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Radiation decontamination of herbs and spices

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Abstract A review regarding application of radiation for decontamination of herbs and spices is presented. General principles of the process and the results of the research on biologically active components are discussed. The report regarding the development in this field in selected countries and regions is included.

Key words herbs • spices • radiation decontamination • bioactive agents

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Introduction

Despite the great advances observed in modern synthesis-based pharmacy, medicinal plants still make an important contribution to health care. There has been a growing interest in the alternative therapies in recent years, especially those from plants. The World Health Organization estimates that about 65–80% of the world's population living in developing countries depends essentially on medicinal plants (herbs) for primary health care.

Spices and herbs are often contaminated with high levels of bacteria, moulds and yeasts. Most often the microorganisms, which are present on the surface of the plant, are a mixture of epiphytic microflora, growing on specific varieties of plants, and the microflora originating from the plant environment, namely soil, water and air. Pathogenic microorganisms may grow on some herbs and spice plants, and quite often they grow not only on the surface, but inside of the plant's tissue as well. If untreated, the herbs and spices will result in rapid spoilage of the products which are supposed to enhance. When contaminated with pathogenic bacteria, they can also result in serious food-borne illnesses.

Spices are usually decontaminated by irradiation or by fumigation with ethylene oxide gas (EtO). Some spices can be also steam treated. There is an extensive body of research available on the irradiation of spices, herbs and vegetable seasonings. Most products have been studied with at least preliminary if not extensive research, and the effects of irradiation on microbial contamination and sensory properties have been quantified. Since irradiation is a clearly preferable sanitation method, its use has been allowed by CODEX and by most countries worldwide [16]. While the use of EtO is allowed in North America, its use is not allowed in many European countries because it is a carcinogen when inhaled and it leaves harmful chemical residues in the spice.

Bacterial contamination

Typical pathogenic microorganisms present in herbs are *Staphylococcus aureus*, *Salmonella*, *Pseudomonas aerginosa, Clastridium perfringens* and *Candia albicans*.

Opportunistic microorganisms are present as well; these are non-pathogenic microorganisms which, in specific conditions, may cause infection. These are *Staphylococcus* type, *Enterococcus* and other rods from the *Enterobacteriaceae* family and *Pseudomonas* type, other nonfermentous rods, aerobic rods *Bacillus* type, most of anaerobic rods *Clostridium* type, fungi imperfecti *Candida* and *Rhodotorula* type, and mould fungi, mostly *Mucor*, *Rhizopus* and *Aspergillus* type.

There are different reasons as to why the infection of the human organisms is caused by a non-pathogenic microorganism. These are metabolic aberrations (diabetes, renal insufficiency etc., long-lasting antibiotic therapy, radiotherapy etc.). Especially sensitive to such a type of infections are children and elderly people.

The other actions of microorganisms which are present in herbs may affect the quality of the final product. The chemical compound being a component of the drug may be destructed or its composition changed. Enzymes produced by microorganisms may react with other components of the drug to produce toxins or compounds having other pharmaceutical effects.

The requirements regarding microbiological purity for herbs were established by WHO [15].

Medicinal plant materials are divided in to three groups:

(a) High microbiological contamination – containing not more than 10,000 rods *E. coli* and 100,000 mould fungi in one gram. Chemical or physical decontamination is necessary.

(b) Medicinal plant materials for preparation of teas and external applications. The number of aerobic bacteria should not be higher than 10,000,000 CFU in one gram, fungi imperfecti (*Saccharomycetes*) and yeast (*Hyphomycetes*) up to 1000 in one gram, *E. coli* rods up to 100 in one gram and *Salmonella* rods should not be present in the material.

(c) Medicinal plant raw materials for preparations for internal use. Aerobic bacteria less than 100,000 in one gram, fungi imperfecti and yeast up to 1000 in one gram, *E. coli* up to 100 in one gram and other rods from enterobacteriae do 1000 in one gram. *Salmonella* rods should not be present in the material.

Comparison of different sterilization methods

High contamination of herbs due to the presence of endopores of *Bacillus* and *Clostridium* and spores which are very resistant to the chemical and physical methods of decontamination, creates significant difficulties in the production of phyto preparations. Application of the physical or chemical methods, which reduce microbiological contamination to the requested level, quite often leads to the reduction of the content of the biologically active components.

