Journal of Food Chemistry & Nanotechnology

https://doi.org/10.17756/jfcn.2022-125

Research Article

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Efficiency Post-Harvest Treatment by Gamma Radiation for the Microbial Safety of Dried Damask Rosa Petals

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Received: April 07, 2022
Accepted: May 29, 2022
Published: June 01, 2022

Citation: Al-Bachir M. 2022. Efficiency Post-Harvest Treatment by Gamma Radiation for the Microbial Safety of Dried Damask Rosa Petals. *J Food Chem & Nanotechnol* 8(2): 43-49.

Published by United Scientific Group

Abstract

The objective of the present study is to evaluate the effect of gamma irradiation on microbial load, and physicochemical properties of dried damask rosa petal (DDRP). Syrian DDRP was irradiated at 5, 10 and 15 kGy doses. Microbial load, physicochemical and sensorial properties of DDRP samples and its extract were investigated. All used doses effect on the reducing of the microbial load was reported (P < 0.05). Complete inactivation of the total viable counts (TVC), total coliform counts (TCC), and total mold and yeast (TMY) was observed at 5 kGy at day 0. Total soluble solids (TSS), total acidity, pH value, total volatile basic nitrogen (TVBN), viscosity, flavor, taste, color and texture of DDRP extract, none of which was affected by the irradiation. Furthermore, the irradiation up to 15 kGy did not alter the color parameters of DDRL including L* (whiteness), a* (redness) and b* (yellowness), respectively. The dose of 5 kGy was considered as suitable to be applied for the purpose of microbial decontamination of DDRP without modifying their composition, physical and chemical properties of its extract.

Keywords

Damask rosa, Gamma irradiation, Physicochemical properties, Microbial load, Sensory properties

Introduction

Damask Rosa (DR) (*Rosa damascene*) is a small plant with aromatic light pink flowers, belonging to the Rosaceae family [1]. DR is the most important crops in the floriculture industry among the Rosaceae family, and has many pharmacological, medical and cosmetically properties and have been used in food industry [2, 3]. DR plant contains several biologically active compounds, which have properties as anti-oxidant, anti-inflammatory, free radical scavenging inhibits the oxidation process [4]. Health-beneficial properties of DR may be attributed to their content of photochemical components such as phenolics flavonoids, anti-microorganisms, and antioxidant activities [5-7]. DR petals, flowers, heads, and oils are useful in medicines for the treatment of different type of health disorders and an infectious disease [8-10]. The industrial processing of DR sets its beginning in the middle of 17th century from the Damascus area of Syria [11]. Dried damask rosa petals (DDRP) for different purposes; its major use is for eating, as it can solve problems with digestive system. Another reason for drying petals is to store them when distilleries cannot accept the whole produced flower anymore. They use them later for distillation [12].

However, plants including herbs easily suffer contaminations that could happen either during production period or after harvest treatment is of a serious problem [13]. Being produced and harvested in a hot and high humid climate conditions, satisfy safe methods must be used for herbs and medical plants. Otherwise, it can lead to spoiling the product and increase the food borne disease with high counts in microorganisms [14]. The use of safe techniques is mandatory both for consumers and environment [15].

Several methods are applied for decontamination of plant products such as fumigation, heating and irradiation techniques [16, 17]. Food products irradiation is one of the new preserving technique that use ionizing radiation treatment to eliminate, prevent growth or reduce the count of unwanted microorganisms [18]. The using of gamma radiation treatment to different plant varieties has proven its effectively to preserve chemical compositions and nutritional characteristics [19, 20]. Irradiation is recommended for the decontamination of the products and is already used in flowers [21], aimed at reducing the use of chemical substances against pests and insects [22]. Several reports have demonstrated that gamma irradiation method of herbs and spices is an alternative effective method for decontamination, quarantine barriers in international trade and for improving safety and storability [16, 23-25]. However, to the best of our knowledge, detailed literature on composition and properties of rosa petal is scarce, and has not been extensively studied. Also, no study has yet compared the effect of gamma irradiation on their chemical composition of DDRP. Therefore, the present study is to evaluate the effectiveness of using gamma irradiation (5, 10 and 15 kGy) on the microbial load of DDRP and evaluate the changes in physical, chemical and sensory characteristics of DDRP.

