A Review of Hygienization Methods of Herbal Raw Materials

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Abstract: This article reviews various decontamination methods of herbal raw materials in Poland. These are the physical and chemical treatments of plant raw materials that remove mineral and microbiological impurities to ensure food safety. There is increasing use of herbal raw materials, and it is important to understand various methods that can be used for hygienization. Techniques used ensure the removal of mechanical impurities and elimination of microorganisms in a plant material. Depending on the method of choice, certain microorganisms are removed to a varying degree, and at the same time, there is a partial loss of the health-promoting properties of the plants subjected to the hygienization. Therefore, there is need to decide on optimal methods for hygienization of herbs that both reduce microbial contamination to the maximum extent and minimize the decrease in valuable ingredients contained in the herbs. Ozonization of plant raw materials is an effective method of removing microbiological contamination from most herbs. A good solution is also to use hybrid methods. In our literature review, ozone is very often mentioned as an effective disinfectant for herbs and spices. However, there is no information about the effect of ozone use on the substances contained in herbs and spices.

Keywords: decontamination of herbs; ozonation; ozone; microbiological safety; aromatic plants; disinfection

1. Introduction

The rapidly growing importance of healthcare, beauty, and vitality, especially in developed countries [1], leads to increase in demand for cosmetics, cosmeceuticals, parapharmaceuticals, diet supplements, and nutraceuticals. A variety of ingredients are used in their production, but most of them, or at least a significant part, are based on herbs [2]. As a result, medicinal herbs for cosmeceutical have evolved to a separate high-growing market segment. The most important producers of herbs are China, India, and Egypt [3]. The area of herbal cultivation in each of these countries amounts to about 20 million hectares. In Poland, such plantations occupy only 30,000 hectares [4]. However, Poland is becoming more and more important producer of the herbal raw materials of good quality—at least in the European Community [5]. In addition to the cultivated herbs, there are also plants with prohealth properties obtained directly from nature [6]. Currently, around 2000 species of medicinal and aromatic plants are traded worldwide and over 200 species are grown and harvested in Poland [7,8]. The processing of herbs and other plants used in medicine, cosmetics and diet supplementation requires maintaining high standards of microbiological purity. At an early stage of production, the herbs are thoroughly selected so that the sick and damaged plants are separated and then they are precleaned in order to remove mineral impurities and insects. The next stage is focused on microbiological purity of production materials—usually there are a lot of microorganisms on the plants that may develop...
during the drying process, storage, or transport. Many of these microorganisms can cause serious disease even death [9,10].

There are many physical and chemical methods that are used to disinfect herbs. Unfortunately, currently used methods have significant disadvantages. A lot of them results in the modification of chemical composition of processed plants so that they loss their valuable components and biological properties. The appropriate method should result in a high level of microbiological purity on the one hand and minimizing the side effect of modifying the chemical composition on the other hand. The aim of this study is to provide an overview of the methods used for herbal hygienization. In this article, we used publications from scientific databases such as Google Scholar, Scopus, Web of Science, PubMed, SIGZ—System Informacji o Gospodarce Żywnościowej, and Food Science Source.

2. Impurities and Contamination

2.1. Removal of Mechanical Impurities

The herbs obtained from the cultivation or from the natural state are submitted to hygienization during the production process. In the first stage, the mineral impurities are removed (mainly the particles of soil), as well as the organic pollutants consisting of fragments of other plants, sticks, dead insects, etc. [11]. Most frequently, the mechanically driven winnowers are used for this purpose, which separate individual fractions on the sieve screens at the same time (grain, lighter grasses, dust, and dust). In addition, different types of separators are used, e.g., the separators intended for sifting loose products with taking into account the size of the particles. They are primarily used in production of mint, hibiscus, chamomile, common valerian, etc. and also in the selection of seasonings. As well, they may be used to separate the parts of plants, e.g., stalks from leaves wet before drying. The other types of machines used for herbal purification are filter cyclones, which trap dust particles [12]. Table 1 shows the types of contamination of herbal raw materials.
Table 1. Types of herbal contamination.

