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Research article

Ozonation – an alternative decontamination method for raw plant materials

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Abstract: *Raw plant materials are vital to our health and well-being because they are furnished with essential vitamins, minerals, fiber, and other health-promoting phytochemicals. Its increasing consumption forces food manufacturers to assure consumers of a proper microbiological purity of their products. Thus, microbiological purity is an important factor during assessing their suitability in the production process. The sources of raw plant material contamination are particularly soil particles which are brought during harvest, transport and storage and also microorganisms (bacteria, moulds and yeasts), which are associated with their living environment. The decontamination methods which have been used so far, cause a significant reduction of infective microflora, though it is observed a change or loss their valuable components such as: essential oils and biologically active substances. Thus, the aim of this paper is to propose an alternative method of decontamination such as ozonation. Microbial status of samples of Elettaria cardamomum (L.) Maton (cardamom) seeds, Juniperus communis (L.) (juniper) berries, Piper nigrum (white pepper) drupes, dried Ribes nigrum (L.) (blackcurrant) berries, and dried Allium cepa (L.) (onion) flakes was determined before as well as after ozonation. The conducted study shows that ozone causes a significant reduction of contaminating microflora. However, the ozone effectiveness depends on the microflora of plant material and its various vulnerability to ozone.*

Keywords: *decontamination, ozone, herbs, spices, raw plant materials, microorganisms.*

Introduction

Increasing consciousness of a healthy life style and hence healthy (natural) food, was the reason for changing consumer's preferences. By now their attention has been directed at products, containing in its composition for instance herbal and spice substances. Because of that fact, it is expected that the quality of products, mentioned before, supposed to be increased. The aim is not only improvement of sensory qualities, but also assurance of a proper microbiological purity. As a consequence, microbiological purity is an important factor during assessing their suitability in the production process. Therefore it is necessary to carry out an insightful microbiological analysis.

The sources of raw plant material contamination are particularly soil particles which are brought at the time of harvest, transport and storage and also microorganisms associated with their living environment. Also, it is known that many plants, for instance spices are grown and harvested in poor sanitary conditions in areas abundant in warmth and humidity [1]. As a result of the high level of microbial contaminant, that kind of substances requires their decontamination.

The decontamination methods which have been used so far (formaldehyde, ethyl alcohol, a high hydrostatic pressure, microwaves, carbon dioxide under pressure, infrared spectroscopy, ionizing radiation, water steam under pressure) cause a significant reduction of infective microflora, though it is observed a change or loss their valuable components such as: essential oils, biologically active substances (polyphenols) [2-4]. Some pros and cons of chemical-physical decontamination methods were given in Table 1.

Another factor of decontamination is ozone, which due to ensuring a good contact with the plant material can be an alternative to existing methods. What is more, the proposed method reduces and even eliminates the majority of reported defects. In addition, removal a dangerous or poisonous substance from plant materials using ozone is beneficial due to its high efficiency, no significant changes in raw material and low organoleptic impact on the environment.

Ozone is a powerful antimicrobial substance because of its potential oxidizing capacity. Ozone is the allotropic variety of oxygen occurring in all states of matter. It is a blue gas at ordinary temperature, but at concentrations at which it is normally produced the colour is not noticeable [3]. It is characterized with pungent odour, similar to sulphur dioxide and chlorine. Ozone is known as the third most potent oxidant (E^o= + 2.076 V) after fluorine and peroxysulphate [5]. The high oxidation-reduction potential causes that Gram-positive bacteria (*Listeria monocytogenes, Staphylococcus aureus, Enterococcus faecalis*), Gram-negative bacteria (*Escherichia coli, Salmonella* Typhimurium, *Yersinia enterocolitica, Pseudomonas aeruginosa*), viruses, yeast (*Candida parapsilosis, Candida tropicalis*), bacterial spores (*Bacillus cereus*) are susceptible to ozone [6].

The conducted studies show that various microorganisms are characterized by a varied sensitivity to ozone. That is why there is a need to optimize process parameters for the raw material. Bacteria are more sensitive than fungi. Gram-positive bacteria are characterized as more sensitive than gram-negative bacteria. However bacterial spores are more resistant than vegetative cells [6, 7].

What is more, ozone rapidly divides to oxygen, without other reaction products, there are created few decontamination by-products. Therefore the use of ozone in food processing plants seems to be a safe and environmentally-friendly technology [7].