Irradiation

Use of ionizing radiation as a physical method of microbiological decontamination of food, including spices and herbs, was approved by the Codex Alimentarius Commission – CDC. The experts agreed that radiation does not cause any toxicological changes or activation of irradiated food products, therefore, toxicological tests for food treated by this method are not needed. Gamma rays or X-rays up to 5 MeV and electrons up to 10 MeV energy can be used for this purposes and dose up to 10 kGy is allowed [9, 17]. The sensory properties of most spices are well maintained between 7.5–15 kGy. Research clearly indicates that irradiation maintains the sensory properties of spices, herbs and vegetable seasonings better than EtO treatment [6]. Generally, the sensory properties of spices are more resistant to irradiation than are some herbs. Also, herbs are more damaged by EtO treatment. Herbs are, therefore, more sensitive to treatment of any kind. At the doses required to control microbial contamination, insects and other pests will be killed in all life stages.

Radiation treatment results in cleaner, better quality herbs and spices compared to that fumigated with ethylene oxide gas (EtO). While both decontamination or sterilization methods result in some changes to some spices, radiation does not change the sensory or functional properties to the same extent as EtO. This is an important export consideration [6].

Gas fumigation

EtO is commonly used to decontaminate spices, with varying degree of success. Use of ethylene oxide is, however, prohibited in many countries (Japan, some countries of EEC, the United Kingdom) because it reacts with organic spice components to leave the harmful residues ethylene chlorohydrin and ethylene bromohydrin on spices. Ethylene chlorohydrin is a known carcinogen that persists in the spice for many months, even after food processing. For this reason, and because of worker safety issues, its use in the United States is under review by the Environmental Protection Agency under the Food Quality Protection Act (residues levels of 50 ppm are currently allowed). In Canada, EtO cannot be used on vegetable seasonings or spice mixtures containing salt (residues of 1500 ppm are currently allowed).

In some countries, propylene oxide is also allowed, although prohibited in other countries because of the residues it leaves in spices. Methyl bromide is also sometimes used to disinfest spices; this use will be phased out as methyl bromide comes under the Montreal Protocol for ozone depleting substances. The use of

Table 1. Comparison of different physical and chemical methods of decontamination of herbal raw materials

Methods of decontamination	Efficiency of decontamination [%]	Number of microorganisms in 1 g of herbal raw material after decontamination
Ethylene oxide	99.960	400
Methyl bromide	96.830	31,700
Ionizing radiation	99.988	120
Steam under pressure	99.994	60

methyl bromide for spices also results in higher levels of the residue ethylene bromohydrin in EtO treated spices.

The use of EtO has recently become more difficult as a result of environmental controls of its use. The instability and flammability of EtO requires it to be mixed with another gas. Formerly, the gas used was CFC but its use is no longer allowed as ozone depleting substance. Now, EtO is stabilized with much less effective CO_2 (usually at 80%) and delivered with steam. Worker safety issues have greatly complicated the use of EtO for all purposes. The World Health Organization has recently upgraded EtO to a known carcinogen. Since EtO leaves spices slowly after the treatment, the product must be stored open to allow the gas to dissipate. The gas is then released in the warehouse environment. The necessity to store EtO treated spices in open containers before shipping or sale results in increased warehousing cost, inconvenient product hold-up and increased risk of recontamination.

When the standards for microbiological contamination of spices and herbs are set very low, as is seen with processors operating under HACCP or ISO standards, EtO can be very inconvenient. After the treatment, the spices should be allowed to outgas EtO (approximately one week). Then, bacteriological tests should be conducted to see if the product meets the standards (it takes 2–3 days). Then, if the spices do not meet the standards, the treatment must be repeated. Some EtO processors try to circumvent this system simply by running the spices through the treatment twice, before even doing the tests. This can easily result in unacceptably high levels of the chemical residues.

Spices and herbs destined for EtO treatment must be packaged in materials that allow the gas to enter the package. Bulk barrels must be opened for treatment. Such packaging requirements allow for recontamination. EtO sanitation is not compatible with never packaging materials that are impervious to gas or are heat-sealed plastics.

Steam

Application of high pressure steam for decontamination of herbs was introduced more than 20 years ago. The procedure is as follows: the air is evacuated, the material is treated with steam at 100–200°C temperature, dried by hot air and quickly cooled. There are other methods that use higher pressure steam. This method is well accepted to treat spices, however the losses of etheric oils are up to 20%.