Materials and Methods

DDRP preparation and irradiation treatment

A study was conducted in food irradiation labs in Syrian Atomic Energy Commission (SAEC). DDRP produced in 2019 were obtained from the organic herb trading market in Damascus, Syria and mixed for packaging in polyethylene plastic bags (150 g bag^{-1}). The measurements were done three time (3 replicates) for each treatment. DDRP samples were divided into two groups: first group was considered as control; the second group was treated at 5, 10 and 15 kGy doses of gamma ray with ^{60}Co source at room temperature (ROBO, Russa). The irradiation processing was done by source strength of 100 kCi. The samples were irradiated at place (15 cm from source) with a dose rate of 9.571 Gy h^{-1}. The experimental and theoretical optimal irradiation time for the dose of 5, 10 and 15 kGy were 31, 63 and 94 min. respectively. The absorbed dose was measured by determination of chloride ions or ions by means Oscillotitrator (OK-302/2, Radelkisz, Budapest, Hungary) [23]. Irradiated and un-irradiated samples were analyzed immediately after irradiation.

Extraction yield

To obtain DDRP extracts from the flower petals, 5 g of DDRP was mixed with 20 ml of distilled water for 20 minutes. The solution was diluted up to 200 ml by adding hot distilled water (75 °C) and mixed for 3 h [23]. The total soluble solids (TSS) were determined in the extracts by using Abbe Refractometer, VEB Carl Zeiss JENA. DDR (0-100) range [26].

Chemical analysis

For proximate analysis, the parameters such as moisture and ash by drying at 105 °C and ashing at 550 °C, crud protein by Kjeldahl method, and crude fat by Soxhlet apparatus, in the different DDRP samples were performed according to the methods the Association of Official Analytical Chemists (AOAC) [23]. Acidity in term of pH values of the DDRP extractions were measured using an HI 8521 pH meter (Hanna Instruments, Woonsocket, RI, USA). The total acidity as (lactic acid %) was measured by a titration with (0.1 N) NaOH and indicated as ml of (0.1 N) NaOH = 0.0090 g lactic acid [26]. Total volatile basic nitrogen (TVBN) in the DDRP sample in terms of mg VBN kg^{-1} DDRP (ppm) was measured [27]. Samples were analyzed in triplicates.

Physical analysis

To evaluate viscosity change, a solution of DDRP was extracted according to methods described by Al-Bachir [23]. The viscosity of the suspensions was determined with HAAKE viscometer 6 R plus Model (RTM) using a R2 column at 200 rpm. Viscosity values were measured in term of mPa.s or cP. The color of DDRP powders extract was evaluated by AvaSpec Spectrometer Version 1, 2 June 2003 (Avantes, Holland) at wave length of 670 nm. The colour changes were expressed as L* (lightness), a* (redness), and b* (yellowness) values were obtained [23].

Microbiological evaluation

The microbiological examination was carried out by using the spread plate technique and pour plate method as proposed by AOAC, [26]. Total viable counts (TVC) were determined by using plat count agar (PCA) (Oxoid, CM 325, UK) (30 °C, 48 h). Total coliform counts (TCC) were determined using Violet Red Bile Agar (VRBA) (Oxoid, CM 485, UK) (37 °C, 48 h). Total mould and yeast (TMY) were determined using Dichloran Rosa-Bengal Chloramphenicol Agar (DRBC) (Merck, 1.00466, Germany) (25 °C, 5 days). Microbial counts were expressed as log 10 cfu g^{-1} DDRP. The survival curve was estimated from irradiation doses of 00, 50, 100, 150, 200 and 250 Gy.

Sensory evaluation

Sensory evaluation was done by 25 persons, using a consumer-type panel. Every person was detecting the sensory alteration between the solution extracted from irradiated and un-irradiated DDPR samples. Each subject independently evaluated a DDRP extract for flavor, taste, color, and texture on a 5-point scale (1: extremely poor, 2: poor, 3: acceptable, 4: good 5: excellent), according to standard method [24].

Statistical analysis

The study followed a completely randomized design of four treatment and three replicates per each treatment. Analysis of variance (ANOVA) was performed using the SUPERANOVA computer package (Abacus Concepts Inc, Berkeley, CA, USA; 1998). The p value less than 0.05

were considered as statistically significant. D10 value were calculated using Cricket graph computer package (40 Valley Stream Parkway Malvern,PA 19355, 1986/87/88 Cricket Software, Copyright, Version 1.3).

