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Quality characteristics and microbiological safety evaluation of oils extracted from gamma irradiated almond (*Prunus dulcis* Mill.) seeds

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RESUMEN

Calidad y seguridad microbiológica de aceites extraídos de semillas de almendras (*Prunus dulcis* Mill.) sometidas a radiación gamma

Se ha evaluado las caracteristicas fisico-químicas y la descontaminación microbiana de aceites extraídos de semillas de almendras (variedades Misión y Price) gamma-irradiadas a dosis absorbidas de 2-10 kGy. La radiación gamma no ejerce ningún efecto considerable en la composición próximal de las semillas. Las características fisico-químicas tales como la densidad y el índice de refracción de los aceites, extraídos a partir de semillas gamma-irradiadas, permanecieron casi sin afectar; el índice de yodo disminuye mientras que el valor de saponificación, la materia insaponificable y los ácidos grasos libres aumentan. El estado oxidativo y el contenido de tocoferoles de los aceites de almendra se vieron afectados negativamente, mientras que el perfil de ácidos grasos se modifica ligeramente debido al estrés de la radiación. Curiosamente, los efectos sobre los atributos de calidad del aceite fueron más pronunciados a dosis de radiación más altas (> 6 kGy). Además, la contaminación microbiana se eliminó por completo en los aceites irradiados a una dosis absorbida de 6,0 kGy. Se puede concluir a partir de los presentes hallazgos que la radiación tiene un efecto positivo o negativo considerable en algunos atributos de la aceite de almendras. Por lo tanto, se debe aplicar una magnitud apropiada de radiación gamma para el tratamiento de semillas de almendra con el fin de retener los máximos beneficios nutritivos.

PALABRAS CLAVE: Ácidos grasos – Composición proximal – Descontaminación microbiana – Dosis absorbida – Estabilidad oxidativa – Radiación gamma – Tocoferoles.

SUMMARY

Quality characteristics and microbiological safety evaluation of oils extracted from gamma irradiated almond (*Prunus dulcis* Mill.) seeds

The physicochemical attributes and microbial decontamination of oils extracted from gamma-irradiated almond (Mission and Price varieties) seeds, to the absorbed doses of 2-10 kGy, have been evaluated. Gamma irradiation exerted no considerable effect on the proximate seed composition. The physicochemical properties such as density and refractive index of the oils, extracted from gamma-irradieted seeds, were almost unaffected; the iodine value decreased while saponification value, unsaponifiable matter and free fatty acids increased. The oxidative status and tocopherol content of almond oils were negatively affected while the fatty acid profile slightly changed due to irradiation stress. Interestingly, these effects on the oil quality attributes were more pronounced at higher irradiation doses (> 6 kGy). Besides, microbial contamination was completely eliminated in the oils irradiated to an absorbed dose of 6.0 kGy. It could be concluded from the present findings that irradiation has a considerably positive or negative effect on some attributes of the almond oil. Therefore, an appropriate magnitude of gamma irradiation should be exercised to treat almond seeds in order to retain maximum nutritive benefits.

KEY-WORDS: Absorbed doses – Fatty acids – Gamma irradiation – Microbial decontamination – Oxidative stability – Proximate composition- Tocopherols.

1. INTRODUCTION

It is well known that vegetable oils undergo oxidative deterioration during processing and storage resulting in the formation of hydroperoxides, aldehydes, ketones, and carboxylic acids which decrease the nutritive and organoleptic value of the products (Richardsa *et al.,* 2005; Bhatti *et al.,* 2010). Oxidation not only causes rancidity in oils and lowers their nutritional value; but the the oxidation products formed exhibit harmful effects on the health of consumers (Muik *et al.,* 2005; Azim *et al.,* 2009; Barros *et al.,* 2011; Rohman *et al.,* 2011).

Irradiation treatment can protect food commodities from oxidation, insects infestation and microbial contamination during storage and processing (Yusof *et al.,* 2007; Alighourchi *et al.,* 2008; Thomas *et al.,* 2008; Braghini *et al.,* 2009). Development of this preservation technique is based on the consideration that high energy irradiation might affect minutely the nutritive value of stored food. However, a study of the relationship between radiation absorbed doses and possible changes in the composition of food stuffs must be carried out in order to comprehensively assess the acceptability of irradiated processed foods (Azim *et al.,* 2009).

Exposing foods to ionizing radiation is similar to a heat treatment, either thermal or microwave, that generates minute and mostly undetectable changes in the chemical composition of food due to its selectivity and high efficiency with which it is

transferred to the orbiting electrons in the atoms constituting food molecules and consequently produce free radicals. The free electrons are rapidly trapped by surrounding atom-forming anions. Most of the absorbed radiant energy is used to generate free radicals and to induce chemical reactions between radicals or between radicals and other molecules. Only a fraction of the absorbed energy is converted into heat, therefore, it is similar to a cold pasteurization technique (Siddhuraju *et al.,* 2002). According to Graham *et al.,* (2002) water is the predominant molecule in all living systems and is the principal primary reactant during radiorization. The free radicals formed in water radiolysis have the ability to recombine with the newly formed radical cations and react with other components in the food matrix to form secondary changes and thus stabilize organic compounds.

