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The effect of gamma irradiation on the microbial load, mineral concentration and sensory characteristics of liquorice (*Glycyrrhiza glabra* L)

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Abstract: Ground liquorice roots were exposed to various doses (0, 5, 10, 15 and 20 kGy) of gamma radiation from a ^{60}Co source. Irradiated and non-irradiated samples were stored at room temperature. Microbial population, viscosity, concentrations of some minerals and the sensory properties of the extracts were evaluated after 0 and 12 months of storage. Tests carried out immediately after irradiation showed that the microbial count had been reduced and that the dose required to reduce the count by 1 log cycle (D_{10}) was about 2 kGy. No effect was observed on the total dissolved solids in extracts of liquorice roots. Glycyrrhizinic acid concentration in the extracts and the viscosities of suspensions produced from irradiated roots were lower than those from non-irradiated ones. Sensory evaluation indicated that there were no significant differences ($P < 0.05$) in colour, taste or flavour between extracts produced from irradiated and non-irradiated roots. However, after 12 months of storage, some mineral ion (Na^+, Ca^{2+} and K^+) concentrations in extracts produced from irradiated roots were lower than in those from non-irradiated ones; no significant differences ($P < 0.05$) in viscosity were found between suspensions of irradiated and non-irradiated roots.

Keywords: decontamination; extract characteristics; irradiation; liquorice; sensory evaluation

INTRODUCTION

Liquorice has been known in Syria for a long time. It is one of the most popular and widely consumed herbs in the world.[1,2] The main active constituent found in liquorice root is glycyrrhizinic acid.[2,3]

Most herbs carry large numbers of micro-organisms capable of causing spoilage or, more rarely, disease. The aerobic plate count of these herbs may sometimes reach $(80–100) \times 10^6\,g^{-1}$.[4]

The most widely used method for decontamination of herbs was, until recently, fumigation with ethylene oxide.[5] Ethylene oxide is a mutagen and is also suspected of causing other chronic or delayed toxic effects.[6–9] Research on herbs has proved that treatment with ionising radiation is an effective process for destroying contaminating organisms.[10,11] Depending on the number and type of micro-organisms and the chemical composition of the commodity, irradiation doses of up to 20 kGy may be required to achieve commercial 'sterility' (ie a total viable cell count of less than $10\,g^{-1}$) for natural herbs; however, doses of 3–10 kGy can reduce viable cell counts to acceptable levels (from 10^5–10^7 to less than $10^4\,g^{-1}$).[10,12] No substantial changes were found in the volatile oil contents or in the amounts of other chemical constituents of most herbs treated with doses of up to 10–15 kGy.[13–17]

There is no information available on microbial loads, the quantity of extract and its characteristics for irradiated liquorice, especially when high irradiation doses (up to 20 kGy) are employed. Therefore the objective of this study was to investigate the effect of gamma radiation on the microbial load of ground roots and on some sensory and chemical properties of liquorice root extract.

MATERIALS AND METHODS

Roots of *Glycyrrhiza glabra* L were harvested in August 1997 from an orchard located in eastern Syria (Raqqa). They were dried in the sun and then ground by the supplier. Samples (1 kg) of these commercially ground roots were transferred into polyethylene bags and treated with various doses (0, 5, 10, 15 and 20 kGy) of gamma rays, using a ^{60}Co source, at a dose rate of $719\,Gy\,h^{-1}$. To determine D_{10}, 1, 2, 3, 4 and 5 kGy doses were used. The absorbed dose was determined using an alcoholic chlorobenzene dosi-

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meter.[18] Each treatment had four replicates. After irradiation the bags containing the ground roots were stored for 12 months at room temperature (18–25 °C) and a relative humidity (RH) of 50–70%.

For microbiological analysis, one bag was opened aseptically and 10 g of ground roots were transferred to a sterilised glass bottle containing 90 ml of sterile physiological water (9 g kg^{-1} NaOH). The bottle was shaken to distribute the organisms homogeneously; further dilutions were made as far as 10^{-6} in the same way (AOAC method 966.23).[19] Total aerobic plate counts (APCs) were performed after plating in duplicate on plate count agar (PCA) medium and incubating at 37 °C for 48 h to determine the total number of mesophilic bacteria.