Results and Discussion

Effect of gamma irradiation in proximate composition of DDRP

Proximate composition of un-irradiated and irradiated DDRP is given in table 1. The present results indicated that the higher portion component compositions of the DDRP such as crude protein (9.80%) and total sugar (75.21%) of DDRF were not substantially affected by gamma irradiation. Moreover, the protein and lipid in a foodstuff have been indicated to be more resistant to radiation treatment than in the pure situation [28]. However, several researches reported that the amounts of macro-nutrients including proteins and sugar were stable against radiation treatment with medium doses (up to 10 kGy) [29]. The limited effect of the gamma radiation treatment on the total sugar percentages in the DDRP could be explained by the low moisture level in DDRP (7.20%). It has been showed that the concentrations of radiolytic materials releasing from the sugars exist in irradiated products are much lower when the moisture percentage is low [30]. However, scarce information in the literatures on the composition of irradiated DDRP. Similar results on other plants indicated that that gamma radiation treatment with doses up to 10 kGy had no considered effect on protein and fiber of plant products [24, 26]. On the other hand, a little but significant ($p<0.05$) different was found in comparison with the irradiated samples for moisture (7.20%), crude fat (0.96%), ash and reducing sugar (3.55%). Whereas, the crude fat content was decreased due to the increasing of reducing sugar. The total crude fat percentages decreased with increasing dosage of gamma irradiation treatment induced by increasing the metabolic reactions and hydrolyzing enzyme activity in irradiated products [31]. The decrease in reducing sugar could be due to the radiation-induced degradation of complex carbohydrates into simple extractable forms [31].

Effect of gamma irradiation on DDRP color

The color parameters of the irradiated and the control samples of DDRP including whiteness (L*), red colour (a*), yellow colour (b*) and over all color differences (ΔE), were measured (Table 2). The irradiated DDRP became brighter and its level of red colour (a* value) and yellow colour (b* value) was less intense. The irradiated samples of DDRP constantly showed not significant ($p<0.05$) higher whiteness (L* value, and lower redness (a* value) and yellowness (b* value) than that of the non- irradiated samples. In this work, the overall differences in color (ΔE) indicated that gamma radiation treatment had not significant ($p<0.05$) effect on the color changes of the DDRP. There is little information available in the published studies on the effect of gamma irradiation on the color of DDRP. However, for the other plant materials, different effects of irradiation on the color of the plant fractions or its extracted solutions have been published. The color differences due to irradiation were reported by Al-Bachir [24] in thyme, Al-Bachir [25] in chamomile, and Rico et al., [32] in dried red pepper. Gamma radiation treatment had a slightly effect on the color indication of the dried lyceum fruit samples [33]. On other hand, Pouya et al. [34] reported that, the colour conservation was better in red pepper powder radiated at 5 kGy, while control sample showed colour loss. The changes in the mean values of a* and b* between the turmeric samples attribute to irradiation was reported to be insignificant ($p<0.05$) [35].

Table 1: Influence of gamma irradiation treatment on composition of dried *Rosa damascena* petal (DRDP).

Treatments	Control	5 KGY	10 KGY	15 KGY	P-level
Moisture (%)	^{a}0.08 ± 7.20	^{b}0.10 ± 6.51	^{a}0.04 ± 7.09	^{a}0.11 ± 7.19	**
Total protein (%)	^{a}0.09 ± 9.80	^{a}0.01 ± 9.63	^{a}0.00 ± 9.75	^{a}0.28 ± 9.90	NS
Total fat (%)	^{a}0.03 ± 0.96	^{a}0.09 ± 0.96	^{b}0.01 ± 0.88	^{c}0.00 ± 0.80	**
Ash (%)	^{a}0.02 ± 3.88	^{c}0.01 ± 3.79	^{c}0.01 ± 3.76	^{b}0.04 ± 3.83	**
Total sugar (%)	^{a}2.59 ± 75.21	^{a}2.80 ± 76.35	^{a}0.87 ± 75.66	^{a}1.27 ± 73.91	NS
Reducing sugar (%)	^{a}0.30 ± 3.55	^{a}0.01 ± 3.89	^{a}0.00 ± 2.86	^{b}0.54 ± 2.86	**

Different superscript uppercase letters show differences between samples ($P < 0.05$).
NS: not significant.
** Significant at $p<0.01$.

