Evaluation of Gamma Irradiation and Storage Period Effects on Polycyclic Aromatic Hydrocarbons Load in Fava Bean (Vicia Faba) Kernels

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Authors' contributions

Article Information

Short Research Article

ABSTRACT

In this work, gamma irradiation at doses of 1, 5, 10 and 15 kGy and storage period effects on polycyclic aromatic hydrocarbons (PAHs) contents of fava bean kernels (FBK) were investigated. PAHs were extracted from FBK (crop year 2017/2018) immediately post-harvest (Mars to late May 2017), and after six months of storage (12/05/2017 to 10/12/2017) and the PAHs, the concentration was determined at each dose using GC-MS analysis. Results demonstrated that the PAHs load in irradiated FBK was dramatically decreased as the applied dose increased. Interestingly, the decrease in the PAHs load six months post storage was less important compared with the post-harvest decrease. Moreover, the decrease in PAHs in kernels was in different trends towards irradiation used doses. Results suggest that a dose of ~ 20 kGy or higher is mandatory for preeminent hygiene of FBK from PAHs load during storage.

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1. INTRODUCTION

Food is the main way for human survival and good health maintenance. However, food could be contaminated by several ubiquitous hazardous substances such as polycyclic aromatic hydrocarbons (PAHs), which present in major environmental components including air, water and soil [1] or during food processing, for instance, heating, roasting, grilling, baking, canning, fermentation, or hydrolysis [2]. PAHs consist of hundreds of compounds, which result mainly from industrial activities and some natural processes as fire and volcanic activity [3]. Moreover, PAHs are considered as major food pollutants [4] due to their accumulation in the human food chain, which can result in short/long-term human health problems. PAHs have genotoxic and carcinogenic effects and benzo[a]pyrene considered as a marker of PAHs contamination. Furthermore, efforts should be made to minimise human exposure to PAHs where food limits have been set by EU especially benzo[a]pyrene and baby foods (4-10 ng/kg bw per day) [5]. This limit can be exceeded through food processing but rarely in raw food. However, the major pathway for PAHs human intakes for non-smokers is food [6,7].

When the food such as wheat and beans makes a large part of the diet [7,8], PAHs intake from food becomes more important. Consequently, food safety is an extremely important issue worldwide for both population and governments, particularly, in developing countries where food contamination set up a big burden. In addition, food contamination has become more serious in recent years due to industrial development and consequent environmental pollution [9]. The diverse sources of nutrient from various kind of inexpensive and hygienic food seems reasonable. Pulses such as peas, lentils and beans are considered the major economic source of nutritional rapport in developing countries. They are a good cheap source of protein, fibre, vitamins, and minerals; with a low-fat proportion content.

From the other hand, several decontamination technologies have been devoted in efforts to remove microbes and other contaminants to improve food safety and the storage period elongations. Among them, heat, chemicals, high pressure and ionising radiation, for instances. Gamma irradiation is recognised as a powerful way to remove such contaminants. The safety of irradiated foods has been confirmed in various animal and human studies [10,11]. These include animal feeding studies lasting for several generations in several different species, including mice, rats and dogs [11]. Recently, gamma irradiation was successfully deployed to remove such PAHs from diverse kinds of food such as wheat grains [12,13] and pea seeds [14,15].

The need to deploy a technique for food decontamination from almost all PAHs contaminants, without any change in chemical properties and quality of food, conducted us to this work to determine the gamma irradiation and storage effects on PAHs contents. Also, PAHs behaviour towards gamma irradiation in bean kernels was studied.

2. MATERIALS AND METHODS

2.1 Sampling and Irradiation Treatments

Fava bean kernels (Vicia faba, crop year 2017/2018) were collected and transferred directly into plastic bags for irradiation treatment (~ 20 g of kernels). Samples were gamma irradiated using different doses (1, 5, 10 and 15 kGy) in a 60Co irradiator (ROBO, Russia, a dose rate of 2.01 kGy. h^{-1}). Irradiation was performed at 20°C and the absorbed dose was determined as described in our previous work [13]. After irradiation, samples were stored for six months in a freezer at -20°C.