To obtain liquorice extracts from the commercially ground roots, 20 ml of distilled water was added to each sample of 10 g from each treatment and mixed for 20 min. The suspension was made up to 200 ml with hot distilled water (80 °C) and mixed for 3 h. Total dissolved solids, Na^+, K^+ and Ca^{2+} contents and glycyrrhizinic acid were determined in the extracts prepared after 0 and 12 months of root storage. According to AOAC method 961.06,[19] 25 g of the extract was clarified by filtering through ashless Whatman No 42 filter paper (Whatman Laboratory Division, Maidstone, UK) and then dried for 6 h at 105 °C to determine the total dissolved solids using the following equation:

$$\text{dissolved solids (g kg}^{-1}\text{ ground roots)} = \frac{\text{dissolved solids (g)} \times \text{volume of total extract (200 ml)} \times 1000}{\text{volume of extract used (25 ml)} \times \text{weight of ground roots used (10 g)}}$$

The concentrations of Na^+, K^+ and Ca^{2+} cations were determined, using a flame photometer (Model ATS 200 MKI, Advanced Technical Services GmbH, Zug, Switzerland), after adding lithium chloride to the solution (25 mg kg^{-1}). Glycyrrhizinic acid was determined by means of a Milton Roy 1201 spectrophotometer (Milton Roy Co, Ivyland, PA, USA) at a wavelength of 258 nm.[20] To determine the viscosity, a suspension of commercially ground liquorice roots was prepared according to Hayashi *et al.* [21] The viscosities of the suspensions were measured using a viscometer (Model RTM, Townson and Mercer, Altrincham, UK) with a U-tube glass capillary ($C = 0.027171$).

For sensory evaluation, 25 ml aliquots of clarified liquorice extracts were placed in numerically coded glass beakers (50 ml). A sensory test (using a consumer-type panel comprising 20 staff members from different departments) was employed to detect sensory differences between irradiated and non-irradiated samples. Each member independently evaluated the extracts for taste, colour and flavour using a five-point scale (5 = very good taste, colour and flavour; 1 = very bad).

The samples consisted of extracts of roots subjected to five irradiation treatments and were presented in a completely randomised design with four replicates. The data were subjected to analysis of variance (ANOVA) using the SUPERANOVA (Abacus Concepts Inc, Berkeley, CA, USA) computer package. A separation test on treatment means was conducted using Fisher's least significant difference (PLSD) method[22] at the 95% confidence level. The D_{10} value was calculated using the Cricketgraph (Cricket Software, Malvern, PA, USA) computer package.

RESULTS AND DISCUSSION

Effect of gamma irradiation on microbial load

The effects of gamma irradiation on the microbial load of liquorice roots are presented in Table 1. The microbial count was $11 \times 10^4\,g^{-1}$ in unirradiated roots. Liquorice roots treated with 5, 10, 15 and 20 kGy of gamma irradiation had significantly ($P < 0.05$) lower counts than the untreated control, both immediately after irradiation and after 12 months of storage. The dose needed to reduce the microbial load by 1 log cycle (D_{10}) was 2.08 kGy. The microbial load ($\sim 10^5\,g^{-1}$) of the commercially ground liquorice roots used is considered unacceptable according to a report by the WHO,[23] which indicated that most untreated herbs, harvested and handled under hygienic conditions and tested by appropriate methods of sampling and examination, should contain no more than 10^4 bacteria g^{-1}. However, the maximum microbial load allowed in marketing liquorice in Syria is $5 \times 10^4\,g^{-1}$ according to the national standard.

The effect of gamma irradiation on the microbial load of commercially ground liquorice roots has not been previously investigated, but similar observations on other kinds of herbs were reported by Eiss and Merel[24] for basil, celery seed, chilli, oregano and thyme, Farkas[10] for sage, Muhammad *et al*[25] for bay leaves and rosemary, Bachman and Gieszczynska[26] for caraway seed, cardamom, charlock, juniper and marjoram, and Munasiri *et al*[27] for coriander.