| Source of Pollution Origin | Types of Contamination                                                                 |
|----------------------------|----------------------------------------------------------------------------------------|
|                            | Physical | Chemical                                                                 | Biological                                                                 |
| Soil                       | ✓ Sand,  | ✓ Residues of fertilizers and plant protection products                      | ✓ Gram-positive bacteria of the genera *Proteus*, *Streptococcus*, *Rhodococcus*, and *Corynebacterium*, as well as spore bacteria of the genus *Bacillus*, especially *B. subtilis*, *B. pumilus*, *B. cereus*, and *B. licheniformis*, and spore-forming anaerobes of the genus *Clostridium*, ✓ Actinomycetes and molds (including *Penicillium*, *Alternaria*, *Fusarium*, and *Cladosporium*) ✓ Sticks of the genus *Escherichia*, *Pseudomonas*, and *Alcaligenes*, ✓ Bacteria of the genus *Mycoplasma*, *Agrobacterium*, and *Xanthomonas* |
|                            | ✓ Glass, | ✓ Wastewater                                                                | ✓ Spoilage bacteria (of the genus *Bacillus*), ✓ Molds (e.g., *Mucor*, *Penicillium*, and *Cladosporium*), ✓ Yeast-like fungi (*Rhodotorula*, *Candida*, *Cryptococcus*, and *Debaromyces*) |
|                            | ✓ Elements of other plants, twigs, sticks, | ✓ Heavy metals                          | ✓ Bacterial spores (e.g., of the genus *Bacillus* and *Clostridium*)✓ Mushroom and mildew spores. |
|                            | ✓ Strings, | ✓ Heavy metals                          |                                                                                |
|                            | ✓ Metals. | ✓ Sulphur dioxide                 |                                                                                |
|                            |          | ✓ Nitrogen dioxide                |                                                                                |
|                            |          |                                  |                                                                                |
| Water                      | ✓ Sand, stones | ✓ Wastewater                                                                      | ✓ Bacterial spores (e.g., of the genus *Bacillus* and *Clostridium*)✓ Mushroom and mildew spores. |
|                            |          |                                  |                                                                                |
| Air                        | ✓ Exhaust gas, | ✓ Heavy metals                          | ✓ The bacteria *Salmonella typhimurium*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Yersinia enterocolitica*, and *Escherichia coli*. |
|                            | ✓ Ashes, |                                  |                                                                                |
|                            | ✓ Dust   |                                  |                                                                                |
| Anthropogenic              | ✓ Clothing elements (buttons) | ✓ Substances added by producers of herbal products in order to improve quality, color, and storage capacity. | ✓ The bacteria *Salmonella typhimurium*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Yersinia enterocolitica*, and *Escherichia coli*. |
|                            | ✓ Jewelry items |                                  |                                                                                |
|                            | ✓ Hair.  |                                  |                                                                                |
| Invalid harvest, storage, and processing. | ✓ Dust, | ✓ Exhaust gases,                       | ✓ *Staphylococcus aureus* |
|                            | ✓ Greases from machines | ✓ Substances that migrate from installations, equipment and packaging |                                                                                |
2.2. Types of Potential Contamination of Herbal Raw Materials