2.2 Chemicals and Reagents

A standard containing the 16 PAHs, which considered by the American Environmental protection agency (EPA) the most abundant in food samples, was used in this work. The mixed standard solution of 16 PAHs (0.1 mg L^{-1}) was purchased from AccuStandard (New Haven, USA), and consists of 16 PAHs namely: Naphthalene (NAP), Acynaphtalene (ACY), Acynyphtalene (ACP), Fluorene (FLR), Phenanthrene (PHE), Anthrancene (ANT), Fluoranthene (FLT), Pyrene (PYR), BenzoAnthrancene (BaA), Chrysene (CHR), Bezo[b]Fluoranthene (BbF), Bezo[k]Fluoranthene (BkF), Benzo[a]Pyrene (BaP), Dibenzo[ah]Anthrancene (DhA), Benzo[ghi]Perylene (BgP) and Indeno[1,2,3-cd]Pyrene (ICP). The working standard solution was prepared in acetonitrile and stored at 4°C in

the dark. Dichloromethane (DCM), n-hexane and acetonitrile (HPLC grade) were supplied by Sigma-Aldrich (St. Louis, USA).

2.3 PAHs Extraction

The extraction of PAHs from FBK samples was achieved using solid phase extraction (SPE) with silica cartridges according to the method reported by Moret and Conte [16]. Briefly, 2 g sample in n-hexane is loaded onto a 5 g silica cartridge, and the PAH fraction is eluted with 8 mL of n-hexane/dichloromethane 70/30. After collection of the PAHs fraction, it was concentrated to near dryness on a rotary evaporator. The residual solvent was allowed to evaporate spontaneously, at room temperature, to minimise volatile PAHs losses. Next, the residue was dissolved in 2 mL of acetonitrile and filtered on a 0.45 µm filter (syringe) before the injection into GS-MS apparatus.

2.4 GS-MS Analysis

An Agilent gas chromatography coupled with mass spectrometry (GC-MS model GC-6890) with an inert selective mass detector 5973 was used in PAHs analysis. The capillary column was DB-35 (30x0.2mm, film thickness 0.25 µm). The operating conditions were as follows: carrier gas, helium, with a flow rate of 1 ml /min; the volume injected was 1 µl of sample extract, and the ionisation mode was electron impact. The GC-MS system was operated under the following conditions: injection temperature 250ºC, source temperature 250°C, fragment energy of 70eV, mass spectra were acquired using an ionisation voltage 70ev. The initial temperature of the column was 50°C (held for 2 min), then heated to 170°C at a rate of 2°C/min (held for 7 min), then heated to 250°C at a rate 4°C/min (held for 10 min). The same conditions of temperature programming were used for pea samples to calculate the retention index (RI). The identification of components in pea seeds was based on RI. Individual components were identified by comparison of both mass spectra and their GC retention data; other PAHs identifications were made by comparison of mass spectra with those in the data system libraries and cited in the literature [17].

2.5 Statistical Analysis

Treatments were distributed in a completely randomised design with three replicates. Data were subjected to the analysis of variance test (ANOVA) using the SUPERANOVA computer package (Abacus Concepts Inc, Berkeley, CA, USA; 1998). The p-value of less than 0.05 was considered statistically significant. The degree of significance was denoted as $p<0.05*$, $p<0.01**$. [18].

3. RESULTS AND DISCUSSION

FBK were treated with 1, 5, 10 and 15 kGy doses of gamma irradiation then GC-MS was successfully employed for FBK-PAHs measurement directly either after harvest or post-storage period (6 months at -20°C in a freezer).

Fig. 1 demonstrates, the PAHs load in FBK (control) that was found to contain 10 PAHs at the beginning of the experiment directly after harvest; namely, Naphthalene (NAP), Acynaphtalene (ACY), Acynyphtalene (ACP), Fluorene (FLR), Phenanthrene (PHE), Anthrancene (ANT), Fluoranthene (FLT), Pyrene (PYR), Chrysene (CHR), Perylene (PER). The initial concentration for PAHs was calculated using the standard concentration as shown in Table 1.