The application of gamma irradiation for food preservation has emerged as an effective tool for imparting longer shelf-life without inducing radioactive contamination (da Trindade *et al.,* 2009; Ehlermann, 2009; Song *et al.,* 2009; Jalili *et al.,* 2010). Worldwide, a number of studies have reported that gamma- irradiated food items such as peanut, sunflower, herbs, almond oil (*Oleum amygdalae*), raw almond, olive oil, fresh meat, pecan nuts, cumin, *Nigella sativa* and pine nuts showed better physico-chemical status as well as biological characteristics verses non-irradiated foods (Lalas *et al.,* 2007; Evren and Gulden, 2008; Mexis and Kontominas, 2009; Ahmad, 2010; Prakash *et al.,* 2010).

Almond (*Prunus dulcis* Mill.), a member of the *Rosaceae* family, is cultivated globally, with about 28% of its worldwide production derived from the Mediterranean region (Chen *et al.,* 2005; Wijeratne *et al.,* 2006). Due to its high nutritional value, the almond is incorporated as an important ingredient in various confectionery products. Regular intake of almonds has been known to reduce cholesterol levels and lipoprotein profiles significantly which might be linked to its high-oleic lipids (Martins *et al.,* 2003; Moure *et al.,* 2007; Cordeiro and Monteiro, 2001). Apart from its nutritive value, the almond is reported to contain a wide variety of phenolics and flavonoids which posses interesting biological effects such as sedative, anti-inflammatory, anti-hyperlipidemic, anti-tumour and antioxidant activities (Chen *et al.,* 2005; Milbury *et al.,* 2006; Esfahlan *et al.,* 2010).

Until now there have been no previous studies reported on irradiated almond seeds. As gamma irradiation is investigated to affect the physico-chemical characteristics and nutritive quality of several foods; this prompts the need to evaluate the effect of such a treatment on the quality characteristics of almond oil. Besides, the oil extracted from the almond kernel, due to its considerably higher moisture content, is more receptive to microbial contamination and rancidity during storage, therefore, it would be worthwhile to improve its shelf-life by using an irradiation process. In this regard, the present research was undertaken to investigate the physico-chemical and biological characteristics of oils extracted from two locally available varieties of gamma irradiated almond seeds.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All the reagents used were of HPLC and analytical grade obtained from Sigma-Aldrich (Buches, Switzerland). Tocopherol standards, DL-α-tocopherol, (+) δ-tocopherol, (+) γ-tocopherol and fatty acid methyl ester (FAMEs) were purchased from Sigma Chemicals Co (St. Louis, MO, USA).

2.2. Sample collection and gamma irradiation

Two varieties of almond, namely Mission and Price, were obtained from the local dry fruit market and further authenticated by the Department of Botany, University of Agriculture, Faisalabad, Pakistan. The samples were packed in polyethylene bags and then exposed to the gamma-radiation for the doses of 2, 4, 6, 8 and 10 KGy using Cs-137 gamma radiation source at the Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan. A non-irradiated sample, kept under the same storage conditions, was used as a control.

2.3. Oil extraction

The irradiated and non-irradiated almond seeds (*var.* Misson and Price) were crushed using an electric grinder and the oils were extracted with *n*-hexane using a Soxhlet extractor for 7-8 hours in a water bath. After extraction, the solvent was evaporated under vacuum in a rotary evaporator (N-N Series) coupled with an aspirator and a digital water bath SB-651 (Eyela, Rikakikai Co. Ltd., Tokyo, Japan) at 45 °C and the extracted oils were stored at 4 °C until further analyses (Wijeratne *et al.,* 2006).

2.4. Proximate seed parameters

After oil extraction from the control and irradiated almond seeds, the residue was subjected to proximate analyses following the standard methods. Protein contents were determined using the Kjeldahl apparatus according to AOAC method (AOAC, 1990). The fiber content was estimated by taking 2 g of defatted ground residual sample. The samples were boiled with 250 mL of 0.25M H_2SO_4, followed by the filtration and washing of insoluble residues. The residues were then boiled with 250 mL of 0.313 M NaOH, followed by separation, washing, and drying. The dried residues were weighed and ashed at 600 °C using a muffle furnace (Eyela, TMF-2100, Tokyo, Japan); the loss in mass was determined gravimetrically

(AOAC, 1990). For determination of ash contents, 2 g residue, left after oil extraction, were carborized and ashed in a muffle furnace at 600 °C until a constant mass was reached (ISO, 1977).

2.5. Physico-chemical characteristics of oils

The physiocochemical parameters such as density, refractive index, iodine value, saponification value, acid value, peroxide value and unsaponifiable matter of the oils extracted form un-irradiated and irradiated almond seeds were measured by IUPAC methods (IUPAC, 1987).