The contamination of plant raw materials is influenced by a number of different factors (Table 1). The herbal contamination may be of the primary and secondary nature [11,13,14]. In the first case, it is caused by the microbes naturally occurring on plants, i.e., belonging to the epiphytic microflora. Herbs may also be subject to secondary contamination during harvesting, transport, and drying. They also may be contaminated at other stages of production and during storage. In particular, it refers to the plants grown in a tropical climate. The most common pollutants in herbal raw materials include the natural habitat of the plant, the place of its growth, and cultivation technology. The soil itself, in which the plant is deposited, can become the main source of pollution of the raw material obtained from it. The soil contains Gram-positive bacteria of the genus Proteus, Streptococcus, Rhodococcus, and Corynebacterium as well as spore-forming bacteria of the genus Bacillus, especially B. subtilis, B. pumilus, B. cereus, and B. licheniformis, and spore-forming anaerobic bacteria of the genus Clostridium, actinobacteria and molds (inter alia Penicillium, Alternaria, Fusarium, and Cladosporium). Furthermore, the soil is also inhabited by the Bacillus of the genus Escherichia, Pseudomonas, and Alcaligenes, as well as bacteria of the genus Mycobacterium, Agrobacterium, and Xanthomonas [15]. Equally often the water used in the processing of the herbal material becomes a determinant of contamination; here, we most often find spore-bearing bacteria (of the genus Bacillus), mold fungi (e.g., Mucor, Penicillium, and Cladosporium), or yeast-like fungi (Rhodotorula, Candida, Cryptococcus, and Debaromyces) [16]. Herbs improperly dried or stored can be attacked by mold fungi, whose growth rate depends on a number of factors (temperature, humidity, etc.). The herbal raw material, most often due to irregular harvesting, may sometimes be infected by Staphylococcus aureus, which causes dangerous food poisoning. The degree of contamination of plant raw materials is very diverse. It can reach $10^6$ microorganisms in 1 g of raw material [9]. Apart from the abovementioned microorganisms, occurring mainly in the form of spore-forming bacteria, human infectious agents were also found in studies on the evaluation of microbiological purity of herbal raw material [17,18]. People can contribute to the contamination of the medicinal herbs and spices with pathogenic bacteria, which are not found (or sporadically) in the raw materials under natural conditions [19]. Cross-contamination of medicinal herbs and spices with excrements of animals or humans can be evidenced by the presence of the genus Salmonella typhimurium, Listeria monocytogenes, Enterococcus faecalis, Yersinia enterocolitica, and Escherichia coli [15].

2.3. Microbiological Contamination of Herbal Plants

When trading in herbal raw materials, the manufacturers put in the quality certificates the information on the share of mineral impurities, but more accurately, the data on the microbiological characteristics are given. The removal and killing of microorganisms are the most important elements of herb decontamination [20,21]. After all, the herbs must be completely safe for the consumers’ health. The results of the microbiological purity analysis determine the suitability of plant raw materials for the production process [15]. According to the regulations in force, in Poland and other countries the herbal products must meet microbiological requirements as defined by the standards. They predict the complete absence of certain types of microorganisms in the samples of defined sizes or they specify the permissible degree of contamination in 1 g of a product.

It is obvious, that the contaminated herbal raw material can have negative consequences for consumers. Especially, herbs, and usually spices, are often not subjected to heat treatment. Some microorganisms (Salmonella) can survive for a long time in the dried herbal raw material. They can cause dangerous poisoning like mycotoxins, a product of metabolism of mold fungi [22]. Mycotoxins are very dangerous. They have teratogenic and carcinogenic effects, and also cause liver damage, reduce immunity, and impair fertility. They also contribute to brain necrosis, as well as intensify the development of kidney and gastrointestinal cancer. Acute mycotoxin poisoning is rare, but low doses of these toxins may have long-lasting effects on a body.
There is no doubt that any herbal material intended for food purposes must be microbiologically safe. However, we know that some of the medicinal plants themselves contain substances in their biochemical structure, which significantly contribute to limiting the multiplication of bacteria, fungi, or viruses. It has been estimated that about 1400 species of herbs and spices belong to such type of plants. The most common and often used by man are peppermint, Melissa, cinnamon, ginger, thyme, tarragon, fennel, stevia, oak, or sage. However, it does not change the fact that every manufacturer is responsible for the microbiological evaluation of the products he manufactures, even if he offers only raw materials and spices with a proven bactericidal or bacteriostatic effect. Only the introduction of such an obligation guarantees consumers safety in using the beneficial properties of herbs and spices.

3. Review of Methods

The decontamination procedures aimed at neutralization of bacteria, parasites, and fungi in plant raw materials (as well as in semifinished products and products) are divided into physical and chemical procedures [23].

3.1. The Physical Methods for the Decontamination of Herbs

One of the methods for the physical decontamination of herbs is to subject them to high hydrostatic pressure, usually with values from 300 to 1000 MPa. Even under the pressure of 100 MPa, the number of bacteria in the colon rod decreases by 99.9% (*Escherichia coli*). The presence of this microorganism, of which certain strains can cause dangerous poisoning (*Staphylococcus aureus* and salmonella) is not allowed by hygienic standards in the products intended for consumption [24]. Fungus from the order of the yeast white litter (*Candida albicans*) dies after 5 min, when subjected to hydrostatic pressure of 500 MPa, as well as the yeast *Saccharomyces cerevisiae*. This method is also highly effective against mold, but unfortunately, it reduces significantly the content of essential oils in plants subjected to it.

