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Ingredients, Morphology and Color Changes of Herbal Drugs and Spices after Mechanical Saturated Steam Decontamination

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Ingredients, Morphology and Color Changes of Herbal Drugs and Spices after Mechanical Saturated Steam Decontamination

Von der Fakultät für Lebenswissenschaften

der Technischen Universität Carolo-Wilhelmina zu Braunschweig

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List of Abbreviations

BC	Before Christ
AD	After Christ
DGHM	German Society for Hygiene and Microbiology
EC	European Commission
LFGB	Lebensmittel- und Futtermittelgesetzbuch
AMG	Arzneimittelgesetzbuch
Ph. Eur.	European Pharmacopoeia
CFU/g	Colony-forming unit per gram
HPLC	High performance liquid chromatography
SD	Standard deviation
GC	Gas chromatography
TBME	tert-Butylmethylether
TMSH	Trimethylsulfoniumhydroxid
TAMC	Total aerobic microbial count
TYMC	Total combined yeasts/moulds count
TCM	Traditional Chinese medicine

1 Introduction

1.1 Utilization and classification of Herbal Drugs and Spices

1.1.1 Herbal Drugs

According to their name, herbal drugs are produced from dried plant parts. These plants have a long tradition and were conducted by experienced herbalists.

It wasn’t until 150 years ago that these drugs and their ingredients became the subject of scientific research. Later the transition of the ingredients in the aqueous phase was investigated. It was recognized that water is not the best solvent for all ingredients. Therefore, some drugs are generally used for making tea e.g fennel and sage, others are used for the preparation of phytopharmaceuticals e.g. nasturtium or ginkgo (7).

The application and description of herbal drugs can be traced back thousands of years. Already in the “Materia Medica” of Mesopotamia (3000 - 2400 BC) comments on oily and aqueous plant extracts are made. Excavations at the library of the Assyrian capital of Nineveh unearthed 22000 clay tablets with the descriptions of 250 medicinally used plants were discovered. In China 4500 years old documents were found proving the use of plants for medicinal purposes. Two well-known sources of Egyptian medicine are the Ebers papyrus and the Edwin Smith papyrus (both 1500 BC). The Ebers Papyrus contains a scroll of over 20 m length with 108 columns of 20-22 lines about the origin of diseases and treatment options with a collection of recipes (89).

In the course of the 5th century BC rational explanations started to replace the believe in magic. Under the influence of pre-Socratic philosophers a healing art flourished with significant contributions to

occidental medicine. Its influences stretched from the periphery of Asia to North Africa and included Sicily, the Greek island of Kos and the Asian peninsula, Knidos. Basically, diseases were explained by an excess of mucus (89).

The transportation of air and blood into the head were believed to be blocked. Hippocrates of Kos (460-375/351 BC) was the most famous physician of his time. He was also, despite his philosophical approach to medicine, the founder of scientific medicine. Diseases were considered the result of an imbalance between man and environment. To restore the balance, the body would fight against harmful influences. The therapeutic aim consisted in restoring the balance and to reinforce the healing efforts of nature. Another important medical historical opus was the "Materia Medica", written by the Greek physician Dioscorides Pedanios from Kilikia (50 AD). His work consists of five books that described about 600 plants and their effects. Until 16th century it was the leading opus on herbs and the basis for many future books on this subject (6).

The philosopher and perhaps most significant physician after Hippocrates, Galen of Pergamum (129-199 AD), wrote a commentary on the Hippocratic Corpus of simple medicines. His work was of great importance, because he established a medical pathology, which is known as "humoral pathology" or "four humours theory" in the history of medicine. It influenced medicine until the 19th century. From the 8th until the 12th century humans and animals were treated with medicinal plants from herb gardens of monasteries (69).

The knowledge about the effects of medicinal plants has been carefully preserved in monastic libraries in the so-called drug arias or herbal recipe books. Among these, the Lorcher Pharmacopoeia of the Carolingian era became especially famous. It contains more than 500 recipes of many drugs (14).

Of particular value was also the opus of Hildegard of Bingen (1098-1179). As the mother superior of a Benedictine monastery she wrote the books "Liber Simplicissimus Medicinae" and "Causae et Cura". In her scriptures

she covered 230 plants, including *Calendula officinalis*, *Salvia officinalis*, *Artemisia absinthium*, *Thymus serpyllum* and many others. The first pharmacopoeia was the “Circa instans”. The original version contains more than 270 monographs and consists of two parts (69).

In the 12th to 14th century it was often the monastery gardens, from which evolved the German medicinal and spice plants cultivation.

Later on pharmacists adapted these cultivation technologies from the monks. It is due primarily to pharmacists, herbalists and traders that many wild-growing medicinal plants were taken over into crop.

These pharmacies gardens have existed over centuries, not only in Germany but also in many other countries. The Schleswig-Holstein-Danish pharmacist order of 1672 even stipulated that a herb garden had to be attached to a pharmacy (69, 14).

There are more than 100 known manuscripts from the 14th and 15 Century in Europe which include culinary recipes. Of course, the level of awareness and the use of spices depended on the social status. But spices were not only used to affect flavor but also to benefit digestion or for medical reasons (89).

In the middle of the 17th century many spices were grown around Nuremberg to a greater extent, e.g. fennel, marjoram, blessed thistle, lemon balm, aniseed and coriander (89).

Finally in the course of 19th century new regions of arable land developed: fennel was grown around Weißenfels and Aschersleben became known as the spice chamber of Germany, mainly through the cultivation of marjoram and peppermint (14).

In 1934 the first national varietal register for medicinal, spice and fragrant plant was established in Leipzig. All currently marketed medicinal plants were registered for testing and examined. In 1938 for the first time cultivars were determined (group and individual varieties) and characterized in a preliminary description of the variety (6, 14).

Since 1950 medicinal and spice plant cultivation is a subarea of agricultural crop farming as an autonomous subject at the agricultural faculty at Martin-Luther-Universität Halle-Wittenberg in Halle (Saale).
Since 1991 the „Bundesanstalt für Züchtungsforschung an Kulturpflanzen" (BAZ) in Quedlinburg has made major contributions to the field of drug and spice research. With effect from 1 January 2008 the BAZ was incorporated into the newly created Julius Kühn Institute (JKI) – Federal Ministry of Food, Agriculture and Consumer Protection.

1.1.2 Spices

Spices, herbs and vegetable seasonings are appreciated for their typical flavor and color (85). International trade in spices goes back many centuries. The most important supplies were shipped to Europe from faraway tropical and subtropical regions. There was a great demand for pepper and cloves, which were as valuable as gold (55).

Nowadays spices are cultivated worldwide in all kinds of climatically suitable areas. Therefore we can buy spices from many different countries. They are traded on the world market like any other product, because the former monopolies no longer exist. Nevertheless some of the originally most important cultivating areas such as Zanzibar (cloves) or Ceylon (cinnamon) are even today among the main exporting countries (33).

These days the consumers can rely on expert-publications and easily comprehensible, detailed information. These publications provide information on the original harvesting and use of imported and domestic spices (77, 9).

The history of spice trade and thus the establishment of trade routes go back to pre-Roman times. Cinnamon, cumin, ginger and pepper came to Europe as early as before Christ (86, 77). In the Eastern Roman Empire, Constantinople was the trading capital. Around 400 AD, spices were also imported more and more into Northern Europe (76). As the balance of power shifted because of the mongol dynasty, the city states of Venice and Genoa became the leading trading centers of Europe. By the mid-15th century the Portuguese monopolized this trade (33). During the 17th century the Dutch took over the spice trade. In the 18th century the English established their trade routes. Since the end of the 18th century the Americans also sailed east to trade spices. From that time on the New York harbour became popular (76).

Spices are not only used for taste and flavor, but for preservation as well (54). An example for preservation would be a meat product that is heavily

spiced in order to preserve it for a longer period of time. Spices can also be used to help digestion, like marjoram in sausage meat products (55).

A feasible definition of spices could be that spices and condiment (81) plant parts are used as flavor- and / or odor-giving ingredients in foods because of their specific content of certain natural substances. Moreover, spices are used because of their antimicrobial properties for preserving food (26). Spices include flowers, fruits, buds, seeds, barks, roots, rhizomes and bulbs or parts of all these, usually in dried form. Spices can be classified either by the type of plant (Taxonomic classification tab. 1.1.2.1) or by the plant parts from which they are obtained (Conventional classification tab. 1.1.2.2). Both are shown in the two tables below (31, 79).

Tab. 1.1.2.1: Conventional classification of spice (79).

Classes	Spices
Hot spices	Capsicum (chillies), Cayenne pepper, black and white peppers, ginger, mustard
Mild spices	Paprika, coriander
Aromatic spices	Allspice (pimento), cardamom, cassia, cinnamon, clove, cumin, dill, fennel, fenugreek, mace and nutmeg
Herbs	Basil, bay, dill leaves, marjoram, tarragon, thyme
Aromatic vegetables	Onion, garlic, shallot, celery

Tab. 1.1.2.2: Taxonomic classification of spices (79).

Angiospermae	*Dicotyledoneae*	*Sympetalae*		*Solanaceae*	chilli, paprika, red pepper
				Pedaliaceae	sesame
			Campalunatae	*Compositae*	camomile, chicory, tarragon
		Archichlamydaeae	*Piperales*	*Piperaceae*	cubeba, long pepper, pepper
			Ranales	*Myristicaceae*	mace, nutmeg
				Lauraceae	bay leaf, cassia, cinnamon
				Magnoliaceae	star-anise
			Rhoeadales	*Cruciferae*	mustard, wasabi
			Myrtiflorae	*Myrtaceae*	allspice, clove
			Umbelliflorae	*Umbelliferae*	anise, caraway, celery, chervil, coriander, cumin, dill, fennel, parsley
	Monocotyledoneae		*Liliiflorae*	*Liliaceae*	garlic, onion
				Iridaceae	saffron
			Scitamineae	*Zingiberaceae*	cardamon, ginger, turmeric
			Orchidales	*Orchidaceae*	vanilla

1.2 Guidelines, Laws and the Resulting Necessity of Microorganism Reduction

1.2.1 Guidelines

Because of the risk of possible intoxication due to microbial load in spices and herbal drugs (*Chap. 1.3*), different guidelines and laws were enacted around late eighties.

The German Society for Hygiene and Microbiology (DGHM) and the European Commission (EC) have developed directional and warning guidelines for the food-producing industry.

Both are not legally binding, but recommend that no *Salmonella spp.* should be found in 25 g of a certain investigated food. About mold and E. coli no details are available from the EC. For *Enterobacteriaceae*, which includes *Escherichia coli* (E. coli), a boundary limit of two decades CFU/g is recommended. The DGHM proposes a warning value of 4 decades CFU/g for E. coli. For *Bacillus cereus* the DGHM issued a lower boundary limit (4 decades CFU/g) as advised by the EC (5 decades CFU/g). In contrast, the EC is stricter in assessing the bacterial content of *Clostridium perfringens*. It recommends a boundary limit of 3 decades CFU/g, while the DGHM only issued a boundary limit of 5 decades CFU/g (18, 27).

1.2.2 Laws

In food, pharmaceutical and cosmetic industries sterilization processes are a necessity in order not to endanger the health of the end user by pathogenic microorganisms. This fact is established by law.

The food and feed law (LFGB) contains following passage: "§ 5 Abs. 1: It is forbidden to produce food or treat, if its consumption is affecting the health ... "(57). The pharmaceutical law (AMG § 95) concerning the pharmaceutical industry states almost the same paragraph: “It is forbidden to bring unsafe drugs on the market.” A violation of this law will draw severe fines or can lead to a prison sentence (4).

In April 2010 the acceptance criteria for microbiological quality of herbal medicinal products for oral use were redefined according to Ph. Eur. 7th Edition, Supplement 6.7, chap. 5.1.8. In this law the herbal medicinal products for oral use are divided into three groups. For each group limits are defined that must be observed by the industry (*Tab. 1.2.2.1*) (25).

Tab. 1.2.2.1: Acceptance criteria for microbiological quality of herbal medicinal products for oral use (25).

Categories	**TAMC**	**TYMC**	**Escherichia coli**	**Bile-tolerant gram-negative bacteria**	**Salmonella**
A	≤ 10^7CFU/g	≤ 10^5 CFU/g	≤ 10^3 CFU/g	-	Absence (25 g)
B	≤ 10^4 CFU/g	≤ 10^2 CFU/g	Absence (1 g)	≤ 10^2 CFU/g	Absence (25 g)
C	≤ 10^5 CFU/g	≤ 10^4 CFU/g	Absence (1 g)	≤ 10^4 CFU/g	Absence (25 g)

The first group covers: "herbal medicinal products containing herbal drugs, with or without excipients, intended for the preparation of infusions and decoctions using boiling water (for example herbal teas, with or without flavouring additives)".

The second group comprises of "herbal medicinal products containing, for example, extracts and/or herbal drugs with or without excipients, where the method of processing (for example, extraction) or, where appropriate, in the case of herbal drugs, of pre-treatment reduces the levels of organisms to below those predefined for this category".

The last group covers: "herbal medicinal products containing, for example, extracts and/or herbal drugs, with or without excipients, where it can be demonstrated that the method of processing (for example, extraction with low strength ethanol or non-boiling water or low temperature concentration) or, in the case of herbal drugs, of pre-treatment, would not reduce the level of organisms sufficiently to reach the criteria required of the second group" (25).

This chapter in the Ph. Eur. 7th Edition is the most important one for herbal drugs because industries must observe these legal limits. Before

the law was amended in April 2010 herbal drugs belonged to section 5.1.4 Ph. Eur. 7th Edition, where all pharmaceutical preparations of plant materials were summarized. With the amendment of the law the hygiene requirements were significantly increased (23).

Apart from regulating the maximum microbial contamination certain germ reduction methods are entirely prohibited.
The EG has issued guidelines about food irradiation in 1999 (22). The current status of provisional authorizations in Member States varies considerably. According to Article 3 paragraph 2 of Directive 1999/2/EC, food and food ingredients are irradiated only in EU relocated and authorized facilities. These directives were recast into the Food Irradiation Regulation (legal since December 21, 2000). The revised regulation allows the treatment of dried herbs and spices with electrons, gamma- and X-rays. The exact specification of these types of radiation can be found in Appendix 1 of this regulation. Booth the technological necessity and the benefit for the consumer are the main reasons for food irradiation. Consequently, for example in France frozen frog legs are usually irradiated to protect consumers from infection. Due to the single market of the EU, frozen irradiated frog legs from Belgium, Netherlands and France can be put on the market in Germany (19, 20).
Herbal drugs are subject to completely different requirements. Since 2007 a new regulation is active for the use of radioactive radiation in connection with pharmaceuticals. This regulation allows exceptions to the prohibition of the use of radioactive rays. But this new regulation excludes herbal medicines. Thus, the earlier law still applies (3) and there it is written that it is forbidden to distribute radiopharmaceuticals or products prepared with the use of ionizing radiation (31).

Since the reactor accident in Fukushima there is a new regulation (EU) No 961/2011, 27th September 2011: for imports of food and feed originating or consigned in Japan special conditions apply to protect the population against possible poisoning. This German regulation states that additional

investigation must be done before medicinal plants may be imported from Japan (13).

Another forbidden microbial reduction method is the treatment with ethylene oxide. Since 1988 it is not allowed to use ethylene oxide in the preparation of pharmaceuticals (95).

Therefore other decontamination methods must be used (*Chap. 1.5*) in the preparation of herbal medicine.

1.3 Microbial Load of Herbal Drugs and Spices

Aromatic and medicinal plants are agricultural products, which are largely imported from e.g. South-East Asia and Africa. Most herbal drug plants need the hot and wet growing conditions there. Because of the hot and humid climate, the contaminants (bird excrement, manure, insects) and the post-harvest treatments (air drying, hand harvesting) there is often a natural contamination (2-8 decades) of herbal drugs and spices (10, 11).

A variety of microorganisms including pathogenic bacteria, molds and yeasts settle on the plant material (85) (Table 1.3.1). Aerobic spores as well as yeasts and molds play a major role in the contamination of herbal drugs and spices (60). As shown in Table 1.3.1, the bacterial loads vary among the different spices. E.g. curry, pepper and marjoram are amongst the most contaminated species (68). Fresh (not dried or treated) melissa (Melissa officinalis) has been proved to have a natural bacterial content of up to 5 decades. Hence, the total bacterial count already exceeds the legal limit (83). The different bacterial loads can be explained by several factors. First, the microbial contamination is highly dependent on the nature and origin of the herbal drug. Another important factor is the inherent anti-microbial effect of the spice (5, 8). Herbs and spices that grow near the ground usually have a higher bacterial load as spices that grow away from the floor (21).

Microbial status is also influenced by drying, transport, processing and storage (43).

Investigations have shown that air-dried plant materials are up to ten times higher contaminated with germs than freeze-dried spices. Similarly, vacuum-packed spice products are more contaminated than air-packed ones, and herbal drugs stored at 35 °C stored have higher bacterial counts than those stored at 25 °C (66).

India is the largest producer and exporter of spices, followed by China and Bangladesh. Western countries have a share of 3% of the world’s spice

production. In countries such as India and China the standards of food hygiene are often much lower compared to western countries. In combination with adverse environmental conditions and poor production conditions, quality problems may occur concerning the spices. Foodborne infections and food poisoning are the result (11, 85).

Various microbial species like bacilli, clostridia, pseudomonas, molds and enterobacteriaceae settle on dried herbal drugs and spices (32). The spores of different bacillus species produce more than 50% of the total bacterial count, including the toxin-producing *Bacillus cereus* as well as the flat-sour-pathogen *Bacillus stearothermophilus* and *Bacillus coagulans* (76).

Molds are the second most common microorganisms on herbal drugs and these are primarily represented by aspergillus species and penicillium species. 25% of the molds (aspergillus species and penicillium species) are able to form toxins (37).

Bacteria and/or molds clinging to spices can become pathogens when added to products in which they are able to grow. In the last twenty years contaminated spices were increasingly detected as cause of diseases (11). From April to September 1993 there was salmonella poisoning throughout Germany caused by salmonella infested paprika potato chips. This incident caused the producer of the chips huge damage, not only economically but also as a result of the tarnished reputation (58). Therefore spices should only be added with extreme caution to foods which are not subjected to additional heating (62).

Tab. 1.3.1: Microbial load of herbal drugs and spices (85).

Herbal drugs and spices	Total plate count CFU/g	Microbial spectrum
Ginger	$10^5 – 10^9$	*Bacillus cereus, Clostridium perfringens, Streptococcus, Staphylococcus, Molds, Yeasts*
Coriander	$10^5 – 10^9$	*Bacillus cereus, Enterobacteriaceae, Streptococcus, Molds, Yeasts*
Caraway	$10^3 – 10^9$	*Enterobacteriaceae, Bacillus cereus, Clostridium perfringens, Yeasts*
Marjoram	$10^1 – 10^6$	*Bacillus cereus, Clostridium perfringens, Staphylococcus*
Cloves	$10^4 – 10^9$	*Bacillus cereus, Clostridium perfringens*
Paprika	$10^5 – 10^7$	*Salmonellae, Molds, Yeasts*
Pepper	$10^6 – 10^9$	*Enterobacteriaceae, Bacillus cereus, Clostridium perfringens*
Rosemary	$\leq 10^5$	*Enterobacteriaceae, Bacillus cereus Streptococcus, Staphylococcus*
Basil	$10^5 – 10^7$	*Enterobacteriaceae, Bacillus cereus, Clostridium perfringens*
Curry	$\leq 10^7$	*Enterobacteriaceae, Bacillus cereus, Molds, Yeasts*

1.4 Microbial Decontamination Methods of Herbal Drugs and Spices

The classification of microorganism reduction methods of herbal drugs and spices is not a simple matter, but it is possible to divide the sterilization methods into three major groups.

The first group covers all chemical decontamination treatments as well as the ozone treatment, the carbon dioxide treatment and the ethylene oxide treatment - which is forbidden since 1988 because of the possible danger of poisoning (95).

The second major group contains the physical decontamination treatments and can be split into two subgroups. Here a distinction is made between the thermal physical and the non-thermal physical decontamination treatments. To the thermal physical decontamination treatments belong the saturated steam decontamination, the steam decontamination with protein addendum „Coating“, the alcohol vapor treatment, the high frequency heat treatment, the microwave decontamination and the infrared decontamination (*Tab. 1.4.1*). All these germ reduction methods kill the microorganism by exposing them to external heat.

The non-thermal physical decontamination treatments kill the microorganisms through radiation where the structure of chemical molecules is decomposed and the extrusion process, where the surface of the microorganisms is scraped off. To this group belong treatments with high-voltage electrical pulses decontamination, high pressure decontamination, extrusion process, ionizing radiation, and UV irradiation.

The third major group covers the combination decontamination treatments like plasma decontamination, alcohol and steam decontamination and mechanical saturated steam decontamination (Lemgo Process). These former methods work with multiple extermination techniques while the Lemgo process works with eradication through heat and the pulling off of microorganism. On the following pages each method will be discussed in more detail (75).

Tab. 1.4.1: Classification of microorganism decontamination methods (75).

Chemical decontamination treatments	Physical decontamination treatments		Combination decontamination treatments
	Thermal	Non-thermal	
Ethylene oxide	Saturated steam decontamination	High-voltage electrical pulses decontamination	Plasma decontamination
Ozone decontamination	Steam decontamination with protein addendum „Coating“	High pressure decontamination	Alcohol and steam decontamination
Carbon dioxide decontamination	Alcohol vapor treatment	Extrusion process	Mechanical saturated steam decontamination (Lemgo Process)
	High frequency heat treatment	Ionizing radiation	
	Microwave decontamination	UV irradiation	
	Infrared decontamination		

1.4.1 Chemical Microorganism Decontamination Methods

1.4.1.1 Ethylene Oxide

For many years, the decontamination with ethylene oxide was regarded as a reliable and practical method. It was first established in the U.S. and only later European countries like Germany began with the germ reduction with ethylene oxide at least for some products, although this method causes a loss of valuable substances, e.g. in pepper and oregano (93). All in all the procedure was reliable and destroyed the microorganisms effectively (59, 91).

The ban on using ethylene oxide can be explained by the mutagenic and carcinogenic decomposition products. The residual ethylene oxide content decreases in the first two weeks, whereas the content of 2-chlorethanol and 2-bromoethanol increases. Both products occur because ethylene oxide reacts with chlorine and bromine of the environment. After reaching a maximum, the concentration of ethylene chlorohydrin declines. Especially dry products with less than 8% moisture reach high levels of ethylene chlorohydrin very fast (28, 90). Since 1988 ethylene oxide has been banned for the sterilization of spices and herbal drugs due to its mutagenic and carcinogenic effects when inhaled (42).

1.4.1.2 Ozone

Ozone is used very successfully to reduce the bacterial load in drinking and bathing water, but it is ineffective in the disinfection of dry media. At least 14% of water content is required for a sufficient reduction of bacteria, but most dried drugs do not contain more than 11% of residual moisture. Otherwise storage stability loss occurs. Leaf-drugs and ground products showed a loss of up to 30% of essential oil when treated with ozone (104). In addition, the ozone created a change in flavor. The sensory results were so poor that all further investigations were terminated (101). It wasn’t until 2008, when new investigations were started. They showed that *E. coli* and *B. cereus*, artificially brought onto

pepper samples can be reduced with ozone. Treatment parameters were ozone concentration of 1 ppm for 360 minutes. This treatment led to a reduction between one and two decades *(*2).

1.4.1.3 Carbon dioxide

Carbon dioxide is of great importance as a protective gas because of its bacteriostatic properties (71). In 1987 it was proven that microorganisms are destroyed when treated with carbon dioxide under high pressure (46). A year later, a patent was submitted in which the carbon dioxide disinfection of spices and herbal drugs was declared. Even vegetables can be freed of microorganisms with the patented process. Further investigations on the patented technology showed that the spices and medicinal drugs must have a water content of 10-40% for the process to be effective.

The decontamination parameters are 60 °C at a pressure of 10-80 bar. Under these conditions molds and enterobacteriaceae are reduced by 2 decades. A drawback was the moistening of the material, which led to clumping during the treatment. Disadvantages of the treatment are drying, high investment costs and the costs of the carbon dioxide treatment itself (54).