From Table 1 we can calculate the sum of these 10 PAHs, which equal to $\sum 10\,\text{PAHs} = 12.27$ µg.kg^{-1}. The latest sum of PAHs load is much smaller than that reported in previous studies conducted on wheat grains [12], pea seeds [14] vegetables and fruits [19].

Figs. 1 and 2 display the 10 PAHs behaviours towards gamma irradiation doses. When kernels were irradiated with 1, 5, 10 and 15 kGy, the concentration of these 10 PAHs was found to decrease as the applied doses of gamma irradiation increased. These results are in agreement with previous studies in wheat grains and pea seeds [12,14,15].

Moreover, gamma irradiation and storage period effects on the 10PAHs, which have found in the control samples (Table 1) are observed in Figs. 1 and 2. Interestingly, at the dose of 1kGy of gamma irradiation, all 10 PAHs were found in the stored kernels except (CHR, PER, PYR) were slightly increased after storage at1kGy dose, which can be explained as the result of the degradation of larger molecules (e.g., CHR, PER, PYR) at this dose.

However, All PAHs were degraded under the effect of 5, 10 and 15 kGy doses of gamma irradiation (Figs. 1 and 2). Actually, this degradation was in a different trend for each

PAHs, it was exponential for PYR, PER, FLT, ACP and PHE with a correlation constant (R^2) higher than 0.926 as shown in Figs. 1, 2 and in Table 1. Therefore, these compounds need a higher gamma dose for better decontamination as predicted from the fitting equations. Whereas, ACP could be degraded for 18.7 kGy doses and PHE could reach a degradation percent of 99% at a dose of 17.2 kGy. Meanwhile, PYR, PER, and FLT could reach a degradation percent of 89.5%, 72.3%, 94.6%, respectively, at the maximum irradiation dose allowed for food decontamination, 30 kGy.

On the other hand, PAHs as CHR, ANT, NAP, ACY, FLR had a linear behaviour towards gamma irradiation doses and could be degraded at doses lower than 29.5 kGy. It is important to report that the correlation constant for the linear experimental fitting was higher than 0.954 (Table 1).

