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IRRADIATION AS AN ALTERNATIVE ENVIRONMENT FRIENDLY METHOD FOR MICROBIOLOGICAL DECONTAMINATION OF HERBAL RAW MATERIAL

P. GORECKI, B. KĘDZIA
Research Institute of Medicinal Plants, Poznań

W. MIGDAŁ, H. B. OWCZARCZYK
Institute of Nuclear Chemistry and Technology, Warsaw

Poland

Abstract

Microbiological contamination of herbals raw materials is a serious problem in the production of therapeutical preparations. A good quality of the product, according to the pharmaceutical requirements may be achieved by applying suitable methods of decontamination. The decontamination treatements should be fast and effective against all microorganisms. It should ensure the decontamination of both packaging and the product in order to act effectively against all the microorganisms present and must not reduce the sensory and technological qualities of the commodities.

In the paper, the results of comparative investigations on the microbiological decontamination of herbal raw materials by chemical (ethylene oxide, methyl bromide) and physical method (irradiation) are presented.

Decontamination of herbal raw materials by irradiation is a method by choice. It is because chemical methods are recognized recently as not safe to the consumer. Irradiation, in turn, is technically feasible, very effective and friendly enough to environment process.

Introduction

Under the prevailing production and handling conditions, most herbs contain a large number of microorganisms what is a serious problem in the production of therapeutical preparations. The microorganisms present are those, which are indigenous to the soil and surroundings in which herbs are grown, and which survive the drying process.

For several years the most widely used methods for decontamination of herbs was fumigation with ethylene oxide or methyl bromide. Both methods are today banned in most countries. Irradiation is an alternative and safe method for effective reducing the microbial contamination of herbal raw materials.

Below the results are presented of comparative investigations on the microbiological decontamination of herbal raw materials by chemical and physical methods.

Results

The effect of ethylene oxide in concentration 1.000 mg/dm^3 , time of action 6h, temperature 56°C , pressure 0,55 MPa and relative moisture 40% on microbiological decontamination of herbal raw materials illustrate Table I.

Table I. Effect of ethylene oxide on the number of microorganisms occurrence in herbal raw material.

Microorganisms	Number of microorganisms in 1g of plant material * A	Number of microorganisms in 1g of plant material * B
Aerobic bacteria	8.670.000	840
Yeast and moulds	69.000	0
Enterobacteriaceae bacilli	27.300	0
Enterococci	1.600	0
Bacillus spores	23.000	710
Clostridium spores	190	58

* Average from 11 samples
A - before decontamination; B - after decontamination.

As seen in the Table I, microbiological decontamination reaches 99,960%. It means, that out of every 1.000.000 microorganisms occuring in 1g of herbal raw material only 400 microorganisms survive after the decontamination.

Fumigation with ethylene oxide effects some biological active substances as present in herbs. The results of investigation as show in Table II prove the decrease in signifficant degree of the content of tannis in Folium Betulae (25%), glycyrhizic acid in Radix Glycyrrhizace (29%) and valepotrates in Radix Valerianae (11%).

In some flavonoid raw materials (Herba Hyperici), glycoside raw materials (Cortex Salicis), saponin raw materials (Semen Foenugraeci) and essential raw materials (Folium menthae Piperitae, Folium Salviae, Fructus Juniperi), the losses of the main biological active substances are up to 10%.

Table III present the results of the microbiological decontamination by fumigation with methyl bromide of some raw herbal materials. Herbal raw materials were exposed to the action of methyl bromide in concentration 100 mg/dm^3, time of action 16h, pressure 0,08 Mpa, temperature 15°C and relative moisture 60%.As seen in the Table methyl bromide decreases the number of microorganisms in herbal raw materials by 96.830%. It means, that out of every 1.000.000 microorganisms occuring in 1g of herbal raw materials 31700 microorganisms remain after the decontamination. Therefore, it is not a process allowing to obtain the high microbiological purity of herbal raw materials.