The use of carbon dioxide under high pressure shows relatively low decontamination efficiency. It does not destroy satisfactorily the bacterial and mold spores [20]. In addition, its effectiveness decreases with the decrease in the moisture content in the plant material [25]. The carbon dioxide also causes the loss of significant amount of aromatic oils and causes changes in the chemical composition of herbs.

In case of vegetative forms of bacteria and fungi, the method using microwaves, i.e., electromagnetic waves of 0.0001–1 m length, turns out to be useful [20,26]. However, spores of Gram-positive hay bacillus (*Bacillus subtilis*) and bacteria of the genus *Clostridium* and mold spores prove to be resistant to their action [27]. In this case, a large loss of the content of essential oils and the changes in their chemical composition take place.

Exposing the herbal raw materials to short-term high temperatures, usually from 110 to 200 °C, and, at the same time, to the pressure of about 20 MPa is called extrusion [28].

This technology does not bring about significant organoleptic changes in the herbs, but it significantly affects their consistency, which is especially unfavorable in the production of seasonings.

In turn, the ionizing radiation has been recognized as safe by the World Health Organization, but it is not accepted by consumers. While the dose of 10 kGy is taken as the upper tolerated dose, the successful disposing of the viruses and microbial spores sometimes requires a dose of up to five times higher [25,29]. Then, the negative chemical and visual changes occur in the herbs that are decontaminated by the use of this technology. However, on the other hand, in case of some plant seasonings, the doses of 5–6 kGy are sufficient to ensure that the amount of microorganisms contained in them meets the requirements of the standards. It should be stressed that the radiation decontamination is only exceptionally associated with an increase in temperature, therefore, it is suitable for heat-sensitive raw materials.

The use of infrared radiation has an effect only on the surface of plants, therefore, in this case, it is difficult to talk about a significant reduction in microorganisms.
The steam is used for many herbal raw materials, but more often it is used in the production of seasonings. The steam temperature is usually between 100 and 200 °C. Then, the plant material is dried with hot air and subjected to rapid cooling. As a result, the significant reduction in microorganisms takes place, primarily, bacilli of the Enterobacteriaceae family, some granulomas, spores of the hay bacillus bacterium, bacteria of the genus Clostridium, as well as yeast and mold [15]. At the same time, however, there is a very significant change in the color of plants containing chlorophyll and carotenoid dyes, and also, the significant material depletion of numerous biologically active compounds [14,29]. This technology is also not suitable for the decontamination of powdered raw materials, because they get lumpy under the influence of water vapor.

A typical device for decontamination with the use of the steam method is a sterilizer. The modern sterilizers, whose operating principle is based on a screw conveyor system, allow sterilization of various plant raw materials of various forms: ground spices (peppers, nutmeg, cloves, etc.), cut to pieces (cut herbs, fix type fraction, grain yield of herbs, and sawn herbs), as well as whole herbs (granular pepper, allspice, marjoram, etc.). The sterilization process in the sterilizer produced by the Polish company Scorpion (Rozdąże, Poland) consists in heating the initial product to a temperature of about 90 °C. From the feeding hopper, the raw material goes to the sterilization chamber, where it is exposed to dry steam at a temperature of 130 °C. The duration of this phase depends on the form of the raw material and sometimes, also on the plant species. Then, the raw material is transferred to the precooling chamber and next, transported to the drying chamber into which the warm air is pumped. In the final stage, the herbs are packaged into bags or big-bags through the receiving feeder. The advantage of this method is the possibility of obtaining very good microbiological parameters of sterilized raw materials with the low loss of aromatic oils, the slight loss of raw material, high efficiency (on the order of 1000 kg/h), and finally, the relatively low sterilization cost. The use of steam is currently the most common way of the sterilization of herbs, and the modern solutions in this field guarantee fairly high microbiological purity and are also ecological.