Table 2: Influence of gamma irradiation treatment on color change of dried *Rosa damascena* petal (DRDP).

Treatments	Control	5 KGY	10 KGY	15 KGY	P-level
L(Lightness)	^{a}0.88 ± 43.36	^{a}0.34 ± 43.04	^{a}0.62 ± 44.76	^{a}5.87 ± 45.99	NS
a (redness/greenness)	^{a}1.81 ± 26.20	^{a}1.22 ± 22.84	^{a}0.70 ± 25.20	^{a}3.58 ± 25.16	NS
b (yellowness/blueness)	^{a}3.47 ± 25.27-	^{a}2.56 ± 25.06-	^{a}1.22 ± 23.90-	^{b}1.11 ± 24.55-	NS
ΔE (Total color difference)	^{a}3.15 ± 35.97	^{c}2.18 ± 31.34	^{ab}1.50 ± 33.92	^{bc}1.81 ± 32.24	NS

Different superscript uppercase letters show differences between samples ($P < 0.05$).
NS: not significant.

Effect of gamma irradiation in microbial load of DDRP

Compared with the control sample of DDRP, all used doses of gamma radiation treatment (5, 10 and 15 kGY) decreased the total viable count (TVC), mold and yeast count (MYC), and total coliform counts (TCC) to less than 10 microbes per gram. However, the TVC, and MYC of non-irradiated DDRP were 2.92 ± 0.04 log cfu g^{-1}, and 2.10 ± 0.17 log cfu g^{-1}, respectively (Table 3). The radiation dose required to reduce the microorganisms load by one log cycle (D_{10}) in DDRP were 227 and 166 Gy for the TVC and MYC respectively. It has been demonstrated that 5 kGy dose of gamma irradiation was enough to control the growth or removal of the all kinds of DDRP microorganisms. Although all irradiated DDRP samples contained no living TVC or MYC after 12 months of storage, therefore 5 kGy was enough for complete decontamination of DDRP. The effect of gamma irradiation treatment on microbial load of DDRP has not been previously evaluated, but similar results on other spices of herbs were investigated including; licorice [16] aniseed [36] chamomile [35], thyme [24]. However, high doses of gamma irradiation (higher than 10 kGy) may be needed to achieve commercial "sterility" (less than 10 microbes per gram), according to the results of previous studies.

The damage of microorganisms may due to irradiation un-direct effects induce by reactive oxygen species (ROS) produced by the radiolysis of water in irradiated medium [22], or in direct action, caused breaking down of important macromolecules, such as DNA and cell membranes of the organism or even causes cell death. [29, 37].

Effect of gamma radiation treatment on TSS in DDRP solution

Extraction yields of DDRP were measured and the results were given in table 4. The solution extracted from control sample of DDRP gave a yield as TSS of 1.77%. Statistical analysis showed that, the concentration of TSS in solution of irradiated DDRP were not significantly ($p<0.05$) alter from those of un-irradiated samples (Table 4). When DDRP at low moisture content (7.20%) are irradiated, the main result is a very slight difference in the number of free monosaccharides. The effect of gamma irradiation treatment on extraction yield of DDRP has not been evaluated. However, previous studies found different results for the effect of gamma irradiation process on the extraction yields of some plant materials. The same results were previously reported in extracts of some other kind of herbs irradiated with 5–20 kGy gamma irradiations, such as licorice root powder [16], aniseed [36], and chamomile

Table 3: Total bacterial (log10cfu.g) and fungal (log10spores.g) count of dried *Rosa damascena* petal (DRDP).

Treatments	Control	5 KGY	10 KGY	15 KGY	P-level
Storage periode/Month	**Total count (log^{10}cfu.g)**				
0 Month	^{b}0.04 ± 2.92	ND	ND	ND	**
12 Month	^{a}0.06 ± 3.32	ND	ND	ND	**
P-level	**				
	Fungal count (log^{10}spores.g)				
0 Month	^{b}0.17 ± 2.10	ND	ND	ND	**
12 Month	^{a}0.04 ± 2.67	ND	ND	ND	**
P-level	**				
	Total coliform (log^{10}cfu.g)				
0 Month	ND	ND	ND	ND	
12 Month	ND	ND	ND	ND	
P-level					

Different superscript uppercase letters show differences between samples ($P < 0.05$).
** Significant at $p<0.01$.
ND: Not Detected.