2.6. Oxidative status of oils

The oxidative status of the oils extracted from non-irradiated and irradiated almond seeds was evaluated spectrophotometrically. The oil samples were diluted using iso-octane and the absorbance which corresponded to conjugated dienes and trienes was recorded at λ_{max} 232 nm and λ_{max} 268 nm, respectively. Using the absorbance data the extinction coefficients were calculated following the IUPAC method (1987). For the measurement of *para*-anisidine value, the oil samples were dissolved in iso-octane and made to react with 5 mL of a *P*-anisidine solution (0.25% w/v in acetic acid) for 10 min (IUPAC, 1987). A colored complex was formed; its absorbance was measured at a wavelength of 350 nm using a double beam spectrophotometer (Cecil, 7200, UK).

2.7. Fatty acid profile of oils

The almond kernel oils were analyzed for fatty acid profiles according to the standard method (IUPAC, 1987). The oils extracted (0.2 g) were transmethylated with potassium methoxide by refluxing at 50 °C in a round bottom flask resulting in fatty acid methyl esters (FAMEs). After the transesterification, the mixture was cooled to room temperature and the contents transferred into a separating funnel. A small volume of *n*-hexane was added into the funnel and the mixture was shaken and then centrifuged for phase separation. The upper FAMEs layer was decanted,washed with distilled water and further dried with anhydrous sodium sulphate. Finally after filtration, the FAMEs recovered were ready for the gas chromatographic analysis. FAMEs were analysed on a SHIMADZU gas chromatograph model 17-A, fitted with an SP-2330 (Supelco) methyl-lignocerate-coated (film thickness 0.20 µm) polar capillary column (30 m × 0.32 mm) and a flame ionizing detector (FID). Oxygen-free nitrogen was used as carrier gas at a flow rate of 3 mL/min. Other analytical conditions were as follow: initial oven temperature, 180 °C; ramp rate, 5 °C min^{-1}; final temperature, 220 °C; injector temperature, 230 °C; detector temperature, 250 °C. The FAMEs were identified by comparing their relative and absolute retention times with those of pure standards. The FA composition was reported as a relative percentage of the total peak area. Nonadecanoic acid was used as internal standard. All of the quantitative measurements were monitored using a Chromatography Station for Windows (CSW32) data handling software (Data APEX, Pague 5, The Czech Republic).

2.8. Tocopherol contents of oils

The tocopherol composition of almond oils (irradiated and non-irradiated) was studied using a high performance liquid chromatograph, model LC-10A series coupled with a liquid pump LS 10AS, a system controller SCL-10A, a Supelco C18 column (250 × 4.6 mm; 5 µm), a fluorescence detector RF-530 and an injector loop of 20-µL (Rheodyne, USA). The column was operated at 30 °C. A mobile phase consisting of a mixture of acetonitrile and methanol (1:1 v/v) at a flow rate of 1.3 mL min^{-1} was used. A sample for the chromatographic analysis was prepared by dissolving 1 g of almond oil into 2 mL of freshly distilled 2-propanal in a 5 mL sample vial. Tocopherol isomers in the eluent were detected using a fluorescence detector set at an emission wavelength of 325 nm and an excitation of 295 nm. Qualitative and quantitative measurements for the individual tocopherols were performed by comparing the retention time and area of the unknown with those of pure standards of α-, γ-, and δ-tocopherols.

2.9. Microbiological analysis of oils

The oil, extracted from non-irradiated and irradiated almond seeds, to the absorbed doses of 2, 4, 6, 8 and 10 kGy, were analyzed for the total bacterial and total fungal count according to the method described by Arici *et al.,* (2007). In each of the three replicate experiments, 1.0 mL oil sample was mixed with 10 mL of 2% autoclaved peptone water. The samples were diluted and plated on agars. Nutritional agar (DIFCO, USA) was used for bacterial counts and potato dextrose agar (Oxoid, UK), acidified with 10% tartaric acid, was used for yeast. Total bacterial counts were performed after incubation at 37 °C for 24 hrs and yeast colonies of the non-irradiated and irradiated oil samples were counted after 3 days of incubation at 28 °C. These microbial analyses were performed at the Bioassay Section, Protein Molecular Biology Lab., Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad.

2.10. Statistical analysis

Almond seeds (irradiated & unirradiated) were analyzed in triplicate and the results were reported as means ± SD. Statistical significance of the difference between mean values was assessed by ANOVA Staistix 8.1 version (Steel *et al.,* 1992).

3. RESULTS AND DISCUSSION

The proximate composition (oil, fiber, moisture, ash and protein contents) of unirradiated and irradiated almond (var. Mission and Price) seeds is given in Table 1. The irradiation up to 10 kGy did not show any significant ($p < 0.05$) effect on the proximate composition (oil, fiber, moisture, ash and protein contents) of almond (var. Mission and Price) seeds (Table 1). The contents of oil and protein of the unirradiated (control) and irradiated almond seeds, with contributions of 40.0-40.1,39.8-40.4% and 22.7-24.8,22.4-24.7%, respectively did not vary significantly between the treated and untreated samples. Similarly, the levels of moisture (7.3-8.2%), ash (4.2-4.8%) and fiber (5.3-5.6%) in the unirradiated seeds was comparable with those (7.3-8.3%,4.1-4.8% and 5.2-5.6%) of the irradiated ones revealing no considerable vartaions between the two types.