The total microbial count in commercially ground liquorice roots was reduced to below $10^2\,g^{-1}$ by a dose of 10 kGy or above. This result is in agreement with

Table 1. Effect of gamma irradiation on microbial load of liquorice roots after 0 and 12 months of storage

	Microbial load (organisms g^{-1})	
Treatment	*0 months*	*12 months*
Control	11×10^4b	65×10^4c
5 kGy	16×10^2a	67×10^0b
10 kGy	11×10a	0a
15 kGy	<10a	0a
20 kGy	<10a	0a

Values within a column followed by the same letter are not significantly different at the 95% confidence level.

the findings of other workers, who reported that a dose of 10 kGy was required to eliminate micro-organisms from coriander.[27–29] In various natural herbs, doses of 3–10 kGy can reduce the viable cell counts to an acceptable level (from 10^5–10^7 to less than $10^4\,g^{-1}$).[10] Eiss and Merel[24] found that unirradiated celery seed, oregano and allspice had standard plate counts of 4.4×10^5, 1.2×10^6 and $2\times10^6\,g^{-1}$ respectively. A 10 kGy dose was capable of reducing all these counts to less than $10\,g^{-1}$. The same dose reduced the standard plate count of thyme from 1.5×10^5 to $40\,g^{-1}$. Akhtar *et al*[30] reported that a radiation dose of 8 kGy completely decontaminated black pepper and chilli.

The effectiveness of irradiation in the destruction of micro-organisms shown in our study in commercially ground liquorice roots ($D_{10}=2.08$ kGy) is in agreement with Ragelis *et al*,[31] who showed that, by using an irradiation dose of 10 kGy, micro-organisms in turmeric powder could be reduced by 6 log cycles. A lower dose of 5 kGy reduced the total plate count of black pepper by 3.4 log cycles and that of chilli by more than 2 log cycles.[27] The standard plate count of mico-organisms in basil was reduced by 3 log cycles at a dose of 10 kGy.[24] The standard plate count of samples of whole and ground red chilli was reduced by 4–6 log cycles with a 10 kGy dose.[32] Microbiological analysis demonstrated that a dose of 5 kGy reduced the total plate count by 2 log cycles in nutmeg.[33]

Our results indicated that the doses of gamma irradiation used interfered with the micro-organisms' reproductive ability and extended the acceptable storage period. A dose of 15 kGy was required to reduce the microbial count to less than $10\,g^{-1}$ immediately after irradiation, whereas the same effect was obtained using a dose of 10 kGy when the liquorice roots had been stored for 12 months after irradiation. These results are in agreement with those of Bachman and Gieszczynska,[26] who found that the survival rate of micro-organisms in caraway seed, marjoram, cardamom and charlock could be reduced to zero by a dose of approximately 15 kGy after 3 days of storage. When caraway seed and marjoram were stored for 2 weeks, total inactivation of micro-organisms was achieved with 12.5 kGy. After 6 weeks of storage, total elimination of micro-organisms occurred at an irradiation dose of 7.5 kGy for caraway seed and charlock and 10 kGy for marjoram and cardamom.

Table 2. Effect of gamma irradiation on total dissolved solids in extracts of liquorice roots after 0 and 12 months of storage

	Total dissolved solids ($g\,kg^{-1}$)	
Treatment	*0 months*	*12 months*
Control	280a	306b
5 kGy	280a	294ab
10 kGy	283a	292ab
15 kGy	284a	277a
20 kGy	285a	298ab

Values within a column followed by the same letter are not significantly different at the 95% confidence level.

Some chemical and physical characteristics of liquorice roots

Ground roots of liquorice consisting of $350\,g\,kg^{-1} < 0.35$ mm diameter, $300\,g\,kg^{-1}$ between 0.35 and 8.0 mm and $350\,g\,kg^{-1} > 8.0$ mm were used. The mean characteristics of the roots were: moisture content, $80\pm8\,g\,kg^{-1}$; organic solids, $814\pm10\,g\,kg^{-1}$); inorganic solids, $106\pm6\,g\,kg^{-1}$.