The content of biological active substances in some herbal raw materials exposed to the action of methyl bromide decreases significantly (Table IV). The content of the essential oils in Folium Menthae piperitae and Folium Salviae decreases for 29% and 25% , respectively.

The effect of irradiation on the microbiological decontamination herbal raw material is given in Table V. The samples were irradiated by electron beam from the accelerator ELEKTRONIKA 10-10 (10 MeV, 10 kW) at the dose 10 kGy. After irradiation, the reduction of microorganisms in herbal raw materials is 99,988%. It means that from 1.000.000 microorganisms as present in 1g of raw material before decontamination, only 120 ones remain alive after using this method.

Table II. Influence of ethylene oxide on the content of main biological active substances in herbal raw materials.

Herbal raw materials	Biological active substances	Content of substances (%) A	Content of substances (%) B	Decrease of substances content (%)
Herba Hyperici	flavonoids	0.67	0.65	3.1
Folium Betulae	flavonoids	1.12	1.12	0
Folium Salviae	flavonoids	0.34	0.34	0
Folium Menthae piperitae	essential oil	1.70	1.66	2.4
Folium Salviae	essential oil	0.78	0.78	0
Anthodium Chamomillae	essential oil	0.63	0.61	3.2
Fructus Juniperi	essential oil	1.95	1.95	0
Semen Foenugraeci	diosgenin	0.54	0.54	0
Cortex Frangulae	glucofranguline	6.09	6.09	0
Cortex Salicis	phen.glucosides	13.31	12.51	6.0
Folium Betulae	tannins	11.10	8.38	24.5
Radix Glycyrrhizae	glycirrhizinic acid	19.58	13.91	29.0
Radix Valerianae	valepotriate	0.73	0.65	11.0

A - before decontamination, B - after decontamination

Table III. Effect of methyl bromide on the number of microorganisms occurrence in herbal raw material.

Microorganisms	Number of microorganisms in 1g plant material * A	Number of microorganisms in 1g of plant material * B
Aerobic bacteria	359.000	9.900
Yeasts and moulds	40.300	2.100
Enterobacteriaceae bacilli	14.200	0
Enterococci	10.100	0
Bacillus spores	37.700	2.200
Clostridium spores	920	800

* Average from 17 samples
A - before decontamination, B - after decontamination

Table IV. Influence of methyl bromide on the content of main biological active substances in herbal raw materials.

Herbal raw materials	Biological active substances	Content of substances (%) A	Content of substances (%) B	Decrease of substances content (%)
Folium Menthae piperitae	essential oil	1.70	1.20	29.4
Folium Salviae	essential oil	1.10	0.80	24.9
Fructus Juniperi	essential oil	0.90	0.90	0
Herba Hyperici	hyperoside	0.78	0.67	14.1
	rutoside	0.40	0.36	10.0
	hypericin	1.96	1.90	3.1
Inflorescentia Tiliae	flavonoids	0.65	0.65	0
Semen Foenugraeci	flavonoids	0.05	0.05	0
	diosgenin	0.52	0.52	0

A - before decontamination, B - after decontamination

Table V. Effect of irradiation on the number of microorganisms occurence in herbal raw materials.

Microorganisms	Number of microorganisms in 1g of plant material * A	Number of microorganisms in 1g of plant material * B
Aerobic bacteria	12.675.000	86
Yeasts and moulds	198.000	5
Enterobacteriaceae bacilli	11.900	0
Enterococci	2.900	0
Bacillus spores	79.600	74
Clostridium spores	2.200	3

* Average from 17 samples.
A - Before decontamination, B - after decontamination

The content of biologically active compounds before and after irradiation the herbal raw materials is given in Table VI. In the most of herbs the content of biological active substances did not change in a signifficant degree after irradiation. The decrease by 10% is considered as typical one. The following raw materials have been examined: Folium Salviae, Fructus Juniperi, Folium menthae piperitae, Rhizoma Calami, Herba Hyperici, Folium Digitalis lanatae, Flos Calendulae, Cortex Hippocastani, radix Glycyrrhizae and Radix Valerianae. Slightly higher changes of the content of the biological active substances from 12.4 to 15% were found in Herba Abrotani, Cortex Salicis, Rhizoma Tormrntillae and Cortex Frangulae.