1.4.2 Thermal Physical Decontamination Treatments

1.4.2.1 Saturated Steam Treatments

Due to the high demand of saturated steam processes in Europe there are now various variants and advanced procedures common.

A well-known procedure is the Micro-Control process: spices and herbal drugs are heated in a warming chamber (50-55 °C). Subsequently the plant material is placed in a heated autoclave and submitted to steam until the final germ reduction temperature is reached. A homogeneous heat transfer is achieved by uniform and gentle rotation. When the reduction of bacteria is completed, the spices are cooled very quickly and

dried even further. With this method a germ reduction of 99.9% can be achieved.

As described in a publication from 1993 the drugs parsley, several varieties of pepper and whole cumin and paprika were treated with the micro-control method. The microbiological analysis after treatment of the different drugs showed that all samples had a maximum contamination of a decade. The loss of essential oil for pepper and cumin amounted to 0.1 to 0.3 mL/100 g raw material (83).

The technology of the EVW-Procedure (Worleè, Hamburg) works by and large in the same way as the Micro-Control process. Temperature and exposure time can be finely tuned to ensure a gentle reduction of bacteria for each drug (70).

Another technique is used by the Zelgerm-Procedure. This batch process is carried out with a pre- and post-vacuum. The treatment chamber is evacuated so that the steam can be rapidly fed to the product, reducing the heating phase. A post-vacuum is created in order to achieve swift drying and cooling of the product. The entire sterilization takes 15 to 20 minutes at a pressure of 1 to 2.7 bar. The treatment temperatures of herbs and medicinal drugs vary between 80 and 135 °C. The sterilization parameters are adjusted individually for each product.

Germ reductions by this method showed that the samples pepper, paprika and parsley do not fall below the limit of 4 decades. Marjoram was indeed just below the limit of 4 decades; however, it turned yellow and showed a loss of essential oil (97).

The technology Hosokawa Microm from the Netherlands also works with a pre- and post-vacuum. In contrast to the Zelgerm Procedure, the sterilization takes place in a vacuum dryer with integrated mixer. McCormick & Co, an US-American company, runs a virtually continuous Micro Master Process which works with pre- and post-vacuum and, if desired, with overheated steam. This technology requires two double-walled cylindrical containers, which are used for the sterilization and drying (97).

A special feature of the continuous process Bactosafe® is the ability to sterilize powdered products. Another feature is the recirculation of steam in order to reduce the loss of quality of spices and medicinal drugs. The prerequisites for this are small treatment rooms and a limited loss of steam during the inward and outward transfer of the products. Finally the product must be dried further.

This variant of the process guarantees a maximum contamination of 4 decades with a loss of 10-15% essential oil. Analyzed were, amongst others, the pharmaceutical drugs peppermint, chamomile, thyme, lemon balm, goldenrod, chamomile flowers, orange peel and flowers (44).

1.4.2.2 Steam Decontamination with Protein Addendum „Coating”

At this point, the "Sterispice" process is emphasized. This method of steam sterilization uses an addition of enveloping proteins which protect the ingredients of spices and medicinal drugs. After filling the autoclave, the protein is added to the product in a quantity of 1% of the plant material dry weight. Again, there are different decontamination parameters for each spice or medicinal drug. The microbiological studies show a reduction of 4 decades. The remaining germ count was therefore always less than 2 decades. Despite a protein envelope there was a loss of essential oil from 3 to 12%. The yellowing of the products cannot be prevented by this "coating” process. Especially chlorophyll was reduced (64).

1.4.2.3 Alcohol Vapor Treatment

There have been attemps to decontaminate various herbal drugs with alcohol vapor. Unfortunately, treatment with pure ethanol vapor could reduce the number of bacteria by only a decade. When a few bars of pressure were added at 100 °C, bacterial reduction by 2 decades could be achieved. On the other side the use of pure methanol improved the sterilization effect, but, methanol leaves residues on the medicinal plant. As methanol can cause health problems, this method cannot be used.

Sensory observations showed a large loss of essential oil in thyme and marjoram. Both drugs had also yellowed after the treatment (76).

1.4.2.4 High Frequency Decontamination

The uniform heating of spices and medicinal products with the aid of electromagnetic oscillations in the high frequency range was investigated in a large scale area. Here, a combined method was used. Once a high-frequency heating was used within a range of two to three minutes, and a hot-holding at 100 °C to 120 °C for 30 minutes to 3 hours. The high frequency treatment was continued until the anode electricity had dropped to its initial value (15, 16, 17).

Investigated were the spices cinnamon, white and black pepper, cumin and marjoram. To achieve decontamination, the water content has to increase up to 5 to 10%. Afterwards the average water amounts to between 12 and 13%. After the decontamination process follows heat drying, whereby the water content is reduced to about 26 to 70%. As a result, some spices had a lower water content after the decontamination than before. The microorganisms were reduced below the detectable limit of 100 CFU/g, but the value-determining ingredients of marjoram and pepper were reduced by half. Unfortunately, the achieved reduction of microorganisms was disproportionate to the loss of ingredients. These results showed that treatment with electromagnetic oscillations in the high frequency range is not suitable for decontamination of spices and pharmaceutical drugs (15, 16, 17).

1.4.2.5 Microwave Decontamination

Studies in laboratory scale with white pepper showed that the optimum water content is 16%. With a treatment period of 60 seconds, the microorganisms are reduced by 3.3 decades with almost no loss of essential oil. If the water content of the pepper is raised only by one percent, the loss of essential oil increases to 7%. Basically, the microwave decontamination is suitable for grain spices and herbal drugs, whereas

with unmilled and leaf drugs the microwave decontamination generates strong sensory changes. An average of two decades of microorganism reduction after a microwave treatment is too little for a heavily contaminated spice to make it a marketable product (15, 16, 17).

1.4.2.6 Infrared Decontamination

In a corporate project of the Swedish Institute for Food and Biotechnology and the Technical University of Berlin it was examined whether the bacterial flora of ground red pepper could be reduced with infrared treatment. The analyzed parameters were temperature, pH, water activity, the total microbial count and the content of *Bacillus cereus*. For the treatment parameters with a temperature of 95 - 100 °C and an a_w-value of 0.88 for a treatment period of 6 minutes there was a microorganisms reduction of 4.5 decades. The subsequent spore concentration was 2 decades. It was shown that the reduction of microorganisms also increases with increasing water activity. At a water activity of 0.96, the reduction of the bacteria was improved by 6 decades (87, 88).

1.4.3 Non-thermal Physical Decontamination Treatments

1.4.3.1 Microorganism Reduction with High-voltage Electrical Pulses

In this method short voltage pulses with high potential are applied over a selected period. The temperature increases very slowly during this process so that heat-sensitive spices and pharmaceutical drugs are handled carefully.

The germ killing effect of the high-voltage electrical pulse treatment is based on the construction of a transmission potential of the cytoplasmic membrane of the microorganisms (41).

In the formation of a transmission potential of 1 volt, voltage-dependent protein channels are opened. Furthermore, a loss of membrane integrity occurs as well as a formation of pores in the membrane and the abolition of membrane permeability. A dielectric breakdown of the cell membrane happens which leads to an outlet of cell components.

This effect is reversible at low field strengths. Therefore, a need for high field strengths and long treatment times are used to destroy germs located on the surface (49).

An additional problem for of the decontamination with high-voltage electrical pulses is that different microorganisms have different sensitivity to the procedure. For example yeast is more sensitive than gram-positive bacteria. Mycotic ascospores and bacterial spores in the stationary phase are not inactivated by this treatment.

As in many other sterilization methods, the water content plays an important role. It became evident in a study about dry spices without a prior addition of water, the microorganisms are reduced by a decade. However, after adding water and using higher temperatures a complete reduction of the microorganisms was achieved. The maximum possible germ reduction was obtained when as the water content was adjusted to 54% (47).

1.4.3.2 High Pressure Decontamination

The studies on decontamination of spices and herbal drugs with the aid of high hydrostatic pressure showed no bacterial reduction. The main idea of this method was the use of protein denaturation, protein coagulation and microorganism's enzyme inactivation injury. Microorganisms in food die when treated with high-pressure. In a pepper-water solution, however, the bacterial load was reduced by 4 decades (50). Due to the complex drying, a great loss of valuable substances was caused (36).

1.4.3.3 Extrusion Process

In this process the microorganisms are exposed to pressures of 2000 to 9000 bar. In addition the seeds are subjected to a shear of several thousand Newton. With this method an average decontamination of 7 decades can be achieved. No sensory changes in pepper and paprika were detected (32).

The "Masterpiece" process of the company "Lucas Ingredients" operates with a starch product during the extrusion so that the herbal drugs are gelatinized. The product is covered in a way that as few ingredients as possible are lost. For each herbal drug certain parameters can be set. These parameters are temperature, pressure, and extruder dimensions. The actual decontamination phase of the "Master Piece" process only takes a few seconds. During this short phase, the critical parameters are a temperature of 140 °C and pressure from 30 to 80 bars._The result is a highly viscous melt, which must be ground afterwards. With this method, a microbial reduction of 4 decades is achieved. It will extract most ingredients but they are not lost, because they are protected by the starch (34).

1.4.3.4 Microorganism Reduction through Ionizing Radiation

The decontamination of herbal drugs and spices through Ionizing Radiation is a field of intensive studies because of the highly controversial discussion of this method.

Electron beams (β-rays) and high frequency electromagnetic waves and high energy (γ-rays) belong to the field of nuclear energy and radioactivity. This fact leads to prejudices and a highly negative attitude among the public. Therefore, an unemotional discussion of this topic is not possible (93).

For the reduction of microorganisms by irradiation the same rules apply as to the reduction through heat treatment. There are different killing curves for different types of germs and the D-values for the spores are higher than for vegetative bacteria. Due to the heterogeneous micro flora and the different surfaces of the spices and herbal drugs a lot of analyses were carried out concerning the efficacy of the γ-rays on different spices like pepper, saffron, thyme, eucalyptus or lavender (38, 78).

Enterobacteriaceae, molds and aerobe spores were already reduced below the detection limit with a γ-rays intensity of 5 kGy. Therefore, the legal limit of 10 kGy was very often not even approached. A preliminary microbiological investigation shows which sterilization intensity is required. At a dose of 5 kGy, there was an average reduction of germs of 3 decades concerning the total number of bacteria. At 10 kGy, 4 decades were reached (92).

Sensory analysis of the various spices and medicinal drugs indicate that no significant change was created in the content of essential oil. Gas chromatography showed changes only in fenugreek, cumin, juniper and marjoram.

For seeds and fruits with high content of fatty oils things look different. Here, these main ingredients turned rancid due to the radiation treatment. For spices and medicinal drugs, where the appearance and the color also play an important role, no significant changes were observed (103).

In the context of decontamination with ionizing radiation, many toxicological studies have been accomplished. In animal experiments, various animals were fed with irradiated spices. The health of these

animals was obviously not affected. In this context it is important to emphasize that humans and animals are different in many ways such as constitution, food reception and metabolism. Results from studies on animals cannot be transferred unconditionally to humans (103).

1.4.3.5 UV Irradiation

The UV irradiation is a less practical procedure for the decontamination of spice and herbal drugs. The antimicrobial effect of the UV radiation is attributed to the changes in nucleic acids. The most effective wavelength range of the UV radiation is 250-260 nm, which coincides with the absorption maximum of the nucleic acids. Because of swirling dust and non-planar surface the radiation reaches only a small portion of the microorganisms which are on the surface. The remaining mold and spores proliferate again (32).

1.4.4 Combination Methods

1.4.4.1 Microorganism Reduction with Plasma

The decontamination of germs with plasma is a relatively new treatment. The outer shells of the germs are destroyed and they leak out. At the same time the plasma and the genotypes of the microorganisms are damaged (66).

Initial studies on the sterilization of spices and medicinal drugs showed greatly varying results. The analysis covers artichoke, chamomile, ginkgo and guarana._For gingko and artichoke a reduction of microorganisms of 3-4 decades could be measured. In contrast the plasma treatment did not reduce the total plate count of the drugs chamomile and guarana (45).

As the plasma decontamination process is a very recent method, more studies will be necessary.

1.4.4.2 Microorganism Decontamination with Alcohol and Steam

The alcohol-water vapor process is toxicologically harmless and also legally permitted. In this combined process, a mixture of alcohol and water is used as saturated steam. Studies have shown that only spices and medicinal drugs with existing plant structures can be sterilized. Inherent in this process is the loss of the valuable ingredients (30).

1.4.4.3 The Mechanical Saturated Steam Vacuum Decontamination

It is extensively described in the next chapter because this procedure is a major part of this PhD thesis.

1.5 Mechanical Saturated Steam Decontamination Lemgo Process

1.5.1 History

Since 1994 a microbial reduction process was discussed, by which the living germs could be pulled off from the surface. The first evidence for this fact showed during the development of a hydro-thermal decontamination process for medicinal plants (106).

A large vacuum buffer tank is attached to the vacuum steam vacuum decontamination system. Fast evacuation of the treatment area is achieved. The product is cooling fast and the thermal stress is low.

Very high bacterial count reductions were detected. 3 to 4 decades were reduced by vapour deposition in only 10 seconds. The thermal decontamination effects alone could not cause such high reductions, because only bacterial spores were on the plant material.

This high decontamination effect drew the attention to a second microbial reduction effect. A process that made use of this effect was developed. This procedure is called Lemgo process. Besides the effect of the thermal inactivation, the microorganisms are ripped off the surface. The microorganisms are pulled off due to flash evaporation of condensate film, which is formed during the steaming of the surface. This is made possible by a sudden evacuation of the treatment room (61).

1.5.2 The Flash-Effect

Basic studies on the reduction of bacteria with a vacuum steam vacuum treatment were performed by Kozempel and Morgan (51, 52, 72, 73).

A plate count reduction was observed due to thermal inactivation. The pre-vacuum is used to remove the air and also a part of the moisture of the process chamber as well as of the product. This leads to a higher heat transfer during steam treatment and thus to a higher bacterial count reduction due to thermal inactivation. The post-vacuum increases the re-evaporation of the condensate formed during the steam treatment and leads to a more rapid cooling of the product. The possibility of tearing off the microorganisms during the re-evaporation of the condensate was not considered (61).

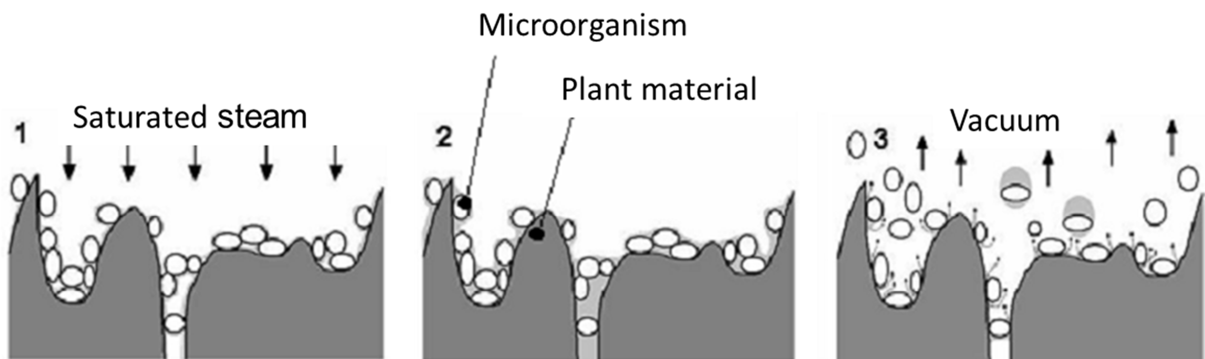

Fig. 1.5.2.1: The principle of the Lemgo process by flash evaporation is shown.

The formation of the flash effects can be split into three phases. At phase one (*Fig. 1.5.2.1*) the steam condenses due to the temperature difference between steam and product. Subsequently a uniform condensate film forms on the product surface. Phase two covers the formation of the condensate film which takes 5 to 10 seconds. Through the condensate film, the capillary and van der Waals forces and thus the adhesive force between the microorganisms and the product surface is reduced (*Fig. 1.5.2.1*) (75).

Phase three is the decisive process step of the method - the flash evaporation. The flash evaporation is an intensive re-evaporation of the

condensate film by sudden application of a vacuum. This causes a tear off of the microorganisms (*Fig. 1.5.2.1*) (75).

In contrast to the single-phase overflow process, the relieving forces are not tangential but vertical to the surface. This results in much larger forces than an overflow.

Unfortunately, it is not possible to determine a breakaway force for this operation which overcomes the corresponding adhesive force. The amount of heat near the surface is used for the re-evaporation of the condensate. Only by very short steaming treatment times it is possible to remove the condensate almost completely by a flash evaporation of the product surface (61).

Long vapor deposition times lead to increased heat transfer into the product. Thereby, the evaporation enthalpy is no longer available for the flash evaporation. The flash evaporation is carried out by a sudden application of a vacuum with high-pressure reduction rates. Due to the rapid pressure reduction, the condensate is a superheated liquid because vapor pressure and ambient pressure are no longer in thermodynamic equilibrium (60).

The extent of mechanical decontamination effects were determined by comparing the results with experimental decontamination of the modeled thermal inactivation. Then, the modelled thermal inactivation of *G. stearothermophilus* at about 135 °C and a vaporization time of 10 seconds lead to a reduction less than a decade of *G. stearothermophilus*. With a determined plate count reduction of about three decades through the Lemgo process the extent of mechanical effects amounts to more than two decades. Based on the results presented, the existences of mechanical effects in this type of decontamination treatment with saturated steam are taken for granted (61).

2 Scope of Work

Because of not avoidable germs, strong quality losses during the microbial reduction and high legal requirements, there is a need for the development and refinement of microbial decontamination methods/systems in Germany. The focus of this work lies on the quality analysis of spices and herbal drugs after application of the mechanical saturated steam decontamination the so-called “Lemgo process”.

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3 Decontamination Equipment

3.1 The 0.7 L Laboratory Decontamination Equipment

The decontamination tests were conducted in a laboratory decontamination facility which was composed of a 0.7 L treatment chamber and an 8 L vacuum buffer tank (Fig. 3.1.1).

Fig. 3.1.1: Construction of the 0.7 L laboratory decontamination equipment.

Both repositories are cylindrical and made of stainless steel. The single devices are connected with pipelines with an inner diameter of 1.27 cm. The record of temperature and pressure is provided by a data acquisition system. Steam treatment and evacuation periods are regulated by

electronically controlled pneumatic angle valves in front of and behind the treatment chamber. The treatment chamber is evacuated by a water ring compressor with gas emitter. A tube bundle heat exchanger between vacuum buffer tank and water ring compressor promotes pressure reduction and provides the condensation of steams extracted from the treatment chamber. The heat exchanger is working with water and has a capacity of 0.4 L (60).

3.2 The 5 L Industry-Similar Decontamination Equipment

For the decontamination reactor, a solid state ploughshare mixer (Lödige Labormischer Typ M5, Lödige Maschinenbau GmbH, Paderborn) was used to provide an adequate disaggregation of the pharmaceutical material. The stirrer has a volume of 5 L and is equipped with a double wall jacket (Fig. 3.2.1). This allows temperature control of the reactor, which in turn provides control over the condensate film. An electric steam generator is attached to the mixer, delivering saturated steam with 8 bar absolute pressure and about 180 °C. Steam pressure is throttled to the desired pressure according to the temperature level via a pressure reduction valve. Steam is introduced into the mixer for vaporization. To establish a pre- or post-vacuum, the equipment also includes a vacuum unit. A 100 L heat exchanger is used as a vacuum buffer to minimize evacuation time. To condense the steam it was cooled down to -20 °C (74).

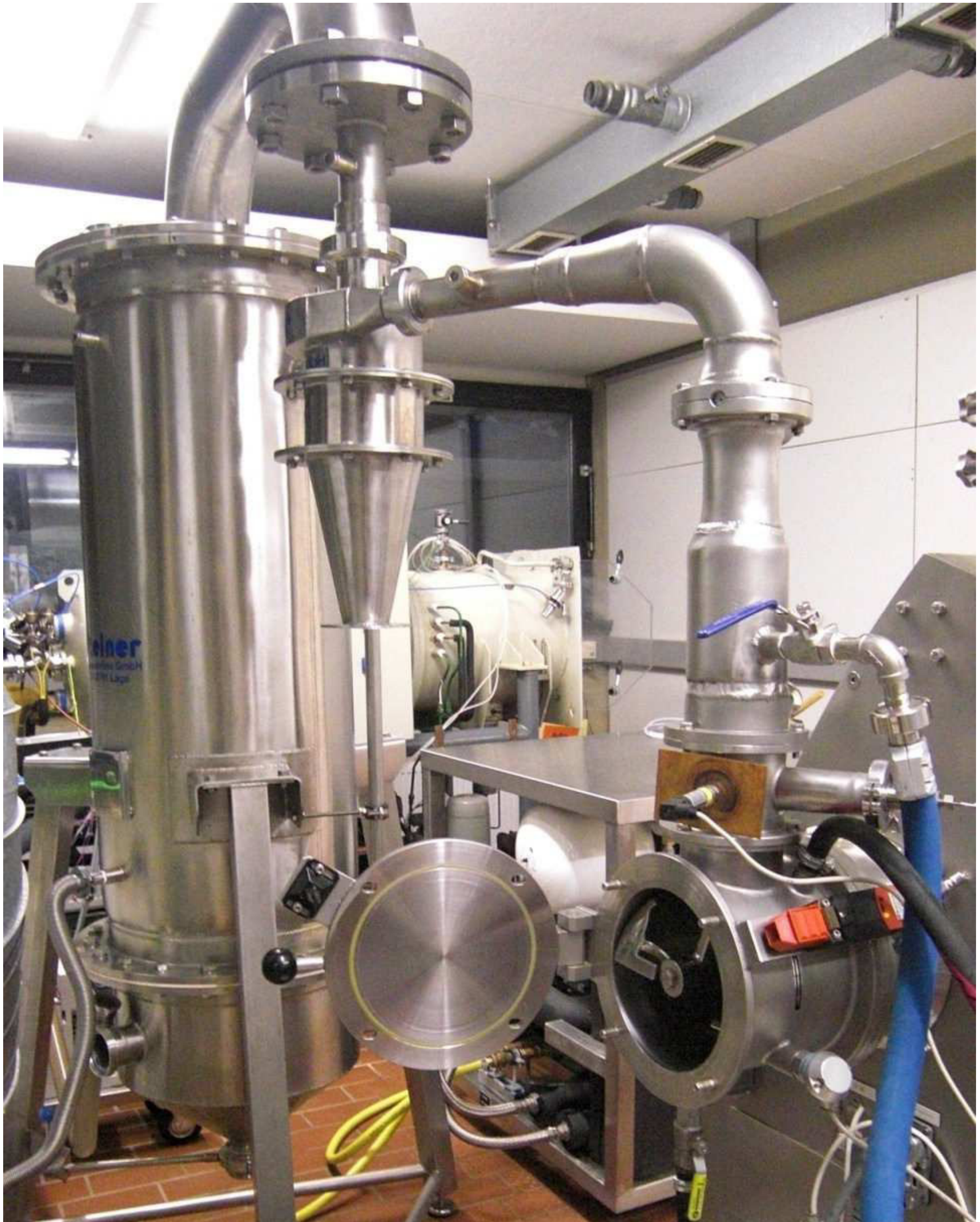

Fig. 3.2.1: Construction of the 5 L industry similar decontamination equipment.

4 Results and Discussion

This chapter presents the achieved and published research results. The focus of this work was the qualitative study of herbal drugs after treatment with the mechanical saturated steam decontamination ("Lemgo process").

In the first release, the pharmaceutical herbal drug nasturtium or Indian cress was treated in a 0.7 L laboratory system. The ingredient losses and the reduction of bacteria were three times (5s, 10s, and 20s) examined at two different temperatures (110 °C and 125 °C).

The second paper shows the superficial changes of six different essential oils within the several drugs after treatment with the "Lemgo process". The treated plant material oregano, marjoram, fennel, eucalyptus, peppermint and thyme were analyzed by scanning electron microscopy and GC.

