds of ethanol extract samples of non-irradiated and irradiated sunflower seeds hull at dose levels of 3, 6 and 9 kGy. From these results, it exhibited that the ethanolic extract of non-irradiated and irradiated sunflower seeds hull samples at dose levels of 3, 6 and 9 kGy contains 10, 10, 11 and 13 components of phenolic compounds, respectively. Meanwhile, the main component were chlorogenic acid, benzoic acid, chlorogenic acid and catechol of non-irradiated and irradiated sunflower seeds hull samples at dose levels of 3, 6 and 9 kGy, respectively. On the other hand, subjecting samples of sunflower seeds hull at dose levels of 3, 6 and 9 kGy induced many changes in the constituents (ppm) of these samples under investigation. It is observed that some components were disappeared as catechol of the ethanolic extract of irradiated samples at dose level of 6 kGy & salicylic acid and cinnamic acid of the ethanolic extract of irradiated samples at dose levels of 3 kGy. While, some new components were appeared as caffeic acid and salicylic acid of the ethanolic extract of irradiated samples at dose levels of 3 kGy & ferulic acid of the ethanolic extract of irradiated samples at dose levels of 3 and 9 kGy & ferulic acid of the ethanolic extract of irradiated samples at dose levels of 3, 6 and 9 kGy.

Generally, the results showed that the ethanolic extract of samples under investigation contains gallic acid, protocatechuic, catechol, chlorogenic acid, caffeic acid, ferulic acid, ellagic acid, coumarin and cinnamic acid may play an important role in the antioxidant activity of the ethanolic extract samples sunflower seeds hull samples. These compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994).

Table (6): Phenolic compounds of the ethanolic extracts of non-irradiated and irradiated sunflower (Helianthus annuus L.) seeds hull.

<table>
<thead>
<tr>
<th>Items</th>
<th>Phenolic compounds (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>13.18</td>
</tr>
<tr>
<td>Protocatechuic</td>
<td>20.78</td>
</tr>
<tr>
<td>Catechol</td>
<td>209.35</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>494.14</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>-</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>21.56</td>
</tr>
<tr>
<td>Caffien acid</td>
<td>-</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>-</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>317.01</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>267.23</td>
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<tr>
<td>Ellagic acid</td>
<td>49.69</td>
</tr>
<tr>
<td>Coumarin</td>
<td>16.94</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>0.75</td>
</tr>
</tbody>
</table>
4. CONCLUSION

From the present study, it can be concluded that the ethanolic extract of sunflower seeds hull of Sakha-153 variety samples under investigation rich in phenolic antioxidants of high polarities and a potential cheap natural source of antioxidant. Meanwhile, the ethanolic extract of irradiated samples at dose level of 6 kGy possess marked antioxidant and antiradical capacities in comparison other samples under investigation. Therefore, the ethanolic extract of sunflower seeds hull at dose level of 6 kGy may be suggested as natural antioxidant for food.

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