3.2. The Chemical Methods for the Decontamination of Herbs

The use of ethyl alcohol or formaldehyde is an extremely effective microbiological technology for chemical contamination of herbs. The disadvantage of this method is a significant reduction in the amount of essential oils in plant materials. This can be counterchecked by using methanol vapor, which is then displaced by ethyl alcohol vapor [30]. However, due to methanol toxicity, the methanol must be completely removed from the herbal raw material, what significantly complicates and rises the cost of this technology. The ethylene oxide very effectively destroys viruses, fungi, bacteria, and their bacterial spores, but also causes the significant loss of biologically active compounds, especially alkaloids, glycosides, and mucus. It also produces carcinogens, among others ethylene glycol. This property of ethylene oxide has become the reason for prohibiting its use in the European Union. However, this method is quite widespread outside Europe and is used by the largest manufacturers of herbs [31,32]. Based on the gas diffusion process, the ethylene oxide neutralizes microorganisms at low cost.

The methyl bromide, until recently used for the chemical decontamination of plant raw materials, is not allowed in the EU countries too. It reduces the harmful microflora, but in the case of bacteria such as Escherichia coli or Staphylococcus aureus, only to a limited extent. The ban on the use of methyl bromide was determined by its harmful effect on the Earth’s ozone layer [33].

3.3. Ozone Treatment of Herbs

Ozone, trioxygen (O₃), is an allotropic variety of oxygen consisting of triatomic molecules. It has strong aseptic and toxic properties. It is used for water disinfection, but also more and more often in the food and pharmaceutical industries (sterilization of food products and production of dietary supplements, with particular emphasis on the herbal range). Ozone is one of the most effective known disinfectants. It has a bactericidal effect at a concentration of about 13 µg/dm³. Ozone is an irritant gas
causing damage to biological membranes through radical reactions with their components. Once it enters the cells, it can inhibit cellular enzymes by inhibiting intracellular respiration. Research on the use of ozone also shows that it can deal with residues of pesticides used in the cultivation and storage of plants [34–36].

However, it is not without disadvantages. Aldehydes and ozone are produced as by-products of ozonization. Moreover, the disadvantage of ozone is its impermanence and thus the risk of secondary contamination of previously disinfected raw materials or products (Table 2). This feature of ozone often becomes the reason for the use of additional forms of decontamination during the processing [6].

| Temperature (°C) | Gaseous Ozone | Aqueous Ozone |
|------------------|---------------|---------------|
|                  | Half-Life (min) | Temperature (°C) | Half-Life (min) |
| −50              | 3 months       | 15             | 30             |
| −35              | 18 days        | 20             | 20             |
| −25              | 8 days         | 25             | 15             |
| 20               | 3 days         | 30             | 12             |
| 120              | 1.5 h          | 35             | 8              |

3.3.1. Application of Ozone

Currently, the use of ozone is considered the most effective method of the chemical decontamination of herbs. This technology is relatively young [34]. Ozone has a strong bactericidal effect, to which both the Gram-positive (Listeria monocytogenes and Staphylococcus aureus) and the Gram-negative (Escherichia coli and Salmonella), as well as viruses and yeasts are susceptible [38–41]. Fungus is slightly less sensitive to ozone than bacteria, and bacterial spores are more resistant than their vegetative cells [42]. Ozone has a high antioxidant potential and is therefore increasingly used in the food industry, among others, during the production and processing of vegetables and fruit [43–47]. It is also used to treat water (drinking and swimming pool water), as well as to sanitize equipment and surfaces, to disinfect rooms, to combat algae, etc. The growing use of ozone is also associated with the production of ozonators for home use. The recommended procedures for the herb decontamination use ozone dissolved in water; a condition for effective purification of the plant raw material is its good contact with this compound.

In Poland, ozone is treated as an allowed means of decontamination of herbal raw materials. Compared to other methods of decontamination of herbal raw materials, the use of ozonators for this purpose seems to be the most advantageous and prospective solution. The leading herb producers in Poland turn to it, among others, the companies such as Dary Natury (Koryciny, Poland) and Herbapol (Wroclaw, Poland).

There is also hybrid technologies of herb hygienization, which combine selected features of the methods mentioned above. Less than 2 years ago, the first in Poland production line for hygienization of seasonings and herbs in a continuous process was launched. This means the introduction of new standards of hygienization and herbal production in general.