Indeed, this value impulses an important finding and gives a clear idea on the behaviour of each PAHs found in FBK under gamma irradiation effects and predicts a possible dose concerning the degradation of each PAH compounds.
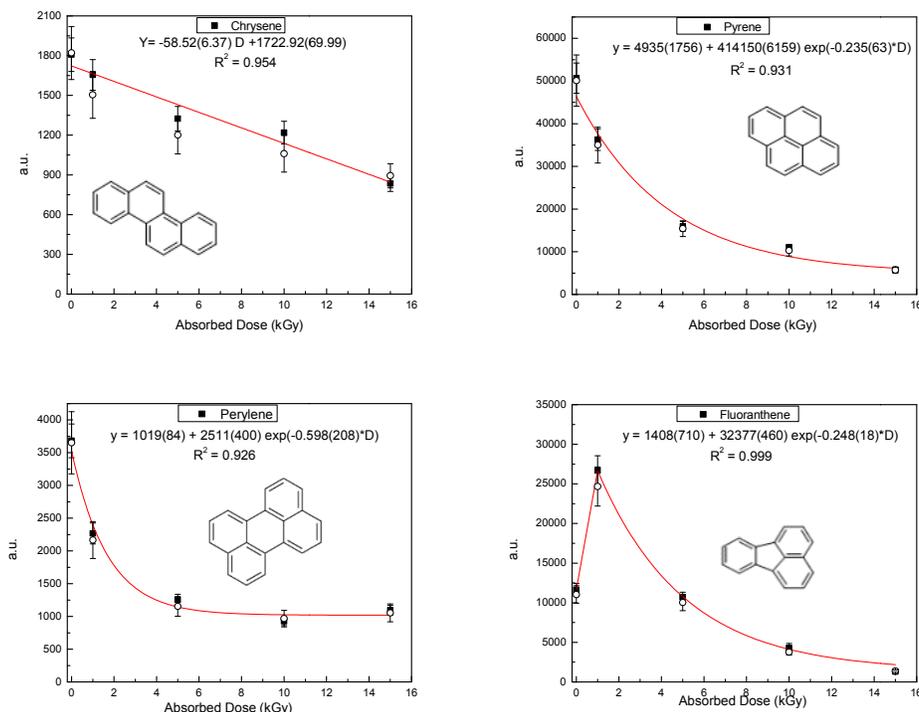
Fig. 1. PAHs (4/5 aromatic cycles) variations versus absorbed dose of irradiated fava bean before and after six months of storage. ■: after storage, o: after irradiation directly (before storage)
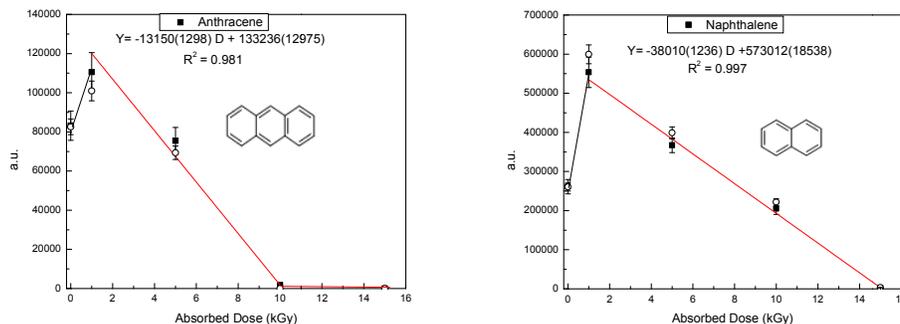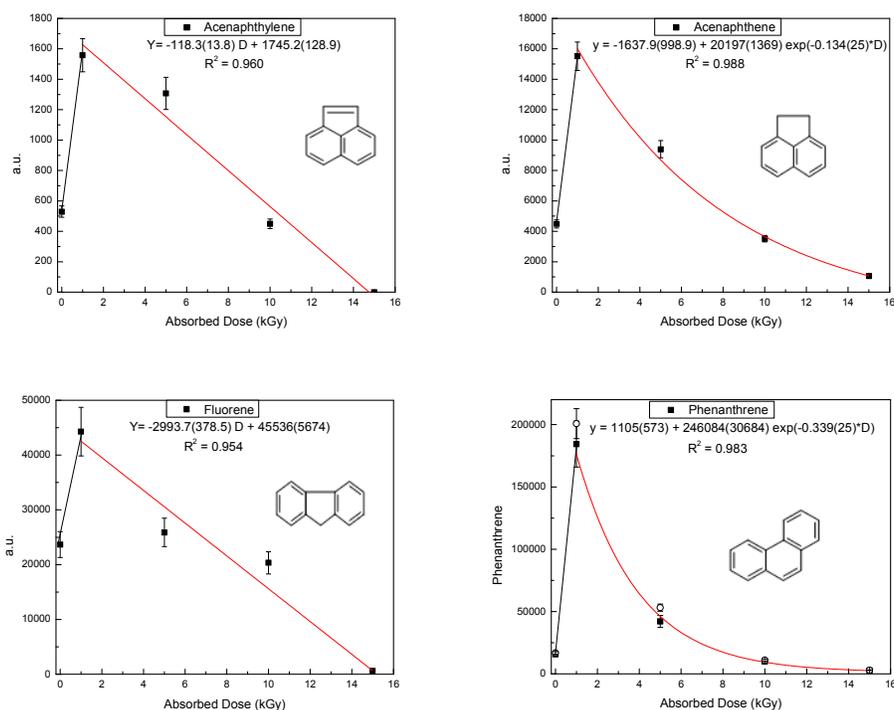
Fig. 2. PAHs (2/3 aromatic cycles) variation versus absorbed dose of irradiated fava bean before and after six months of storage. ■: after storage, o: after irradiation directly (before storage)

Table 1. The fitting function and correlation constant for the fava bean PAHs concentration behaviour under gamma irradiation