The third publication deals with the five herbal drugs linseed, fennel, nasturtium, marjoram and onion which were treated in a 5 L small-scale saturated steam system. This system is also working on the principle of the mechanical saturated steam decontamination "Lemgo process". Therefore the primary ingredients and the color changes off all drugs were studied.

4.1 Mechanical Saturated Steam Decontamination of Nasturtium

*This chapter summarizes the article "**Entkeimung von Kapuzinerkresse (Tropaeolum majus L.) mittels »Mechanischer Sattdampfentkeimung«**" submitted by H. Lange, H. Krüger, A. Dammann, K. Schwarzer, R. David und U. Müller in Z Arznei-Gewurzpfla 15(2): 81-85 (2010). The manuscript can be viewed in Appendix 1.*

Nasturtium (*Tropaeolum majus* L.) is known as a medicinal plant in its home countries, Brazil, Peru, Colombia, Venezuela, Chile, and Easter Island for more than 450 years (98). From historical records it is known that nasturtium was applied for external skin diseases, inflammations and injuries. Later, the plant has found application in other diseases such as bronchitis and scurvy (29). Nasturtium was first introduced to Europe in 1684 as an ornamental plant. Today, nasturtium is used in supportive treatment for bacterial infections of the urinary tract and respiratory tract. The pharmaceutical product portfolio ranges from tablets to liquid preparations to spray. These products are based on the antimicrobial and antiviral effects of glucosinolates present in nasturtium (39, 82). Glucotropaeoline is the active ingredient. Nasturtium contains the cleavage enzyme (myrosinase) in closed cell compartments which upon contact with the substrate easily degrade the glucotropaeoline. This is why it must be ensured that these cell compartments are not destroyed during the treatment of nasturtium (82).

For the production of medicines based on herbal drugs, not only plants with high glucosinolate content are important but also such with low bacterial load (*Chap. 1.3*). Therefore, plant materials with a high total plate count will be decontaminated with several germ reduction methods (*Chap. 1.4*). Although microbial reduction has been going on for many years there are still plants that suffer high losses of active ingredients. For this reason, in this study, a modern germ reduction method, the Lemgo process (*Chap. 1.5*), is tested regarding its germ-reduction potential and ingredient conservation.

4.1.1 Decontamination Results

Microbial analyses were conducted following the European Pharmacopoeia (7th edition) (23). All microbial results, except for Enterobacteriaceae, are stated as colony-forming units per g (CFU/g). The quantitative detection limit was 10^2 CFU/g. Quality detection was only done for Enterobacteriaceae. Very mild experimental conditions of single treatment with 110 °C saturated steam for 5 s resulted in a reduction of the total bacterial count of 2.5 decades (Tab. 4.1.1.1). The nasturtium were treated in the 0.7 L treatment chamber described above with a filling degree of 40% using steam temperatures of 110 °C and 125 °C and an exposure time of 20 s. For the optimization of the germ reduction in some treatments two cycles of steam addition and vacuum ("double treatment") were applied (Tab. 4.1.1.2).

In a double treatment of nasturtium with the same process parameters, the microorganisms are reduced by at least 4 log steps. After the treatment only 5.0E ± 0.2 cells/g are detectable. This severe and in some cases complete reduction can be explained by mechanical decontamination, but not through inactivation due to heat (4.1.1.2).

The Lotus effect (48) amplifies the reduction rate. This can be observed when nasturtium is treated for 10 s with 110 °C steam which leads to a reduction of 3 decades. A further, slight reduction could be justified by a heat effect (inactivation), but is difficult to capture by measurement. When using saturated steam at 125 °C initially a similar result to 110 °C is reported. This suggests that the mechanical effects even at 110 °C (but min 10 s) are fully formed, which in view of the already mentioned lotus effect also appears credible. Only higher temperatures (125 °C) show an increase in bacterial reduction. A prolonged treatment time (e.g. 20 s) does not result in a significantly better outcome. A single treatment at 125 °C for 20 s results in a germ reduction of 4 decades. Thus the quantitative detection limit of 100 CFU/g was reached. After a double 125 °C

treatment of nasturtium, the total number of bacteria is below the quantitative detection limit (*Tab. 4.1.1.1*). The microbial reduction of two consecutive 5 s steam phases, each followed by flash evaporation, has a significantly better bacterial reduction than a single vapor deposition of 10 s or 20 s. This is another indication that the flash evaporation provides the main contribution towards a heat thermal decontamination.

Tab. 4.1.1.1: Comparison of glucotropaeoline content (mg/g), total plate count and mold count (colony-forming units, CFU/g) as well as Enterobacteriaceae (minus = qualitatively negative and plus = only qualitatively detectable) after single treatment of nasturtium at temperatures of 110°C and 125°C and vaporization times of 5 s, 10 s and 20 s.

Treatments	Glucotropaeoline (mg/g)	Tukey-Grouping	Total plate count (CFU/g)	Mold count (CFU/g)	Enterobacteriacee
control	14.49 ± 0.22	A	$4.6 \cdot 10^{6}$	$1.0 \cdot 10^{3}$	+ + +
110 °C / 5 s	13.94 ± 0.27	A	$5.8 \cdot 10^{3}$	$<1.0 \cdot 10^{2}$	- - -
110 °C / 10 s	13.43 ± 0.59	A	$4.7 \cdot 10^{3}$	$<1.0 \cdot 10^{2}$	+ - +
110 °C / 20 s	12.54 ± 0.39	B	$3.0 \cdot 10^{4}$	$<1.0 \cdot 10^{2}$	- - +
125 °C / 5 s	13.13 ± 1.05	A	$1.9 \cdot 10^{4}$	$<1.0 \cdot 10^{2}$	+ + +
125 °C / 10 s	11.97 ± 0.61	B	$1.9 \cdot 10^{3}$	$<1.0 \cdot 10^{2}$	- + +
125 °C / 20 s	10.79 ± 0.60	C	$<1.0 \cdot 10^{2}$	$<1,0 \cdot 10^{2}$	- - -

4.1.2 Chemical Analysis Results

The ingredient glucotropaeoline was measured by HPLC (84). All saturated steam treatments were carried out three times and each treatment was analyzed twice. For testing significance, a one-way ANOVA was carried out with Sigmaplot 11 (Systat Software Inc). For group comparison a Tukey-Test was performed. Values represent means ± SD, n = 3. Means and SD followed by the same superscript within are not significantly. After treatment with the Lemgo process and subsequent freeze-drying the loss of the value-giving ingredients of nasturtium is between 5% and 37% (*Tab. 4.1.1.1 and 4.1.1.2*). There was a significant loss of glucotropaeoline

in the single treatments with a temperature of 110 °C and 5-10 s of treatment duration; the same applies to 125 °C and 5 s. Steam at 110 °C and 20 s exposure time results in the loss of 13%. The loss of glucotropaeline increases in double treatments with 110 °C and 125 °C, 5 - 20 s evaporation to 15 - 25% (*Table 4.1.1.2*). Double treatment at 125 °C for 20 s leads to a loss of 37%. The active ingredient is reduced more with increasing temperature and evaporation time. The treatment option with a high bacterial reduction (4 log steps) and a minor loss of glucotropaeoline (13%) is a double treatment with 110 °C steam temperature with a treatment time of 5 s (Tab. 4.1.1.2).

Tab. 4.1.1.2: Comparison of glucotropaeoline content (mg/g), total plate count and mold count (colony-forming units, CFU/g) as well as Enterobacteriaceae (minus = qualitatively negative and plus = only qualitatively detectable) after double treatment of nasturtium at temperatures of 110 °C and 125 °C and vaporization times of 5 s, 10 s and 20 s.

Treatments	Glucotropaeoline (mg/g)	Tukey-Grouping	Total plate count (CFU/g)	Mold count (CFU/g)	Enterobacteriacee
control	13.28 ± 0.03	A	$2.1 \cdot 10^6$	$<1.00 \cdot 10^2$	+ + +
110 °C / 2*5 s	11.48 ± 1.26	B	$2.3 \cdot 10^2$	$<1.00 \cdot 10^2$	- - -
110 °C / 2*10 s	10.81 ± 0.26	C	$9.3 \cdot 10^2$	$<1.00 \cdot 10^2$	- - -
110 °C / 2*20 s	10.05 ± 0.12	D	$1.3 \cdot 10^2$	$<1.00 \cdot 10^2$	- - -
125 °C / 2*5 s	10.76 ± 0.51	C	$<1.0 \cdot 10^2$	$<1.00 \cdot 10^2$	- - -
125 °C / 2*10 s	10.01 ± 0.41	D	$<1.0 \cdot 10^2$	$<1.00 \cdot 10^2$	- - -
125 °C / 2*20 s	8.39 ± 0.11	E	$<1.0 \cdot 10^2$	$<1,00 \cdot 10^2$	- - -

4.2 Changes in Essential Oil Content and Leaf Surface Structures of Herbal Drugs

*This chapter represents the results for the publication "**Effects of steam and vacuum administration during decontamination on essential oil content in herbal medicines**" submitted by H. Lange, K. Schwarzer, A. Dammann, U. Müller, K. R. Richert-Pöggeler und H. Krüger in Journal of Applied Botany and Food Quality 85: 34-40 (2012). The manuscript can be viewed in Appendix 2.*
Additionally results for thyme and peppermint are presented.

The decontamination studies were conducted in a laboratory decontamination facility, which comprises a 0.7 L treatment chamber and a 8 L vacuum buffer tank as described in chapter 3.1. Only a maximum of 5 g of the appropriate herbal drugs marjoram, fennel, oregano, thyme, peppermint and eucalyptus could be decontaminated in the small treatment room. In the course of saturated steam decontamination Lemgo Process (Chap. 1.5), a steaming with 100 °C (Treatment 2) and 120 °C (Treatment 1) heated vapor was conducted with the accordant equilibrium pressure and lasted 20 s. For achieving saturated steam conditions, a pre-vacuum of 20 s was generated. The period of the flash-vacuum, which is critical for decontamination, was also 20 s. To investigate how "steam" and "vacuum" individually affected the plant material the following treatments (*Tab. 4.2.1*) were performed. The herbal drugs were treated with saturated steam only (Treatment 3). After 20 s of steam treatment the chamber was opened slowly to ambient atmosphere. Compressed air, instead of steam, was introduced into the treatment chamber to identify only the impact of vacuum on herbal drug tissue (Treatment 4).

Tab. 4.2.1: The parameters of applied treatments to study effects of steam decontamination.

Treatment Type	Steam temperature	Steam time	Air pressure	Pre- and postvacuum Time
Treatment 1	120 °C	20 s	-	20 s
Treatment 2	100 °C	20 s	-	20 s
Treatment 3	120 °C	20 s	-	-
Treatment 4	-	-	2.5 bar	20 s

Microbial analyses were conducted following the European Pharmacopoedia (7th edition). The total plate count results are stated as colony-forming units per g (CFU/g). The quantitative detection limit was 10^2 CFU/g. For the chemical analysis of the six medicinal plants (marjoram, oregano, peppermint, thyme, fennel and eucalyptus) 100-200 mg of dried plant material were weighed into a 100 mL centrifuge tube and homogenized in iso-octane with an Ultra-Turrax. This mixture had been low speed centrifuged at 3000 rpm in a table top centrifuge and the supernatant was analyzed by gas chromatography (Hewlett Packard HP 5890 Series II GC).

The essential oil content in mL/100g (air dried mass) is defined as the total value of the specific individual components determined by gas chromatography (53).

To control how the surface will change during the saturated steam decontamination the lower and upper leaf surface of steam treated material has been applied for scanning electron microscopy.

The samples were mounted accordingly with double-sided, nonconductive sticky tape on aluminium stubs and coated with gold. The chamber of the sputter coater was filled with argon during deposition of heavy metal. Images were collected using the scanning electron microscope Quanta 250 (FEI Worldwide Corporate Headquarters) equipped with a tungsten cathode. The acceleration voltage was 10 kV. On average 20 gland scales were analyzed and representative images selected. Images were adjusted in brightness and contrast using Adobe Photoshop CS4. Each saturated steam treatment was carried out three times and was analyzed twice for essential oil (n=3). Statistical evaluation was accomplished with Systat Software Inc SigmaPlot 11. For testing significance, a one-way ANOVA was carried out. A direct comparison of groups was performed with a Tukey-Test.

4.2.1 Marjoram

The plant material for *Majorana hortensis* L. (marjoram) was provided by Majoranwerke Aschersleben and had been cultivated and harvested in 2009. The control sample was characterized by an initial value of essential oil of 0.91 mL/100g dry product (*Tab. 4.2.2*). Marjoram belongs to the family of Lamiaceae which stores the essential oil in peltate glandular hairs, where the storage compartment is protected by an elevated cuticle (*Fig. 4.2.1*) covered by a thin wax layer (34, 98). It is known that essential oils evaporate at a very low rate as long as the wax coated cuticle is intact. Less than 10% essential oil loss per year has been observed in dried leaf material when stored at room temperature (12). The upper panel shows that the peltate glandular hair of untreated marjoram are not embedded in the leaf matrix, but exposed with a sphere-like appearance (*Fig. 4.2.1, A*).

Tab. 4.2.2: Essential oil content (mL/100g) and total plate count (colony-forming unit, CFU/g) of marjoram after the saturated steam decontamination Lemgo process with four different treatment parameters. Same superscript means no significant difference between groups.

Marjoram				
Treatment type (Compare Tab. 4.2.1)	Oil content Mean ± SD	Loss of oil [%]	See panel in Fig. 4.2.2	CFU/g
Control	0.91 ± 0.04^{A}		A0	5.1×10^{5}
Treatment 1	0.07 ± 0.04^{D}	93	A1	2.2×10^{3}
Treatment 2	0.13 ± 0.04^{C}	86	A2	4.8×10^{5}
Treatment 3	0.07 ± 0.04^{D}	93	A3	1.2×10^{5}
Treatment 4	0.84 ± 0.04^{A}	0	A4	5.3×10^{5}

However, the wax-coated cuticle of Lamiaceae can be broken by mechanical forces or by high temperatures (35). The holes visible in peltate glandular hairs of marjoram after exposure to 100 °C and 120 °C heated steam (*Fig. 4.2.2, A0-A2*) provide direct evidence that the wax-coated cuticle has been destroyed. We assume that also the severe shrinkage of peltate glandular hairs after such heat treatment (*Fig. 4.2.2*) resulted in distortion of the protective cell layer promoting release of

essential oil. Exposing marjoram to 120°C saturated steam for 20 s with a subsequent 20 s vacuum results in a loss of 93% of essential oil content. As depicted in Figure 4.2.2, A2 this is due to severe damages of gland scales during the decontamination procedure. The cuticle of the glandular trichome becomes perforated releasing essential oil that most likely got evaporated with the water layer. The loss of content is visible by severe shrinkage from the outer area that leads to almost complete flattening in the middle region of the original sphere like appearance of the cell. Steam temperatures of 100°C do not have as dramatic effects as observed after treatment 1 conditions (*Tab. 4.2.1*). The peltate glandular hairs in Figure 4.2.2, A3 show less shrinkage and no disruptions in its cuticle are visible resulting in reduced essential oil loss of 86%. Comparisons of the parameters "steam" and "vacuum" provided evidence that the observed damages in marjoram gland scales and accompanied ingredient losses were more pronounced by steam exposure rather than vacuum exposure (*Fig. 4.2.2, A0 and A4*). As Figure 4.2.2, A3 illustrates incubation of leaf tissue, with steam heated to 120°C caused isolated surface disruptions of the peltate glandular hairs.

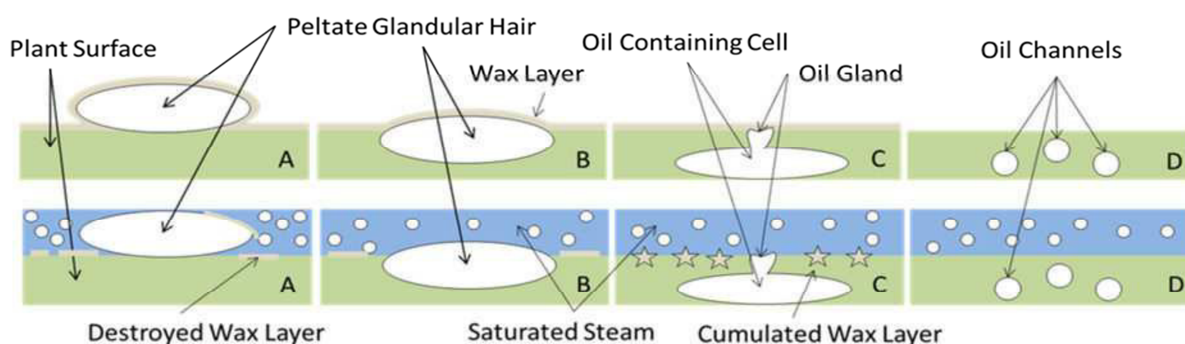

Fig. 4.2.1: Upper panel = Peltate glandular hair of marjoram with epidermis in a plane (A), sunken peltate glandular hair of oregano (B), oil containing cell and oil gland of eucalyptus (C) and oil channel of fennel (D) before the steam deposition phase in which the wax layer is fully functional. Lower panel = (A), (B), (C) and (D) during steam deposing phase when the condensate layer is formed.

In contrast application of vacuum showed for the majority of visible cells deformations in peltate glandular hairs shape leading to more flattened appearance but did not show perforations of the cuticle surface. The

measured oil content of 0.84mL/100g was similar to untreated leaves which contained 0.91mL/100g. The saturated steam decontamination shows only for treatment 1 a reduction of two decades of total plate count. Treatments 2 and 3 reduce the microorganisms on marjoram very sparsely and the last treatment 4 provides no reduction.

4.2.2 Oregano

Among the investigated Lamiaceae *Oreganum vulgare* L. provided by Majoranwerke Aschersleben carried the highest terpene content of 1.82 mL per 100 g dry mass.

In contrast to marjoram, the peltate glandular hairs are not exposed but embedded into the leaf matrix as revealed by scanning electron microscopy (*Fig. 4.2.1, B and 4.2.2, B0*). Thus, peltate glandular hairs of oregano seem to be less exposed to destruction by external forces which may account for the observed lower loss of essential oil after the decontamination treatments. Deformation of peltate glandular hairs occurs naturally as indicated in the control (*Fig. 4.2.2, B0*).

The different steam temperatures during decontamination had a significant effect on the terpene content as shown for marjoram, thyme and peppermint. Treatment with 120 °C steam and vacuum resulted in a loss of 59% and 43% during treatment of 100 °C followed by vacuum (*Tab. 4.2.3*).

Tab. 4.2.3: Essential oil content (mL/100g) and total plate count (colony-forming unit, CFU/g) of oregano after the saturated steam decontamination Lemgo process with four different treatment parameters. Same superscript means no significant difference between groups.

Oregano				
Treatment type (Compare Tab. 4.2.1)	Oil content Mean ± SD	Loss of oil [%]	See panel in Fig . 4.2.2	CFU/g
Control	1.82 ± 0.09^{A}		B0	6.1×10^{6}
Treatment 1	0.75 ± 0.09^{C}	59	B1	2.5×10^{3}
Treatment 2	1.05 ± 0.10^{B}	43	B2	3.5×10^{6}
Treatment 3	0.74 ± 0.04^{C}	59	B3	9.7×10^{5}
Treatment 4	1.92 ± 0.11^{A}	0	B4	7.1×10^{6}

Most peltate glandular hairs are deformed by saturated steam treatments as revealed in scanning electron microscopy (*Fig. 4.2.2, B1-B3*). Images collected from leaves exposed to treatment 3 and 4 confirmed the destructive forces by steam rather than vacuum on oregano peltate glandular hairs. In the picture for saturated steam treatment without

vacuum (*Fig. 4.2.2, B4*) it resulted in multiple broken, some intact, but also semi-broken peltate glandular hair cells.

Apparently the sunken appearance of oregano secretory cells protected better against external forces and thus preserved the essential oil better. Only half of the terpene content was lost during decontamination in contrast to marjoram tissue which showed an almost complete loss of terpenes during decontamination. The microbial reduction results of oregano are similar to marjoram. Treatment 1 is the only treatment variation which decreases the total plate count of oregano by about 3 decades. For treatments 2, 3 and 4, no appreciable degradations of the colony-forming units of oregano are found.

4.2.3 Thyme

Similar to oregano, *Thymus vulgare* L. (thyme) was provided by Majoranwerke Aschersleben features peltate glandular hairs embedded into the lower leaf surface (*Fig. 4.2.1, B*). Thus, gland scales seem to be protected from applied forces during the decontamination procedure. Before application of the Lemgo Process, thyme holds a control value of 1.02 mL/100 g essential oil content.

Thyme denotes a high loss of 64% of essential oil after the treatment with 120 °C saturated steam and flash-vacuum (*Fig. 4.2.2, C1*). Test conditions of 120 °C steam alone caused a loss of 65% (*Fig. 4.2.2, C3*) and demonstrated the damaging effects of higher steam temperatures. The decrease of steam temperature to 100 °C significantly improved terpene content. Two third of initial essential oil content is preserved when the samples are exposed to 100 °C steam temperature during decontamination. Comparing Figure 4.2.2 panel C2 and C3 the specific effects resulting from the applied temperature treatments become obvious. Figure 4.2.2, C2 shows completely collapsed gland scales, while in image C3 of Figure 4.2.2 only half of the gland scales display the phenotype observed in panel C2. The other half of gland scales maintained residual shape probably due to the presence of remaining ingredients. This

is in accordance with the fact that almost double of essential oil content can be measured (*Tab. 4.2.4*). The parameter vacuum without steam did not affect terpene concentration and resulted in intact peltate glandular hairs as displayed in Fig. 4.2.2, C4.

The total plate count of thyme is reduced to 8.4×10^2 CFU/g and therefore below the statutory minimum limit. All other decontamination treatments change the microbial load insignificantly.

Tab. 4.2.4: Essential oil content (mL/100g) and total plate count (colony-forming unit, CFU/g) of thyme after the saturated steam decontamination Lemgo process with four different treatment parameters. Same superscript means no significant difference between groups.