Our results, related to the proximate analyses, are consistent with previous reports which also reveal non significant difference in moisture, fat, ash and protein contents between irradiated and unirradiated almond seeds (Al-Bachir, 2004). Similarly, Bela *et al.,* (2008) also reported that the protein and crude fiber contents of almonds did not change after irradiation. In agreement with our present results, Bhatti *et al.,* (2010) and Yaqoob *et al.,* (2010) also determined that gamma irradiation (2-10 kGy) did not affect the lipids, protein, fiber and ash contents of either sunflower nor maize seeds significantly ($p < 0.05$).

The refractive index, density, iodine value, saponification value, unsaponifiable matter and free fatty acid contents determined for the oils produced from unirradiated and irradiated almond seeds, to the absorbed doses from 2-10 kGy, are given in Table 2. There was no difference for the values of refractive index and density between the irradiated (1.4600-1.4700, 0.89-0.90 mg mL^{-1}) and non-irradiated oil samples (1.4599-1.4700,0.90-0.91 mg/mL), respectively, indicating that γ-radiation up to doses of 10 kGy did not exert a significant negative effect on these parameters of the oils. Our results are in accordance with the previously reported findings of Bhatti *et al.,* (2010) and Yaqoob *et al.,* (2010) who also did not observe any significant change in refractive indices and densities between the controls and irradiated peanut, sunflower and maize oils. The refractive index and density values depend on thermal degradation and percentage of polar compounds formed during oxidation and hydrolytic reactions (Benedito *et al.,* 2007).

As a result of exposure to gamma radiation, the iodine values of the control almond oils (105.7-110.7 g of I $100g^{-1}$ oil) were found to be substantially decreased to the levels as low as 61.3-79.3 g of I $100g^{-1}$ oil (the values for samples exposed to 10 kGy dose). Generally, at higher doses the decline in the oils' iodine value was more remarkeable. The decreasing trend in the oil iodine value upon irradiation in this study might refer to the saturation of the oil as a result of the breakdown of double bonds due to oxidative deterioration in the fatty acids. A similar decreasing trend in iodine value has already been seen (Al-Bachir, 2004; Anjum *et al.,* 2006; Bhatti *et al.,* 2010; Yaqoob *et al.,* 2010). The oils saponification values increased upon irradiation (an increase from 185-187 to 204-231 mg KOH g^{-1} of oil) which indicated that large original molecules of oils containing long-chain fatty acids degraded to smaller molecules as a result of oxidation and cleavage of bonds (Agatemor,

Table 1
Effect of gamma irradiation on proximate composition (%)[a] of almond seeds of different varieties

Contents	Variety	Radiation doses					
		Control	2 kGy	4 kGy	6 kGy	8 kGy	10 kGy
Oil	**Mission**	40.0 ± 1.5	40.0 ± 2.0	39.8 ± 1.0	40.3 ± 1.5	40.4 ± 1.0	40.0 ± 2.0
	Price	40.1 ± 2.0	40.0 ± 1.8	40.0 ± 2.0	39.8 ± 1.4	40.3 ± 2.0	39.9 ± 1.7
Moisture	**Mission**	7.3 ± 0.9	7.3 ± 0.5	7.6 ± 0.6	7.6 ± 0.8	7.6 ± 0.8	7.8 ± 0.7
	Price	8.2 ± 0.5	8.3 ± 0.8	8.2 ± 1.0	8.2 ± 0.4	8.2 ± 0.8	8.2 ± 1.0
Protein	**Mission**	22.7 ± 1.5	22.5 ± 1.0	22.5 ± 1.1	22.4 ± 1.0	22.5 ± 1.1	22.7 ± 1.1
	Price	24.8 ± 0.9	24.3 ± 1.5	24.3 ± 1.2	24.7 ± 1.0	24.7 ± 1.7	24.2 ± 1.0
Fiber	**Mission**	5.6 ± 0.3	5.5 ± 0.5	5.6 ± 0.3	5.5 ± 0.6	5.5 ± 0.8	5.5 ± 0.4
	Price	5.3 ± 0.5	5.2 ± 0.4	5.3 ± 0.3	5.3 ± 0.5	5.3 ± 0.7	5.3 ± 0.9
Ash	**Mission**	4.2 ± 0.6	4.1 ± 0.5	4.4 ± 0.5	4.6 ± 0.4	4.6 ± 0.8	4.5 ± 0.5
	Price	4.8 ± 0.5	4.8 ± 0.5	4.8 ± 0.6	4.8 ± 0.6	4.8 ± 0.2	4.8 ± 0.7

Mean ± SD calculated from three replicates.
[a] The mean values of oil, moisture, protein, fiber and ash within the same row are non-significantly ($P < 0.05$) varied among radiation doses.
Control (non-irradiated sample).

