INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 10, p. 97-103, 2015

RESEARCH PAPER OPEN ACCESS

Quality characteristics and microbial safety evaluation of gamma irradiated almonds (*Prunus dulcis* L.)

Faiza Saleem[1], Rukhama Haq[1], Neelma Munir[1*], Shagufta Naz[1], Mehral Aslam[1], Rabia Aslam[1], Imran Altaf[2]

[1]*Lahore College for Women University, Jail Road, 011464, Lahore, Pakistan*

[2]*QOL, WTO Laboratories, University of Veterinary and Animal Sciences, Lahore, Pakistan*

Key words: *Prunus dulcis* L., Gamma irradiation, PARAS (Pakistan atomic radiation services), Micro flora, Nutritional value.

http://dx.doi.org/10.12692/ijb/6.10.97-103 Article published on May 30, 2015

Abstract

Almond (*Prunus dulcis* L.) is one of the important stone fruit grown in Pakistan and is a tremendous source of magnesium, copper, phosphorus, fiber, riboflavin, monounsaturated fatty acids and protein. Efficacy of gamma irradiation on Pakistani almonds was studied during the present work. The samples were treated with four different doses (1, 3, 5 and 7kGy) at radiation services of PARAS. Screening and evaluation of native micro flora on almonds was performed and the viable counts of *Salmonella* was determined before and after gamma treatment. Furthermore, nutritional composition of irradiated and non-irradiated almonds was also compared by their ash, moisture, fat, fiber, protein and carbohydrate content. Results showed that 3 kGy is the optimum dose for almonds at which complete elimination of *Salmonella* was recorded with no significant effect on nutritional value.

* **Corresponding Author:** Neelma Munir ✉ neelma.munir@yahoo.com

Introduction

Dry fruits are very important and the most valued food items used as light meals, snack foods or as sweets. Due to the importance of nuts, their quality control must be strictly observed for successful marketing(Yahia, 2009). Almonds (*Prunusdulcis*L.*)*, local name "badam" are edible tree nuts, belonging to the family *Rosacea* and is very delicious dry fruit with high nutritional value because of its oil contents (Ali, 2012).Almonds are highly rich in their nutritional value as they contain manganese 45 %, copper 20 %, vitamin E 44.8 %, vitamin b2 17.6 %, magnesium 24.6 %, phosphorus 16.8 %, tryptophan 21.8 % and calories 11 % (Chen, 2006) and are declared as an excellent source of vitamin E and manganese by US Food and Drug Administration (Chen, 2006). Results from microbiological and chemical studies show the mould*Aspergillusflavus*to be greater than the bacteria *Salmonella enteritidis* on stored nuts particularly on low moisture foods like almonds (Scott, 2009). Almond along with other nuts are less likely to be spoiled by bacteria due to extremely high fats and protein content and low level of water content. But moulds can grow depending upon storage conditions (FAO, 2011). Gamma rays are also used for irradiation processing of food are radioactive fission products of ^{60}Co and ^{137}Cesium. A major problem of raw almonds is cross contamination of *Salmonella entericas*erovar*enteritidis*, which probably occurs prior to processing. *Salmonella enteritidis* is an important food borne pathogen that normally causes diarrhea, fever, nausea, or vomiting. Investigations confirm whole natural almonds as the source of contamination by an unusual strain, *S. enteritidis* PT 30, which occurs when almonds fall to the ground during harvest. Starting from 2007, therefore, the US Department of Agriculture (USDA) authorized pasteurization of raw almonds prior to export. Several possible treatment methods are under progress for almonds; including propylene oxide fumigation, high pressure, steam and infrared heating. These methods might reduce *S. enteritidis* population on almond surfaces (Gao *et al.* 2011). In case of almonds to check the effect of gamma rays on their nutrient composition and to remove and control the growth of microbes on them they are being irradiated at different doses which range from 36 µC (Micro cowry) from ^{60}Co and radiated at different doses to observe the results (Mexis, 2009). Proximate Analysis is a partitioning of compounds in a feed into six categories based on the chemical properties of the compounds which include moisture content, ash content, crude protein (Kjeldahl protein), crude lipid, crude fiber and nitrogen-free extracts (digestible carbohydrates). The proximate composition of almonds is that they consists of the protein content ranging up to 36%, dietary fiber 12% to 36%, crude fats up to 66%, and carbohydrates up to 6% (Anonymous, 2011). Food irradiation is the use of ionizing radiation to enhance food storage life, lessen post-harvest food losses and abolish food poisoning microorganisms. The efficiency of ionizing radiation, its penetrating power and its straight forward kinetics make it much simpler in practice, to use than heat. Moreover, irradiation fulfills other criteria for CCP (critical control point) i.e., critical limits (minimum and maximum doses) can be developed or monitored, and process control is well known. Corrective actions can also be taken when required. Food sterilization by gamma can destroy microorganisms, bacteria, viruses, or insects that might be present in the food. Irradiated food does not become radioactive, but in some cases there may be subtle chemical changes. Food irradiation is currently permitted by over 50 countries, and the volume of food treated is estimated to exceed 500,000 metric tons annually worldwide (Aquino, 2012). In general, irradiated food accordingly to the Codex Alimentary Commission regulation, the International Atomic Energy Agency, the WHO and FAO regulation for food irradiation treatment is wholesome. Hence the present study was focused to optimize such a dose for almonds which is safe for the consumption without harming the nutrient content and minimizing the microbial load particularly *S. enteritidis*.