Table 1. Decontamination methods of plant materials [7]

Method	Decontamination factor	Cons	Pros
Chemical	methyl bromide	limited spectrum of activity the ozone layer destruction	–
	ethylene oxide	a loss of biologically active substances carcinogenic compounds formation	broad spectrum of activity
	formaldehyde ethyl alcohol	a loss of essential oils	high effectiveness
	ozone	diverse microbiological susceptibility	high effectiveness broad spectrum of activity a lack of environmental damage
Physical	carbon dioxide under pressure	no effect on bacterial spores and mould spores activation of bacterial spores a loss of essential oils changes in the composition of the essential oils effectiveness depends on the moisture content	–
	microwaves	no effect on bacterial spores and mould spores a loss of essential oils changes in the composition of the essential oils	–
	high temperature and pressure	consistency change	high effectiveness slight changes in colour, taste, smell
	high pressure	possible changes in the composition of the essential oils	high effectiveness
	water steam at temperature 100-200°C	colour changes a loss of biologically active substances caking of powdered substances	high effectiveness
	ionizing radiation	high cost sensory changes dependent on the dose of radiation reduction the antioxidant properties of plant materials	high effectiveness
	infrared spectroscopy (IR)	low effectiveness a loss of essential oils	quickness

Microflora of raw plant materials

Numerous studies have confirmed the high microbial load of raw plant materials, which can become a serious problem for food manufacturers [1]. It has been proved that the level of microflora, which contaminates these products, is variable and depends primarily on the analyzed material. It is believed that the total number of microorganisms is usually formed at 10^3-10^7 cells per 1 gram [7].

Furthermore, according to the literature the number of spores of aerobic mesophilic bacteria is between 10^2 and 10^7 spores per 1 g. However, the number of moulds which are present in the plant material ranges between 10^1-10^6 colony forming units per 1 gram (cfu/g). Moreover, it is revealed that the occurrence of bacteria from the family *Enterobacteriaceae* is at the level 10^1-10^5 cfu/g, and sometimes even at the level 10^8 cfu/g. In addition, the identification results of pathogenic bacteria such as *Bacillus cereus, Clostridium perfringens, Staphylococcus aureus* are presented at the level: 10^1-10^2 cells per 1 gram; 10^1-10^2 cfu/g; less than 10^2 cfu/g, respectively (Table 2).

Table 2. The microorganisms presented in raw plant materials [7]

Group of microorganisms		Genus or Species
Bacteria	Gram-positive	*Bacillus* sp., *Clostridium Corynebacterium* sp., *Enterococcus faecalis, Listeria monocytogenes, Micrococcus* sp., *Mycobacterium* sp., *Rhodococcus* sp., *Staphylococcus aureus*
	Gram-negative	*Acinetobacter* sp., *Aerobacter* sp., *Aeromonas* sp., *Agrobacterium* sp., *Alcaligenes* sp., *Chromobacter* sp., *Chromobacterium* sp., *Enterobacter* sp., *Erwinia* sp., *Escherichia coli, Flavobacterium* sp., *Pseudomonas* sp., *Salmonella Typhimurium, Spirillum* sp., *Vibrio* sp., *Xanthomonas* sp., *Yersinia enterocolitica*
Yeast		*Candida* sp., *Saccharomyces cerevisiae*
Moulds		*Alternaria* sp., *Aspergillus* sp., *Chaetomium* sp., *Cladosporium* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Trichoderma* sp., *Verticillium* sp.
Actinomycetes		*Actinomyces* sp., *Micromonospora* sp., *Streptomyces* sp., *Thermoactinomyces* sp.

It is maintained that thyme and marjoram which belong to herbal raw materials are characterized by the highest microbial contamination level: both bacterial and saprophytic microflora [8]. Besides, the literature data indicates the presence of moulds in the number of 10^5 cfu/g.

These examples illustrate not only the microbiological status of raw plant materials, but also demonstrate the danger of introduction along with them the saprophytic microflora and pathogens to food [9, 10].

Thus, the aim of this paper is to propose an alternative method of plant materials decontamination such as ozonation.

Experimental

Materials

Materials used in present study are commonly used in cooking, and popular in herbal medicine. Research material consisted of *Elettaria cardamomum* (L.) Maton (cardamom) seeds, *Juniperus communis* L. (juniper) berries, *Piper nigrum* (white pepper) drupes, dried *Ribes nigrum* L. (blackcurrant) berries, and dried *Allium cepa*

L. (onion) flakes. Research materials, which were investigated herein, have been provided by private supplier.

Methods

Microbiological analysis

At the beginning naturally contaminated samples were analyzed microbiologically. The aim was to get the knowledge of microorganism quantities in plant materials. Microbiological screening of plant materials and the monitoring of the survival of microorganisms after decontamination treatment were performed according to the existing regulations.

