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CONTROL OF NATURAL MICROORGANISMS IN CHAMOMILE (*CHAMOMILLA RECUTITA* L.) BY GAMMA RAY AND ELECTRON BEAM IRRADIATION

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ABSTRACT

Background. Microbial contamination levels and corresponding sensitivities to gamma rays (GR) and electron beam (EB) irradiation were tested in chamomile (*Chamomile recutta* L.).
Material and methods. Chamomile powders were treated with 10 and 20 kGy by GR and EB, respectively. Microbiological and chemical analyses were performed on controls and treated samples immediately after irradiation, and after 12 months of storage.
Results. The control samples of chamomile exhibited rather high microbiological contamination, exceeding the levels of 4 $\log_{10}$ CFU g^{-1} (CFU – colony forming units) reported by national and international authorities as the maximum permissible total count level. Irradiation with GR and EB was found to cause a reduction in microbial contamination proportionate to the dose delivered. The sterilizing effect of EB on microorganisms was higher than the GR one. A dose of 10 kGy of GR and EB significantly ($p < 0.05$) reduced the total bacterial, total coliform and total fungal contamination. A dose of 20 kGy of GR significantly ($p < 0.05$) reduced the total bacterial and total fungal contamination, while a 20 kGy dose of EB reduced the initial bacterial, total coliform and total fungal contamination to below detection level when the analysis was carried out immediately after irradiation treatment or after 12 months of storage.
Conclusion. The comparative study demonstrated that electron beam was more effective for decontamination of chamomile powder than gamma irradiation.

Keywords: chamomile, decontamination, electron beam, gamma ray, moisture storage

INTRODUCTION

Medicinal plant research is universally on the rise. Researchers, as well as the general public, recognize that natural products, predominantly those derived from plants, may exhibit health benefits (Petronilho et al., 2012). Chamomile (*Chamomilla recutita* L.) is one of the most widely used and well-documented medicinal plants in the world (Lohse et al., 2006). Chamomile is used both internally and externally to treat an extensive list of conditions and is also extensively consumed as a tea or tonic (Al-Bahtiti, 2012). Chamomile ingredients function mostly as fragrance ingredients and as skin conditioning agents in cosmetic products (Gottschalck and Breslawec, 2012). Chamomile is reportedly listed as an official drug in the Pharmacopoeias of 26 countries (Ross, 2008).

Herbs including chamomile contain a high level of bacteria spores and fungi. These spores are mainly mesophilic aerobes, and sour thermophilic aerobes

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(Sharma, 2006). The presence of invisible microorganisms, many of which could cause disease risks to human health, especially when spices or herbs are added to food after cooking (Sharma, 2006).

In the post harvest processing of chamomile, drying is an important process for preserving plant material, in that it inhibits enzymatic degradation and limits microbial growth (Harbourne et al., 2009). A number of methods have been tried for decontamination of herbs such as heat treatment, UV irradiation and fumigation (Gupta et al., 2011).

Ionizing irradiation is now gaining recognition throughout the world as phyto-sanitary treatment of herbal materials (Farkas, 1998). Some studies indicate that gamma irradiation (Al-Bachir, 2007; Aquino et al., 2010; Gupta et al., 2011; Khattak, 2012) and electron beam (Nemtanu et al., 2008; Ramathilaga and Murugesan, 2011) are effective treatment for microbial decontamination of medicinal plants. However, to the best of our knowledge, the effect of gamma ray or electron beam irradiation on the microbial load of chamomile has not yet been investigated. At present, there are limited studies on the comparative effects of gamma irradiation and electron beam processes on microbiological properties of commercial herbs, except for a study on tamarind seed (Choi et al., 2009). Thus, this study was intended to compare and evaluate the effects of gamma irradiation and the electron beam process on the microbiological properties of chamomile powders during post-treatment storage.

MATERIAL AND METHODS

Treatment and analysis performed

Dried chamomile plant powder (4.5 kg) was purchased from an organic herb trading company in Damascus, "Syria" and mixed for packaging in polyethylene plastic bags (150 g bages^{-1}). They were divided into three groups: the first group was non-irradiated control, the second group was irradiated at 10 and 20 kGy of gamma ray (GR) with a ^{60}Co source with a dose rate of 719 Gy h^{-1} at room temperature (ROBO, Russa). The source strength was approximately 55 kCi. The absorbed dose was assured by alcoholic chlorobenzene dosimeter. This absorbed dose was determined by the measurement of chloride ions or hydrogen ions by means of Oscillotitrator (OK-302/2, Radelkisz, Budapest, Hungary). The uncertainty of the real absorbed dose for gamma irradiation was less than 5%, according to the certificate of international dose assurance system IDAS program of the International Atomic Energy Agency (IAEA, 2002). The third group was irradiated at (10 and 20 kGy) of accelerated electron beam (EB). Irradiation was carried out using a linear electron accelerator facility (D-EPS-T30-30-002V, VIVARAD, France). The conditions of the accelerator were: beam energy 2.3 mega electron volt (MeV) (variable), beam power 2.6 kW, the beam current was 10 mA. The absorbed dose was measured employing film dosimetry (B3, GEX, corporation, USA). The absorbed dose was determined by measurement of optical density by means of a spectrophotometer (Genesys 20, Thermo Fisher Scientific, USA). The uncertainty of the average absorbed dose of the electron beam is (1.62) for (20 kGy).