Thyme				
Treatment type (Compare Tab. 4.2.1)	Oil content Mean ± SD	Loss of oil [%]	See panel in Fig. 4.2.2	CFU/g
Control	1.02 ± 0.04^{A}		C0	9.2×10^5
Treatment 1	0.37 ± 0.03^{C}	64	C1	8.4×10^2
Treatment 2	0.74 ± 0.04^{B}	28	C2	5.6×10^5
Treatment 3	0.34 ± 0.01^{C}	67	C3	6.7×10^5
Treatment 4	1.01 ± 0.05^{A}	0	C4	7.5×10^5

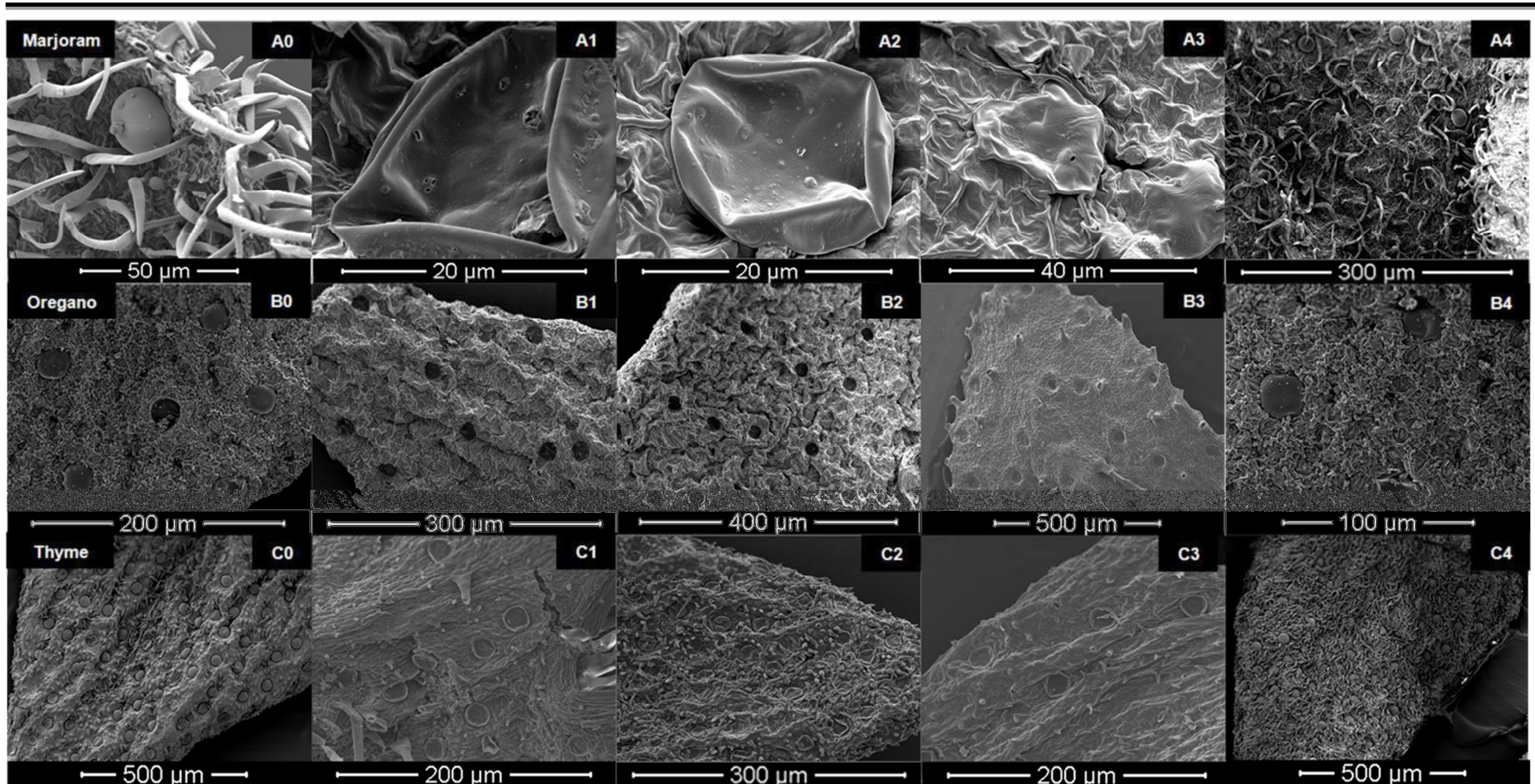

Fig. 4.2.2: Scanning electron microscopy of marjoram, oregano and thyme. A0 = on the surface-fitting intact peltate glandular hair, A1 = collapsed peltate glandular hair with large holes in cuticle after, A2 = collapsed peltate glandular hair with minor holes, A3 = collapsed peltate glandular hair with large hole in cuticle, A4 = intact peltate glandular hair / B0 = peltate glandular hairs embedded in leaf surface on the control sample of oregano, B1 – B3 = many collapsed peltate glandular hairs, B4 = two intact and one collapsed peltate glandular hairs / C0 = intact peltate glandular hairs, C1 – C3 = several collapsed peltate glandular hairs, C4 = not corrupted peltate glandular hairs.

4.2.4 Peppermint

The *Mentha x piperita* (peppermint) sample was provided by Majoranwerke Aschersleben and showed an initial value for the essential oil content of 1.55 mL/100 g and thus represented the second highest oil concentration in leaves of the studied Lamiaceae. Incubation of peppermint with 120 °C for 20 s and subsequent vacuum causes the highest loss of 96% of essential oil (*Tab. 4.2.5*). This may be explained by the position of peltate glandular hairs on the lower epidermis of peppermint, which is, similar to marjoram's peltate glandular hairs, more exposed rather than embedded into the lower epidermis matrix similar to oregano (*Fig. 4.2.3, D0*). The cell deformation after 120 °C steam exposure was the same as noticed before with other Lamiaceae.

Tab. 4.2.5: Essential oil content (mL/100g) and total plate count (colony-forming unit, CFU/g) of peppermint after the saturated steam decontamination Lemgo process with four different treatment parameters. Same superscript means no significant difference between groups.

Peppermint				
Treatment type (Compare Tab. 4.2.1)	Oil content Mean ± SD	Loss of oil [%]	See panel in Fig.4.2.3	CFU/g
Control	1.55 ± 0.02^{A}		D0	5.3×10^{6}
Treatment 1	0.06 ± 0.01^{C}	97	D1	2.8×10^{2}
Treatment 2	0.14 ± 0.01^{B}	91	D2	9.8×10^{5}
Treatment 3	0.17 ± 0.03^{B}	89	D3	3.4×10^{6}
Treatment 4	1.59 ± 0.06^{A}	0	D4	4.1×10^{6}

The decrease of temperature to 100 °C caused no improvement in oil content preservation as was observed for oregano and thyme. The loss at 100 °C steam temperature including flash vaccum comprises 92% and is as high as the loss after a treatment with 120 °C saturated steam without vacuum (*Tab. 4.2.5*). Actually, at 120 °C steam temperature the vacuum had an even slightly enhancing effect on the oil loss in the case of peppermint. Such an effect had not been observed in the case of oregano and thyme. In those samples no additive effects of the parameters steam and vacuum on oil content reduction have been found. Similarly to the

other drugs, solely application of flash-vacuum resulted in no loss of essential oils in leaf tissue.

The effects on morphology of peltate glandular hairs were similar to changes observed in the other drugs investigated. Figure 4.2.3, D2 shows a sunken peltate glandular hair after a treatment with 120 °C for 20 s and subsequent vacuum. The decrease of temperature to 100 °C reduced the deforming effects on peltate glandular hairs (*Fig. 4.2.3, D2*). Several broken and sunken peltate glandular hairs are seen after a treatment with 120 °C steam without vacuum (*Fig. 4.2.3, D3)*. After treatment without steam but only with vacuum, glandular scales are completely intact.

4.2.5 Fennel

Foeniculum vulgare Mill. (fennel) fruits also provided by Majoranwerke Aschersleben accumulate the valuable essential oils in a number of channels inside the kernel as is shown in Figure 4.2.3, E0-E4. The channel structure allows an increase in storage capacity. Each compartment is embedded in several cell layers derived from the fruits coat (*Fig. 4.2.3, E0–E4*). They are spaced throughout the upper and lower fruits coat surrounding the endosperm. Such a protective shell additional to the oil channel cell wall (*Fig. 4.2.3, E4*) provides optimal protection against environmental influences like sun, rain, wind and animals. In comparison to the control (8.79 mL/100 g), fennel had an essential oil value of 8.21 mL/100g after the treatment with 120 °C saturated steam for 20 s and a subsequent vacuum of 20 s. This means a loss of 7% (*Tab. 4.2.6*). From all parameters tested, only a minor, significant reduction in terpene concentration was evident in treatments comprising 120 °C steam temperatures (*Tab. 4.2.6*).

Tab. 4.2.6: Essential oil content (mL/100g) and total plate count (colony-forming unit, CFU/g) of fennel after the saturated steam decontamination Lemgo process with four different treatment parameters. Same superscript means no significant difference between groups.

Fennel				
Treatment type (Compare Tab. 4.2.1)	Oil content Mean±SD	Loss of oil [%]	See panel in Fig. 4.2.3	CFU/g
Control	8.79 ± 0.15^{A}		E0	5.3×10^{5}
Treatment 1	8.21 ± 0.14^{B}	7	E1	1.0×10^{2}
Treatment 2	8.51 ± 016^{A}	3	E2	3.5×10^{5}
Treatment 3	8.14 ± 0.13^{B}	8	E3	8.7×10^{4}
Treatment 4	8.62 ± 0.12^{A}	0	E4	5.1×10^{5}

No effect on gland scale anatomy was visible in the treated samples (*Fig. 4.2.3, E1-E4*) compared to the unexposed control tissue (*Fig. 4.2.3, E1*). In contrast to marjoram and oregano, treatment 1 reduces the total plate count below the detection limit. In treatment 2 and 4 the number of microorganisms does not change. Treatment 3 reduces the total plate count by about 0.5 decades.

4.2.6 Eucalyptus

Eucalyptus grandis (eucalyptus) leaves were purchased from the company "Martin Bauer" (Vestenbergsgreuth, Germany) in 2009, which have lacking gland scales with oil containing cells that are located in the leaf mesophyll. Thus the positioning of storage cells is sub dermal in contrast to the external structure of gland scales among Lamiaceae (40).

The temperature effects on deformation of thick wax layers were clearly illustrated on the eucalyptus leaf surface (*Fig. 4.2.3, F1-F3*). This is due to the different leaf structure harboring internal oil glands that are not modified during heat exposure (*Fig. 4.2.1, C*) (39).

The oil content of eucalyptus is 1.54 mL/100 g (*Tab. 4.2.7*), which is similar to concentrations found in the leaves of studied Lamiaceae. In eucalyptus a preservation of essential oil has been found in all treatments. The images in Figure 4.2.3, F1-F3 display gland openings which do not show morphological changes after treatments with saturated steam. The Lemgo process is a superficial treatment and thus hardly affects the essential oil concentration which is located inside the plant material. Eucalyptus holds a uniformly developed spicular wax film on the leaf surface (63) which was found to be distorted after hot steam exposure. Independent of treatment type 1, 2 or 3 (*Tab. 4.2.1*) the spicules were accumulated forming discrete clusters on the leaf surface. Only after application of vacuum alone, the wax spicules were retained in their original form (*Fig. 4.2.3, F4)*. The microorganism reduction outcomes are identical to marjoram. Treatment 1 reduces the total plate count below the detection limit and treatment 3 decreases the germs by one decade. The other reduction methods 2 and 4 show no decrease.

Tab. 4.2.7: Essential oil content (mL/100g) and total plate count (colony-forming unit, CFU/g) of eucalyptus after the saturated steam decontamination Lemgo process with four different treatment parameters. Same superscript means no significant difference between groups.

Eucalyptus				
Treatment type (Compare Tab. 4.2.1)	Oil content Mean±std	Loss of oil [%]	See panel in Fig. 4.2.3	CFU/g
Control	1.54 ± 0.06^{A}		F0	2.6×10^{6}
Treatment 1	1.48 ± 0.08^{A}	0	F1	1.0×10^{2}
Treatment 2	1.43 ± 0.07^{A}	0	F2	4.7×10^{5}
Treatment 3	1.54 ± 0.08^{A}	0	F3	1.3×10^{5}
Treatment 4	1.52 ± 0.02^{A}	0	F4	1.8×10^{6}

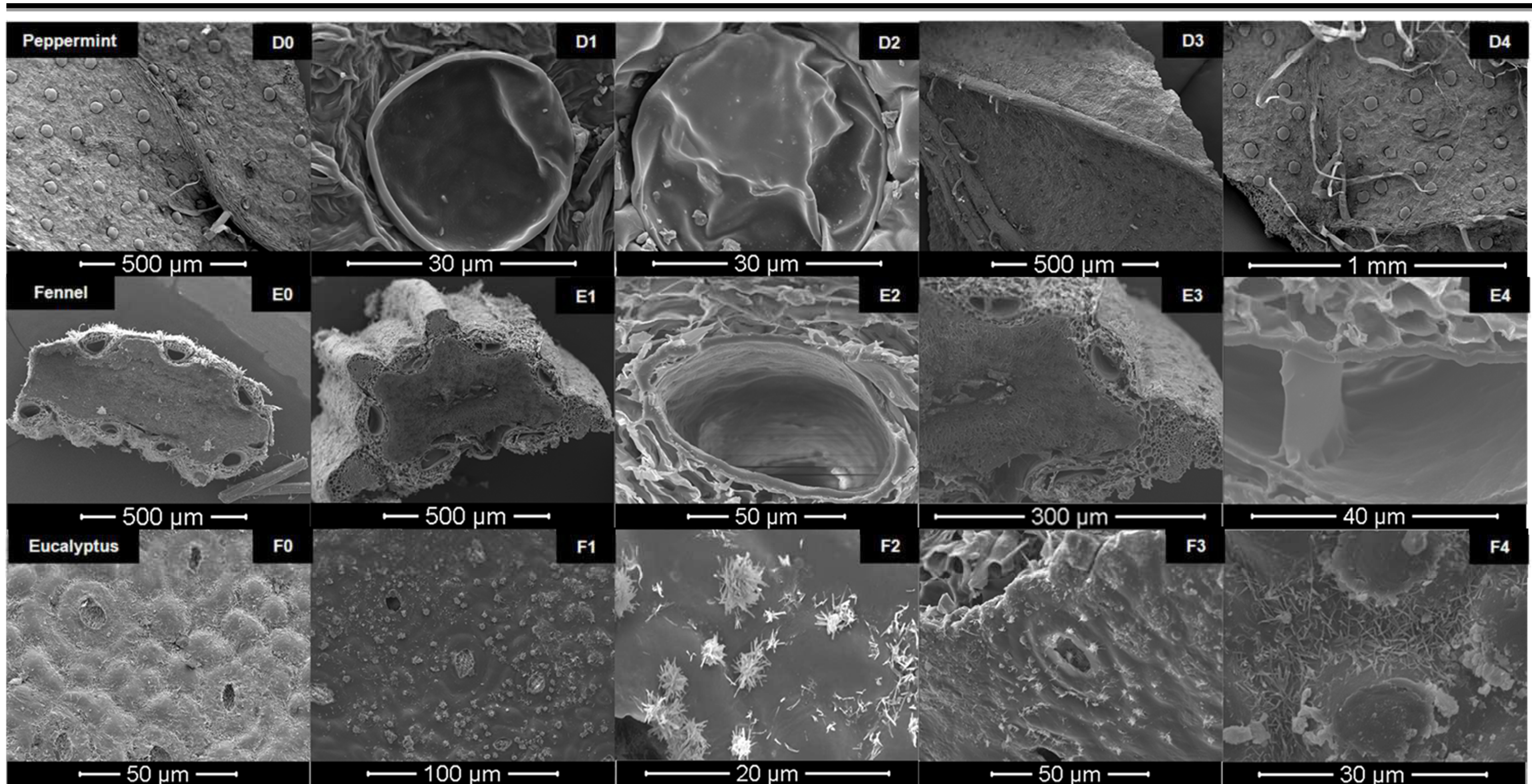

Fig. 4.2.3: *Scanning electron microscopy of peppermint, fennel and eucalyptus: D0 = intact peltate glandular hairs, D1 = complete corrupted peltate glandular hair, D2 = partial damaged peltate glandular hair, D3 = many corrupted peltate glandular hairs, D4 = a lot of intact peltate glandular hair / E0 – E1= cross-sectional fennel fruit with oil channels, E3= cross-sectional fennel fruit with three oil channels, E2 - E4 = oil channel with thick channel wall and partition of fennel fruit / F0 = three oil gland vents with intact wax layer on the control sample of eucalyptus, F1 = one oil gland vent with cumulated wax layer around it, F2 = close cumulated wax layer of eucalyptus after treatment 2, F3 = cumulated wax layer, F4 = normal wax layer.*

4.3 Changes of Herbal Drug Ingredients and Color during a Saturated Steam Decontamination

*This chapter summarizes the article "***Chemical, physical and sensory changes of herbal drugs after saturated steam decontamination** *" submitted by H. Lange, A. Dammann, K. Schwarzer, U.Müller und H. Krüger in Z Arznei- Gewurzpfl 17(4):174-181(2012). The manuscript can be viewed in Appendix 3.*

Additionally fatty acid and essential oil spectra were determined.

The five pharmaceutical drugs (linseed, fennel, marjoram, nasturtium, onion) were treated in the 5 L ploughshare stirrer described in chapter 3.2 with a filling degree of 40% using steam temperatures of 110 °C and 125 °C and an exposure time of 20 s. The double jacket was heated to 20 °C, 40 °C, and 80 °C (Table 4.3.1). For the optimization of the germ reduction in some treatments not only one cycle of steam followed by vacuum (and flash evaporation) but two cycles (double treatment) were applied. For details in treatment see Table 4.3.2.

Tab. 4.3.1: Temperatures of steam and double jacket in "single" and "double treatments" to study chemical and sensory effects of steam decontamination on herbal drugs.

Treatment	Steam Temperature	Double Jacket Temperature
Control	-	-
Treatment 1 and 11	110 °C	20 °C
Treatment 2 and 12	110 °C	40 °C
Treatment 3 and 13	110 °C	80 °C
Treatment 4 and 14	125 °C	20 °C
Treatment 5 and 15	125 °C	40 °C
Treatment 6 and 16	125 °C	80 °C

Tab. 4.3.2: Sequences of steam and vacuum in "single" and "double treatments" to study chemical and sensory effects of steam decontamination on herbal drugs (20v = 20 s vacuum, 20s = 20s steam).

Treatment Type	Treatment	Sleeve Temperature
Control	-	-
Single treatment	1 - 6	20v – 20s – 20v
Double treatment	11 - 16	20v – 20s - 20v – 20s – 20v

The Lemgo process creates a mechanical stress on the surface of the drugs. This could lead to the destruction of surface structures and leakage

of the ingredients (*Chap. 4.2.*). For the marketability, besides the valuable ingredients, other criteria are important, too. First of all, the color should be fresh and unchanged. Therefore the color was defined with the Hunter´s color values L (degree of whiteness, white + 100, black 0), a (a, degree of redness, red + 100, green -80), and b (degree of yellowness, yellow + 70, blue -80) using a colorimeter (Minolta CR-200). Averages were reported. A numerical total color difference ΔE ($\Delta E = \Delta L^2 + \Delta a^2 + \Delta b^2$) was calculated from the Hunter´s values obtained. Photos of all plant materials were made after the several saturated steam treatments. The photos of the various herbal drugs were taken with an SLR camera (Canon EOS 1000D).

By using a holder it was ensured that all samples were photographed from the same distance and under the same lighting conditions to enable a comparison. Another critical point after a saturated steam process is the sensory quality. Especiall linseed is known to be easily deteriorated. For that linseed was evaluated for color, odor, pungent taste, and overall acceptability by 10 trained judges after six months. Approximately 2 g of linseed in a disposable Petri dish was given to each panelist. All samples were coded with three-digit numbers. The panelists were instructed to record their rating using a five-point hedonic scale (5 like extremely and 0 dislike extremely for color, odor, and overall acceptability; and 5 extremely strong and 0 extremely weak for pungent taste).

4.3.1 Linseed

Linum usitatissimum L. (linseed), which was supplied by SALUS Haus GmbH & Co. KG (Bruckmühl, Germany) with no pre-treatment having been done, was analyzed employing Soxhlet extraction according to LFGB § 64. An amount of 1 g ground flaxseed is weighed into 26x60 mm Soxhlet thimble and sealed cotton wool. For the extraction 150 mL n-hexane was added in a round bottom flask with about 15 boiling chips. The extraction time was four hours. After that the n-hexane is evaporated and the crude fat is dried by 115 °C for about 1 hour (57).

For the fatty acid spectrum (palmitic acid = C16:0, stearic acid = C18:0, oleic acid = C18:1, linoleic acid = C18:2, alpha linolenic acid = C18:3) two milliliter of n-hexane were added. An aliquot of 20 µL was transfered in a GC vial (allow the n-hexane to flash-off) and 750 µL of tert-butyl methyl ether (TBME) and 250 µL of TMSH solution were added (57).

The crude fat of linseed is very stable with a high boiling point and for that reason it is comprehensible that fat contents (Tab. 4.3.5) and the fatty acid spectrum do not change after the single saturated steam decontamination with all tested steam and jacket temperatures (Tab. 4.3.3 and 4.3.4).

Tab. 4.3.3: Fatty acid spectrum of linseed after "single treatment" of saturated steam decontamination. *Same superscript means no significant difference between groups.

Linseed fatty acid spectrum [% in oil]					
	palmitic acid	stearic acid	oleic acid	linoleic acid	alpha linolenic Acid
110 °C					
control	4.93±0.24[1]	3.64±0.12[1]	18.13±0.78[1]	17.67±0.28[1]	55.63±1.42[1]
20°C	5.04±0.07[1]	3.70±0.05[1]	18.72±0.26[1]	17.89±0.12[1]	54.65±0.17[1]
40°C	5.06±0.08[1]	3.74±0.10[1]	18.91±0.38[1]	17.92±0.09[1]	54.37±0.59[1]
80°C	4.93±0.01[1]	3.63±0.07[1]	18.36±0.25[1]	17.76±0.25[1]	55.33±0.44[1]
125 °C					
control	5.01±0.14[1]	3.66±0.02[1]	18.84±0.01[1]	17.84±0.09[1]	54.63±0.06[1]
20°C	4.93±0.04[1]	3.57±0.04[1]	18.30±0.14[1]	17.64±0.10[1]	55.56±0.20[1]
40°C	4.84±0.14[1]	3.48±0.11[1]	17.93±0.62[1]	17.49±0.23[1]	56.26±0.94[1]
80°C	4.90±0.05[1]	3.55±0.05[1]	18.18v0.19[1]	17.57±0.28[1]	55,80±0.40[1]

Certainly, L- and b-color values of the Lab color space change after a single treatment with 110 °C and 125 °C steam temperature. The changes

are not temperature dependent. The lightness L decreases by 4 degrees (*Fig. 4.3.1, L1-L6*) so that linseed is darker after the treatment. B-value declines by 2 degrees, resulting in an increased blue content of linseed. Both color changes are not visible to the naked eye, see Figure 4.3.1, L1-L6 (*Tab. 4.3.5*). But what is apparentis that the linseed grains are glued to the decontamination treatment. The more condensation occurs the more pronounced is the bonding for example at treatment 1 (Tab. 4.3.5/Fig. 4.3.1, L1).

Waje et al. show that pepper manifested no significant changes in its sensory values after 6 months. Although it often happens that linseed goes deteriorated after a treatment with saturated steam (96). All trained judges give decontaminated linseed 3.7 points out of 5 possible points. Thus, no rancidity was detected after single saturated steam decontamination (*Tab. 4.3.5*). In summary, single treatment of linseed caused only slight color changes and no changes in the content or composition of valuable ingredients occurred. At a comparable steam treatment of pepper similar color changes were noticed (96).

For linseed a double treatment was solely conducted with 125 °C saturated steam temperature. Due to condensation water in the plough share stirrer, linseed initiates swelling and becomes a thick, inertial mass. The lower the temperature of saturated steam, the more condensate water develops and seeds initiate swelling. Even at 125 °C, the bonding of the grains is clearly visible (*Fig. 4.3.1, L14-L16*) (24).

Tab. 4.3.4: Fatty acid spectrum of linseed after "single treatment" of saturated steam decontamination. *Same superscript means no significant difference between groups.

Linseed fatty acid spectrum [% in oil]					
	palmitic acid	stearic acid	oleic acid	linoleic acid	alpha linolenic Acid
2 x 125 °C					
Kontrolle	5.02±0.11[1]	3.66±0.04[1]	18.62±0.15[1]	17.56±0.11[1]	54.21±0.29[1]
20°C	4.89±0.10[1]	3.57±0.02[1]	18.41±0.19[1]	17.59±0.15[1]	55.50±0.37[1]
40°C	4.98±0.06[1]	3.48±0.10[1]	17.73±0.33[1]	17.39±0.28[1]	56.32±0.76[1]
80°C	5.03±0.07[1]	3.55±0.07[1]	18.25±0.29[1]	17.59±0.39[1]	55.29±0.39[1]

Additionally, no changes in crude fat content are detectable at double treatment of linseed. In comparison with single treatment, linseed shows similar color changes at double treatment with 125 °C. The L-value decreases by 4 degree and b-value is reduced by 2 degree. Thus, saturated steam treatment causes no visible darkening of the sample (*Tab. 4.3.5/Fig. 4.3.1, L14-L16*). Also in the sensory analysis and color analysis with a colorimeter at 125 °C there were no changes compared with the 110 °C variant.

Finally, double treatment of linseed has no advantages over single treatment. Double treatment reduces total viable count and powerful adhering aerobe spores just as single treatment. Furthermore, no changes of ingredients or sensors and only slight changes of color are recorded.