Table VI. Influence of irradiation on the content of main active sunstances in herbal raw materials.

Herbal raw materials	Biological active substances	Content of substances (%) A	Content of substances (%) B	Decrease of substances content (%)
Herba Hyperici	flavonoids	0.77	0.77	0
	hypericin	0.20	0.20	0
Folium Digitalis lanatae	lanatoside C	0.28	0.28	0
Flos Calendulae	oleanosides	3.71	3.71	0
Cortex	esculin	5.60	5.30	5.4
Hippocastani	fraxin	3.00	2.90	3.3
Radix Glycyrrhizae	glycirrhizinic acid	1.80	1.80	0
Radix Valerianae	valerenic acid	94.30	85.90	8.9
Rhizoma	essential oil	4.15	4.15	0
Calami	α-asarone	0.38	0.38	0
	β-asarone	3.71	3.71	0

A - before decontamination, B - after decontamination.

Table VII. Project of microbiological requirements for herbal plants assigned for Polish Pharmacopoeia Vth ed.

Group of the raw materials	Microorganisms	Content of microorganisms in 1g of the raw materials
I.		
The raw materials designed for preparing granulates, tablets, dragees, capsules, aqueous extracts, infusions, macerations and preparations for external use.	Aerobic bacteria	< 10.000
	Yeast and moulds	<100
	Bacilli from the *Enterobacteriaceae* familly	<100
	S. aureus, *P. aeruginosa*, *Salmonella*	absent
II.		
The raw materials assigned for preparing alcoholic preparations, isolated compounds, oil preparations and essential oils.	Aerobic bacteria	<1.000.000
	Yeast and moulds	<10.000

Discussion

The medical herbs and herbal raw materials before their use in the production of herbal drugs ought to have an acceptable high microbiological purity level. The Polish Pharmacopoeia [1] defines a permissible level of microbiological contamination of plants preparation. In the Research Institute of Medicinal Plants in Poznañ the microbiological requirements for herbal plants were elaborated to be included in Pharmacopoeia, Vth ed. [2]. Microbiological requirements for herbal raw materials are given in Table VII.

In herbal raw materials from the first group, according to international requirements [3] the total count of aerobic bacteria can not exceed 10000 in 1g. Fungi and bacilli from the Enterobacteriaceae family should not be higher than 100 in 1g. The absence of E.coli, S.aureus and P.aeruginosa are the obligatory requirement.

Our investigations have shown conclusively that herbal raw materials in Poland are contaminated by microorganisms in a large extent. In all samples number of aerobic bacteria, yeast and moulds, Enterobacteriaceae baccili exceed the permissible level.

Among investigated methods, decontamination with methyl bromide is not a process allowing to obtain a proper microbiological purity of herbal raw materials. In addition, this process causes a decrease of essential oil content in some fumigated materials. Because methyl bromide destroys the ozone layer at atmosphere, the total prohibition of the usage this compound as a fumigant will obey from 2010 (Montreal Protocol).

Decontamination with ethylene oxide is an effective process. It gives bacteriostatic and bactericidal effect without more pronounced unfavourable changes in biological active substances in raw herbal materials. However, ethylene oxide is known of its high toxicity and is considered as a human carcinogen, and the use of ethylene oxide for fumigation is prohibited in EU. In the near future the same situation will be in Poland.

Conclusions

Deconamination by irradiation is one of the most effective method for decreasing of the number of microorganisms in herbal raw materials. The content of biological active substances in many raw herbal materials did not change in a signifficant degree after irradiation. The process is technically feasible, very effective and friendly to environment. Radiation treatment can be applied to hermetically packed product, thereby excluding recontamination.

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