PAHs	Initial concentration ($\mu g.kg^{-1}$, *n=6*)	Fitting function	R^2	Decontamination expected dose (kGy)
CHR	1.12	y = -58.5 D +1722.9	0.954	29.5
PYR	1.02	y = 4935+ 414150 $e^{-0.235 D}$	0.931	30 (89.5%)
PER	1.32*	y = 1019 + 2511 $e^{-0.598 D}$	0.926	30 (72.3%) +
FLT	1.21	y = 1408 + 32377 $e^{-0.248 D}$	0.999	30 (94.6%) +
ANT	1.14	y = -13150 D + 133236	0.981	10.1
NAP	1.9**	y = -38010 D + 573012	0.997	15.1
ACY	1.03	y = -118.3 D + 1745.2	0.960	14.8
ACP	0.73	y = -1638 + 20197 $e^{-0.134 D}$	0.988	18.7
FLR	1.5**	y = -2993.7 D + 45536	0.954	15.2
PHE	1.3*	y = 1105 + 246084 $e^{-0.339 D}$	0.983	17.2 (99%) +
NAP, 1-methyl	-	y = -9192 D +143005	0.918	15.6
PHE, 3, 6-dimethyl	-	y = -173.1 D +3386.4	0.934	19.6
PHE, 2-methyl	-	y = -738.4 D +14016.5	0.966	19.0
PYR, 1-methyl	-	y = -58.3 D +1057	0.901	18.1

$*P<0.05; **P<0.01$
+ Expected degradation percent at maximum dose allowed for food decontamination (30 kGy)

Results of the present work demonstrate that the PAHs load of FBK was reduced due to gamma irradiation effects, except with the dose of 1 kGy, somewhat there was an increase in all PAH compounds. However, these PAHs continued to decrease with the others used doses, and even

some PAHs required higher doses for their total elimination.

Besides, the result of GC-MS analysis also reveals that there are newly formed compounds derived from NAP, PHE and PYR under gamma irradiation effects. These new compounds were restructured during the storage period, namely Naphthalene, 1-methyl; Phenanthrene, 3, 6-dimethyl; Phenanthrene, 2-methyl and Pyrene, 1-methyl. It is important, to notice that these compounds did not exist in the outstanding levels in the control samples of FBK (Fig. 3).

As PAHs exist in a mixture, we could explain the formation of PAHs derived compounds from NAP, PHE and PYR by destruction and re-combination of some PAHs degradation products during the post-irradiation and storage period. These derived methylated-PAHs compounds have similar PAHs carcinogenicity [20].

Anyhow, Fig. 3 demonstrates that methylated-PAHs recreated in a smaller quantity by the increase of the applied doses of gamma irradiation after the storage period. In this event, we can notice that 15 kGy of gamma irradiation

is not sufficient to reduce these methylated-PAHs as with the main PAHs (Figs. 1 and 2) as demonstrated in previous works [12-15]. Moreover, fitting of experimental data showed that these undesirable compounds could be no longer detectable at a dose higher than 19.6 kGy (Table 1).

Consequently, we can propose to increase the applied dose of gamma irradiation, for storage purpose of FBK, since high doses of gamma irradiation have been applied for decontamination and improving the hygienic quality of dried food. FBK is one of several food groups approved for irradiation in different countries [21].