Materials and methods

Sample collection and gamma irradiation

Unshelled almonds were collected from the local market of Lahore. They were apparently of good

quality and without any physical injury. Almonds were then packed in polythene bags and carried to the radiation unit of PARAS (Lahore) for irradiation. The doses administered were 1kGy, 3kGy, 5kGy and 7kGy. During the present work, Harwell Amber 3042 dosimeter was used for dose measurement. The measurement uncertainty was 3% at 95% confidence level.The dose uniformity ratio for irradiated sample of almonds during the present work was 1.6 which was achieved by multi-sided irradiation. Un-irradiated control was kept under identical conditions for comparison. Both control and irradiated almonds were stored at ambient temperature (30-37°C). Period of storage was a few weeks.

Proximate analysis

Almonds were being analyzed to find out moisture content, ash, fat, protein and carbohydrates. Official methods of AOAC manual (2005) were used for proximate analysis of sample, which are listed in table 1.

Microbial analysis

Irradiated almonds were analyzed weekly to determine the microbial load. Four growth media were used for the enumeration and identification of bacteria and fungi associated with almonds. Nutrient agar (for bacterial isolation), MacConkey agar (for Gram-negative enteric bacilli isolation), Potato dextrose agar (for fungi isolation) and *Salmonella-Shigella* Agar (for *Salmonella* spp. and *Shigella*spp. isolation), were used. Isolation of micro flora was carried out by suspending 1 almond kernel in 9ml of sterilized distilled water. 100 µl of aliquots were transferred in petri plates containing sterilized nutrient agar media. Plates were incubated at 37°C for 24 hours. The crowing or excessive stacking of plates was avoided to permit rapid equilibration of plates with incubator temperature. The colonies were counted promptly after incubation period. The average colony count (arithmetic mean) of all replicates was calculated. Viable bacterial count is determined by standard formula of Colony Forming Unit per ml (CFU/ml) (Gent and Schwartz 2005).

$$CFU/ml = \frac{\text{Colony count on plate}}{\text{total dilution of tube(used to make plate)} \times \text{amount plated}}$$

Identification tests were performed to determine characterizing aspects of bacteria. Bacterial species were identified by referring to Berge's Manual of Determinative Bacteriology (Krieg *et al*., 1994). After obtaining pure culture bacterial isolates were subjected to gram staining. For further identification of Gram negative bacterial isolates, the API 20 E method was used, and the tested isolates identified using 20 E analytical profile index. Molds were identified from the macroscopic morphology of the colony grown in PDA plates.

Statistical analysis

All the experiments were arranged in a completely randomized design and data was analyzed using the Costat software (version 6.3) data (mean + SD) and was collected from experiments with five replicates based on Duncan's new multiple range test (Steel, 1997).