Table 2
Effect of gamma irradiation on physico-chemical parameters of oils extracted from almond seeds of different varieties

Parameters	Variety	Radiation doses					
		Control	2 kGy	4 kGy	6 kGy	8 kGy	10 kGy
Refractive index (40 °C)	Misson	1.4700 ± 0.002^{a}	1.4700 ± 0.002^{a}	1.4600 ± 0.003^{a}	1.4600 ± 0.001^{a}	1.4700 ± 0.004^{a}	1.4699 ± 0.002^{a}
	Price	1.4600 ± 0.002^{a}	1.4699 ± 0.003^{a}	1.4600 ± 0.002^{a}	1.4600 ± 0.002^{a}	1.4599 ± 0.002^{a}	1.4600 ± 0.003^{a}
Density (mg/mL) (25 °C)	Misson	0.89 ± 0.04^{a}	0.90 ± 0.05^{a}	0.90 ± 0.05^{a}	0.90 ± 0.04^{a}	0.91 ± 0.05^{a}	0.91 ± 0.05^{a}
	Price	0.91 ± 0.05^{a}	0.90 ± 0.05^{a}	0.91 ± 0.05^{a}	0.91 ± 0.05^{a}	0.91 ± 0.05^{a}	0.90 ± 0.05^{a}
Iodine value (g of I/100 g oil)	Misson	105.7 ± 4.1^{a}	101.7 ± 4.3^{ab}	97.8 ± 5.0^{abc}	94.0 ± 4.7^{bc}	86.6 ± 4.3^{cd}	79.3 ± 3.7^{d}
	Price	110.7 ± 4.0^{a}	101.8 ± 3.0^{ab}	93.0 ± 4.0^{abc}	86.6 ± 4.3^{bc}	74.0 ± 3.7^{cd}	61.3 ± 3.0^{d}
Unsponifiabale matter (%)	Misson	0.43 ± 0.02^{a}	0.43 ± 0.02^{ab}	0.53 ± 0.03^{bc}	0.54 ± 0.03^{bc}	0.62 ± 0.03^{c}	0.76 ± 0.04^{c}
	Price	0.38 ± 0.02^{a}	0.38 ± 0.02^{ab}	0.45 ± 0.02^{bc}	0.45 ± 0.02^{bc}	0.48 ± 0.02^{c}	0.52 ± 0.03^{c}
Saponification value (mg KOH/g of oil)	Misson	185.0 ± 4.5^{a}	190.0 ± 3.5^{ab}	194.0 ± 3.7^{abc}	197.0 ± 2.9^{bc}	201.0 ± 4.0^{bc}	204.0 ± 3.5^{c}
	Price	187.0 ± 3.5^{a}	188.7 ± 4.4^{ab}	190.0 ± 3.5^{abc}	208.0 ± 4.5^{bc}	219.5 ± 2.9^{bc}	231.0 ± 4.0^{c}
Free fatty acids (% as oleic acid)	Misson	1.11 ± 0.06^{a}	1.17 ± 0.06^{a}	1.28 ± 0.06^{ab}	1.28 ± 0.10^{bc}	1.34 ± 0.07^{cd}	1.34 ± 0.07^{d}
	Price	1.61 ± 0.08^{a}	1.64 ± 0.08^{a}	1.67 ± 0.08^{ab}	1.78 ± 0.09^{bc}	1.84 ± 0.09^{cd}	1.90 ± 0.01^{d}

Mean ± SD calculated from three replicates.
The means with different superscript letters within the same row vary significantly ($P < 0.05$) among radiation doses.
Control (non-irradiated sample).

2006). Similarly, in the case of unsaponifiable matter, an increasing trend was observed with gamma radiation absorbed doses. These results are in accordance with Yaqoob *et al.,* (2010) who reported an increase in unsaponifiable matter in sunflower and maize oil extracted form gamma irradiated seeds. In our experiments, the highest unsaponifiable matter (0.52-0.76%) was observed for the samples irradiated to 10 kGy, which might be due to the existence of high contents of hydrocarbons, sterols and triterpenols at this stage (Uquiche *et al.,* 2008). The increase in free fatty acids from 1.11-1.61% (for control oils) to 1.34-1.90% for oils extracted from γ-irradiated almond seeds, to a final dose of 10 kGy, might be due to slight and random hydrolysis of triglycerol molecules to free fatty acids and diacylglycerols (Al-Bachir, 2004; Boonchoo *et al.,* 2005; Anjum *et al.,* 2006; Badr, 2006).