3.3.2. Negative Aspects of the Ozonation of Plant Raw Materials

The conducted experiments [48,49] prove that in case of some species and parts of plants, the impact of ozone causes permanent tissue damage.

The seedlings of common basil (Ocimum basilicum) and sage (Salvia officinalis) subjected to a 2-week ozonation showed no noticeable changes; also, the chlorophyll fluorescence parameters (indicating a deterioration of the physiological state) of the leaves of both of these plants, compared with the parameters of seedlings of the control lots, not treated with ozone, did not differ significantly. However,
in case of lemon balm (*Melissa officinalis*), a species related to those mentioned above (all the three belong to the *Labiatae* family and have similar morphology and life cycle), whose seedlings were provided with identical conditions as in case of basil and sage, noticeable changes were observed after 6 days of exposure to the same doses of ozone. Apart from significant discrepancies in the chlorophyll fluorescence values in the lemon balm leaves treated with ozone, in relation to the control samples, a visible damage took place. Necrotic spots up to 2 mm in diameter appeared on both sides of the leaves, resembling the symptoms of hypersensitive-like response. The symptoms of ozone poisoning were particularly bright in older leaves that turned yellow and withered. Both lemon balsms, sage and basil, belong to common and important herbal raw materials, but only the former showed significant sensitivity to ozone. The mechanism of resistance of basil and sage to the toxic impact of ozone should probably be explained by the rapid reaction of the antioxidant system of these plants, because ozone, after having penetrated through stomata, creates various reactive oxygen forms that damage the inside of cells. From among the plants of herbal importance, among others, plantain, dandelion, nettle, and soap dish are ozone resistant [23]. The described experiment refers to naturally living plants—a separate issue, requiring thorough examination, is to determine whether ozone has a negative impact, e.g., on the level of terpenoids in oil obtained from lemon balm.

4. Advantages and Disadvantages of Herbal Hygienic Methods

The selection of the correct method for the decontamination of herbs is dictated not only by concern for obtaining maximum microbiological purity of the raw material but also by preservation of the valuable properties of these beneficial plants [28,50]. They contain a number of active substances, i.e., compounds that have healing effects or in other ways affect human health and well-being. These are vitamins, tannins, flavonoids, phytoestrogens, carotenoids, antioxidants, polyphenols, mucus, etc. The herbs have anti-inflammatory, anti-fungal, anti-bacterial, anti-atherosclerotic, and antioxidant properties [51]. In many cases, the effects and efficacy of individual medicinal plant species have not been sufficiently studied yet [52]. The manufacturers of herbs must ensure the high quality of their products including preservation of their properties (that is, biologically active substances present in plants) and the sensory attractiveness, that is such qualities as the right taste, aroma, and color [53]. Unfortunately, during the decontamination of herbal raw materials, the medicinal benefits and other valuable attributes are often reduced and the drastic change in appearance takes place. More serious loss of the antioxidant properties of plants or the concentration of polyphenols in herbs occur more often under the influence of long-term storage, than as a result of the use of decontamination. In the extracts of thyme, oregano, marjoram, or basil, after a year of storage, the share of polyphenols is reduced by up to 10 times [54–56]. Table 3 shows a comparison of currently used methods for herbal hygiene.

Each of these methods has advantages and disadvantages related to the decontamination process. The common disadvantage of all these methods seems to be their influence on the chemical composition of plants. Chemical compounds can transform into other derivatives, degrade, undergo polymerization, or rearrangement processes, e.g., the use of solvents, i.e., methanol or ethanol, may cause the leaching of some of the biologically active compounds from plants. Extensive ozone usage may cause the formation of new carbonyl compounds. Therefore, during this process, we can observe a reduction in the content of compounds containing a double bond [48,57]. The studies carried out showed that the use of ethylene oxide is associated with the loss of tannins, triterpenic spikes, and iridoid in plant material. On the other hand, ionizing radiation in dose 10 kGy reduces the concentration of tannins, phenolic glycoside, and sulfur compounds. Due to its properties, steam will significantly affect the content of essential oils in the plant material [33,58]
Table 3. Advantages and disadvantages of decontamination methods.