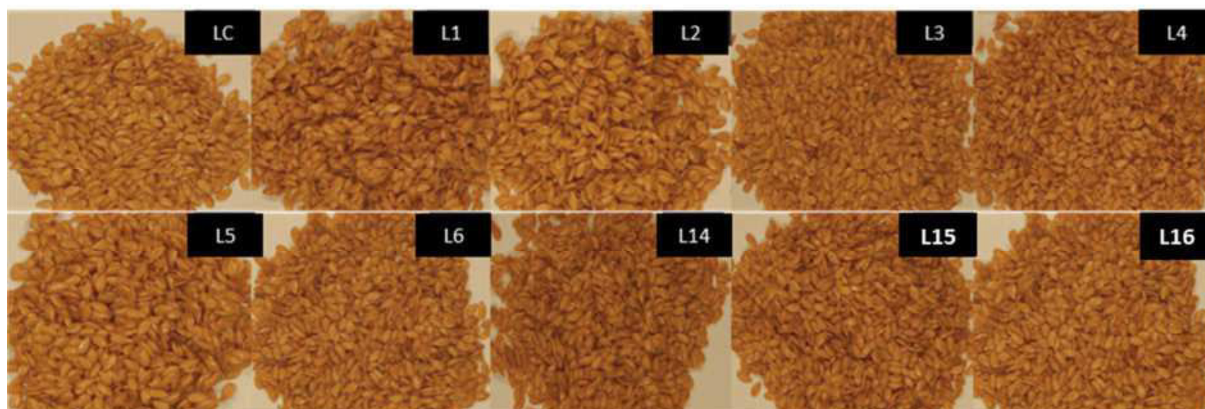

Fig. 4.3.1: Linseed before and after mechanical saturated steam decontamination. LC = Control, L1 – L6 = Single treatment with saturated steam, L14 – L16 = Double treatment with saturated steam. For details of the treatment see Table 4.3.1 and 4.3.2.

Tab. 4.3.5: Crude fat content, "Lab" color space values, sensory parameter and swelling ability before and after "single" and "double treatment" of saturated steam decontamination of linseed.*: Same superscript means no significant difference between groups.

Linseed single and double treatment parameters										
Treatments	Control	1	2	3	4	5	6	14	15	16
Ingredients analysis										
Crude fat % *	42.48 ± 0.95^{1}	43.74 ± 1.39^{1}	42.83 ± 1.30^{1}	42.76 ± 0.61^{1}	43.43 ± 0.65^{1}	42.98 ± 0.53^{1}	43.55 ± 1.25^{1}	43.34 ± 0.65^{1}	43.44± 1.53^{1}	43.19± 1.25^{1}
„Lab" color space values										
L *	36.59 ± 0.57^{1}	32.25 ± 0.48^{2}	34.09 ± 0.52^{3}	34.94 ± 0.29^{3}	33.36 ± 0.63^{3}	33.95 ± 0.54^{3}	34.22 ± 0.39^{3}	32.67 ± 0.28^{2}	32.98 ± 0.45^{2}	34.21 ± 0.52^{3}
a *	4.93 ± 0.73^{1}	4.94 ± 0.58^{1}	4.80 ± 0.72^{1}	4.95 ± 0.67^{1}	4.75 ± 0.45^{1}	4.96 ± 0.56^{1}	5.01 ± 0.39^{1}	4.60 ± 0.18^{1}	4.66 ± 0.28^{1}	5.01 ± 0.34^{1}
b *	19.49 ± 0.82^{1}	17.45 ± 0.45^{2}	17.86 ± 0.67^{2}	18.38 ± 0.49^{2}	17.69 ± 0.19^{2}	17.95 ± 0.68^{2}	17.97 ± 0.57^{2}	16.84 ± 0.37^{2}	16.96 ± 0.56^{2}	17.97 ± 0.38^{2}
ΔE	-	4.8	2.99	1.99	3.7	3.06	2.82	4.74	4.42	2.83
Sensory analysis										
Color *	3.6 ± 0.5^{1}	3.9 ± 0.6^{1}	3.3 ± 0.6^{1}	3.6 ± 0.7^{1}	3.8 ± 0.7^{1}	3.5 ± 0.6^{1}	3.7 ± 0.5^{1}	3.5 ± 0.4^{1}	3.9 ± 0.5^{1}	3.3 ± 0.6^{1}
Odor *	3.2 ± 0.4^{1}	3.7 ± 0.5^{1}	3.6 ± 0.5^{1}	3.8 ± 0.4^{1}	3.9 ± 0.6^{1}	3.7 ± 0.4^{1}	3.6 ± 0.3^{1}	3.2 ± 0.4^{1}	3.5 ± 0.6^{1}	3.4 ± 0.5^{1}
Pungent taste *	3.1 ± 0.4^{1}	3.2 ± 0.5^{1}	3.4 ± 0.5^{1}	3.9 ± 0.5^{1}	3.3 ± 0.5^{1}	3.8 ± 0.5^{1}	3.1 ± 0.4^{1}	3.3 ± 0.4^{1}	3.8 ± 0.6^{1}	3.2 ± 0.6^{1}
Overall acceptability	3.3 ± 0.3^{1}	3.8 ± 0.3^{1}	3.5 ± 0.4^{1}	3.4 ± 0.4^{1}	3.5 ± 0.5^{1}	3.2 ± 0.5^{1}	3.3 ± 0.5^{1}	3.4 ± 0.5^{1}	3.9 ± 0.4^{1}	3.1 ± 0.4^{1}
Swelling index										
Swelling Index in mL	6.33 ± 0.29^{1}	6.83 ± 1.15^{1}	6.67 ± 0.58^{1}	6.83 ± 1.15^{1}	6.83 ± 1.15^{1}	6.33 ± 1.32^{1}	6.75 ± 0.58^{1}	5.67 ± 0.29^{2}	5.67 ± 0.29^{2}	5.17 ± 0.28^{2}

4.3.2 Fennel

Foeniculum vulgare Mill. (fennel) fruits cultivated in 2009 were provided by MAWEA (Majoranwerke Aschersleben GmbH, Germany). The cultivation locality was Aschersleben in Saxony-Anhalt, Germany.

The essential oil of fennel is included in the grain and thus protected from a short superficial heating.

In the essential oil analysis of fennel 100-200 mg plant material were weighed into a 100 mL centrifuge tube and homogenized in iso-octane with an Ultra-Turrax (IKA). This mixture had been low speed centrifuged at 3000 rpm in a table top centrifuge and the supernatant solution was analyzed by gas chromatography (Hewlett Packard HP 5890 Series II GC). The essential oil content in mL/100g is defined as the total value of the specific individual components determined by gas chromatography (52).

As it is shown in Table 4.3.10, there is only a diminishment of essential oil by 7% when 125 °C steam and 80 °C jacket temperature were used. There are no differences in the percentages of the composition of the ingredients in all treatment variations (Tab. 4.3.6).

Tab. 4.3.6: Essential oil composition of fennel after "single treatment" of saturated steam decontamination. *Same superscript means no significant difference between groups.

Fennel oil composition [%/100g]			
	Fenchon	Estragol	Anethol
110 °C			
Control	1.73±0.02[1]	0.21±0.01[1]	5.62±0.22[1]
20°C	1.87±0.06[1]	0.21±0.01[1]	5.76±0.28[1]
40°C	1.76±0.13[1]	0.20±0.01[1]	5.50±0.29[1]
80°C	1.78±0.11[1]	0.21±0.02[1]	5.57±0.22[1]
125 °C			
Controll	1.73±0.02[1]	0.21±0.01[1]	5.62±0.22[1]
20°C	1.86±0.22[1]	0.21±0.01[1]	5.58±0.33[1]
40°C	1.87±0.16[1]	0.21±0.01[1]	5.79±0.18[1]
80°C	1.80±0.14[1]	0.21±0.01[1]	5.76±0.18[1]

When the customer purchases the drug as raw material, not only the content of essential oil is important, but also the appearance. For that, L*a*b*-color values were determined. The results of fennel after single

treatment show significant changes in L- and b-values. The L-value is diminished by two degrees from 33.91 ± 0.65 to 31.05 ± 0.59 degree on average and b-value is increased from 14.63 ± 0.42 to 16.89 ± 0.25 degree on average (*Tab. 4.3.10*). Thus, the color of fennel shows a minor lightness and higher yellow content. These changes were not visible to the naked eye (*Fig. 4.3.2, F1-F6*).

The double thermal exposure of fennel causes a 6% loss of essential oil already at the low steam temperature of 110 °C and 80 °C jacket temperature. Using the higher steam temperature of 125 °C, losses of 8% appears at all three jacket temperatures (*Tab. 4.3.10*). Just as in the single treatment, there are also no changes in the ingredients composition (Tab. 4.3.7).

Tab. 4.3.7: Essential oil composition of fennel after "single treatment" of saturated steam decontamination. *Same superscript means no significant difference between groups.

Fennel oil composition [%/100g]			
	Fenchon	Estragol	Anethol
2 x 110 °C			
Control	1.85±0.03[1]	0.21±0.02[1]	5.60±0.20[1]
20°C	1.82±0.05[1]	0.22±0.01[1]	5.68±0.29[1]
40°C	1.79±0.04[1]	0.18±0.03[1]	5.55±0.31[1]
80°C	1.81±0.08[1]	0.20±0.02[1]	5.63±0.39[1]
2 x 125 °C			
Controll	1.82±0.06[1]	0.19±0.02[1]	5.59±0.33[1]
20°C	1.80±0.12[1]	0.18±0.03[1]	5.64±0.25[1]
40°C	1.77±0.09[1]	0.22±0.01[1]	5.73±0.23[1]
80°C	1.86±0.11[1]	0.24±0.02[1]	5.69±0,15[1]

Color values of fennel are changing after the double treatment with the saturated steam decontamination, too. The L-value decreases by 4 degrees from 45.10 ± 0.72 to 40.15 ± 0.39 (*Tab. 4.3.10*). Thus, the double treated fennel loses lightness. The darkening of the sample is not visible to the naked eye, as it was noticed at single treatment (*Fig. 4.3.2, F11-16*. Finally, the loss of essential oil increases to 8%. Color changes are exclusively perceivable in a slight diminishment of lightness, but, again, not visible to the naked eye. The gained results confirm findings of

several studies concerning other plants or inoculated model systems in a 0.7 L facility (60, 61).

Fig. 4.3.2: Fennel before and after mechanical saturated steam decontamination. FC = Control, F1 – F6 = Single treatment with saturated steam, F11 – F16 = Double treatment with saturated steam. For details of the treatment see Table 4.3.1 and 4.3.2.

4.3.3 Nasturtium

REPHA GmbH Biologische Arzneimittel (Langenhagen, Germany) supplied *Tropaeolum majus L.* (nasturtium), which was cultivated in 2009 in Germany (Odenwald). The nasturtium plant material was industrial dried at 35 °C for 120 hours. The resulting nasturtium sample is a mixture of first and second cut of 2009. The saturated steam-treated nasturtium samples were air-dried and the glucotropaeoline content was measured by HPLC (84).

Degradation of glucotropaeoline is caused by the swelling due to the saturated steam. Swelling leads to cell disrupture. Thus the thioglucosidase myrosinase is released and causes degradation of glucotropaeoline. The first step produces a non-stable intermediate (aglycones). Subsequently a spontaneous rearrangement takes place giving rise to a formation of *inter alia* isothiocyanate.

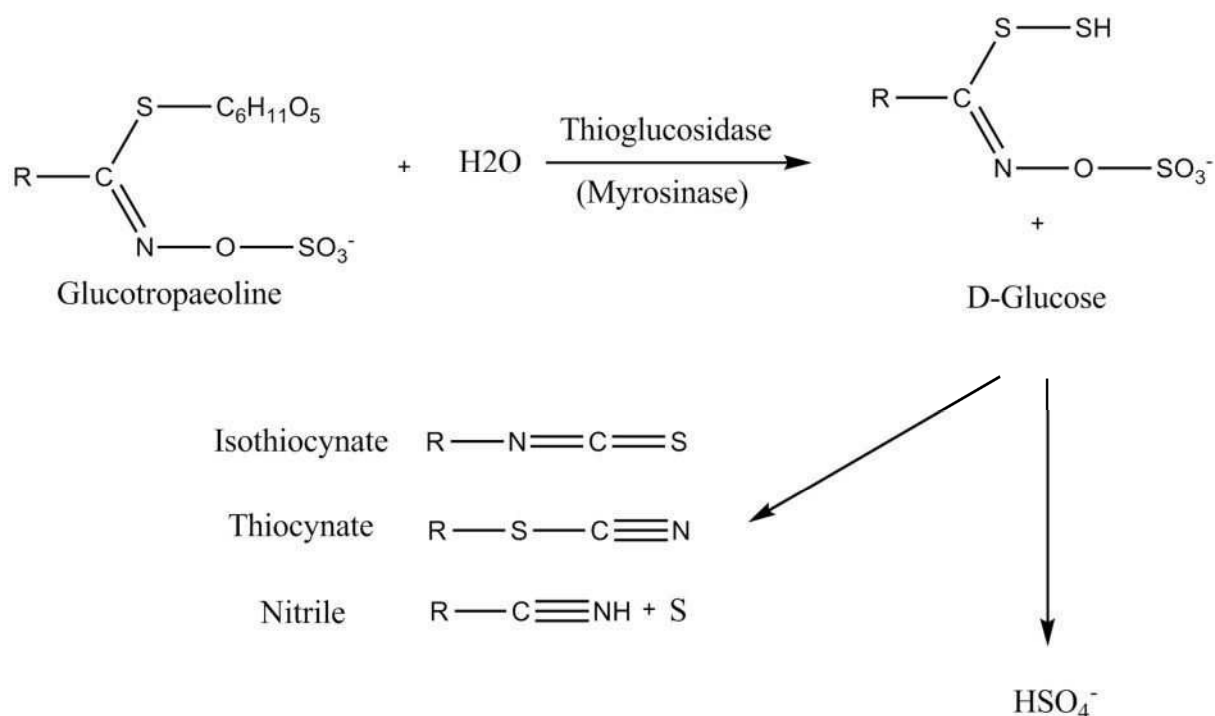

Fig. 4.3.3 Degradation of glucotropaeoline by myrosinase

After a treatment with single cycle saturated steam decontamination and successive thermal (35 °C) air drying, the loss of nasturtium valuable ingredients comprises between 8% and 28% (*Tab. 4.3.10*) depending on the particular test conditions. A single treatment with 110°C steam and 20 °C jacket temperature results in a significant loss of 8%. Jacket temperatures of 40 °C or 80°C combined with 110 °C saturated steam displayed a glucotropaeoline loss of 12% (*Tab. 4.3.10*).

One cyclical treatment with 125 °C steam temperature caused a glucotropaeoline loss of 14-16% at all three jacket temperatures. Color changes after a single treatment of nasturtium is less considerable. L*a*b*-values show only slight deviation from the control (*Tab. 4.3.10)*. At the investigation of samples, it was visible to the naked eye that stems are brighter and leafs are darker in comparison with the control (*Fig. 4.3.4, NC-N6*). Therefore, a single treatment of nasturtium with both saturated steam temperatures and a jacket temperature of 80°C is assessed as successful, since a reduction of total viable count and aerobe spores below the detection limit was accomplished.

Investigations in a 0.7 L facility gained no reduction below the detection limit at a single treatment with 110 °C. In this context it has to be noticed that at the 0.7 L facility no heatable jacket was available (*Chap. 3.1*). As a result, a lower pressure in the treatment chamber is possible in the smaller facility. Thus, the speed of pressure reduction is diminished and the flash-effect is lower. Qualitative changes regarding color and valuable ingredients were only observed in a minor degree. At the highest jacket temperature a loss of 12% glucotropaeoline was recorded for 110 °C steam temperature, while at 125 °C a loss of 16% glucotropaeoline was observed. Thus, a marketable product is obtained after decontamination.

At double saturated steam decontamination, nasturtium shows a higher loss of glucotropaeoline. This effect may be explained with an increased swelling and augmented breaks of the drug cell walls due to an elevated formation of condensate water. In addition, there is an augmented heat input into nasturtium.

At the double treatment, L*a*b*-values deviate more from the control (*Tab. 4.3.10*). The difference stated above is definitely more obvious after a double treatment with the "mechanical saturated steam decontamination". Stems loose significantly the green color and leafs become dark green (*Fig. 4.3.4, N11-16)*.

Finally, a double treatment with 110 °C saturated steam and 20 °C or 40 °C jacket temperature show the same loss as a single treatment with 125 °C and 40 °C. Additionally, elevating jacket temperatures increased losses of glucotropaeoline. The double cyclic alternative with 80 °C jacket temperature and 110°C saturated steam causes a reduction of valuable ingredients by 12%, whereas the loss increases to 28% when 125 °C was used with the same jacket temperature.

The 12% loss of glucotropeoline is tolerable. Additionally, color changes are considerably better distinguishable after double than single treatment.

Fig. 4.3.4: Nasturtium before and after mechanical saturated steam decontamination. NC = Control, N1 – N6 = Single treatment with saturated steam, N11 – N16 = Double treatment with saturated steam. For details of the treatment see Table 4.3.1 and 4.3.2.

4.3.4 Onion

The *Allium cepa* L. (onion) specimens were reaped and processed in 2009 and delivered by the company Kräutermix GmbH (Abtswind, Germany). The Onions were air-dried at 40-50 °C and chopped subsequently to 1 cm² large pieces. An amount of 1 g of the industrially chopped onion was weighed into a round bottom flask and 10 mL of methanol were added. After the mixture boiled under refluxed for 10 min water is added. Further 15 min later the methanol/water mixture was evaporated to dryness. The remaining solid is taken up with 1 mL of water and measured by HPLC / MS (105). Methiin, alliin and propiin were analyzed separately.

The cysteine sulfoxides are eliminated mainly by enzymatic means (Fig. 4.3.5). The enzyme alliinase is stored in cell compartments, which are separated from the cysteine sulfoxides, and is released only when the cell is damaged (100). It is such cell damage that can occur during saturated steam decontamination. Upon moistening of the vegetable material, swelling occurs and as a result cracks emerge in the cell compartments and this leads to enzymatic degadation of cysteine sulfoxides. In the first reduction step the intermediate sulfonic acid and α-aminoacrylic acid arise. The end products are thiosulfinates and pyruvate. This leads to the steady reduction of the three cysteine sulfoxides alliin, methiin and propiin (Tab. 4.3.8 and 4.3.9).

The losses constitute up to 17% of cysteine sulfoxide when 125 °C saturated steam temperature was applied.

Thermal treatments often cause color changes of plant material (1, 80). These are clearly distinguishable in the case of onion. The single treatment with 110 °C saturated steam causes a considerable darkening of the sample at all of the three jacket temperatures (*Fig 4.3.6, O1-O6*). L-values decrease by 30 °C, while a- and b-values increase (a from 0.89 ± 0.01 degree to 9.75 ± 0.28 and b from 14.70 ± 0.58 to 31.50 ± 0.86) (*Tab. 4.3.10*). The onion appears noticeably more red and brown to the naked eye after the treatment with the saturated steam decontamination (*Fig. 4.3.6,O11-O16*).

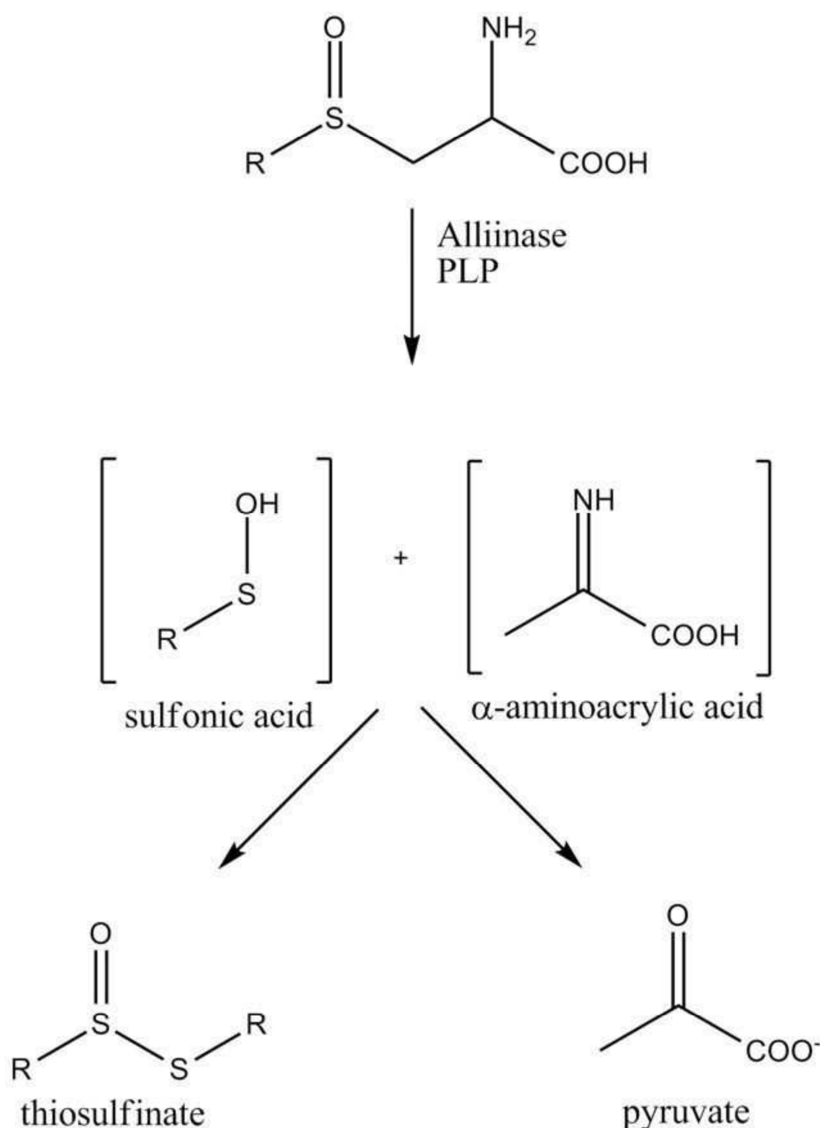

Fig. 4.3.5: Degradation of cysteine sulfoxide by alliinase

The double treatment is connected with an increased heat input into the onion, which causes larger color changes. The b and L values of the double-treated onion samples are reduced. For this reason, it can be clearly seen a darkening of the sample in Figure 4.3.6 O12-O16.

No higher loss of cysteine sulfoxide was noticed after the double treatments with 110 °C and 125 °C.

Fig. 4.3.6: Onion before and after mechanical saturated steam decontamination. OC = Control, O1 – O6 = Single treatment with saturated steam, O11 – O16 = Double treatment with saturated steam. For details of the treatment see Table 4.3.1 and 4.3.2.

Tab. 4.3.8: Cysteine sulfoxide composition of onion after "single treatment" of saturated steam decontamination. *Same superscript means no significant difference between groups.

Onion cysteine sulfoxide composition [ng/g]			
	Methiin	Alliin	Propiin
110 °C			
Control	88.85±0.96[1]	131.21±1.20[1]	155.68±1.96[1]
20°C	89.73±1.09[1]	130.96±0.83[1]	154.84±1.69[1]
40°C	84.04±5.03[1]	130.77±0.93[1]	153.91±1.91[1]
80°C	89.92±0.92[1]	130.73±1.12[1]	155.86±1.58[1]
125 °C			
Control	87.34±2.71[1]	130.73±2.55[1]	155.92±3.02[1]
20°C	88.21±1.66[1]	130.85±3.06[1]	154.93±2.28[1]
40°C	86.85±3.12[1]	129.27±0.81[1]	155.74±1.46[1]
80°C	84.6±1.33[1]	129.35±2.37[1]	156,10±2.19[1]

Tab. 4.3.9: Cysteine sulfoxide composition of onion after "single treatment" of saturated steam decontamination. *Same superscript means no significant difference between groups.