Samples were prepared according to ISO 6887-4. Total mesophilic bacteria count (TMC) was determined on Plate Count Agar (PCA) medium (incubation at 30°C, aerobically). *Enterobacteriaceae* count (EE) was determined on VRBG agar following incubation at 30°C for 24 h. Total count of fungi (TFC) was isolated and cultured on Czapek-Dox Agar and incubated as 25°C for 7 days. The colonies that appeared on the selected plates were counted as cfu/g of weight sample. The results were presented as mean ± standard deviation.

Decontamination method with using ozone

The procedure of ozone decontamination was as follows: ozone treatments were performed in a round bottom glass reactor at a room temperature (20±1°C). Ozone, previously generated from oxygen bottle by a laboratory Ozone Generator BMT 803 N (BMT Messtechnik Berlin), was transferred to research samples (40.0 g). Samples were decontaminated at following parameters: ozone concentration 150 160 g/m^3; flow rate 0.1 L/min; pressure 0.5-0.8 atm; time 10 min. The ozone concentration in the reactor was controlled by digital Ozone Analyzer BMT 964 (BMT Messtechnik Berlin).

Then, the samples were transferred to sterile packages by pneumatic transport.

Statistical analysis

All analyses were carried out in triplicate. Mean values with standard deviations (±SD) were reported for each case. Significance differences for multiple comparisons were considered by Mann-Whitney's test (Statistica 10.) at the $p < 0.05$ level.

Results and Discussion

The results of microbial analysis of plant material samples are summarized in Table 3. No considerable variations were observed in the microbial counts, even between samples of the same kind. International Commission on Microbiological Specifications for Foods [11] set up maximum limits of 10^6, 10^4 and 10^4 cfu/g of total mesophilic bacteria (TMC), yeasts and moulds, respectively. Before decontamination the obtained results indicate a medium level of contamination (< 10^4 cfu/g).

At present there are not specified maximum contaminant levels of plant materials in European Union. But, the microbiological contamination of them should be as limited as ensure food safety for consumers.

Results of the experiments showed that there were no statistically significant differences at $p < 0.05$ between the total mesophilic count in cardamom seeds, dried onion flakes and white pepper drupes before and after ozonation. Therefore, the effectiveness of decontamination process depends on the microflora of plant material and its various vulnerability to ozone [7]. It was noticed that ozone treatment was sufficient to reduce mesophilic bacteria (< 10 cfu/g) in juniper and dried blackcurrant berries and total fungal count (< 10 cfu/g) in cardamom seeds, dried blackcurrant berries, and white pepper drupes. Additionally, the study confirmed that ozone was very effective in reduction *Enterobacteriaceae* count – decontaminated samples revealed the highest microbial purity. Therefore, for this reason, method of decontamination with using ozone is justified.

Table 3. Occurrence of microbial contamination in plant materials before and after ozonation

Material	**Ozonation**	**Total mesophilic bacteria count**	**Total fungal count**	***Enterobacteriaceae* count**
Juniper berries	Before	2.90 ± 0.01	3.59 ± 0.02	2.97 ± 0.03
	After	< 1*	2.58 ± 0.05	< 1*
Cardamom seeds	Before	4.98 ± 0.03	2.15 ± 0.04	2.04 ± 0.02
	After	5.26 ± 0.03	< 1*	< 1*
Dried onion flakes	Before	4.41 ± 0.02	2.53 ± 0.03	3.26 ± 0.01
	After	4.11 ± 0.03	2.81 ± 0.03	2.76 ± 0.02
Dried blackcurrant	Before	2.18 ± 0.01	2.32 ± 0.02	2.11 ± 0.03
	After	< 1*	< 1*	< 1*
White pepper	Before	5.11 ± 0.04	2.88 ± 0.02	< 1*
	After	5.30 ± 0.05	< 1*	< 1*

All results are given as log (cfu/g). The results obtained were expressed as Mean ± SD with n=3.
*not detected at the level 10 cfu/g

Conclusions

Ozone decontamination of plant materials can be carried out to the desired level of microbial purity, provided that the initial contamination level in a representative sample, and sensitivity the contaminating microflora to ozone are known. At any rate, sufficient knowledge exists about some raw materials to allow for a reasonable estimate of the required dose.

The proposed decontamination method has many advantages. First of all, ozonation method is much cheaper in comparison to radiation. Introduction into the market plant materials with a high microbiological purity, will not only protect the consumer's health, but also improve the health safety. Moreover, the alternative method of decontamination, mentioned in this article, seems to be environmentally-friendly technology.

This study was an introduction to later experiments. Obtained results will be used for further investigations. It is hoped that further studies will allow to develop a complete method of decontamination with using ozone and, as a consequence receive plant products with unchangeable quality.

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