Results and discussion

Proximate analysis

The proximate analysis (Moisture content, Ash content, Fiber content, Fat content, Protein content and carbohydrates) of non-irradiated and irradiated almonds is given in detail with values is given in the table 1. The radiation dose from 1 kGy to 7kGy did not show any significant effect on the proximate components of almonds. The moisture content of non-irradiated and irradiated almonds ranges from 7.49-7.25 g $100g^{-1}$ respectively. A variation in the ash content of non-irradiated and irradiated almonds ranging from 3.31-4.00 g $100g^{-1}$ were recorded in the samples but this change was statistically non-significant. The fiber content of non-irradiated and irradiated almonds ranges from 16.86-17.20 g $100g^{-1}$ respectively. The obtained mean of fat content of non-irradiated and irradiated almonds at various dises ranges from 66.99-66.28 g $100g^{-1}$ are also depicted in table 1 indicating no statistical differences. The protein content of non-irradiated and irradiated almonds ranges from 2.75-2.76 g $100g^{-1}$ respectively. The carbohydrate content of non-irradiated and

irradiated almonds ranges from 2.60-2.51 g $100g^{-1}$ respectively. The results of this study related to proximate analysis is steady with the previous literature which conclude that there is no significant difference in all the proximate components of irradiated and non-irradiated almonds and was previously reported by (Kartiz , 2005, Mexis, 2008). Similarly, Abbey*et al.* (2008) also reported that the protein and crude fiber contents of almonds did not change after irradiation.

Table 1. Weekly analysis on the effecton colour of almonds before and after radiation.

Radiation Doses (kGy)	Color change		
	Week 1	Week 2	Week 3
Control	Light Brown	Light Brown	Light Brown
3.0	Shiny golden Brown	Shiny golden Brown	Shiny golden Brown
5.0	Shiny golden Brown	Shiny golden Brown	Shiny golden Brown
7.0	Dark Brown	Dark Brown	Dark Brown

Microbial analysis

The fungal colonies gradually decrease with increasing gamma radiation doses (Fig. 1). The control samples exhibited the maximum fungi with a total viable fungal count of 2×10^3 cfu/g on the 1^{stday} of storage which increased with further storage and was found 5.6×10^3 cfu/g after 30 days.

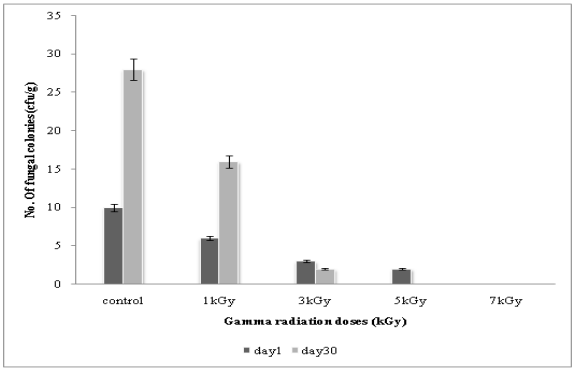

Fig. 1. Impact of different gamma radiation doses on total viable fungal count of almonds using PDA as testing medium. Each value is the mean of five parallel replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$. Incubation period 72 hours, temperature of incubation 30°C.

The most effective results were noted for the highest dose of 7 kGy. The initial total viable fungal counts of 1.2×10^3cfu/g, 0.6×10^3cfu/g and 0 cfu/g were obtained for the doses of 1 kGy, 3 kGy and 5 -7kGy respectively. The count increased to 16.32×10^3cfu/g for dose 1kGy and decreased to 0.4×10^3cfu/g for dose 3kGy till the end of the month.The macroscopic and microscopic analysis of fungal growth showed the species identified to be *Aspergillusniger, Aspergillusflavus Fusariumoxysporum*and also some *yeast* species *Saccharomyces cerevisiae*.Considerable difference of total viable bacterial count was observed between controls and irradiated samples on nutrient agar (Fig. 2). Radiation dose of 7 kGy greatly reduced bacterial count to 0.8×10^3 cfu/g as compared to bacterial count on control samples 4.7×10^3 cfu/g during first week of storage.

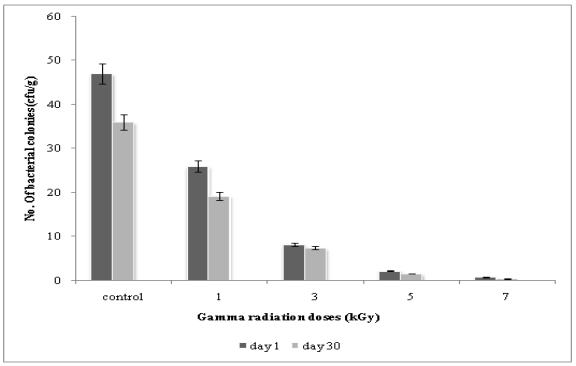

Fig. 2. Impact of different gamma radiation doses on total viable bacterial count of almonds using Nutrient agar as testing medium. Each value is the mean of five parallel replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$. Incubation period 24 hours, temperature of incubation 37°C.