Comparison of different physical and chemical methods is presented in Table 1 [3].

Conditions of treatment:

- EtO – 1 g/L; 6 hours,
- methyl bromide – 0.1 g/L; 16 hours,
- ionizing radiation – 10 kGy,
- steam – 121°C; 15 minutes.

Efficiency is defined as the percentage of microorganisms which survive the treatment at specified conditions.

Influence of radiation on bioactive agents

A study on the influence of different dose of radiation on medical properties of radiation decontaminated medicinal plants was performed in Brazil [5]. *Rosmarinus officinalis* Linné is a plant that is eatable and demonstrates medicinal properties. Rosemary extract is already commercialized as antioxidant, and its actions are due to the phenolic compounds. *Nasturium officinale*, also known as watercress, is largely cultivated in Brazil. Despite the nutritional value, this vegetable has important medicinal properties as depurate and diuretic. Artichoke (*Cynara scolymus*) is used as diuretic in the treatment of diabetes, due to the flavonoidic property. The leafy parts of basil (*Ocimum basilicum*) have great uses in cooking, besides the tonic and antiseptic properties. Local herb companies in São Paulo, Brazil, provided dehydrated samples of rosemary (*Rosmarinus officinalis* Linné), watercress (*Nasturium officinale* R. Br), artichoke (*Cynara scolymus* Linné) and sweet basil (*Ocimum basilicum* Linné). The samples were irradiated in plastic packages in a ^{60}Co Gammacell 220 (AECL) installed in IPEN (São Paulo, Brazil). The applied radiation doses were 0, 10, 20 and 30 kGy, and the dose rate was 5.8 kGy/h.

Thin layer chromatography was used to analyze flavonoids and essential oils.

The determination of the concentration of phenolic compounds was performed and the tannins were quantified as beta-carotene analysis: the decontamination of medicinal plants by the open-column chromatographic method was performed to analyze and quantify total beta-carotene and its provitamin A activity.

From the described pharmacological tests carried out by this study, it is concluded that phytotherapy showed identical therapeutical action as non-irradiated preparations after exposure to a dose of 10, 20 and 30 kGy of ionizing radiation [5].

Evaluation of the effects of ionizing radiation on the microbial burden and on the active ingredients in two medicinal herbs (ginkgo and guarana) was reported by Soriani *et al*. [13]. The total aerobic microorganism count was, on the average, 10^6 CFU/g for both herbs, and the fungi count was about 10^3 and 10^6 CFU/g for ginkgo and guarana, respectively.

As for the enterobacteria count, it was observed that all ginkgo samples presented undetectable quantity. An inherent resistance of the drug cannot be ignored, since some works have revealed antimicrobial action of ginkgo. Although the samples of guarana from two suppliers showed an average count of 10^3, enterobacteria could not be detected in the sample from the third supplier, which may be explained by the differences in the procedures concerning cultivation, harvest, and post harvest handling. The possible use of any decontamination method can also cause this absence. Concerning research on specific microorganisms, *E. coli* was found in guarana from some suppliers and *P. aeruginosa* in guarana from others. After irradiation, all samples showed reduction of total aerobic counts to a level of $\leq$ 10 CFU/g when submitted to an average dose of 11.4 kGy. As for fungal contamination, an average dose of 5.5 kGy was enough to reduce the counts to acceptable levels. Enterobacteria are relatively sensitive to radiation and, in most cases, a dose of about 5 kGy is sufficient for their elimination. After irradiation up to 17.8 kGy, the content of the main biologically active substances was not modified [13].

Medicinal plants play a very important role in the traditional medicine of the East Asia region. In the test performed in Vietnam, HaSinh (*Polygonum multiflorum Thumb* and *Rehnannia glutinosa Libosch*), *Rheumatine* (sea-snake extract, *Lapenis hardiwickii Gray*), snake extract based medicine (mixture with anthraglycoside, tannin, saponin, alkaloid, essence and fatty oils) and *Samcotagiao* (*Radix codonopsic, Radix angelica sinensis*, *Polygonum multiflorum Thumb* and animal bones), were treated by gamma radiation with doses of 22, 33, 18 and 22 kGy, respectively. The changes of physical and chemical properties after irradiation with these doses were shown to be not over the accepted levels [2].