Irradiation was performed at room temperature and ambient pressure and the distance from the beam source to samples was 25 cm. One side irradiation (exposure from one sides) was performed. Chamomile powder was irradiated to 30 mm of thickness due to the limited penetration of the electron beam in the irradiated products. All samples were stored for 12 months at room temperature (18 to 25°C) under a relative humidity (RH) of 50 to 70%. Microbiological and chemical analyses were performed on controls and treated samples immediately after irradiation, and after 12 months of storage.

Microbiological analysis

Three replicates from each treatment were aseptically opened, and 10 g of whole chamomile powder was transferred to prepare serial dilutions according to standard methods (AOAC, 2010). The medium used for determining the total bacterial plate counts (TBPC) was agar plate counts (APC) (Oxoid, CM 325, UK) (48 h incubation at 30°C). Total coliform was determined on Violet Red Bile Agar (VRBA) (Oxoid, CM 485, UK) at 37°C for 48 h. Fungus was enumerated on Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) (Merck, 1.00466, Germany) after incubation at 25°C for 5 days. The microbiological contaminations were calculated as $\log_{10}$ CFU g^{-1} (CFU – colony forming units).

Chemical analysis

For chemical analysis, each sample of chamomile plant powder was homogenized and analyzed in triplicate to determine moisture and dry mass content (drying for 6 h at 105°C). The same samples were mineralized in order to determine the ash content for 4 h at 550°C (AOAC, 2010).

Statistical analysis

The five treatments and two storage periods were distributed in a completely randomized 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). A separation test on treatment means was conducted using Fisher's least significant differences (LSD) method (Snedecor and Cochran, 1988) at 95% confidence level.

RESULTS AND DISCUSSION

Microbiological qualities of chamomile

The control samples of chamomile exhibited rather high microbiological contamination. The initial contamination loads of chamomile samples were 7.25, 4.71 and 3.73 log 10 CFU g^{-1} of the total bacterial, total coliform and total fungi count, respectively (Table 1). After storing chamomile at ambient temperature for 12 months, the bacterial, coliform and fungal count increased significantly in the case of non-irradiated control samples. High bacterial, coliform and fungal growth was visible after 12 months of storage. It could be attributed to the high storage temperature, which helps to increase the growth of bacteri, coliform and fungi during the long period of storage. Therefore, ambient temperature storage is not suitable for the shelf-life extension of chamomile.

Table 1. Effect of gamma irradiation and electron beam on total bacterial count of chamomile stored at room temperature (18–25°C), $\log_{10}$ CFU g^{-1}

Storage period months	Dose		
	0	12	LSD 5%
Control	7.25 ±0.09	9.26 ±0.19	0.33
10 KGY-EB	4.24 ±0.12	4.23 ±0.05	0.21
20 KGY-EB	<1	<1	–
10 KGY-GI	5.17 ±0.19	5.24 ±0.07	0.33
20 KGY-GI	2.43 ±0.11	2.47 ±0.02	0.19
LSD 5%	0.22	0.09	

EB – electron beam, GI – gamma irradiation.

The microbiological population of used chamomile plant powder was found to be comparatively high, which was not in accordance with Syrian microbial standards for foods such as chamomile powders, which include less than 5.0×10^4 CFU g^{-1} in total aerobic bacteria and negative in coliform (coliform not detected) (SASMO, 2010). Moreover, the microbial load of chamomile exceeded the levels of 1.0×10^4 CFU g^{-1} (4 $\log_{10}$ CFU g^{-1}) reported by the WHO (1998) as the maximum permissible total count level of bacteria. The high contamination level could be attributed to the high natural micro flora of the chamomile plants, as well as general conditions during their cultivation, harvesting, drying, handling processing, storage, distribution and sales. However, it was reported that the chamomile could carry various microbial contaminants due to environmental factors, inappropriate harvesting and handling or transport (Martins et al., 2001). The probability of contamination depends on the available surface of the materials and processing history: flowers and leaves usually contain more contamination than fruits and seeds, while crude herbs contain more than extracts (Razem and Katusin-Razem, 2002). The high microbial contamination of chamomile powders and that increased throughout storage may be attributed to the high concentration of moisture (10.21%; Table 4). Our previous studies also indicated that the microbial contamination level was above the international and national limits in commercially available herbs such as licorice root powders and products (Al-Bachir and Zeinou, 2005; Al-Bachir et al., 2004) and aniseed (Al-Bachir, 2007). Similar microbial contamination levels have been reported in certain herbs (Kumar et al., 2010), and herbal cosmetic products (Neramitmansook et al., 2012).