The bacterial colony count was 26×10^3 cfu/g, 8.2×10^3 cfu/g and 2.2×10^3 cfu/g on 1st day on other three doses 1kGy, 3 kGy and 5 kGy, respectively which was also less than those of controls. Again, the highest dose 7 kGy reduced the total viable bacterial count (0.4×10^3 cfu/g) as compared to the lower doses 1 kGy

(19.2 × 10^3 cfu/g), 3kGy (7.4× 10^3 cfu/g) and 5 kGy (0.4 × 10^3 cfu/g) and controls (36 × 10^3 cfu/g) after 30 days. The colonies observed were circular/filamentous having convex elevations and smooth or undulate margins. Most of the bacteria were gram positive rods. The inhibitory effect of gamma irradiation on the bacterial growth on almond surface was also observed on MacConkey agar (Fig. 3).

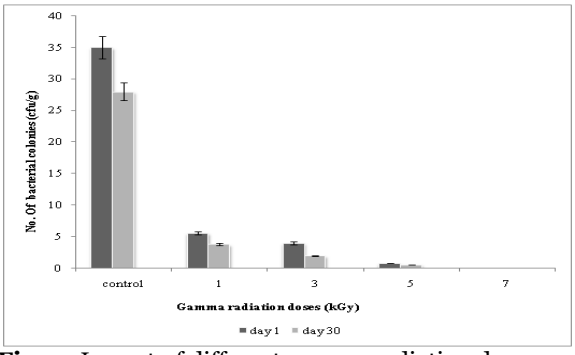

Fig. 3. Impact of different gamma radiation doses on total viable gram negative bacterial count of almonds using MacConkey Agar as testing medium. Each value is the mean of five parallel replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$. Incubation period 24 hours, temperature of incubation 37°C.

The control group showed bacterial count of 35.0 × 10^3cfu/g which increased progressively with storage time till the end of the month (28 × 10^3cfu/g). Again the irradiated group of onion showed reduced total viable bacterial count than the control one and this effect was more evident with the increase in the applied doses of gamma radiation. The almond samples irradiated at 1 kGy, 3kGy and 5kGy exhibited a reduction level with total viable bacterial count of 5.6 × 10^3cfu/g, 4 × 10^3cfu/g and 0.8 × 10^3cfu/g on the 30th day of storage. The total viable bacterial count was completely absent at 7 kGy during whole period of storage. The inhibitory effect of gamma irradiation on the bacterial growth on almond surface was also observed on blood agar medium (Fig. 4). Non-irradiated samples show maximum of 36 × 10^3cfu/g. the growth of hemolytic bacteria was observed to reduce at comparatively lower doses of 1kGy, 3kGy (1.8 × 10^3cfu/g and 1 × 10^3cfu/g). At higher doses of 5kGy and 7kGy no hemolytic bacterial growth.

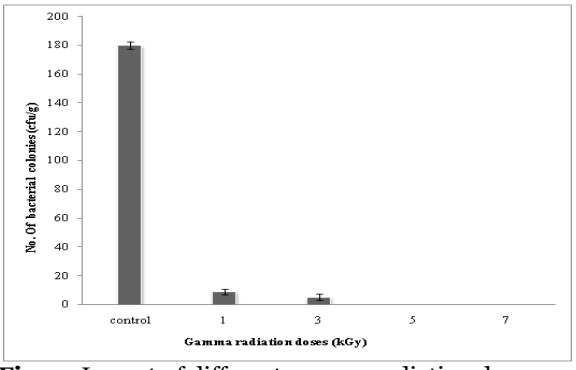

Fig. 4. Impact of different gamma radiation doses on haemolytic bacterial count of almonds using Blood agar as testing medium. Each value is the mean of five parallel replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$. Incubation period 24 hours, temperature of incubation 37°C.