The results regarding oxidative status of the oils produced from γ-irradiated and control almond seeds of both varieties are shown in Table 3. Irradiation significantly increased (an increase from 2.26-3.46 to 5.18-6.21 meq O_2 kg^{-1} oil) the peroxide value (PV) of the oils tested which might be attributed to the excessive formation of hydroperoxide as a result of oxidation, dehydration and polymerization reactions due to the interaction of γ radiation with fat molecules (Evren and Gulden, 2008). Our results are in accordance with Badr, (2006) and Yusof *et al.,* (2007) who also observed an increase in the peroxide values in gamma irradiated egg yolks and coconut oil samples. However, few previous reports are available where no significant increase upon irradiation was observed in the PV of different lipids (Hampson *et al.,* 1996, Al-Bachir, 2004; Bhatti *et al.,* 2010) which might be linked to the breakdown of primary oxidation products including hydroperoxide into smaller stable fragments such as carbonyl compounds, alcohols and hydrocarbons. Peroxide value characterizes the quantity of peroxides formed in the oils as intermediates of oxidative reactions after irradiation (Uquiche *et al.,* 2008). Peroxide value characterizes the quantity of peroxides formed in the oils as intermediates of oxidative reactions after irradiation (Uquiche *et al.,* 2008). The effect of γ irradiation (up to 10 kGy) on the *para*-anisidine value (an increase from 20.10 to 23.50) in this study was found to be significant in accordance with the findings of Yaqoob *et al.,* (2010). The extinction coefficients corresponding to λ_{max} 232 nm and λ_{max} 268 are related to the conjugated diene and trienes, respectively. The magnitude of these oxidation products is reflected as purity index and depicts the oxidative degradation of oil. These values were affected slightly at low radiation doses but increased rapidly at doses higher than 6 kGy. The oxidative stability of oils predicts their resistance to the formation of conjugated dienes, trienes and peroxides. Conjugated diene and triene values correspond to bond shifting as a result of oxidation (Deiana *et al.,* 2002). The increase in the conjugated diene and triene contents for both varieties of almond seed oils observed in the present investigation might be linked to the lipid oxidation caused by irradiation (Bhatti *et al.,* 2010).

Table 4 depicts the values of tocopherol homologues of the oils extracted from unirradiated

Table 3
Effect of gamma irradiation on oxidative stability of oils extracted from almond seeds of different varieties

Contents	Variety	Radiation dose					
		Control	2 kGy	4 kGy	6 kGy	8 kGy	10 kGy
$\varepsilon_{1cm}^{1\%}$(λ 232)	Misson	1.91 ± 0.10^{a}	2.09 ± 0.10^{ab}	2.28 ± 0.11^{bc}	2.97 ± 0.15^{cd}	3.45 ± 0.17^{de}	3.93 ± 0.20^{e}
	Price	1.71 ± 0.09^{a}	2.14 ± 0.11^{ab}	2.53 ± 0.13^{bc}	2.80 ± 0.14^{cd}	3.07 ± 0.15^{de}	3.34 ± 0.17^{e}
$\varepsilon_{1cm}^{1\%}$(λ 268)	Misson	0.22 ± 0.01^{a}	0.36 ± 0.03^{ab}	0.49 ± 0.02^{b}	0.68 ± 0.03^{c}	0.73 ± 0.04^{d}	0.78 ± 0.04^{e}
	Price	0.21 ± 0.01^{a}	0.31 ± 0.03^{ab}	0.45 ± 0.02^{b}	0.51 ± 0.03^{c}	0.61 ± 0.03^{d}	0.77 ± 0.04^{e}
PV(meqO_2 kg^{-1} of oil)	Misson	3.46 ± 0.17^{a}	3.75 ± 0.19^{b}	4.03 ± 0.20^{c}	4.37 ± 0.22^{d}	5.29 ± 0.26^{e}	6.21 ± 0.31^{f}
	Price	2.26 ± 0.15^{a}	2.63 ± 0.17^{b}	3.00 ± 0.20^{c}	3.34 ± 0.22^{d}	4.26 ± 0.30^{e}	5.18 ± 0.40^{f}
*P*ara-anisidine Value	Misson	20.30 ± 1.00^{a}	20.75 ± 1.04^{a}	21.20 ± 0.99^{b}	21.70 ± 1.09^{b}	22.60 ± 1.30^{c}	23.50 ± 1.18^{d}
	Price	20.10 ± 1.10^{a}	20.55 ± 1.03^{a}	21.00 ± 1.00^{b}	21.50 ± 1.28^{b}	22.40 ± 1.12^{c}	23.30 ± 1.15^{d}

Mean ± SD calculated from three replicates.
The means with different superscript letters within the same row vary significantly ($P < 0.05$) among radiation doses.
Control (non-irradiated sample).

Table 4
Effect of gamma irradiation on tocopherols content of oils extracted from almond seeds of different varieties

Tocopherols (mg/Kg)	Variety	Radiation doses					
		Control	2 kGy	4 kGy	6 kGy	8 kGy	10 kGy
α-Tocopherol	Mission	436.0 ± 13.1^{a}	410.0 ± 12.3^{ab}	390.0 ± 11.7^{ab}	375.0 ± 11.3^{ab}	340.4 ± 10.2^{ab}	425.0 ± 12.8^{b}
	Price	485.0 ± 14.6^{a}	452.0 ± 13.2^{ab}	439.0 ± 13.2^{ab}	416.0 ± 12.5^{ab}	405.0 ± 12.2^{ab}	385.0 ± 11.6^{b}
γ-Tocopherol	Mission	8.8 ± 0.3^{a}	7.8 ± 0.2^{b}	6.1 ± 0.2^{c}	6.0 ± 0.2^{c}	4.1 ± 0.1^{d}	3.0 ± 0.1^{e}
	Price	9.0 ± 0.3^{a}	7.4 ± 0.2^{b}	6.3 ± 0.2^{c}	5.3 ± 0.2^{c}	4.1 ± 0.1^{d}	2.9 ± 0.1^{e}
δ-Tocopherol	Mission	2.3 ± 0.1^{a}	2.2 ± 0.1^{b}	2.1 ± 0.1^{bc}	1.9 ± 0.1^{c}	1.6 ± 0.1^{d}	1.3 ± 0.1^{e}
	Price	2.8 ± 0.1^{a}	2.4 ± 0.1^{b}	2.3 ± 0.1^{bc}	2.1 ± 0.1^{c}	1.9 ± 0.1^{d}	1.5 ± 0.1^{e}
Total Tocopherol	Mission	447.1 ± 13.4^{a}	420.0 ± 12.6^{b}	398.2 ± 11.9^{b}	382.9 ± 11.5^{c}	346.0 ± 10.4^{d}	429.3 ± 12.9^{e}
	Price	496.8 ± 14.9^{a}	461.8 ± 13.9^{b}	447.5 ± 13.4^{b}	423.3 ± 12.7^{c}	411.0 ± 12.3^{d}	389.4 ± 11.7^{e}