Korean medicinal herbs are known to include high amounts of antioxidants such as tocopherols, ascorbic acid and carotenoids. Flavonoids are phenol derivatives synthesized in substantial amounts (0.5–1.5%) and widely distributed in plants. These compounds have been found to possess antioxidant and free radical scavenging activity in foods. They are hydroxylated, methoxylated, and/or glycosylated derivatives of the 2-phenylbenzo(a)pyrane ring, which consist of two benzene rings combined by conjunction of the oxygen-containing pyrane ring. In general, the leaves, fruit, roots and other tissues of the plant contain glycosides and the woody tissues contain aglycons. Phenolic acids are phenolic antioxidants in foods and are often esterified with flavonoids. The results of the tests indicated that gamma irradiation for hygienic quality of Korean medicinal herbs at 10 kGy had no effect on antioxidative capacity [11].

Effects of gamma irradiation on hygienic quality and extraction yields for twenty one kinds of Korean medicinal herbs are reported [8]. Gamma irradiation at 5–10 kGy inactivated contaminating microorganisms. The total extraction yield in the fifteen kinds of the investigated medicinal herbs increased by 5–25% with a dose of 10 kGy. These results show that gamma irradiation up to 10 kGy is an effective method for increasing extract yields as well as for reducing microbial contamination of Korean medicinal herbs.

To consider the possibility of the application of radiation technology for the Korean traditional medicinal herbs, the genotoxicological safety and stability of the active components of the γ-irradiated *Paeoniae Radix* were studied. The herb was irradiated with γ-rays at a practical dosage of 10 kGy, and then was extracted with hot water. The genotoxicity of the extract of the irradiated herb was examined in two short-term *in vitro* tests: (1) Ames test in *Salmonella typhimurium*; and (2) micronucleus test in cultured Chinese hamster ovary (CHO) cells. The extract of the irradiated herb did not show mutagenicity in the Ames test of the *Salmonella* reverse mutation assay, and did not show cytogenetic toxicity in the culture of the CHO cells. HPLC chromatogram of paeoniflorin in the irradiated *Paeoniae Radix* was similar with that of the non-irradiated sample; the quantity of paeoniflorin did not change significantly with irradiation. These results suggest that γ-irradiated *Paeoniae Radix* is toxicologically safe and chemically stable [19].

Several thousand tons of medicinal herbs are produced annually by the pharmaceutical industry in Poland. This product should be of high quality and microbial purity. Recently, chemical methods of decontamination are recognized as less safe, thus irradiation technique was chosen to replace them. At the Institute of Nuclear Chemistry and Technology, Warsaw, Poland, research on the microbiological decontamination of herbs by irradiation is being carried out since 1996, when the national programme on the application of radiation to the decontamination of medicinal herbs was implemented. The purpose of the programme was to elaborate, on the basis of research work, on the facility standards and technological instructions indispensable for the practice of radiation technology. It was shown that the use of ionizing radiation (a dose of 10 kGy) can ensure satisfactory results of microbiological decontamination of these products [7]. The content of essential biologically active substances such as essential oils, flavonoids, glycosides, anthocyanins, anthra-compounds, polyphenoloacids, triterpene saponins, oleanosides and plants mucus did not change significantly after irradiation. Pharmacological activity of medicinal herbs has been found satisfactory after microbiological decontamination by irradiation [12].

One of the best report on the subject was prepared in the frame of this programme [3]. Influence of different methods of decontamination on the stability of the main biologically active substances is listed in Table 2. Conditions of treatment as specified at Table 1.

Detection

The essential control procedures have to be developed and adopted in the countries that approve irradiation treatment of spices, herbs and other food items [14].

Table 2. Influence of different methods of decontamination on the stability of the main biologically active substances in herbal raw materials

Main substances in herbal raw materials	Methods of decontamination			
	Ethylene oxide	Methyl bromide	Ionizing radiation (dose 10 kGy)	Steam under pressure
Flavonoids	+	+		+
Polyphenylo-compounds				+
Tannins	–		–	+
Essential oils	+	–	+	–
Steroid saponins	+	+		
Anthra-compounds	+		–	+
Triterpene saponins	–		+	+
Iridoids	–		+	+
Phenolic glycosides	+		–	+
Coumarins		+	+	+
Cardic glycosides			+	
Oleanosides			+	+
Sulphur compounds			–	
Flavonoligands				+
Alkanoids				+
Plants mucus				–

+ Stable substances. – Unstable substances.