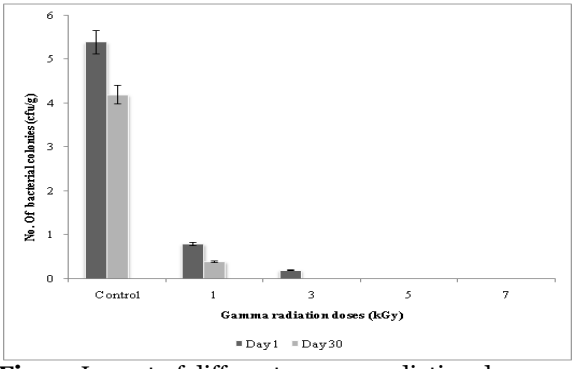

Fig. 5. Impact of different gamma radiation doses on total viable enteric bacterial count of almonds using SS agar as testing medium. Each value is the mean of five parallel replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$. Incubation period 24 hours, temperature of incubation 37°C.

The total viable bacterial count of the irradiated and control group of almond was observed on *Salmonella Shigella*Agar. The colony count of controls on 1st day was 5.4 × 10^3cfu/g whereas samples radiated at the doses of 1 kGy, and 3 kGy showed microbial load of 0.8 × 10^3 and 0.2× 10^3cfu/g, respectively. The highest doses of 5 kGy and 7kGy completely inhibited the growth of bacteria and no colonies were found on growth medium (Fig. 3). The bacterial colony count increased after every week, however, substantial

difference was observed between control and irradiated samples. The growth of *salmonella* was observed on *Salmonella Shigella*agar medium at control but not on the first dose 1kGy.

Colonies of *Escherichia coli* and *Salmonella* species were identified by using API 20 E strips. These colonies were found on *Salmonella Shigella*Agar on non-irradiated samples only.

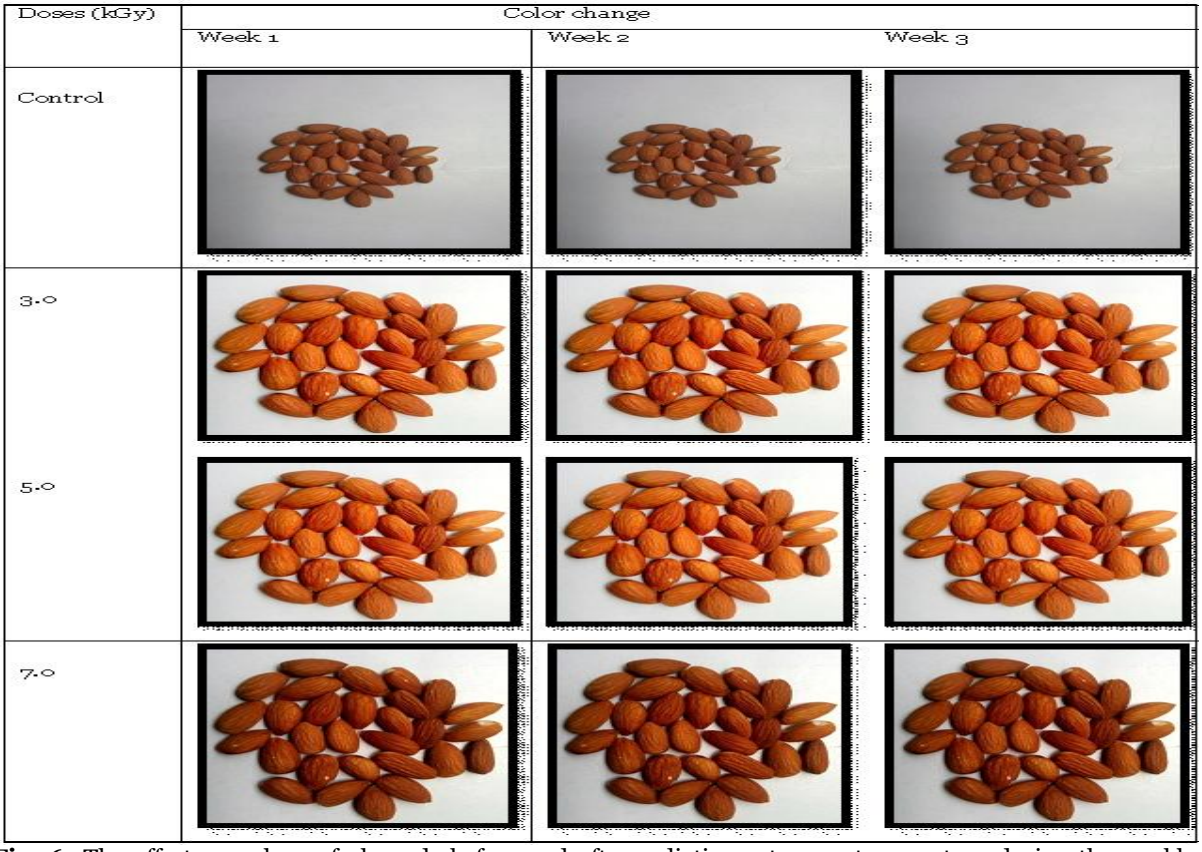

Fig. 6. The effect on colour of almonds before and after radiations at room temperature during the weekly evaluation.