Table 4: Influence of gamma irradiation treatment on chemical properties of dried *Rosa damascena* petal (DRDP).

Treatments	Control	5 KGY	10 KGY	15 KGY	P-level
Total soluble solid (°Brix)	^{a}0.15 ± 1.77	^{a}0.06 ± 1.77	^{a}0.10 ± 1.80	^{a}0.06 ± 1.77	**NS**
Total acidity (% Lactic acid)	^{a}0.03 ± 0.77	^{a}0.01 ± 0.75	^{a}0.02 ± 0.79	^{a}0.04 ± 0.77	**NS**
PH value	^{a}0.04 ± 5.06	^{a}0.03 ± 5.06	^{a}0.03 ± 5.04	^{a}0.04 ± 5.06	**NS**
Volatile basic nitrogen (ppm)	^{a}0.62 ± 6.11	^{a}1.07 ± 5.35	^{a}1.24 ± 5.74	^{a}0.62 ± 4.67	**NS**
Viscosity (mP.Sc)	^{a}0.58 ± 21.67	^{a}1.00 ± 22.00	^{a}1.00 ± 22.00	^{a}1.53 ± 21.67	**NS**

Different superscript uppercase letters show differences between samples ($P < 0.05$).
NS: not significant

[23]. Also, gamma irradiation process led to the increase the extraction yield of several kinds of medical herbs with the increase in radiation doses [38]. In contrast of our results, Polovka and Suhaj [39] showed small reduction in extraction yield of caraway treated with high doses of gamma irradiation (30 kGy). The increase in the dry weights of extracted solutions due to irradiation treatment might be attributing to break down of some high molecular weight substances, and modifying these substances from non-soluble to soluble forms in the test solutions [40]. The contrast in increase the extraction yield comparing to that published in the literatures may be attributing to the alteration in chemical composition of the tested plants [38].

Effect of gamma irradiation on total acidity and pH value of DDRP extract

The results of the effect of gamma irradiation on total acidity and pH value of DDRP extract are listed in table 4. The total acidity (TA) and pH value of the water solution extracted from the control samples of DDRP was 0.77% and 5.06 respectively. The TA and pH value are one of the main factors influencing the quality of medicine. It always controls many chemical and microbiological reactions [41]. When the TA and pH value is low (presence of acidic substances), the bacterial count could be low, but at neutral or higher TA and pH value, the level of contamination of the herbal preparations could be observed to be higher. This suggests that a neutral or alkaline TA and pH favored high contamination levels of the herbal preparation. This agrees with observation that bacterial growth is optimal at more or less natural pH, around pH 5.0-8.5 [42].

Regarding TA and pH value, not significant ($p>0.05$) alterations were detected between un-irradiated and irradiated samples. Total acidity of the extract produced from DDRP irradiated with 0, 5, 10 and 15 kGy was 0.77, 0.75, 0.79, and 0.77 respectively. Whereas, the pH values of the solution prepared from irradiated DDRP with 0, 5, 10 and 15 kGy were 5.06, 5.06, 5.04, and 5.06, respectively (Table 4). Previous studies reported that irradiation process with dose up to 10 kGy did not significantly affect on the pH value of the dried red pepper [32]. No significant differences in pH value of lycium fruit were determined due to gamma irradiation treatment with 4, 8 and 15 kGy [33]. Also, pH value of four medical herbals, namely, Rosa, guggul, chirata and gulvel and four medical plant formulations were not affected by processing with 10kGy of gamma irradiation [17]. Increased acidity of irradiated organic plant materials may be attributing to a degradation of organic acids substances, and it might be due to the production of carboxylic groups [43].

Effect of gamma irradiation treatment on TVBN of DDRP extract

The effect of different doses of gamma irradiation treatment (5, 10 and 15 kGy) on TVBN contents of DDRP is shown in table 4. The data demonstrated that the concentration of the TVBN in control sample of DDRP was 6.11 g TVBN/kg (part per million, ppm). There were no significant ($p>0.05$) alterations in the concentration of TVBN between irradiated and un-irradiated DDRP samples. These results are in agreement with previous results in faba bean [44], and loquat kernel [29], they found that gamma irradiation treatment had no significant effect on the TVBN concentration of faba bean and loquat kernel.