Onion cysteine sulfoxide composition [ng/g]			
	Methiin	**Alliin**	**Propiin**
2 X 110 °C			
Control	88.85±0.96[1]	131.21±1.53[1]	155.68±2.45[1]
20°C	88.45±0.99[1]	130.96±2.07[1]	154.84±3.16[1]
40°C	85.39±2.05[1]	130.77±1.97[1]	153.91±3.01[1]
80°C	88.72±2.76[1]	130.73±2.23[1]	155.86±3.48[1]
2 X 125 °C			
Control	87.34±0.95[1]	130.73±1.48[1]	155.92±2.38[1]
20°C	85.21±2.57[1]	129.85±1.89[1]	152.32±3.21[1]
40°C	83.85±3.04[1]	127.27±2.32[1]	151.65±3.02[1]
80°C	82.61±1.98[1]	126.35±2.10[1]	151.13±2.98[1]

Tab. 4.3.10: Content of valuable ingredients of fennel (essential oil), nasturtium (glucotropaeoline) and onion (cysteine sulfoxide), as well as the "Lab" color space values before and after "single" and "double treatment" of saturated steam decontamination. *:Same superscript means no significant difference between groups.

Fennel single and double treatment parameters													
Treatment	Control	1	2	3	4	5	6	11	12	13	14	15	16
Ingredients analysis													
Essential Oil mL/100g *	8.79 ± 0.20^{1}	8.85 ± 0.22^{1}	8.39 ± 0.20^{1}	8.46 ± 0.31^{1}	8.44 ± 0.35^{1}	8.64 ± 0.23^{1}	8.19 ± 0.15^{2}	8.53 ± 0.23^{1}	8.51 ± 0.19^{1}	8.16 ± 0.12^{2}	8.17 ± 0.15^{2}	8.04 ± 0.13^{2}	8.02 ± 0.21^{2}
„Lab“ color space values													
L*	33.91 ± 0.65^{1}	31.17 ± 0.76^{3}	31.90 ± 0.35^{3}	32.83 ± 0.41^{2}	31.50 ± 0.93^{3}	31.16 ± 1.02^{3}	31.05 ± 0.59^{3}	41.43 ± 0.65^{4}	40.69 ± 0.81^{4}	41.47 ± 0.32^{4}	40.72 ± 0.45^{4}	41.38 ± 0.65^{4}	40.15 ± 0.39^{4}
a*	1.74 ± 0.28^{1}	2.13 ± 0.11^{1}	2.18 ± 0.24^{1}	1.73 ± 0.29^{1}	2.31 ± 0.32^{1}	1.83 ± 0.14^{1}	2.08 ± 0.27^{1}	4.95 ± 0.81^{2}	4.79 ± 0.32^{2}	4.93 ± 0.41^{2}	4.76 ± 0.48^{2}	4.82 ± 0.72^{2}	4.94 ± 0.53^{2}
b*	14.63 ± 0.42^{1}	16.80 ± 0.34^{2}	15.60 ± 0.19^{2}	16.42 ± 0.49^{2}	16.05 ± 0.62^{2}	16.89 ± 0.25^{2}	15.65 ± 0.28^{2}	14.39 ± 0.63^{1}	13.75 ± 0.29^{1}	14.39 ± 0.45^{1}	14.04 ± 0.68^{1}	14.49 ± 0.55^{1}	13.75 ± 0.28^{1}
ΔE	-	3.52	2.27	2.09	2.85	3.56	3.06	3.7	4.5	3.65	4.42	3.73	5.04
Nasturtium single and double treatment parameters													
Ingredients analysis													
Glucotropaeoline mg/g *	12.01 ± 0.36^{1}	11.05 ± 0.38^{2}	10.57 ± 0.53^{2}	10.63 ± 0.31^{2}	10.04 ± 0.35^{2}	10.36 ± 0.45^{2}	10.06 ± 0.17^{2}	12.45 ± 0.40^{2}	10.35 ± 0.45^{2}	10.07 ± 0.28^{2}	9.83 ± 0.29^{2}	10.22 ± 0.33^{2}	9.27 ± 0.37^{3}
„Lab“ color space values													
L*	38.21 ± 0.83^{1}	36.25 ± 0.75^{2}	36.24 ± 0.37^{2}	35.99 ± 0.95^{2}	37.86 ± 0.56^{2}	37.36 ± 0.89^{2}	36.40 ± 1.09^{2}	41.33 ± 0.74^{3}	37.06 ± 0.89^{2}	37.37 ± 0.48^{2}	36.65 ± 1.08^{2}	36.65 ± 0.93^{2}	35.36 ± 0.84^{2}
a*	-1.56 ± 0.25^{1}	0.10 ± 0.06^{2}	0.06 ± 0.12^{2}	0.05 ± 0.08^{2}	0.03 ± 0.11^{2}	0.12 ± 0.09^{2}	0.12 ± 0.09^{2}	-2.27 ± 0.10^{3}	-0.50 ± 0.05^{3}	-0.29 ± 0.03^{4}	-0.29 ± 0.02^{4}	-0.29 ± 0.02^{4}	0.07 ± 0.01^{2}
b*	6.32 ± 1.11^{1}	5.07 ± 0.98^{1}	5.10 ± 0.92^{1}	5.08 ± 0.89^{1}	6.29 ± 1.04^{1}	5.85 ± 0.50^{1}	5.09 ± 0.69^{1}	9.34 ± 0.34^{2}	6.74 ± 0.29^{1}	6.90 ± 0.28^{1}	6.74 ± 0.30^{1}	6.74 ± 0.23^{1}	6.03 ± 0.28^{1}
ΔE	-	2.86	2.83	3.01	1.63	1.94	2.76	-	5.30	5.06	5.71	5.71	7.22
Onion single and double treatment parameters													
Ingredients analysis													
Cysteine sulfoxide µg/g *	51.05±2.09^{1}	50.67±3.12^{1}	50.53±2.87^{1}	49.59±2.59^{1}	42.48±2.11^{1}	43.94±2.52^{1}	44.22±2.39^{1}	51.05±3.10^{1}	49.98±2.55^{1}	48.54±3.83^{1}	50.91±2.89^{1}	41.67±2.92^{1}	42.19±2.05^{1}
„Lab“ color space values													
L	45.16 ± 0.89^{1}	35.84 ± 1.29^{2}	38.03 ± 0.97^{3}	25.59 ± 1.10^{4}	16.83 ± 1.91^{4}	16.93 ± 0.95^{4}	15.27 ± 1.43^{4}	45.16 ± 0.98^{1}	36.69 ± 0.83^{2}	30.08 ± 0.37^{5}	26.98 ± 0.73^{4}	15.86 ± 0.82^{4}	14.73 ± 0.34^{4}
a*	0.89 ± 0.01^{1}	3.57 ± 0.21^{2}	3.78 ± 0.13^{2}	3.42 ± 0.23^{2}	8.59 ± 0.47^{3}	9.25 ± 0.37^{3}	9.75 ± 0.28^{3}	0.89 ± 0.01^{1}	3.66 ± 0.39^{2}	3.74 ± 0.42^{2}	4.76 ± 0.84^{2}	8.45 ± 0.56^{3}	8.32 ± 0.87^{3}
b*	14.70 ± 0.58^{1}	17.77 ± 0.45^{2}	18.94 ± 0.49^{2}	18.72 ± 0.73^{2}	30.01 ± 0.49^{3}	31.30 ± 0.38^{3}	31.50 ± 0.86^{3}	14.70 ± 0.28^{1}	13.15 ± 0.31^{1}	13.45 ± 0.82^{1}	14.13 ± 0.15^{1}	20.45 ± 0.62^{4}	22.89 ± 0.92^{5}
ΔE	-	10.17	8.78	20.14	33.11	33.80	35.41	-	9.05	15.40	18.60	30.80	32.38

4.3.5 Marjoram

Leafs of marjoram are mostly used as pharmaceutical, since they contain essential oils as valuable ingredients. The essential oil is produced in peltate glandular trichomes at the leaf surface and thereby is very sensitive to a superficial, humid heat treatment. This fact is reflected by the results of Kutchan 2005. The choice of lowest sleeve and steam temperatures already caused a loss of 86% essential oil. Elevated temperatures led to an even higher loss of valuable ingredients (56). With 110 °C steam and 40 °C or 80 °C jacket temperature, the loss of essential oil amounted to 93% (*Tab. 4.3.11*). Elevating saturated steam temperature to 125 °C shows a further increase of the loss in comparison with the 110 °C treatment. Test conditions with 125°C saturated steam and 20 °C jacket temperature show the same loss as the treatment with 110 °C steam and 40 °C jacket temperature. But, both higher jacket temperatures, 40 °C and 80 °C, result in an oil diminishment of 95%, which is higher than after the treatment with 110 °C (*Tab. 4.3.11*).

Tab. 4.3.11: Essential oil content and "Lab" color space values before and after "single treatment" of saturated steam decontamination of marjoram. *Same superscript means no significant difference between groups.

Marjoram single treatment parameters							
Treatments	Control	1	2	3	4	5	6
Ingredients analysis							
Essential oil mL/100g *	1.64±0.07[1]	0.23±0.02[2]	0.16±0.03[3]	0.12±0.02[3]	0.15±0.04[3]	0.11±0.02[3]	0.09±0.01[3]
„Lab" color space values							
L	40.02 ± 0.82[1]	37.61 ± 0.79[2]	37.55 ± 0.65[2]	37.65 ± 0.72[2]	36.10 ± 0.91[2]	36.79 ± 0.90[2]	36.80 ± 0.86[2]
A	0.42 ± 0.22[1]	1.18 ± 0.43[1]	1.27 ± 0.32[1]	1.24 ± 0.38[1]	1.07 ± 0.42[1]	1.13 ± 0.45[1]	1.09 ± 0.39[1]
B	7.26 ± 0.81[1]	6.19 ± 0.75[1]	6.02 ± 0.93[1]	6.10 ± 0.91[1]	5.34 ± 0.85	5.60 ± 0.79[1]	5.57 ± 0.65[1]
ΔE	-	2.74	2.89	2.76	4.41	3.70	3.70

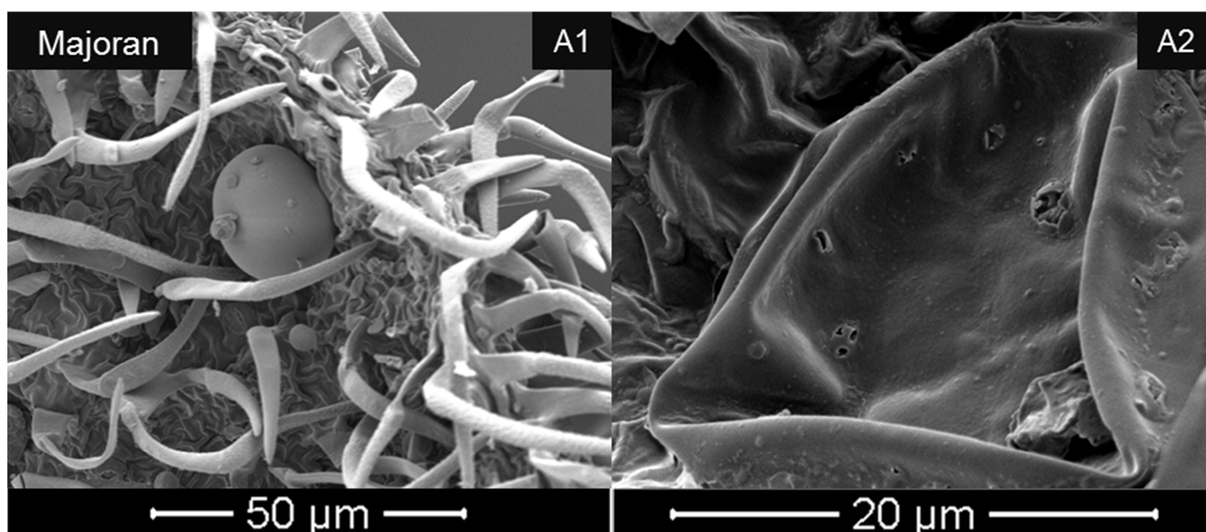

Fig. 4.3.7: Scanning electron microscopy image of marjoram. A1 = intact peltate glandular trichomes, A2 = damaged peltate glandular trichomes after "mechanical saturated steam treatment" with 125 °C and 80 °C jacket temperature.

Due to the high losses of essential oil, marjoram and superficial peltate glandular trichomes were investigated via scanning electron microscopy. These analyses revealed that peltate glandular trichomes, which are normally set as a ball or cyst on the surface, not only lost the essential oil completely, but also manifested holes after the saturated steam treatment. A double treatment was not accomplished for marjoram. The loss of essential oil was too large after single treatment so that marjoram was not marketable anymore. Marjoram appears obviously greyer after saturated steam treatment. In contrast to the changes of nasturtium where the leaves became darker, leaves of marjoram lost the color. Thus, marjoram appears older and more desiccated than the control (*Fig 4.3.8, M1-M7*).

Fig. 4.3.8: Marjoram before and after mechanical saturated steam decontamination. MC = Control, M1 – M6 = Single treatment with saturated steam. For details of the treatment see Table 4.3.1 and 4.3.2.

Heat during decontamination of several medicinal drugs is known to cause reduction or conversion of essential oil components (65). The analysis of individual ingredients of marjoram shows exactly this outcome (Tab. 4.3.12). The most significant changes in the ingredient spectrum is due to treatment with 110 ° C steam and 80 ° C jacket temperature (Tab. 4.3.12).

Following observations regarding de/increase were made with this treatment method:

- Sabinene: decrease from 10% to 5%
- cis-Sabinene hydrate: decrease from 29% to 17%
- cis-Sabinene hydrate acetate: decrease from 20% to 7%
- Terpinen-4-ol: increase from 3% to 6%.

The Figure 4.3.9 shows a saponification of cis-Sabinene hydrate acetate to cis-Sabine hydrate and afterwards a rearrangement and dehydration to α–terpinene, γ–terpinene or terpinen-4-ol. This also explains the changes of the oil components.

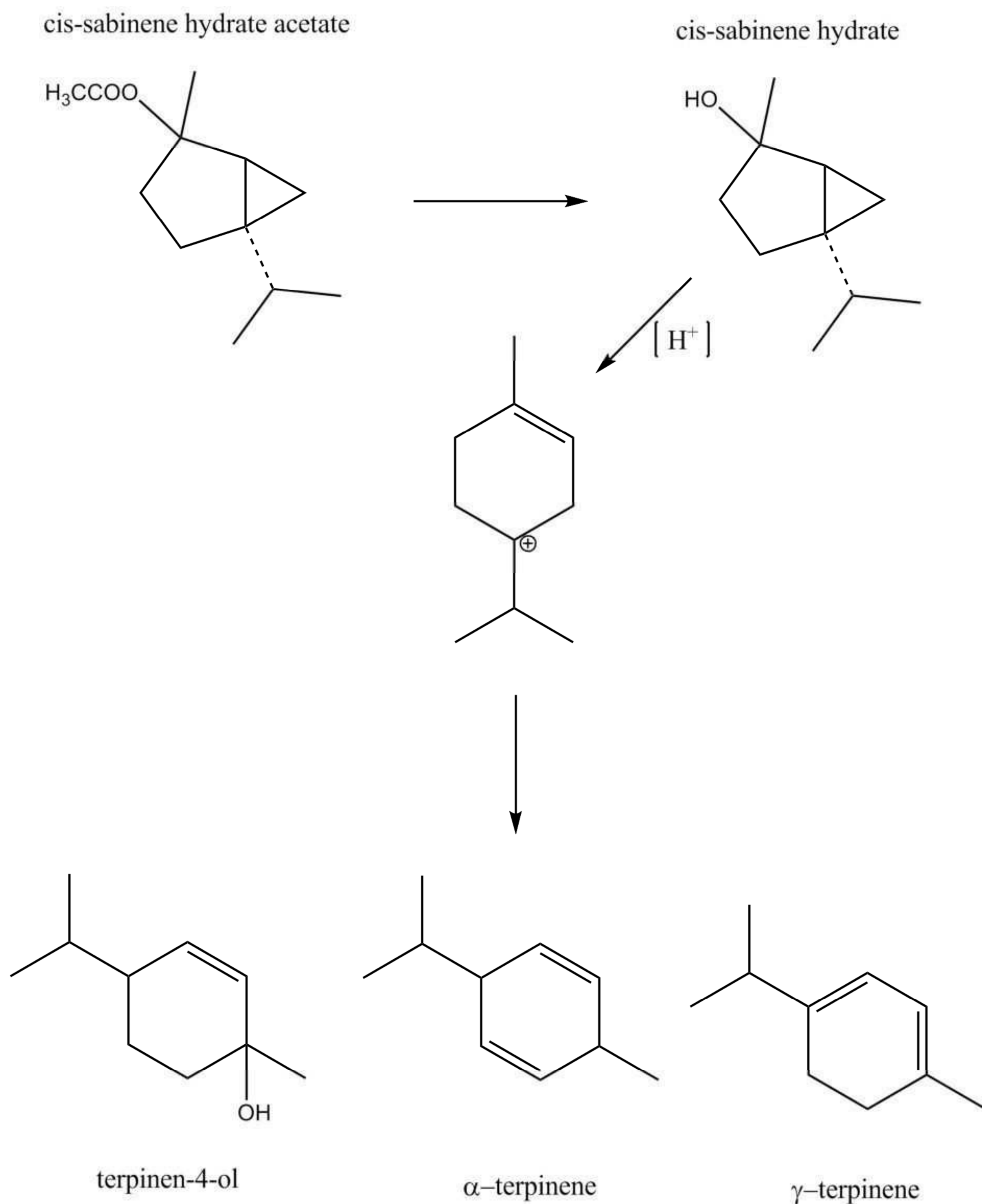

Fig. 4.3.9: Saponification, Rearrangement and Dehydration of cis-Sabinene hydrate acetate to α–terpinene, γ–terpinene or terpinen-4-ol

Tab. 4.3.12: Essential oil composition of marjoram after "single treatment" of saturated steam decontamination. *Same superscript means no significant difference between groups.

Marjoram oil composition [%/100g]								
	Sabinene	α-Terpinene	γ-Terpinene	trans-SH	cis-SH	Terpinen-4-ol	Linalyl- acetate	cis-SH- acetate
110 °C								
Control	11.50±0.12^{1}	2.33±0.20^{1}	4,05±0,16^{1}	4,45±0,08^{1}	29,12±0,43^{1}	3,03±0,49^{1}	3,07±0,10^{1}	20,16±1,63^{1}
20°C	9,72±0,50^{2}	2,54±0,18^{1}	4,16±0,28^{1}	2,21±0,12^{2}	14,27±1,15^{2}	3.40±0.15^{1}	3.73±0.20^{2}	20.13±2.06^{1}
40°C	8.88±0.09^{3}	2.74±0.07^{1}	4.41±0.22^{1}	1.27±1.46^{3}	11.98±2.06^{2}	3.70±0.30^{1}	4.02±0.08^{2}	15.95±2.54^{2}
80°C	5.27±0.36^{4}	0.00±0.00^{2}	3.63±0.19^{1}	3.06±0.44^{4}	17.77±1.38^{3}	6.54±0.76^{2}	3.10±0.22^{1}	6.92±1.91^{3}
125 °C								
Control	11.50±0.12^{1}	2.33±0.20^{1}	4.05±0.16^{1}	4.45±0.08^{1}	29.12±0.43^{1}	3.03±0.49^{1}	3.07±0.10^{1}	20.16±2.11^{1}
20°C	10.82±0.39^{2}	2.62±0.07^{1}	4.22±0.12^{1}	2.70±0.11^{2}	17.20±0.89^{2}	2.69±0.12^{1}	4.12±0.12^{2}	23.88±1.87^{1}
40°C	10.11±0.44^{2}	2.58±0.14^{1}	4.38±0.28^{1}	2.22±0.47^{2}	13.81±2.41^{3}	3.02±0.29^{1}	4.17±0.31^{2}	21.73±1.79^{1}
80°C	7.59±0.20^{3}	2.39±0.02^{1}	3.92±0.06^{1}	2.88±0.16^{2}	17.43±1.37^{4}	5.10±0.16^{2}	3.64±0.22^{3}	14,36±2.35^{2}

5 Summary

Today spices and medicinal plants are used by many different industries. For instance spices are widely used by the food or fragrance industry or as raw materials for medicinal products.

It is very important in all the different applications that the raw plant materials are free of microorganisms. In Germany saturated steam decontamination is the most common method for reducing germs on herbal drugs and spices as the use of ionizing radiation is not allowed.

This study tested a new method of mechanical saturated steam decontamination called “Lemgo process” which is used for tearing off microorganisms from the surface of spices and medicinal plants. This method of saturated steam decontamination is characterized by an explosion-like evaporation of a condensate film on the plant material caused after the sudden vacuum creation. This process leads to a prompt and gentle decontamination by saturated steam.

As a first example *Tropaelum majus* L. (nasturtium) was treated with the “Lemgo process” in a small laboratory scale (0,7 L). Due to this effect and in contrast to the classical applied saturated steam procedures it could be shown that this treatment decreased the microbial drug contamination of nasturtium up to 4 decades, along with an only small loss in the content of glucotropaeoline (13 %).

Further investigations respectively focused on measuring the effects of steam temperatures at 120 °C and 100 °C, for a duration of 20 s with a subsequent flash vacuum of 20 s. Control treatments, flash-vacuum as well as saturated steam heated at 120 °C were tested separately.

The impact of these incubation parameters on essential oil content and on the surface of different medicinal plants were analyzed using gas chromatography and scanning electron microscopy. During this study the following plant materials were used as an example: *Foeniculum vulgare* Mill. (fennel), *Majorana hortensis* L. (marjoram), *Origanum vulgare* L. (oregano), *Thymus vulgaris* L. (thyme), *Eucalyptus grandis* (eucalyptus), and *Mentha x piperita* L. (peppermint).

Especially in herbal drugs with peltate glandular hairs, such as marjoram and peppermint, a heavy loss in essential oil content is observed (93% for marjoram and up to 97% for peppermint). Oregano and thyme, however, have peltate glandular hairs that are embedded into the leaf surface and therefore less loss of oil occurs (59-64%). The oil loss of pharmaceutical plants like fennel and eucalyptus was very low. Application of saturated steam decontamination did not only destroy the oil-including peltate glandular hairs, it also clotted the existing wax layer of the eucalyptus afterwards.

In another studie five different pharmaceutical drugs were investigated: *Linum usitatissimum* L. (linseed), *Foeniculum vulgare* Mill. (fennel), *Origanum majorana* L. (marjoram), *Tropaeolum majus* L. (nasturtium), *Allium cepa* L. (onion). These herbal drugs were chosen because of their different morphologies and ingredients.

The analysis considered applications in a 5 L ploughshare stirrer of the "Lemgo process" with saturated steam temperatures of 110 °C and 125 °C and an exposure time of 20 s followed by the mechanical displacement of the condensate film. A double treatment, comprising of a repeated vaporization and evacuation was also examined. The total plate count and aerobic spore count was reduced up to 4 decades.

Valuable ingredients such as the essential oil of fennel and marjoram were measured before and after the treatment. The results showed a heavy

loss of essential oil from marjoram (93%) and the analysis of the essential oil components indicated a dehydration of cis-sabinene hydrate, cis-sabinene hydrate acetate and sabinene into terpinen-4-ol, γ-terpinene and α-terpinene.

In contrast fennel only showed a small essential oil loss (7%) and no modifications in its percentage composition.
Distinct color changes of the plants were apparent: whereas marjoram looked dry and pale after the treatment, fennel did not undergo any changes in its appearance.

Linseed is the only medicinal plant in this study that suffered no loss of value-adding ingredients, i.e. no changes of the fatty acid spectrum and no change in flavor and color.
Both onion and nasturtium have ingredients which can be enzymatically degraded. As soon as the plant material has contact with saturated steam, it begins to swell and cell disrupture occurs. As a consequence the enzymes myrosinase (nasturtium) and alliinase (onion) degrade the value-adding ingredients. Onion loses 18% of cysteine sulfoxide. The percentage ratio of methiin, propiin and alliin stay the same but the color changes from white to reddish brown. Nasturtium appears darker after the "Lemgo process" treatment and loses 17% of glucotropaeoline.