Effect of gamma irradiation on liquorice extract and its characteristics

Immediately after irradiation, all treatment means were not significantly different from that of the unirradiated control. However, after 12 months of storage the total dissolved solids extracted from the 15 kGy-irradiated roots were significantly ($P<0.05$) lower than those produced from the unirradiated ones (Table 2). Because the moisture content in the roots was about $80\,g\,kg^{-1}$, the main effect of gamma irradiation on microbial loads could only be a direct one. Farkas *et al*[34] reported that the direct and indirect effects of ionising radiation on plant materials in general are 10 and 90% respectively. The doses of gamma irradiation used are, at a lower level, sufficient to induce degradation of large molecules in the tissues of liquorice roots. However, Al-Masri and Zarkawi[35,36] observed degradation of the complex carbohydrates in barley straw, sorghum straw, wheat chaff and maize cobs when doses of more than 50 kGy were used.

Effect of gamma irradiation on mineral ions in liquorice extract

Table 3 shows that immediately after irradiation the sodium levels in extracts of liquorice produced from irradiated roots were significantly ($P<0.05$) higher in the 5 and 20 kGy treatments than in that of the unirradiated control. However, after 12 months of storage, all the doses used reduced the concentration of sodium in the extracts significantly compared with the control.

For calcium concentration, significantly ($P<0.05$) lower values were obtained at 15 and 20 kGy immediately after irradiation. However, after 12 months of storage, 10, 15 and 20 kGy all had the same effect (Table 3). Regarding potassium, the different doses had no effect immediately after irradiation. However, the potassium concentrations in the extracts increased significantly ($P<0.05$) during storage for all doses of irradiation.

The increases in K^+ and Ca^{2+} in extracts produced from irradiated liquorice roots after 12 months of storage may be attributed to the degradative effect of gamma irradiation on insoluble macromolecules,

Table 3. Effect of gamma irradiation on mineral ions (Na^+, Ca^{2+} and K^+) in extracts of liquorice roots after 0 and 12 months of storage

	Mineral ions (mg l^{-1})					
	Na^+		Ca^{2+}		K^+	
Treatment	0 months	12 months	0 months	12 months	0 months	12 months
Control	44.3a	38.3c	50.0b	84.4c	220.0a	278.0c
5 kGy	54.6b	23.7b	50.0b	77.8bc	217.0a	255.0b
10 kGy	45.6a	16.3a	50.0b	68.8a	215.0a	261.0bc
15 kGy	48.0a	15.0a	40.0a	71.1ab	211.0a	248.0b
20 kGy	54.7b	17.3ab	40.0a	64.4a	222.0a	229.0a

Values within a column followed by the same letter are not significantly different at the 95% confidence level.

glycyrrhizinic acid and soluble components produced, such as glycyrrhizinic salts. Our results are in agreement with those of Al-Masri,[37] who indicated that high doses of gamma irradiation (50 kGy) induced degradation of macromolecules in some feedstuffs.

Glycyrrhizinic acid evaluation

Glycyrrhizinic acid concentrations in the extracts produced from irradiated and unirradiated ground liquorice roots after 0 and 12 months of storage are shown in Table 4. All the doses used decreased the glycyrrhizinic acid concentration significantly in extracts obtained immediately after irradiation. However, after 12 months of storage the concentration of glycyrrhizinic acid in the extract from roots irradiated with the highest dose (20 kGy) was lower than in extracts produced from unirradiated roots or from roots receiving lower levels of irradiation.

The reduction of glycyrrhizinic acid content in the extracts obtained immediately after irradiation may be attributed to the oxidising effect of gamma irradiation on glycyrrhizinic acid.