Effect of gamma ray and electron beam on microbial load of chamomile

Total bacterial load: Irradiation was found to cause a reduction in microbial contamination proportionate to the dose delivered. A dose of 10 kGy of gamma ray (GR) and electron beam (EB) irradiation significantly ($p > 0.05$) reduced the bacterial contamination to 2 and 3 log cycle, respectively. A dose of 20 kGy of GR irradiation reduced the bacterial contamination to 5 log cycle, while a 20 kGy dose of EB reduced the initial bacterial population to below the detection level when the analysis was carried out immediately after irradiation treatment or after 12 months of storage (Table 1). No statistically significant levels in the population of aerobic bacteria were observed in irradiated samples after 12 months of storage at room temperature, but the number of bacteria significantly increased (2 log cycle) in non-irradiated control samples stored at the same conditions. Both doses of GR (10 and 20 kGy) and the lower dose of EB (10 kGy) were not sufficient to eliminate all mesophilic bacteria, because the raw materials (chamomile plant powders) have a high contamination level and the local environmental conditions were suitable to support the rapid growth of such contaminants.

Effect of gamma ray and electron beam on total coliform

The viability of coliform microorganisms in chamomile powder, following irradiation with doses of 10 and 20 kGy of GR and EB, was evaluated (Table 2). The dose of 10 kGy of GR and EB significantly ($p > 0.05$) reduced the total coliform contamination to a 2 log cycle, while a 20 kGy dose of GR or EB reduced the initial coliform contamination to below the detection level when the analysis was carried out immediately after irradiation treatment or after 12 months of storage. The total coliform counts (TCC) of control samples increased significantly ($p < 0.05$) from 4.71 to 5.76 log cycle CFU^{-1} after 12 months' storage. It was noted that the number of total coliform slightly increased (>0.05) with the storage period for samples irradiated at 10 kGy of GR or EB, similar to non-irradiated samples because of the re-growth of the surviving microbial population. Samples treated with 20 kGy of GR or EB irradiation remained completely free of coliform thorough the storage period.

Table 2. Effect of gamma irradiation and electron beam on total coliform count of chamomile stored at room temperature (18–25°C), $\log_{10}$ CFU g^{-1}

Storage period months	Dose		
	0	12	LSD 5%
Control	4.71 ±0.23	5.76 ±0.16	0.44
10 KGY-EB	2.44 ±0.20	2.73 ±0.06	0.33
20 KGY-EB	<1	<1	–
10 KGY-GI	2.88 ±0.45	2.91 ±0.15	0.75
20 KGY-GI	<1	<1	–
LSD 5%	0.44	0.55	

EB – electron beam, GI – gamma irradiation.

Effect of gamma ray and electron beam on total fungal contamination

Regarding fungal contamination, immediately after radiation treatment, no fungal colonies were detected in samples irradiated with 10 and 20 kGy of electron beam, while 10 and 20 kGy doses of GR significantly ($p > 0.05$) reduced the fungal contamination to 2.87 and 1.53 log cycle, respectively (Table 3). The total fungal counts (TFCs) of the control samples increased from 3.73 to 6.44 log CFU^{-1} after 12 months' storage. It was noted that the number of fungal colonies

Table 3. Effect of gamma irradiation and electron beam on total fungal count of chamomile stored at room temperature (18–25°C), $\log_{10}$ CFU g^{-1}

Storage period months	Dose		
	0	12	LSD 5%
Control	3.73 ±0.11	6.44 ±0.08	0.21
10 KGY-EB	<1	2.49 ±0.04	0.06
20 KGY-EB	<1	<1	–
10 KGY-GI	2.87 ±0.17	3.11 ±0.13	0.35
20 KGY-GI	1.53 ±1.32	2.57 ±0.02	2.12
LSD 5%	1.09	0.13	

EB – electron beam, GI – gamma irradiation.

increased with the storage period. This increase was significant ($p < 0.5$) for samples irradiated at 10 kGy of electron EB, and not significant ($p > 0.05$) for samples irradiated at 10 and 20 kGy of GR, similar to non-irradiated samples, because of the re-growth of the surviving microbial population. Samples treated with 20 kGy of EB remained completely free of fungi thorough the storage period.