| Type of Method                                      | Advantage                                                                 | Disadvantage                                                                 |
|-----------------------------------------------------|----------------------------------------------------------------------------|------------------------------------------------------------------------------|
| A. The method of mechanical removal of contaminants.| - To get rid of the largest particles of pollutants such as soil particles, sand, other plant grains, dust and dirt. | - Low efficiency of decontamination and the raw material requires further cleaning steps. |
| B. Physical decontamination method:                  |                                                                           |                                                                              |
| 1. High hydrostatic pressure                         | - High biocide efficacy against both bacteria and fungi.                   | - Loss of essential oils from the raw material.                              |
| 2. Carbon dioxide under pressure                      | - The method is cheap and easy to carry out, does not require any specialized equipment. | - Low biocidal efficiency and loss of essential oils from plants subjected to this method. |
| 3. Electromagnetic wave                               | - Low application costs and good availability of equipment.               | - It is not effective against all microorganisms—Gram-positive spores *(Bacillus subtilis)* and bacteria of the genus Clostridium and mold spores are resistant to their action, - also causes significant loss of essential oils from the plant. |
| 4. Short-term high temperature application with high pressure at the same time | - The technology does not involve significant organoleptic changes in the herbs. | - Significantly affects the consistency of the raw material, which may be unfavorable especially in the production of spices. |
| 5. Ionizing radiation                                | - A safe method for the consumer, accented by the WHO.                    | - It induces negative chemical and visual changes in the raw material, -dose-dependent effectiveness. |
| 6. A wave with an infrared light spectrum            | - Easily accessible, low cost, and easy to use method.                    | - This method has an effect only on the surface of the plants, resulting in a low microbial reduction potential. |
| 7. Steam                                            | - Obtaining very good microbiological parameters of sterilized raw materials with low losses of essential oils, - small losses of raw material and high capacity (about 1000 kg/h), - low-cost method and no complicated generators or reagents are needed, just a simple sterilizer. | - The method brings very clear changes in color of plants containing chlorophyll and carotenoid dyes, - significantly impoverishes the material with numerous biologically active compounds, - not suitable for decontamination of powdered raw materials—it causes caking. |
| Type of Method                              | Advantage                                                                 | Disadvantage                                                                 |
|--------------------------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| **C. Chemical methods**                    |                                                                           |                                                                              |
| 1. Compounds: ethyl alcohol, methyl alcohol | - High efficiency of raw material sterilization.                           | - Significant reduction in the amount of essential oils in plant raw materials |
| 2. Ethylene oxide                          | - Very effectively destroys viruses, fungi, bacteria, and their spores.    | - Causes significant losses of biologically active compounds, especially alkaloids, glycosides, and mucus, - produces carcinogenic substances, including ethylene glycol. |
| 3. Methyl bromide                          | - High solubility in organic substances.                                  | - Shows selective influence on the reduction in microorganisms in the raw material, - harmful effect on the Earth’s ozone layer. |
| 4. Ozonation                                | - It has a strong bactericidal effect, to which both Gram-positive (Listeria monocytogenes, Staphylococcus aureus) and Gram-negative (Escherichia coli, Salmonella) bacteria are susceptible, as well as viruses and yeasts, - does not cause changes in the chemical composition of the essential oils they contain. | - The method is relatively young, - a technological line for mass sterilization of herbal material in processing plants. |
5. Conclusions

This article highlights the need for a unified herb purification protocol. Currently, there is no uniform procedure to control the microbiological purity of herbal products in their processing. Among the methods of herbal hygiene, ozonation seems to be the most promising. The conducted analyzes of the literature show that the ozonation process used achieves a high degree of reduction in microbiological contamination. Low energy consumption of ozone generators favors their wide application. This technology is also characterized by multidimensional innovation. All this is a strong argument for high efficiency and no influence of the ozone concentration used on the composition and expected biological activity of herbal products. Therefore, they confirm the validity of developing an effective method of herbal decontamination with the use of ozone. The producers of herbs also use other ways of depriving the plant raw material of microbiological impurities (e.g., the steam sterilization). It seems that the combination of different methods should be the most effective solution.

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