No information is available concerning the effect of gamma irradiation on the glycyrrhizinic acid of liquorice roots. However, chemical analysis, after irradiation at similar doses, of other herbs and spices has been carried out. There is contradictory information about such effects on other kinds of plants, but some of them are in agreement with ours. Bachman *et al*[38] reported that a dose of 15 kGy decreased the yield of essential oils of cardamom from 48.6 to 39.3 g kg^{-1}. The same authors observed a slight decrease in VOSD (volatile oil content extracted by steam distillation) after irradiating celery seed (25.8 g kg^{-1} control, 25 g kg^{-1} irradiated). In addition, Eiss and Merel[24] showed a decrease in VOSD in irradiated allspice. Other studies were contrary to these and no significant differences were seen in VOSD between irradiated and unirradiated samples of thyme, oregano and pepper[24] or in the GC volatile profiles of coriander and cumin.[28] There were no marked differences in VOSD and NVMCE (non-volatile oil content derived by methylene chloride extraction) between untreated and irradiated pepper and chilli at a dose of 10 kGy.[39] No significant change in the contents of chemical constituents, including essential oils, fatty acids and sugars, was observed in caraway seed irradiated at doses of 7.5–10 kGy.[38] Also, in paprika, non-volatile oils were not significantly altered by irradiation.[13] A 10 kGy irradiation dose did not significantly affect the content of essential oils of eight kinds of spice.[12] Irradiation doses of less than 10 kGy can be used for processing cardamom, nutmeg, clove and pepper without producing any changes in volatile components.[40]

Viscosity

The effects of gamma irradiation on the relative viscosity of liquorice suspensions are shown in Table 5. It can be seen that, immediately after irradiation, all the doses used reduced the viscosity of liquorice suspensions significantly as compared with the control. However, after 12 months of storage, no signi-

Table 4. Effect of gamma irradiation on glycyrrhizinic acid in extracts of liquorice roots after 0 and 12 months of storage

	Glycyrrhizinic acid (mg g^{-1})	
Treatment	0 months	12 months
Control	20.64b	8.06b
5 kGy	16.37a	8.21b
10 kGy	16.42a	8.45b
15 kGy	15.60a	7.78ab
20 kGy	14.12a	6.73a

Values within a column followed by the same letter are not significantly different at the 95% confidence level.

Table 5. Effect of gamma irradiation on relative viscosity of liquorice suspensions after 0 and 12 months of storage

	Relative viscosity (cP)	
Treatment	0 months	12 months
Control	5.30c	1.44a
5 kGy	2.66b	1.36a
10 kGy	1.96a	1.41a
15 kGy	1.91a	1.41a
20 kGy	1.95a	1.38a

Values within a column followed by the same letter are not significantly different at the 95% confidence level.

Table 6. Effect of gamma irradiation on sensory characteristics of liquorice roots extracted after irradiation[a]

Treatment	Taste	Flavour	Colour
Control	3.14b	3.50b	3.32bc
5 kGy	3.04b	3.06ab	3.41bc
10 kGy	3.32b	3.24b	3.70ac
15 kGy	3.29b	3.25b	3.75c
20 kGy	2.25a	2.49a	2.75a

[a] Data represent a five-point scale ranging from 1 (very bad) to 5 (very good).
Values within a column followed by the same letter are not significantly different at the 95% confidence level.

ficant differences were seen among suspensions prepared from irradiated and unirradiated liquorice roots.

The differences in viscosity between irradiated and non-irradiated roots immediately after irradiation may be attributed to the degradation and gelatinisation of starch and glycyrrhizinic acid in the roots. Nene *et al*[41] found that starch is degraded by ionising radiation, resulting in a decrease in viscosity. Our results are also in agreement with Hayashi *et al*,[21] who found that the viscosity of extracts of irradiated peppers (5 kGy) was higher than that of non-irradiated samples.

Sensory evaluation

The consumer panel data indicated that no significant ($P < 0.05$) differences in taste, flavour or colour were observed between irradiated (5, 10 and 15 kGy) and control liquorice extracts (Table 6), whereas the highest dose (20 kGy) significantly decreased the taste, flavour and colour. The effect of gamma irradiation on the sensory characteristics of liquorice extract has not been reported previously. We found that the 20 kGy dose caused noticeable changes in these characteristics, but doses of 5–15 kGy, which were sufficient for 'pasteurisation', did not influence these properties. Farkas[10] reported that organoleptic changes in more than 15 crops were not detectable by sensory panels until dose levels exceeded 10 kGy.

CONCLUSION

The results of this study indicated that a 10 kGy dose of gamma irradiation is effective for microbial decontamination of commercially ground liquorice roots. Immediately after irradiation the doses used reduced the viscosity of liquorice suspensions. However, after 12 months of storage, no significant differences were seen among suspensions prepared from irradiated and unirradiated liquorice roots.

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