Our results showed that the total microbial contamination of chamomile decreased linearly with the radiation dose of GR and EB absorbed, and this evolution is in agreement with the results reported by Katušin-Ražem et al. (1983) and Nemtanu et al. (2008). An irradiation dose of 20 kGy of GR or EB irradiation allowed the acceptable microbial levels for herbs based on Syrian microbial standards (SASMO, 2010) to be reached. Our results illustrated that EB irradiation is a better decontamination method than GR irradiation treatment in disinfecting chamomile plant powders. It was reported that EB irradiation was proven very effective for inactivation of microorganisms contaminated in chamomile powders (Nemtanu et al., 2008). This result was in good agreement with our previous findings on microbial quality of licorice root powders (Al-Bachir and Zeinou, 2005), licorice root products (Al-Bachir et al., 2004), and aniseed (Al-Bachir, 2007) after irradiation at 10 kGy.

Table 4. Effect of gamma irradiation and electron beam on moisture and ash of chamomile stored at room temperature (18–25°C), %

Storage period months	Dose		
	0	12	LSD 5%
Moisture, %			
Control	10.21 ±0.89	6.45 ±0.02	1.43
10 KGY-EB	10.03 ±0.57	6.41 ±0.04	0.91
20 KGY-EB	9.64 ±0.06	6.33 ±0.05	0.12
10 KGY-GI	8.92 ±1.17	6.33 ±0.02	1.87
20 KGY-GI	9.55 ±0.03	6.31 ±0.04	0.08
LSD 5%	1.28	0.06	
Ash, %			
Control	1.95 ±0.03	5.00 ±0.85	1.36
10 KGY-EB	1.95 ±0.17	4.18 ±0.35	0.63
20 KGY-EB	1.99 ±0.11	3.26 ±0.66	1.08
10 KGY-GI	2.17 ±0.17	3.86 ±0.51	0.86
20 KGY-GI	2.01 ±0.22	4.01 ±0.29	0.59
LSD 5%	0.29	1.04	

EB – electron beam, GI – gamma irradiation.

Effect of gamma ray and electron beam on moisture and ash content of chamomile

The effect of gamma ray (GR) and electron beam (EB) treatment and post irradiation storage on the moisture content of chamomile is shown in Table 4. The moisture content of the non-irradiated control sample of chamomile powder was 10.25%. The data showed that both GR and EB irradiation doses led to the decrease in the moisture content in chamomile powder. These decrements were higher in GR-irradiated samples than EB-irradiated ones.

In order to improve the purity and safety of the herb products, monitoring of basic hygiene during preparation and standardization of some physical characteristics such as moisture are desirable (Abba et al., 2009). Drying is the most common and fundamental method for post-harvest preservation of medicinal plants, because it allows for the quick conservation of the medicinal qualities of the plant material in an uncomplicated manner (Rocha et al., 2011). The optimization of the drying process contributes to the physical, chemical and microbiological stability of the medicinal herbs (Rocha et al., 2011). The moisture content of herbs may vary from 6–12%, depending on the extent of drying and climatic conditions (Sharma, 2006). Drying at high temperature decreases the total aerobic microbial count in herbs, and can lower the water activity to the level required for preventing the growth of microorganisms (Kulshrestha et al., 2008). The high moisture reduction in the sample treated with GR is probably due to the temperature of the products and long period of irradiation comparing with EB treatment. The temperature of the product may rise by 5°C at a dose of 10 kGy (Sádecká, 2007). Irradiated chamomile powder showed a low moisture content compared with the control. Rico et al. (2010) found that irradiation of dried red pepper with 10 kGy

promoted a reduction in the moisture. On the other hand, moisture of lotus remained unchanged following gamma irradiation (6 kGy) as compared to the control (Khattak et al., 2009). Radiation is known to induce de-polymerisation of polysaccharides (Wilkinson and Gould, 1996) and this radiation-induced damage causes considerable changes in the cell membranes and connective tissues (Josephson and Peterson, 1982), leading to softening and easier water release in foods. The ash content of the un-irradiated control sample of chamomile powders was 1.95%, and no differences were found in ash content due to EB treatment. Gamma ray irradiation resulted in an increased ash content in the chamomile powder. The increase in the ash content of chamomile powders following GR irradiation might be due to a decrease in moisture and other organic compounds. In contrast, Al-Bachir (2007), Al--Bachir and Zeinou (2005), and Al-Bachir et al. (2004) reported that the ash of irradiated (10 kGy of gamma ray) licorice powder and aniseed were not significantly different from those of un-irradiated ones.

CONCLUSION

A comparative study demonstrated that the electron beam was more effective for decontamination of chamomile powder than gamma irradiation. From these results, we confirmed that a dose of 20 kGy of electron beam is effective in the case of chamomile plant powder to achieve the required level of decontamination and meet food safety standards.

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