Mean ± SD calculated from three replicates.
The means with different superscript letters within the same row vary significantly (P<0.05) among radiation doses.
Control (non-irradiated sample).

and γ-irradiated almond seeds to the absorbed doses of 2-10 kGy. There was a noticeable difference in tocopherol contents (α, γ and δ) of the oil samples derived from irradiated verses non-irradiated (control) seeds. The values of α-tocopherol in unirradiated almond oils were predominant (436 mg kg^{-1} and 485 mg kg^{-1}) in Mission and Price variety, respectively). Alpha-tocopherol contents were higher in the case of the non-irradiated Price verity as compared to Mission. Meanwhile, γ- and δ-tocopherols were detected in small amounts with contributions of 8.81-8.95 mg kg^{-1} and 2.32-2.80 mg kg^{-1}, respectively in both the varieties of almond oils. Overall, the tocopherol values were decreased by increasing the irradiation dose and the trend was in accordance with the studies of Bhatti et al. (2010) and Yaqoob *et al.,* (2010) who studied a decreasing trend in the amounts of total tocopherols of irradiated sunflower, maize and peanut oil up to absorbed doses of 10 kGy. The decreasing trend in the tocopherol contents of irradiated oil samples might be linked to the degradation of these antioxidant componenets during irradiation. Furthermore, due to the thermal oxidation of the oil, the tocopherol values may also decrease (Yaqoob *et al.,* 2010). The effect of gamma radiations on the tocopherol contents of the oils from both almond varieties was similar. Previous studies conducted by Lalas *et al.,* (2007), Lakritz and Thayer (1994), and Lakritz *et al.,* (1995) showed that there was a slight decrease in tocopherol contents of seed oils, fresh chicken breast muscle, and red meat, respectively with an increase in gamma radiation doses. While another study on cooked minced chicken showed that there was no considerable

effect of irradiation on the tocopherol contents up to 4 kGy (Galvin *et al.,* 1998). Furthermore, the concentration of tocopherols depends on genotype, cultivar traits as well as agroclimatic conditions of the harvest (Kodad *et al.,* 2011). It is known that α-tocopherol along with tocotrienols (Vitamin E) are important liposoluble metabolites and due to their strong anti-oxidative effects, they retard the oxidation of unsaturated fatty acids in foods and biological systems. The most active form of vitamin E in *vivo* is α-tocopherol, while γ-tocopherol is an active form of vitamin E in *vitro* (Gimeno *et al.,* 2000; Uquiche *et al.,* 2010). Suhaj *et al.,* (2006) reported that antioxidant activity was influenced by radiation treatment which can be attributed to the degradation and peroxidation of unsaturated fatty acid in the oils (Hassanein *et al.,* 2003). Lalas *et al.,* (2007) also reported a decrease in the total tocopherol contents of sunflower and soybean oils after irradiation at higher doses, while no significant change for tocopherol concentration was observed at lower doses. Similar results have been reported earlier in case of irradiated peanut, sunflower and maize seeds oils (Bhatti *et al.,* 2010; Yaqoob *et al.,* 2010). The contents of α-, γ- and δ-tocopherols were slightly affected in the oils extracted from peanut, sunflower and maize seeds irradiated up to 6 kGy, however, the effect was comparatively pronounced at higher dosages.

Radiation-induced changes in the fatty acid profile for Mission and Price varieties of almond seeds are shown in Table 5. The effect of γ radiation (dose 2-10 kGy) on fatty acid composition was found to be almost insignificant for palmitic acid (16:0), the content of stearic acid (18:0) and oleic acid (18:1) increased, while the concentration of linoleic acid (18:2) decreased significantly ($p < 0.05$) by increasing the absorbed dose. The increasing trend for stearic acid (an increase from 2.18-2.37 to 2.88-3.00%) and oleic acid (an increase from 69.51-70.00 to 70.25-71.5%) and decreasing trend of linoleic acid (a decrease from 22.0-22.10 to 20.05-20.90%) might be due to the preferential cleavage of double bonds. Radiation treatment caused the saturation of double bonds of linoleic acid which increased with the absorbed dose. Our findings are in agreement with Yaqoob *et al.,* (2010) who reported that the effects of irradiation on the fatty acid composition of sunflower oil showed a significant ($p < 0.05$) change in the amounts of stearic, oleic and linoleic acids, while the concentration of palmitic acid was unaffected even at 10 kGy.