Eight types of spices and herbs or their mixtures of Asian origin have been investigated for the detection of the irradiation treatment using thermoluminescence (TL) of insoluble mineral contaminants adhering to the samples. These samples were irradiated by ^{60}Co-rays (at absorbed doses of 1, 5 and 10 kGy) as well as by 10 MeV electrons using an accelerator (at a dose of 5.4 kGy). The integrated TL intensity of the glow curve for all the irradiated samples was found to be much higher than that from the unirradiated samples. These results were normalized by administering a re-irradiation dose of 1 kGy and calculating the ratio of the integral of the first glow curve (of unirradiated or irradiated samples) to that of the second glow curve (after re-irradiation). This ratio is less than 0.02 for all unirradiated samples and more than 0.3 for all irradiated samples (more than 1 for samples irradiated to 5 kGy or at higher doses), thereby making discrimination between irradiated and unirradiated samples possible. If one also compares the different temperature regions of the glow-curve maxima of the unirradiated and irradiated samples, unequivocal discrimination is achieved for those previously irradiated to doses equal to or greater than 1 kGy [4].

Non-irradiated and gamma-irradiated dry herbs savoury (*Savoury*), wild thyme (*Thymus serpollorium*) and marjoram (*Origanum*) with an absorbed dose of 8 kGy have been investigated by the methods of electron paramagnetic resonance (EPR) and thermoluminescence (TL). Non-irradiated herbs exhibit only one weak singlet EPR signal, whereas, its intensity increases in the irradiated samples, and, in addition, two satelite lines are recorded. This triplet EPR spectrum is attributed to cellulose free radical generated by irradiation. It has been found that upon keeping the irradiated samples under normal storage conditions, the life-time of the cellulose free radical in the examined samples is ~ 60–80 days. Thus, it can be concluded that the presence of an EPR signal of cellulose free radical is an unambiguous indication that the sample under study has been irradiated, but its absence cannot be considered as the opposite evidence. In the case when the EPR signal is absent, the method of TL should be used to give the final decision about the previous radiation treatment of the sample [18].

Developments in different countries

Regulatory situation

The Joint FAO/WHO Codex Alimentarius Commission and many other regulatory authorities have established principles for the irradiation of foods together with the essential control procedures. Worldwide, over 41 countries have approved food irradiation for more than 60 food products. The irradiation of spices is allowed in most countries. International standards such as CODEX have accepted it as a beneficial treatment. Most countries require irradiated spices to be labeled with the international symbol for irradiation, and sometimes words to describe the process and/or the effect (such as treated to destroy harmful bacteria).

Major producing countries such as India allow the irradiation of spices; major importing countries such as the United States and Canada both accept and use the process commercially [10]. In the US, over 30.00 Mg of spices, herbs and dry ingredients are irradiated each year. In the United States and Canada, irradiated spices have to be labeled with the international symbol for irradiated foods and wording. However, in Canada, irradiated ingredients in the processed food are required to be labeled.

In Australia and New Zealand, the food regulator have given the go-ahead to irradiate herbs and spices at a level much higher than most other countries [1].

The Brazilian pharmaceutical industry has a US$9 billion market, and is among the 10 largest in the world. Around 30% of the marketed drugs registered by the Federal Health Office are classified as phytopharmaceutical, representing 20–25% of the local pharmaceutical market. In addition, Brazil exports considerable quantities of medicinal plants, extracts and isolated substances, reaching the value of US$22 million per year.

Despite their importance, 70% of the phytopharmaceutical resources have not been sufficiently studied to provide the necessary confirmation of their efficacy and safety, as every medicine actually demands. Since ethylene oxide is no more allowed as a sterilizing agent for herbs, use of irradiation process is increasing in Brazil.

In Europe, EU Directive 1999/2/EC provides the regulations concerning foods and food ingredients treated with ionizing radiation. To date, only one food category – dried herbs, spices and vegetable seasonings – has been included in the list of foods that may be irradiated, although other food categories have been nominated. The Directive specifies provisions including the source of ionizing radiation, controls of the levels of radiation permitted and food labeling requirements. Conditions are also specified for the importation of irradiated foods.

Conclusions

Radiation decontamination of medicinal plants and spices is a safe and very effective method. The losses of the biologically active substances is lower than in the case of other decontamination methods.

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