Similar results were reported by Thomas *etal.* (2008), who studied colony formation in black tea irradiated up to 10 kGy absorbed dose. Similarly,Alighourchi*et al.* (2013) reported a progressive reduce in the microbial load of pomegranate juice irradiated to 0.5-10 kGy.

Conclusion

The results of this study showed that gamma irradiation up to an absorbed dose of 3kGy did not significantly alter the nutritional components of the almonds whereas the microbial load was nullified completely at this treatment level. Hence it can be concluded that gamma radiation works well for enhancing shelf life by reducing the microbial load. So in order to preserve the almonds from disinfection as well as from some other quality oriented deteriorative effects, an appropriate gamma irradiation treatment should be given to the samples before introducing them in the market.

Acknowledgement

The authors are obliged to Mr. Shoaib of Pakistan Radiation Services (PARAS), Lahore, Pakistan, for providing the ^{60}Co, gamma irradiator, facility.

References

Abbey M, Noakes M, Belling GB, Nestel PJ. 1994. Partial replacement of saturated fatty acids with almonds or walnuts lowers total plasma cholesterol and low-density-lipoprotein cholesterol. The American journal of clinical nutrition**59 (5),** 995-

999.

Ali E, Anjum FS, Khan BA, Zubair A. 2009.Aflatoxin contamination and mineral profile of almond seeds.Mycopath**7(1), 39-44**.

Alighourchi HR, Barzegar M, Sahari MA, AbbasiS. 2013. Effect of sonication on anthocyanins, total phenolic content, and antioxidant capacity of pomegranate juices.International Food Research Journal **20(4),** 1703-1709.

Anonymous. 2013/ Webmaster@nutsforalmonds Nuts and Farms.

AOAC. 2005. Official Methods of Analysis of the Association of Analytical Chemists. Eighteenth Ed. AOAC, Washington.

Aquino DS. 2012. Sterilization by gamma irradiation. INTECH Open Access Publisher. Croatia: InTech Europe.

Chen CY, Lapsley K, Blumberg J. 2006. A nutrition and health perspective on almonds. Journal of the Science of Food and Agriculture**86,** 2245-2250..

FAO. 2011. a. Forests for improved nutrition and food security. Rome. www.fao.org/forestry/2797602c09ef000fa99932eefa37c22f76a055.pdf.

Gao M, Tang J, Rojas RV, Wang Y, Wang S. 2011.Pasteurization process development for controlling Salmonella in in-shell almonds using radio frequency energy. Journal of Food Engineering **104(2),** 299-306.

Yahia EM. 2009. Dried fruits and tree nuts. Modified and controlled atmospheres for the storage, transportation and packaging of horticultural commodities. CRC press, 507-526.

KatzerK,Khetarpaul N, Bishnoi S. 2005. HCl-extractability of minerals from almonds: Influence of domestic processing methods. Innovative Food Science Emerging Technology **2(4),** 323-325.

Mexis S, Badeka A, Chouliara E, Riganakos K, Kontominas M. 2009. Effect of γ-irradiation on the physicochemical and sensory properties of raw unpeeled almond kernels . Innovative Food Science & Emerging Technologies **10,** 87-92.

Scott VN, Chen Y,Freier TA, Kuehm J, Moorman M, **Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J.** 2009.Control of Salmonella in low-moisture foods I: Minimizing entry of Salmonella into a processing facility. Food Protection Trends **29(6),** 342-353.

Steel R, Torrie J, Dickey D. 1997. Principles and procedures of statistics: A biometrical approach. WCB, ed.:McGraw-Hill, New York.

Thomas J, Senthilkumar RS, Kumar R, Mandal AKA, Muraleedharan N. 2008. Induction of gamma irradiation for decontaminationand to increase the storage stability of black teas. Food Chemistry **106,** 180–184.