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7 Appendix 1

H. Lange, H. Krüger, A. Dammann, K. Schwarzer, R. David und U. Müller

Entkeimung von Kapuzinerkresse (*Tropaeolum majus* L.) mittels »Mechanischer Sattdampfentkeimung«

Originalbeiträge

Einleitung

Die Kapuzinerkresse (*Tropaeolum majus* L.) ist als Arzneipflanze in ihren Heimatländern Brasilien, Peru, Kolumbien, Venezuela, Chile sowie auf den Osterinseln seit mehr als 450 Jahren bekannt (18). Aus damaligen Aufzeichnungen geht hervor, dass die Kapuzinerkresse bei äußerlichen Hautkrankheiten, Entzündungen und Verletzungen angewandt wurde. Später fand die Pflanze Anwendung bei Krankheiten wie Bronchitis und Skorbut (3). Nach Europa wurde die Kapuzinerkresse 1684 zunächst als Zierpflanze eingeführt. Heutzutage wird Kapuzinerkresse zur unterstützenden Behandlung bei bakteriellen Infektionen der ableitenden Harnwege und Atemwege genutzt. Die pharmazeutische Produktpalette reicht von Tabletten aus gemahlener Kapuzinerkresse über Flüssigpräparate bis hin zu Sprays. All diese Medikamente beruhen auf antimikrobiellen und antiviralen Effekten der in der Kapuzinerkresse vorliegenden Glucosinolate (4, 14), wobei Glucotropaeolin die wertgebende Komponente darstellt. Wirkstoff ist allerdings das aus dem Präkursor Glucotropaeolin unter Einfluss von Myrosinase gebildete Isothiocyanat.

Für die Produktion von Arzneimitteln auf pflanzlicher Basis werden nicht nur Drogen mit hohem Glucosinolatgehalt, sondern auch mit geringer Keimbelastung benötigt. Um die rechtlichen Vorgaben der Höchstkeimzahl einzuhalten (Europäisches Arzneibuch 6. Auflage), müssen oft keimreduzierende Maßnahmen angewendet werden. Chemische Entkeimungsmethoden wie beispielsweise die Verwendung von Ethylenoxid sind aufgrund ihrer fehlenden gesundheitlichen Unbedenklichkeit verboten (1). Ebenso ist bei der Herstellung von Arzneimitteln die Bestrahlung des Pflanzenmaterials mit ionisierender Strahlung verboten, auch wird diese seitens der Verbraucher nicht akzeptiert.

Die Methode der Wahl zur Keimreduzierung bei Arzneidrogen ist in Deutschland zurzeit die Sattdampfentkeimung (17, 11). Aufgrund einer gewissen Befeuchtung der Pflanzenoberflächen und eines intensiven Wärmeübergangs kann dieses Verfahren zu einer effizienten und gut steuerbaren hitzethermischen Abtötung der Mikroorganismen auf pflanzlichen Materialen mit mikrobieller Belastung führen. Hierfür ist eine längere Behandlungsdauer von mehreren Minuten gerade bei Anwesenheit von bakteriellen Sporen nötig, und es kommt im Fall der Kapuzinerkresse zu produktschädigenden Effekten (5).

Die an der Hochschule Ostwestfalen-Lippe entwickelte »Mechanische Sattdampfentkeimung« nutzt bei verkürzter Bedampfungsdauer eine anschließende Flash-Verdampfung zur Entfernung der noch lebenden Keime (10). Dieser Flash-Effekt wird durch eine extrem schnelle Evakuierung des Behandlungsraumes hervorgerufen und benötigt zur Umsetzung besondere apparative Voraussetzungen (*Abb. 1*). Typisch für das mechanische Verfahren ist auch, dass der keimabtrennende Effekt erst ab einer Mindestsattdampftemperatur von üblicherweise um die 115 °C auftritt. Unter diesen Bedingungen sind die ablösenden Kräfte bei der Flashverdampfung groß genug, um die Haftkräfte zwischen Keim und Pflanzenoberfläche zu überwinden und eine Keimentfernung zu ermöglichen.

Alle bisher gemessenen Entkeimungskinetiken weisen einen typischen Verlauf auf, der innerhalb der ersten 10 s eine starke Keimzahlreduzierung erkennen lässt. In diesen ersten 10 s wird ein Wasserfilm ausgebildet, der dann zusammen mit den vorhandenen Keimen durch Flashverdampfung wieder entfernt wird. Danach wird eine relative Keimzahlkonstanz erreicht. Eine zusätzliche Reduktion ist lediglich auf eine klassische hitzethermische Abtötung zurückzuführen.

In einer 0,7 L-Laborapparatur wurden bisher Untersuchungen zur Keimreduzierung bei künstlich verkeimten Modellsystemen (8) wie auch natürlich verkeimten Gewürzen wie Pfeffer (9) und Drogen wie Kamillenblüten (12) durchgeführt. Die oben erwähnte typische Kinetik wurde hierbei bestätigt. Bei der schonenden Keimzahlreduktion von Kamillenblüten wurde mit dem Verfahren der »Mechanischen Sattdampfentkeimung« eine Gesamtkeimzahlreduktion von 4 – 5 Dekaden bei lediglich 120 °C Behandlungstemperatur und Behandlungszeiten von 10 bis 20 s erzielt. Zwar gab es hierbei eine Auffeuchtung von ca. 10 bis 35 %, aber der Gehalt an ätherischem Öl in der Droge veränderte sich durch die Behandlung nicht (12).

Zusammenfassung

Am Beispiel von Kapuzinerkresse (*Tropaeolum majus* L.) wird die »mechanische Sattdampfentkeimung« als neuartiges und schonendes Entkeimungsverfahren für Arzneidrogen vorgestellt. Das Verfahren beruht auf einer kurzen Bedampfung, gefolgt von einem extrem schnellen Evakuieren des Behandlungsraumes. Dies bewirkt eine flashartige Verdampfung des auf dem Pflanzenmaterial ausgebildeten Kondensatfilms und damit eine mechanische Ablösung oberflächlich vorhandener Mikroorganismen. Betrachtet wurden Applikationen in einer 0,7 L – Festbettapparatur mit Sattdampftemperaturen von 110°C und 125°C und Einwirkungszeiten von 5, 10 und 20 s, gefolgt von der mechanischen Entfernung des Kondensatfilms. Eine Zweifachbehandlung – bestehend aus wiederholter Bedampfung und Evakuierung – wird ebenfalls untersucht. Es konnte gezeigt werden, dass bei weitgehendem Erhalt von Glucotropaeolin, dem wertgebenden Inhaltsstoff der Kapuzinerkresse, eine Reduzierung der Keimzahl von bis zu 4 Zehnerpotenzen möglich ist.

Schlagwörter

Glucotropaeolin, Keimreduzierung, Mechanische Sattdampfentkeimung, Produktschonung, *Tropaeolum majus*

Reduction of microbial contamination of nasturtium (*Tropaeolum majus* L.) by »mechanical saturated steam decontamination«

Abstract
Taking nasturtium (*Tropaelum majus* L.) as example, a new and less stressful process of microorganism reduction in herbs and spices is presented. It is demonstrated that the »mechanical saturated steam decontamination« is a compound-friendly process for decontamination of medicinal drugs. This special method of saturated steam decontamination is characterized by the application of a vacuum accompanied by an explosion-like evaporation of a condensate film applied to the plant material. Due to this effect and in contrast to the usually applied saturated steam procedures with the thermal killing of the microorganisms naturally occurring on the plant surface, the microorganisms are mechanically removed by the mechanical saturated steam decontamination. It could be shown that this process of nasturtium treatment decreased the microbial drug contamination up to 4 decades, along with a small loss of glucotropaeoline of 13 %.

Keywords
Germ reduction, glucotropaeoline, mechanical saturated steam decontamination, product preservation, *Tropaelum majus*

Im Rahmen eines derzeit mit der Forschungsvereinigung der Arzneimittel-Hersteller e. V. (FAH) durchgeführten Projektes entsteht gegenwärtig eine weitere Keimreduzierungsanlage auf Basis eines 5 L-Pflugscharmischers. Die in dieser Studie dargelegten Entkeimungsversuche an Kresse liefern die Datengrundlage zur Nutzung des Verfahrens zunächst im 5 L-Maßstab und später in größeren, industriell einsetzbaren Feststoffmischern.

Material und Methoden

Pflanzenmaterial

Die in der Studie verwendete Kapuzinerkresse (*Tropaeolum majus* L.) geht auf das Saatgut der Firma »Saatgut Quedlinburg GmbH« zurück und wurde im Odenwald von der Firma »REPHA GmbH Biologische Arzneimittel« angebaut. Das Planzenmaterial wurde bei 35 °C ca. 120 Stunden getrocknet und anschließend geschnitten. Die erhaltene Probe ist eine Mischung aus erstem und zweitem Schnitt des Jahres 2008.

Mechanische Sattdampfbehandlung

Die Entkeimungsversuche wurden in einer Laborentkeimungsapparatur, die im Wesentlichen aus einem 0,7 L-Behandlungsraum und einem 8 L-Vakuumpuffertank besteht, durchgeführt (*Abb. 1*). Beide Behälter sind zylindrisch und bestehen aus nichtrostendem Stahl. Die Verbindung der einzelnen Apparatekomponenten erfolgt über Rohrleitungen mit einem Innendurchmesser von 1,27 cm (0,5 Zoll). Die Aufnahme von Temperatur und Druck wird durch ein Messwerterfassungssystem (DASYLab V.10.0, Messfrequenz 1 kHz) gewährleistet. Dampfbehandlungs- und Evakuierungszeiten werden durch elektronisch gesteuerte pneumatische Schrägsitzventile (Bürkert Fluid Control Systems, Ingelfingen) vor und hinter dem Behandlungsraum reguliert. Das Evakuieren des Behandlungsraums erfolgt durch einen Wasserringverdichter mit Gasstrahler (LEMA 25 AZ, Sterling SIHI GmbH, Itzehoe, p_{min}≈4 000 Pa).

Ein Rohrbündelwärmeüberträger zwischen Vakuumpuffertank und Wasserringverdichter unterstützt die Druckerniedrigung und gewährleistet die Kondensation der aus dem Behandlungsraum entzogenen Dämpfe. Der Wärmeüberträger (Bitzer, Sindelfingen, Deutschland) arbeitet mit Wasser (3,8 L, 5 – 10 °C) und hat eine Kapazität von 0,4 L.

Im Zuge der mechanischen Sattdampfentkeimung wurde die Bedampfung mit 110 °C und 125 °C heißem Dampf bei dem entsprechenden Gleichgewichtsdruck durchgeführt und dauerte 5, 10 oder 20 s. Zur Erreichung von Sattdampfbedingungen wurde ein Vorvakuum von 20 s angelegt. Auch die Haltezeit des für die Keimreduzierung entscheidenden Flashvakuums beträgt 20 s.

Bei Zweifachbehandlungen werden Bedampfungsphase und anschließendes Vakuum wiederholt.

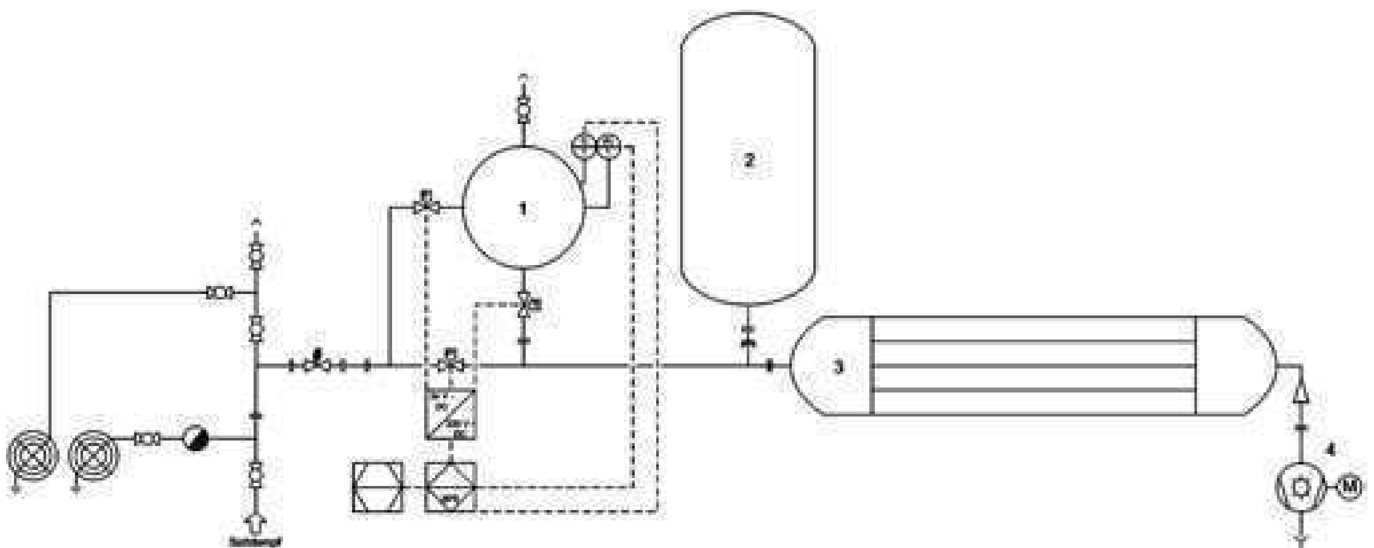

Abb. 1: Fließbild der Laborapparatur: 1) Behandlungsraum, 2) Vakuumpuffertank, 3) Wärmeübertrager, 4) Verdichter.
Fig. 1: Flow chart of the laboratory equipment: 1) treatment chamber, 2) vacuum buffer tank, 3) heat transducer, 4) concentrator.

Glucosinolatanalytik
Die keimreduzierten Proben wurden gefriergetrocknet. Damit wurde sichergestellt, dass der Glucosinolatverlust ausschließlich von der Entkeimung hervorgerufen wird. Da großtechnisch eine Gefriertrocknung nicht realisierbar ist, wurden ausgesuchte Proben bei 40 °C in einem Trockenschrank luftgetrocknet.
Glucotropaeolin wurde mittels HPLC nach Schütze et al. (15) analysiert.

Bestimmung der Keimzahlen

Alle mikrobiologischen Untersuchungen wurden nach dem Europäischen Arzneibuch (2) durchgeführt. Die Ergebnisse werden in koloniebildenden Einheiten pro g (KbE/g) angegeben. Gesamtkeimzahl und Schimmelpilze wurden quantitativ bestimmt, *Enterobacteriaceae* halbquantitativ. *Escherichia coli*, *Salmonella* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* und *Clostridium* sp. wurden in 10 g qualitativ nachgewiesen. Da das Ausgangsmaterial nur wenige Sporen (10^3 KbE/g) aufwies, wurde auf eine nachfolgende Bestimmung der Sporenzahl verzichtet. Alle mikrobiologischen Ergebnisse sind die Mittelwerte aus Dreifachbestimmungen.

Statistische Berechnung
Die statistische Auswertung bezüglich des Glucotropaeolingehalts erfolgte mit dem Programm Sigmaplot 11. Zur globalen Signifikanzanalyse wurden einfaktorielle Varianzanalysen durchgeführt. Beim direkten Gruppenvergleich kam ein Tukey-Test zur Einteilung der Glucotropaeolin-Gehalte in Gruppen von A bis E zum Einsatz (siehe *Tab. 1* und *2*).

Ergebnisse und Diskussion

Keimreduzierung
Bereits unter den sehr milden Versuchsbedingungen einer Einfachbehandlung mit 110 °C heißem Sattdampf und einer Einwirkzeit von nur 5 s erfolgt eine Reduzierung der Keimbelastung um 2,5 Dekaden (*Abb. 2*), die bei längerer Dampfeinwirkung von 10 und 20 s nicht verbessert wird. Dies entspricht der bereits bekannten Kinetik mit mechanischer Keimabtrennung, wobei im Falle der Kapuzinerkresse der bekannte Effekt bereits bei der sehr niedrigen Dampftemperatur von 110 °C eintritt. Dies könnte auf geringe Haftkräfte zwischen Keimen und Pflanzenoberfläche hinweisen. Die stärkere Keimreduzierung bei 125 °C und längeren Bedampfungszeiten (10 und 20 s) (*Abb. 2*) lässt auf eine zusätzliche thermische Abtötung der hauptsächlich vegetativen Keime schließen. Bei der höheren Dampftemperatur (125 °C) und den Bedampfungszeiten von 10 bzw. 20 s ist außerdem eine bessere Ausbildung des Kondensatfilmes gegeben.

Durch eine Zweifachbehandlung mit einer Sattdampftemperatur von 110 °C und 5 s Bedampfungsdauer wird im Vergleich zur Einfachbehandlung eine Absenkung der Gesamtkeimzahl um 4 Dekaden bis unter die Nachweisgrenze erreicht, ebenso wie bei Zweifachbehandlung mit der Bedampfungsperiode von 20 s (*Abb. 2, Tab. 2*). Ob eine Zweifachbehandlung mittels Sattdampf von 125 °C gegenüber 110 °C eine weitere Verbesserung der Entkeimung bringt, kann nicht festgestellt werden, da alle Ergebnisse unter der Nachweisgrenze lagen.
Begründet werden kann dieser Entkeimungserfolg damit, dass durch die erste Flash-Verdampfung, verbunden mit nochmaliger Befeuchtung, weitere Keime gelockert werden, die dann beim zweiten Evakuieren abgesaugt werden.
Alle im Zusammenhang mit der Kapuzinerkresse ermittelten Entkeimungskinetiken weisen also einen typischen Verlauf auf, der innerhalb der ersten 10 s eine starke Keimzahlreduzierung erkennen lässt. Dies ist in der Bildung des für die Entkeimung notwendigen Wasserfilms begründet, der die Grundlage für die Flashverdampfung der Mikroorganismen darstellt. Hiernach werden nahezu konstante Keimzahlen erreicht, eine zusätzliche geringe Keimzahlreduktion findet aufgrund von hitzethermischen Effekten statt (10). Positiv auf die Flash-Entkeimung wirkt sich auch die Oberflächenstruktur des Kapuzinerkresseblattes aus, die zu einem Lotuseffekt führt, der die Haftkräfte der Keime zusätzlich verringert (6).

Veränderungen im Glucosinolatgehalt
Der Verlust der wertgebenden Inhaltsstoffe der Kapuzinerkresse nach einer Behandlung mit der »Mechanischen Sattdampfentkeimung« und anschließender Gefriertrocknung liegt je nach Auslegung des Keimreduzierungsverfahrens zwischen 4 und 37 % (*Tab. 1* und *2*). Bei den Einfachbehandlungen mit 110 °C Behandlungstemperatur und 5 – 10 s Behandlungsdauer kommt es zu keinem signifikanten Verlust, gleiches gilt für eine Sattdampftemperatur von 125 °C und einen Applikationszeitraum von 5 s. Bei einer Sattdampftemperatur von 110 °C über einen Zeitraum von 20 s beträgt der Verlust lediglich 13 %.
Die Minderung des Glucotropaeolingehalts steigt bei Zweifachbehandlun-

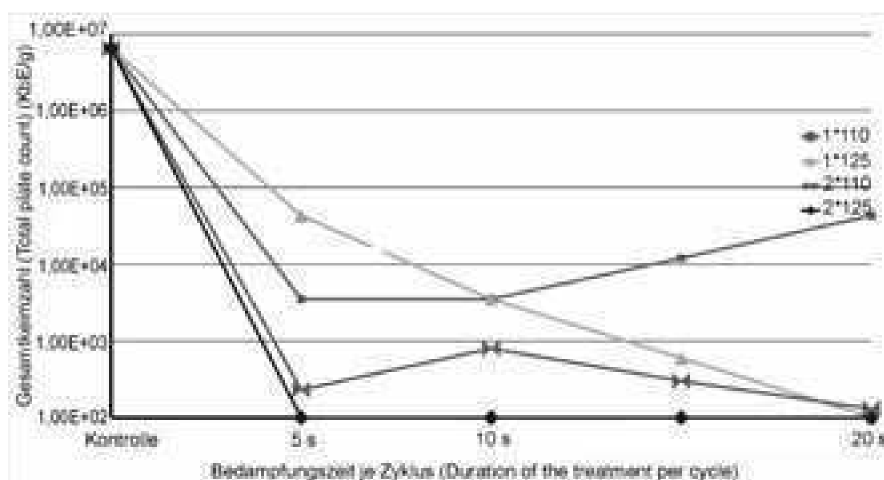

Abb. 2: Gesamtkeimzahl (KbE/g) von Kapuzinerkresse in Abhängigkeit von der Bedampfungsdauer, der Dampftemperatur und der Zahl der Bedampfungen (10^2 KbE stellt die Nachweisgrenze dar)
Fig. 2: Total plate count (CFU/g) of nasturtium depending on the duration, temperature and frequency of saturated steam (10^2 CFU is the detection limit)

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Tab. 1: Gegenüberstellung der Gehalte an Glucotropaeolin in mg/g und der mikrobiologischen Gesamtkeimzahl in KbE/g von Schimmelpilzen und Enterobacteriaceae nach Einfachbehandlung mit »Mechanischen Sattdampfentkeimung« bei 110 °C und 125 °C sowie 5 s, 10 s und 20 s Bedampfungszeit (Minus = qualitativ negativ, Plus = nur qualitativ nachweisbar)
Tab. 1: Comparison of glucotropaeoline content (mg/g), total plate count and mold count (colony forming units, CFU/g) as well as Enterobacteriaceae (minus = qualitatively negative and plus = only qualitatively detectable) after single treatment of nasturtium at temperatures of 110 °C and 125 °C and vaporization times of 5 s, 10 s and 20 s and quality tests of Enterobacteriaceae

Behandlungsvarianten (Different variants of treatment)	Glucotropaeolin (Glucotropaeoline) (mg/g)	Tukey-Grouping	Gesamtkeimzahl (KbE/g) (Total plate count) (cfu/g)	Schimmelpilzgehalt (KbE/g) (Moldcount) (cfu/g)	Enterobacteriaceae (Enterobacteriacee)
Kontrolle	14,49 ± 0,22	A	$4{,}6 \cdot 10^6$	$1{,}0 \cdot 10^3$	+ + +
110 °C für 5 s	13,94 ± 0,27	A	$5{,}8 \cdot 10^3$	$<1{,}0 \cdot 10^2$	– – –
110 °C für 10 s	13,43 ± 0,59	A	$4{,}7 \cdot 10^3$	$<1{,}0 \cdot 10^2$	+ – +
110 °C für 20 s	12,54 ± 0,39	B	$3{,}0 \cdot 10^4$	$<1{,}0 \cdot 10^2$	– – +
125 °C für 5 s	13,13 ± 1,05	A	$1{,}9 \cdot 10^4$	$<1{,}0 \cdot 10^2$	+ + +
125 °C für 10 s	11,97 ± 0,61	B	$1{,}9 \cdot 10^3$	$<1{,}0 \cdot 10^2$	– + +
125 °C für 20 s	10,79 ± 0,60	C	$<1{,}0 \cdot 10^2$	$<1{,}0 \cdot 10^2$	– – –

Tab. 2: Gegenüberstellung der Gehalte an Glucotropaeolin in mg/g und der mikrobiologischen Gesamtkeimzahl in KbE/g, Schimmelpilze und Enterobacteriaceae nach Zweifachbehandlung mit »Mechanischen Sattdampfentkeimung« bei 110 °C und 125 °C sowie 5 s, 10 s und 20 s Bedampfungszeit (Minus = qualitativ negativ, Plus = nur qualitativ nachweisbar)
Tab. 2: Comparison of glucotropaeoline content (mg/g), total plate count and mold count (colony forming units, CFU/g) as well as Enterobacteriaceae (minus = qualitatively negative and plus = only qualitatively detectable) after double treatment of nasturtium at temperatures of 110 °C and 125 °C and vaporization times of 5 s, 10 s and 20 s and quality tests of Enterobacteriaceae

Entkeimungsparameter (Degermination parameters)	Glucotropaeolin (Glucotropaeoline) (mg/g)	Tukey-Grouping	Gesamtkeimzahl (KbE/g) (Total plate count) (cfu/g)	Schimmelpilzgehalt (KbE/g) (Moldcount) (cfu/g)	Enterobacteriaceae (Enterobacteriacee)
Kontrolle	13,28 ± 0,03	A	$2{,}1 \cdot 10^6$	$<1{,}00 \cdot 10^2$	+ + +
110 °C für 2*5 s	11,48 ± 1,26	B	$2{,}3 \cdot 10^2$	$<1{,}00 \cdot 10^2$	– – –
110 °C für 2*10 s	10,81 ± 0,26	C	$9{,}3 \cdot 10^2$	$<1{,}00 \cdot 10^2$	– – –
110 °C für 2*20 s	10,05 ± 0,12	D	$1{,}3 \cdot 10^2$	$<1{,}00 \cdot 10^2$	– – –
125 °C für 2*5 s	10,76 ± 0,51	C	$<1{,}0 \cdot 10^2$	$<1{,}00 \cdot 10^2$	– – –
125 °C für 2*10 s	10,01 ± 0,41	D	$<1{,}0 \cdot 10^2$	$<1{,}00 \cdot 10^2$	– – –
125 °C für 2*20 s	8,39 ± 0,11	E	$<1{,}0 \cdot 10^2$	$<1{,}00 \cdot 10^2$	– – –

gen mit 110 °C und 125 °C und einer Bedampfungszeit von 5 bis 20 s auf 15 – 25% (*Tab. 2*). Nur die Zweifachbehandlung mit 125 °C heißem Dampf und Bedampfungszeiten von je 20 s führt zu einem erhöhten Wertstoffverlust von 37 %. Grundsätzlich kann gesagt werden, dass die Glucosinolate mit zunehmender Temperatur und Bedampfungszeit verstärkt abgebaut werden.
Im Vergleich zur Gefriertrocknung ergab die anschließende Trocknung mit Warmluft bei 40 °C außerhalb der Entkeimungsanlage ebenfalls keinen zusätzlichen Verlust an Glucotropaeolin.