The effect of gamma irradiation on the microbial inactivation of oils extracted from irradiated and unirradiated seeds of both varieties of almond are shown in Table 6. The bacterial populations in non-irradiated oil samples were 4.30×10^3 CFU/g and 3.87×10^3 CFU/g while the fungal spores 3.87×10^2 /mL and 3.87×10^2 mL^{-1} for Mission and Price varieties, respectively. After radiation treatment of 2 kGy the bacterial load was reduced to 3.91×10^2 CFU g^{-1} for Mission variety and 3.21×10^2 CFU/g (Price) and the fungal count levels were 3.45×10^1 spores mL^{-1} (Mission) and 2.84×10^1 spores mL^{-1} for Price variety. A significant reduction of microbes was observed at an absorbed dose of 4 kGy whereas at 6 kGy irradiation increasingly hampered the microbial growth and no population wentundetected. Similar results as observed in the present investigation were reported by Thomas *et al.* (2008), who studied colony formation in black tea irradiated up to 10 kGy absorbed dose. Likewise, Alighourchi *et al.,* (2008) reported a progressive decrease in the microbial load of pomegranate juice irradiated to 0.5-10 kGy.

4. CONCLUSIONS

The results of this study showed that gamma irradiation up to an absorbed dose of 6 kGy did

Table 5
Effect of gamma irradiation on the fatty acidS profile (g/100 g FA) of oils extracted from almond seeds of different varieties

Contents (%)	Variety	Radiation doses					
		Control	2 kGy	4 kGy	6 kGy	8 kGy	10 kGy
Palmitic acid	Mission	6.10 ± 0.24^{a}	6.13 ± 0.25^{a}	6.14 ± 0.25^{a}	6.14 ± 0.25^{a}	6.15 ± 0.25^{a}	6.16 ± 0.25^{a}
	Price	6.17 ± 0.25^{a}	6.21 ± 0.25^{a}	6.21 ± 0.25^{a}	6.27 ± 0.25^{a}	6.35 ± 0.25^{a}	6.37 ± 0.29^{a}
Stearic acid	Mission	2.18 ± 0.09^{a}	2.18 ± 0.09^{b}	2.12 ± 0.08^{b}	2.55 ± 0.10^{c}	2.60 ± 0.10^{c}	2.88 ± 0.12^{c}
	Price	2.37 ± 0.09^{a}	2.45 ± 0.10^{b}	2.61 ± 0.10^{b}	2.71 ± 0.11^{c}	2.79 ± 0.11^{c}	3.00 ± 0.12^{c}
Oleic acid	Mission	69.51 ± 2.78^{a}	69.7 ± 2.79^{ab}	69.89 ± 2.80^{bc}	69.9 ± 2.88^{c}	70.10 ± 2.80^{cd}	70.25 ± 2.81^{d}
	Price	70.00 ± 2.83^{a}	70.68 ± 2.83^{ab}	70.77 ± 2.83^{bc}	70.95 ± 2.84^{c}	71.20 ± 2.85^{cd}	71.5 ± 2.86^{d}
Linolieic acid	Mission	22.10 ± 0.88^{a}	21.80 ± 0.88^{a}	21.60 ± 0.86^{a}	21.40 ± 0.86^{a}	21.00 ± 0.84^{a}	20.90 ± 0.84^{a}
	Price	22.00 ± 0.90^{a}	22.10 ± 0.88^{a}	21.85 ± 0.87^{a}	21.40 ± 0.86^{a}	20.00 ± 0.83^{a}	20.05 ± 0.80^{a}

Mean ± SD calculated from three replicates.
The means with different superscript letters within the same row vary significantly ($P < 0.05$) among radiation doses.
Control (non-irradiated sample).

Table 6
Bacterial (CFU/g) and fungal (spores/mL) count of oils extracted from almond seeds of different varieties

Dose (kGy)	Total bacterial count (CFU/g)		Total fungal count (spores/mL)	
	Mission	Price	Mission	Price
Control	4.30×10^3	3.87×10^3	3.87×10^2	3.87×10^2
2	3.91×10^2	3.21×10^2	3.45×10^1	2.84×10^1
4	2.41×10^1	2.84×10^1	< 10	< 10
6	ND	ND	ND	ND
8	ND	ND	ND	ND
10	ND	ND	ND	ND

Mean calculated from three replicates, ND (not detected).
Control (non-irradiated sample).
CFU: Colony-forming unit.

not significantly alter the routine physiochemical characteristics of the almond (Mission and Price verity) oils, while the microbial load was nullified completely at this treatment level. The fatty acid and tocopherol profiles as well as the oxidation status of the irradiated oils were negatively affected especially at higher irradiation doses (> 6 kGy). It can be concluded that higher gamma irradiation doses might lead to the deterioration of some valuable components such as tocopherols and essential fatty acids in the oils. In order to preserve the almond seed oil from disinfection as well as from some other quality-oriented deteriorative effects, an appropriate gamma irradiation treatment should be sought.

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