The concentration of some volatile substances increased, whereas the concentration of a few other main substances decreased after irradiation due to the high sensitivity of hydrocarbon compounds to irradiation process [45]. The TVBN is connected with protein breakdown, and the reported increases may be due to the production of ammonia or other basic substances due to activity of microorganisms [31].

Effect of gamma irradiation on viscosity of DDRP extract

The effect of gamma irradiation treatment on the relative viscosity of DDRP extracted solutions are shown in table 4. The viscosity of the solution extracted from control sample of DDPR was (21.67 m.Pa.S). Irradiation treatment with 5kGy, 10 kGy and 15 kGy of gamma irradiation had no real effect on viscosity. In agreement with our results, Al-Bachir [23] reported that, the viscosity of the solution extracted from control samples of chamomile was 60 m.Pa.S, and irradiation with 10 kGy of gamma irradiation had no significant effect on viscosity of chamomile extract. The effect of irradiation treatment seems to be connected to the degradation of starch in irradiated spices [23]. The previous experiments in our labs showed that, irradiation treatment with 5, 10, 15, and 20 kGy doses of gamma ray decreased significantly ($p>0.05$) the viscosity of licorice root extracts comparing with control [46].

Effect of gamma irradiation in sensorial properties of DDRP extract

Sensory evaluation data as affected by gamma irradiation are presented in table 5. The extract of control samples of DDRP showed score of 2.83, 2.69, 2.86 and 3.52 for flavor, taste, color, and aroma respectively. Moreover, the flavor, taste, color, and aroma of DDRP extract was not affected by used gamma irradiation doses (5, 10 and 15 kGy). There

Table 5: Effect of gamma irradiation on the taste, texture, color and flavor of dried *Rosa damascena* petal (DRDP).

Treatments	Control	5 KGY	10 KGY	15 KGY	P-level
Flavor	^{a}1.17 ± 2.83	^{a}1.28 ± 2.72	^{a}1.33 ± 2.55	^{a}1.32 ± 2.66	NS
Taste	^{a}0.97 ± 2.69	^{a}0.94 ± 2.66	^{a}0.94 ± 2.38	^{a}1.09 ± 2.52	NS
Color	^{a}0.99 ± 2.86	^{a}1.09 ± 2.86	^{a}0.99 ± 2.52	^{a}1.08 ± 2.66	NS
Texture	^{a}0.99 ± 3.52	^{a}0.98 ± 3.38	^{a}1.20 ± 3.17	^{a}1.07 ± 3.28	NS

Different superscript uppercase letters show differences between samples ($P < 0.05$).
NS: not significant.

is no information available in the literature on the effect of gamma irradiation on the sensory characteristics of DDRP. However, several authors have indicated the effect of gamma irradiation treatment on the sensory characteristics of other kind of medical herpes and spices. Rahman et al. [47] studied sensory attributes for irradiated and non-irradiated red chilli. The attribute from the sensory panelists observed that both radiated and control samples had insignificant difference and were acceptable in terms of sensory attributes including colour, texture, and flavour. Similar results were also reported by Jung et al. [48]. The researcher observed sensory scores odor was slightly affected for radiated red pepper [32]. Our group determined the effect of gamma irradiation treatment on licorice [49], chamomile [23], and thyme meal [24]. The results of those studies reported that gamma irradiation of licorice root powders and aniseed at dose of 10 kGy conserved the sensorial characteristics of its extract unchanged. Park et al., [50] stated that the use of 10 kGy of gamma irradiation should be applying for reducing or eliminating microorganisms' populations with no undesirable effect on property and most of sensorial attribute (color, chewiness, and taste).

Conclusion

Gamma irradiation can be used for the decontamination of microorganisms such TVC, TMYC, and TCC in DDRP. A dose of 5 kGy can inactivate all kinds of microorganisms immediately after exposure to gamma irradiation. The applied dose (5, 10 and 15 kGy) of gamma irradiation treatment did not induce any significant differences in the physico-chemical characteristics and sensorial properties of the DDRP.

Acknowledgements

The author wishes to express deep appreciation to the Director General of the AECS and the staff member working in labs of food irradiation division.

Conflict of Interest

The author reports no conflict of interest, and he is responsible for the content of the manuscript.

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