It important to report, that Benzo[a]pyrene, B[a]P which, classified the most carcinogens of PAHs [22,23] and its presence reflected as an indicator for PAHs pollution in certain region [24] doesn't exist in FBK samples (Table 1 and Fig. 1) which, drove us to say that this area is somehow clean, and not polluted with PAHs. Also, the 10 PAHs founded in the control kernels samples may originate from the deposition from the atmosphere or deposition and transfer from PAHs polluted particles.
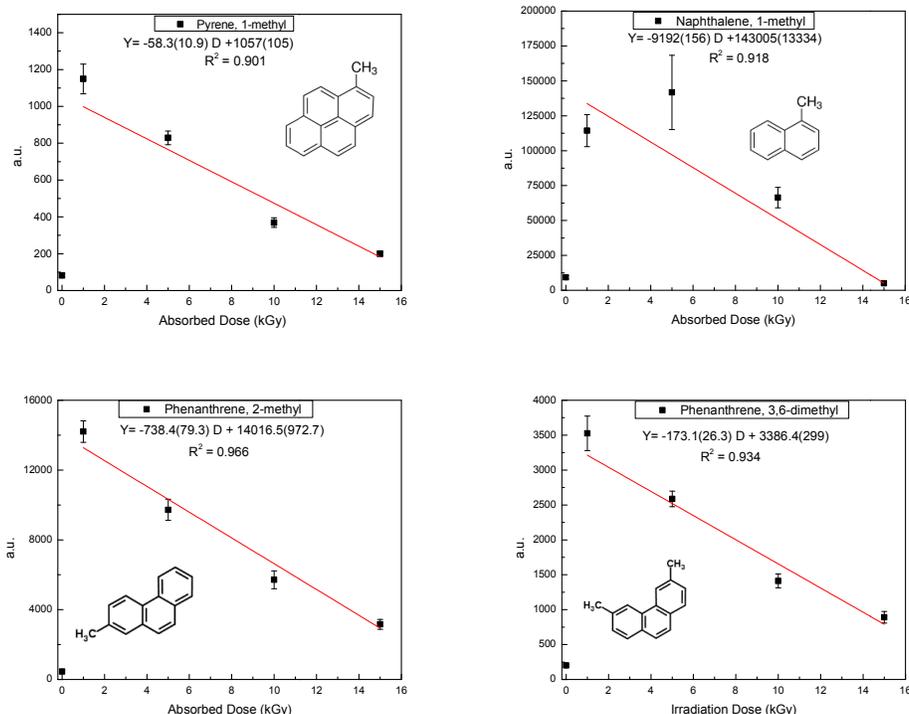
Fig. 3. PAHs derivatives versus absorbed dose of gamma irradiation of irradiated fava bean after six months of storage

4. CONCLUSION

The current work demonstrated that gamma irradiation is a simple technique for decontamination of FBK from probable PAHs load. The GC-MS analysis could be, successfully employed to observe the gamma irradiation and storage period effects on FBK decontamination from PAHs and their derivatives by monitoring the peaks of the GC-MS chromatograms. The highest dose (15 kGy) has been demonstrated as an effective treatment but was not a sufficient dose for FBK- PAHs content removal after the six months of storage. Experimental data fitting appeared to be a good practice to predict the appropriate dose of maximal PAHs elimination from fava bean kernels.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zelinkova, Z. Wenzl T. The occurrence of 16 EPA PAHs in food-a review. Polycycl Aromat Comp. 2016;35:248-284.
   DOI: 10.1080/10406638.2014.918550
2. Nerín C, Aznar M, Carrizo D. Food contamination during food process. Trends Food Sci Technol. 2016;48:63-68.
   DOI: 10.1016/j.tifs.2015.12.004
3. Howsam M, Jones K. Sources of PAHs in the environment in PAHs and related compounds, ed. A. Neilson, (Berlin Heidelberg: Springer, 1998). 1998;137-174.
   DOI: 10.1007/978-3-540-49697-7_4
4. Phillips DH. Polycyclic aromatic hydrocarbons in the diet. Mut Res. 1999; 443:139-147.
   DOI: 10.1080/10590501.2011.629974
5. Joint FAO/WHO Expert Committee on Food Additives (JECFA), Polycyclic Aromatic Hydrocarbons.
   Available:http://www.inchem.org/documents/jecfa/jeceval/jec_1941.htm
6. Scientific Committee on Food (SCF). Opinion of the Scientific Committee on Food on the Risks to Human Health of Polycyclic Aromatic Hydrocarbons in Food. (Brussels: Scientific Committee on Food); 2002.
   Available:https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out153_en.pdf
7. Scientific Committee on Food (SCF). Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. EFSA. 2008;724:1-114.
   Available:http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main.../724.pdf
8. Food Standards Australia New Zealand. Survey of Polycyclic Aromatic Hydrocarbons (PAH) in Australian Foods. Dietary Exposure Assessment and Risk Characterization (Food Standards Australia New Zealand; 2004.
   (Accessed 30 July 2017)
   Avalable:http://www.foodstandards.gov.au/science/monitoring/surveillance/Pages/surveyofpolycyclicar4818.aspx
9. Song Q, Zheng YJ, Xue Y, Sheng WG, Zhao MR. An evolutionary deep neural network for predicting morbidity of gastrointestinal infections by food contamination. Neurocomputing. 2017; 226:16-22.
   DOI:10.1016/j.neucom.2016.11.018
10. WHO. World Health Organization (WHO). High-Dose Irradiation: Wholesomeness of Food Irradiated with Doses above 10 kGy. Report of a Joint FAO/IAEA/WHO Expert Committee; 1999.
    Available:http://www.who.int/foodsafety/publications/food-irradiated/en/
11. Hazekamp A. Evaluating the effects of gamma-irradiation for decontamination of medicinal cannabis. Front Pharmacol. 2016;7-108.
    DOI:10.3389/fphar.2016.00108
12. Khalil A, Al-Bachir M. The deployment of γ-irradiation for reducing polycyclic aromatic hydrocarbons and microbial load in wheat kernels. Toxicol Environ Chem. 2015;97: 857-867.
    DOI: 10.1080/02772248.2015.1070159
13. Khalil A, Al-Bachir M. Decontamination of polycyclic aromatic hydrocarbons in pea seeds by gamma irradiation. Food Measure. 2017;11:1167-1173.
    DOI: 10.1007/s11694-017-9493-6
14. Khalil, A., Al-Bachir, M. Monitoring of gamma irradiation effects on polycyclic aromatic hydrocarbons and microbial load in pea seeds using GC-MS analysis. The Annals of the University Dunarea de Jos of Galati Fascicle VI-Food Technology. 2017; 41:9-20.

15. Khalil A, Albachir M, Odeh A. Effect of gamma irradiation on some carcinogenic polycyclic aromatic hydrocarbons (PAHs) in wheat grains. Polycycl Aromat Comp. 2016;36:873-883.
DOI: 10.1080/10406638.2015.1042551

16. Moret S, Conte LS. A rapid method for polycyclic aromatic hydrocarbon determination in vegetable oils. J Sep Sci. 2002;25:96-104.
DOI: 10.1002/1615-9314(20020101)

17. Adams, R.P. Identification of essential oil components by gas chromatography/mass spectrometry. 4ed. Allured Publishing Corporation, Carol Stream, Illinois USA. 2007;804.

18. Snedecor G, Cochran W. Statistical methods. The Iowa State University Press, Ames, Aiwa. 1988;221-221.

19. Bishnoi NR, Mehta U, Pandit GG. Quantification of polycyclic aromatic hydrocarbons in fruits and vegetables using high performance liquid chromatography. Indian J Chem Tech. 2005;13:30-35.

20. LaVoie EJ, Bedenko V, Tulley-Freiler L, Hoffmann D. Tumour initiating activity and metabolism of polymethylated phenanthrenes. Cancer Res. 1982;42: 4045-4049.

21. IAEA. International atomic Energy Agency: Food irradiation clearances database; 2008.
Available:http://nuclues.iaea.org/NUCUES/content/applications/FICdb/DatabaseHome.jsp
(Accessed 30 July 2008)

22. IARC. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC Monogr Eval Carcinog Risks Hum. 2010;92:1-853.
Available:https://www.ncbi.nlm.nih.gov/books/NBK321712/

23. Canadian Council of Ministers of the Environment (CCME). Canadian soil quality guidelines for potentially carcinogenic and other PAHs: scientific criteria document. CCME: Winnipeg; 2010.
Available:http://ceqg-rcqe.ccme.ca/download/en/320

24. Ohura T, Amagai T, Fusaya M, Matsushita H. Polycyclic aromatic hydrocarbons in indoor and outdoor environments and factors affecting their concentrations. Environ Sci Technol. 2004;38:77-83.
DOI: 10.1021/es030512o