Schlussfolgerung

Die beste Variante der mechanischen Sattdampfentkeimung zur Keimreduzierung von Kapuzinerkresse wird durch eine Zweifachbehandlung mit 110 °C heißem Sattdampf und einer Bedampfungsdauer von je 5 s erreicht; hierbei wird eine Minderung der Keimzahl um 4 Dekaden bei einem Glucotropaeolinverlust von nur 13% erzielt.

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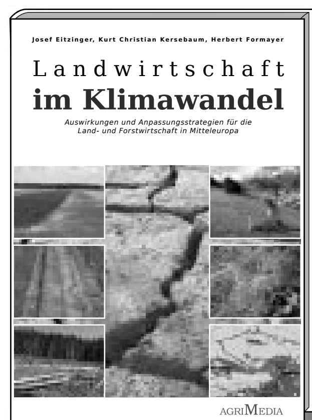
Landwirtschaft im Klimawandel



Die land- und forstwirtschaftlichen Betriebe sind in besonderem Maße vom Klimawandel betroffen, da sie weitgehend von den natürlichen Standortverhältnissen abhängen. Dieses Buch zeigt konkret und verständlich auf, welche Auswirkungen zu erwarten sind und welche Anpassungsstrategien geeignet erscheinen. Ein wichtiges Buch, das den aktuellen Stand der Erkenntnisse auf den Punkt bringt.

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8 Appendix 2

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Effects of steam and vacuum administration during decontamination on essential oil content in herbal medicines

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Summary

Saturated steam decontamination is an application for elimination of microorganisms from the surface of different materials. This technique has been optimized for the treatment of dried spices or pharmaceuticals, which could have been contaminated with microorganisms during cultivation, processing, storage or transport. The described saturated steam decontamination is based on the Lemgo process. This method does not kill microorganisms, but removes them physically from the surface.

Our investigation focused on measuring the effects of steam temperatures at 120 °C and 100 °C, respectively, for 20 s with a subsequent flash vacuum of 20 s. Applications of flash vacuum as well as saturated steam heated to 120 °C were also tested separately. The impact of these parameters on the essential oil content and on the surface of different medicinal plants such as marjoram, oregano, fennel and eucalyptus was analysed using gas chromatography and scanning electron microscopy.

Especially in herbal drugs with glandular trichomes such as marjoram and oregano severe surface destruction was visible accompanied by high losses of essential oil from 93 % in marjoram tissue to 59 % in oregano tissue. For fennel and eucalyptus that possess protected essential oil storage cells only minor or no reduction of volatiles has been observed during exposure to saturated steam. The experiments show clearly a positive correlation between stability of essential oil cavities and essential oil content preservation.

Introduction

Essential oils are stored in oil storage cavities that can be found distributed in tissues of blossoms, leaves, seeds, pericarps, roots, resins, barks or wood. Several genera of *Lamiaceae* accumulate essential oil in glandular hairs. In this case, the oil is protected solely by a cuticle layer with a resinous film (GUENTHER, 1949). These essential oils play an important role at chemical-ecological interactions of plants and their environment. Numerous monoterpenes protect the plant from direct or indirect attack by herbivores and microorganisms. On the other hand, some monoterpenes serve as attractant for insects (HALLAHAN et al., 2000; HARBORNE et al., 1991; LANGENHEIM et al., 1994; PICKETT et al., 1991; WISE et al., 1999).

Glandular hairs can be further divided into the group of peltate glandular hairs and the group of capitate glandular hairs. Both types of glandular hairs are present on leaf surfaces of oregano and marjoram belonging to the *Lamiaceae* family. These groups differ in anatomy and mode of secretion (BURBOTT and LOOMIS, 1969). Peltate hairs consist of a basal cell, a broad and short stalk cell with cutinized outer walls, and a round broad head of secretory cells. Secretory cells of peltate hairs show different positioning depending on the leaf surface morphology. As shown for marjoram in the right panel of Fig. 1 secretory cells can be exposed or as shown for oregano in the left panel secretory cells can be sunken in a pit formed by epidermal tissue (BRUNI and MODENESI, 1983).

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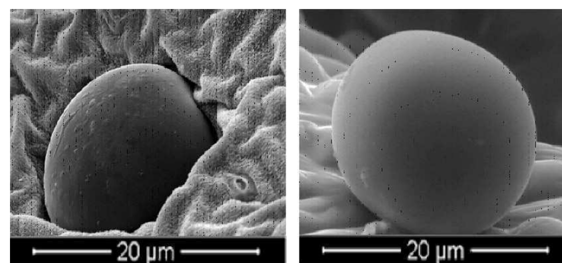

Fig. 1: Scanning electron microscopy (SEM) images of peltate glandular hairs of oregano left (*Origanum vulgare*) and marjoram right (*Majorana hortensis*).

The second large group of hairs is represented by capitate hairs. They consist of a basal cell, a stalk cell and a uni- or bi-cellular head (SCHNEPF et al., 1972; HEINRICH et al., 1973; AMELINXEN et al., 1965). Capitate hairs are much smaller than peltate hairs (Werker et al., 1985(A); Werker et al., 1985(B)) and start very early in development with secretion. At this stage peltate glandular hairs are not yet fully developed (FAIRBANKS et al., 1971) and functioning.

Saturated steam decontamination is the preferred method for decontamination of herbs and spices in Germany (WEBER, 2003). Due to the intensive heat transfer and moisturization of herbal surfaces, this process leads to an efficient and well applicable thermal decontamination of microorganism containing material. For plants with spore-formers on their surface, an extended treatment time up to 20 min is required to kill them, as spores are highly resistant to heat (KABELITZ, 1996).

The Lemgo process, that has been developed at the University of Applied Science Ostwestfalen-Lippe, Germany, uses a reduced vaporisation time (20 s) followed by a subsequent flash evaporation for removing microbes (MÜLLER et al., 2002). This so-called flash-effect is caused by an extremely rapid evacuation of the treatment chamber and requires special technology for achieving appropriate conditions. This mechanical decontamination method is characterised by a minimum saturated steam temperature of 80 °C, which is needed for the decontamination effect. The physical forces during flash evaporation are strong enough for overcoming adhesion of microbes to herbal surfaces resulting in decontamination (LILIE, 2009; LILIE et al., 2006). Previous decontamination studies were conducted in a 700 mL laboratory equipment for inoculated model systems (LILIE et al., 2009) as well as naturally contaminated plant material such as pepper (*Piper nigrum*)(LILIE et al., 2007; LILIE et al., 2004) and camomile (*Matricaria chamomilla)* (RUMKE et al., 2006). The decontamination of camomile using the Lemgo process resulted in a reduction up to 5 decades of total microbial count using a steam temperature of 120 °C for 10 to 20 s. Though the material was humidified up to 35 %, the concentration of essential oil had not been affected (RUMKE et al., 2006). In further investigations it was clearly demonstrated that the process also reduces bacterial spores quickly (MÜLLER, 2010). This study describes the individual volatiles in selected aromatic plants using gas chromatography and it reflects morphological changes of essential oil cells using scanning electron

microscopy. Furthermore, the impact of the applied decontamination method on the essential oil concentration is measured and observed losses of volatiles are correlated with differences in overall leaf structure.

Materials und methods

Plant material

The plant material for marjoram (*Majorana hortensis* L., Lamiaceae), oregano (*Origanum vulgare,* Lamiaceae) and fennel (*Foeniculum vulgare,* Apiaceae) was provided by Majoranwerke Aschersleben and was cultivated and harvested in 2009. Eucalyptus leaves (*Eucalyptus grandis,* Myrtaceae) were purchased from the company "Martin Bauer" (Vestenbergsgreuth, Germany) in 2009. All plant materials were air-dried before saturated steam treatment (Lemgo process) and all results of essential oil composition refer to air-dry mass.

Lemgo process

The decontamination tests were conducted in a laboratory decontamination facility, which comprises a 0.7 L treatment chamber and an 8 L vacuum buffer tank. Both repositories are cylindric and made of stainless steel. The connection of the single devices is carried out with pipelines with an inner diameter of 1.27 cm. The record of temperature and pressure is provided by a data acquisition system. Steam treatment and evacuation periods are regulated by electronically controlled pneumatic angle valves before and behind the treatment chamber. The evacuation of the treatment chamber is conducted by a water ring compressor with gas emitter. A tube bundle heat exchanger between vacuum buffer tank and water ring compressor encourages pressure reduction and provides the condensation of steams extracted from the treatment chamber. The heat exchanger is working with water and comprises a capacity of 0.4 L (LILIE et al., 2007).

Decontamination treatment of marjoram, oregano, fennel and eucalyptus

Only a maximum of 5 g of the appropriate plant material could be decontaminated in the small treatment room. In the course of saturated steam decontamination, a steaming with 100 °C and 120 °C heated vapor was conducted with the accordant equilibrium pressure and lasted 20 s. For achieving saturated steam conditions, a prevacuum of 20 s was generated. The period of the flash-vacuum, which is critical for decontamination, was also 20 s.

To investigate how "steam" and "vacuum" individually affected the plant material the following treatments (see Tab. 1) were performed:

The herbal drugs were treated with saturated steam (treatment 3). After 20 s of steam the chamber was opened slowly to ambient atmosphere.

Compressed air, instead of steam, was introduced into the treatment chamber to identify only the impact of vacuum on herbal drug tissue (treatment 4).

Tab. 1: The parameters of applied treatments to study effects of steam decontamination

Treatment Type	Steam Temperature	Steam Time	Air Pressure	Pre- and Postvacuum Time
Treatment 1	120 °C	20 s	-	20 s
Treatment 2	100 °C	20 s	-	20 s
Treatment 3	120 °C	20 s	-	-
Treatment 4	-	-	2.5 bar	20 s

Determination of microbial count

Microbial analyses were conducted following the European Pharmacopoeia (6th edition). The total plate count results are stated as colony forming units per g (CFU/g). The quantitative detection limit was 10^2 CFU/g.

Essential oil analysis

100-200 mg of dried plant material (marjoram, oregano, fennel and eucalyptus) were weighed into a 100 mL centrifuge tube and homogenized in iso-octane with an Ultra-Turrax. This mixture had been low speed centrifuged at 3000 rpm in a table top centrifuge and the supernatant was analyzed by gas chromatography (Hewlett Packard HP 5890 Series II GC). The essential oil content in mL/100 g (air dried mass) is defined as the total value of the specific individual components determined by gas chromatography (KRÜGER et al., 1998).

Scanning electron microscope analysis

The lower and upper leaf surface of steam treated material has been applied for scanning electron microscopy. The samples were mounted accordingly with double-sided, non conductive sticky tape on aluminium stubs and coated with gold. The chamber of the sputter coater was filled with argon during deposition of heavy metal. Images were collected using the scanning electron microscope Quanta 250 (FEI Worldwide Corporate Headquarters) equipped with a tungsten cathode. The acceleration voltage was 10 kV. On average 20 gland scales were analysed and representative images selected. Images were adjusted in brightness and contrast using Adobe Photoshop CS4.

Statistical analysis

Each saturated steam treatment was carried out three times and was analysed twice for essential oil (n=3). Statistical evaluation was accomplished with Systat Software Inc SigmaPlot 11. For testing significance, a one-way ANOVA was carried out. A direct comparison of groups was performed with a Tukey-Test.

Results

Changes in content of ingredients and leaf surface structures

Changes of essential oil content and surface structure modifications were investigated under four different treatment conditions (Tab. 1) in marjoram, oregano, fennel and eucalyptus.

Marjoram

The control sample was characterised by an initial value of essential oil of 0.91 mL/100 g dry product (Tab. 2). Marjoram is an herbal drug with high sensitivity against decontamination methods with saturated steam. Figure 2, A0 shows that the peltate glandular hairs of untreated marjoram are not embedded in the leaf matrix, but exposed with a sphere-like appearance.

Exposing marjoram to 120 °C saturated steam for 20 s with a subsequent 20 s vacuum results in a loss of 93 % of essential oil content. As depicted in Fig. 2, A1 this is due to severe damages of glandular scales during the decontamination procedure. The cuticle of the glandular trichome becomes perforated releasing essential oil that most likely is evaporated with the water layer.

The loss of content is visible by severe shrinkage from the outer area that leads to almost complete flattening in the middle region of the original sphere-like appearance of the cell. Steam temperatures of 100 °C do not have as dramatic effects as observed after treatment 1 conditions (Tab. 1). The peltate glandular hairs in Fig. 2, A2 show less shrinkage and smaller disruptions in its cuticle resulting in reduced essential oil loss of 86 %. Comparisons of the parameters "steam" and "vacuum" provided evidence that the observed

damages in marjoram glandular scales and accompanied ingredient losses were more pronounced by steam rather than vacuum exposure (compare Fig. 2, A3 and A4). As Fig. 2, A3 illustrates incubation of leaf tissue, with steam heated to 120 °C causing isolated surface disruptions of the peltate glandular hairs. In contrast application of compressed air showed for the majority of visible cells deformations in peltate glandular hairs shape leading to more flattened appearance but did not show perforations of the cuticle surface. The measured essential oil content of 0.84 mL/100 g was similar to untreated leaves which contained 0.91 mL/100 g (Tab. 2).

The saturated steam decontamination shows only for treatment 1 a reduction of two decades of total plate count. Treatments 2 and 3 reduce the microorganisms on marjoram very sparsely and the last treatment 4 provides no reduction.

Tab. 2: Comparison of essential oil content of marjoram in mL/100 g and total plate count (colony forming units, CFU/g) after the Lemgo process with four different treatment parameters. Same superscript means no significant difference between groups.

Marjoram				
Treatment parameter (compare Tab. 1)	Essential oil content Mean ± std	Loss of essential oil [%]	See panel in Fig. 2	CFU/g
Control	0.91 ± 0.04^{A}		A0	5.1×10^5
Treatment 1	0.07 ± 0.04^{D}	93	A1	2.2×10^3
Treatment 2	0.13 ± 0.04^{C}	86	A2	4.8×10^5
Treatment 3	0.07 ± 0.04^{D}	93	A3	1.2×10^5
Treatment 4	0.84 ± 0.04^{A}	0	A4	5.3×10^5

Oregano

Among the investigated *Lamiaceae* species oregano presented the highest essential oil content of 1.82 mL per 100 g dry mass. In contrast to marjoram, the peltate glandular hairs are not exposed but embedded into the leaf matrix as revealed by scanning electron microscopy (Fig. 1 and 2, B1). Thus, peltate glandular hairs of oregano seem to be less exposed to destruction by external forces, which may account for the observed lower loss of essential oil after the decontamination treatments. Deformation of peltate glandular hairs occurs naturally as indicated in the control (Fig. 2, B0). The different steam temperatures during decontamination had a significant effect on essential oil content as shown for marjoram. Treatment with 120 °C or 100 °C steam and vacuum caused a loss of 59 % and 43 %, respectively (Tab. 3). Most peltate glandular hairs are deformed by saturated steam treatments as revealed in scanning electron microscopy (Fig. 2, B1-B3). Images collected from leaves exposed to treatment 3 and 4 confirmed the destructive forces by steam rather than vacuum on oregano peltate glandular hairs. In the picture for saturated steam treatment without vacuum (Fig. 2, B3), the treatment/application resulted in multiple broken, some intact, but also semi-broken peltate glandular hair cells. Apparently the sunken appearance of oregano secretory cells protected superior against external forces. Only half of essential oil content was lost during decontamination in contrast to marjoram tissue which showed an almost complete loss of terpenes during decontamination.

The microbial reduction results of oregano are similar to marjoram. Treatment 1 is the only treatment variation which decreases the total plate count of oregano by about 3 decades. For methods 2, 3 and 4, no appreciable degradations of the colony forming units of oregano are found.

Tab. 3: Comparison of essential oil content of oregano in mL/100 g and total plate count (colony forming units, CFU/g) after the Lemgo process with four different treatment parameters. Same superscript means no significant difference between groups.

Oregano				
Treatment parameter (compare Tab. 1)	Essential oil content Mean ± std	Loss of essential oil [%]	See panel in Fig.2	CFU/g
Control	1.82 ± 0.09^{A}		B0	6.1×10^6
Treatment 1	0.75 ± 0.09^{C}	59	B1	2.5×10^3
Treatment 2	1.05 ± 0.10^{B}	43	B2	3.5×10^6
Treatment 3	0.74 ± 0.04^{C}	59	B3	9.7×10^5
Treatment 4	1.92 ± 0.11^{A}	0	B4	7.1×10^6

Fennel

Fennel seeds accumulate the valuable essential oils in a number of channels inside the fruit as shown in Fig. 3, C0-C4. The channel structure allows an increase in storage capacity 4-9 fold magnitude compared to the sphere-like peltate glandular hairs. Each compart-

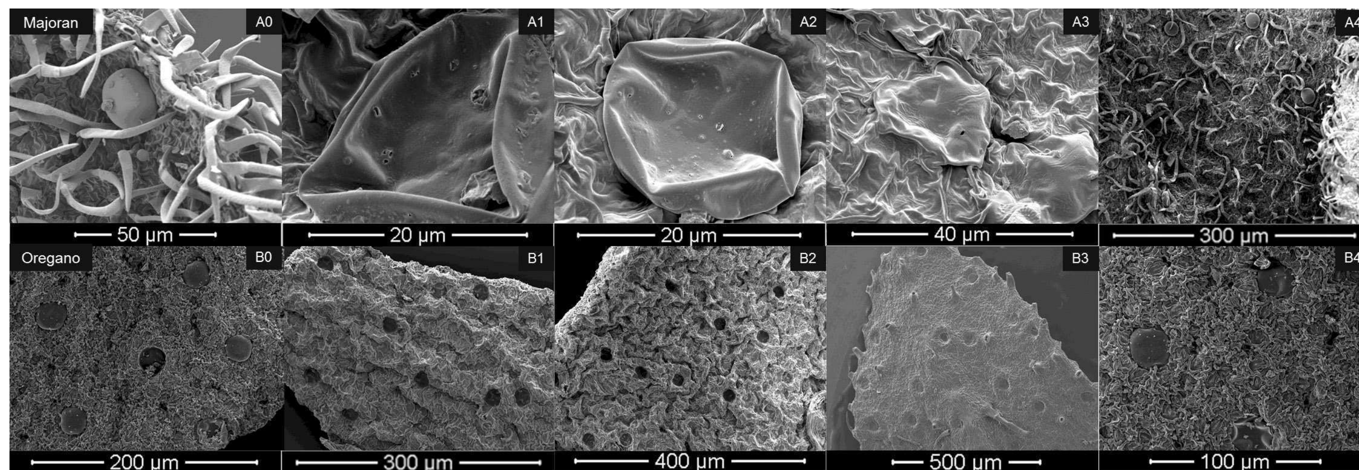

Fig. 2: ***Scanning electron microscopy of marjoram and oregano.*** A0 = on the surface-fitting intact peltate glandular hair, A1 = collapsed peltate glandular hair with large holes in cuticle after, A2 = collapsed peltate glandular hair with minor holes, A3 = collapsed peltate glandular hair with large hole in cuticle, A4 = intact peltate glandular hair / B0 = peltate glandular hairs embedded in leaf surface on the control sample of oregano, B1 – B3 = many collapsed peltate glandular hairs, B4 = two intact and one collapsed peltate glandular hairs.

ment is embedded in several cell layers derived from the fruit coat. They are spaced throughout the upper and lower fruit coat surrounding the endosperm. In addition to the essential oil channel cell wall (Fig. 3, C4) such protective shell offers optimal protection against environmental influences like sun and rain. In comparison with the control (8.79 mL/100 g), fennel had an essential oil value of 8.21 mL/100 g after the treatment with 120 °C saturated steam for 20 s and a subsequent vacuum of 20 s, this means a loss of 7 % (Tab. 4). From all parameters tested a minor, significant reduction in essential oil content was only evident in treatments comprising 120 °C steam temperatures (Tab. 4).

No effect on gland scale anatomy was visible in treated samples (Fig. 3, C1-C4) compared to the unexposed control tissue (Fig. 3, C0). In contrast to marjoram and oregano, treatment 1 reduces the total plate count below the detection limit. In treatment 2 and 4 the number of microorganisms does not change. Treatment 3 reduces the total plate count by about 0.5 decades.

Tab. 4: Comparison of essential oil content of fennel in mL/100 g and total plate count (colony forming units, CFU/g) after the Lemgo process with four different treatment parameters. Same superscript means no significant difference between groups.

Fennel				
Treatment parameter (compare Tab. 1)	Essential oil content Mean±std	Loss of essential oil [%]	See panel in Fig.2	CFU/g
Control	8.79 ± 0.15^{A}		C0	5.3×10^{5}
Treatment 1	8.21 ± 0.14^{B}	7	C1	1.0×10^{2}
Treatment 2	8.51 ± 016^{A}	3	C2	3.5×10^{5}
Treatment 3	8.14 ± 0.13^{B}	8	C3	8.7×10^{4}
Treatment 4	8.62 ± 0.12^{A}	0	C4	5.1×10^{5}

Eucalyptus

Eucalyptus lacking gland scales (Carr and Carr, 1970) possesses essential oil containing cells that are located in the leaf mesophyll (King et al., 2006). Thus the positioning of storage cells is subdermal in contrast to the external structure of gland scales among *Lamiaceae* species. The oil content of eucalyptus is 1.54 mL/100 g (Tab. 5), which is similar to concentrations found in the leaves of studied *Lamiaceae*. In eucalyptus, preservation of essential oil has been found in all treatments. The images in Fig. 3, D0-D3 display gland openings which do not show morphological changes after treatments with saturated steam. The Lemgo process is a superficial treatment and thus hardly affects the amount of essential oil, which is located inside of the phytopharmaceutical. Eucalyptus holds a uniformly developed spicular wax film on the leaf surface (Louro, 2002) which had been distorted after hot steam exposure. Independently of treatment type 1, 2 or 3 (Tab. 1) the spicules were accumulated forming discrete clusters on the leaf surface. Only after application of vacuum alone, the wax spicules were retained in their original form (Fig. 3, D4).

The microorganism reduction outcomes are identical to marjoram. Treatment 1 reduces the total plate count below the detection limit and treatment 3 decreases the germs by one decade. The other reduction methods 2 and 4 show no decrease.

Tab. 5: Comparison of essential oil content of eucalyptus in mL/100 g and total plate count (colony forming units, CFU/g) after the Lemgo process with four different treatment parameters. Same superscript means no significant difference between groups.

Eucalyptus				
Treatment parameter (compare Tab. 1)	Essential oil content Mean±std	Loss of essential oil [%]	See panel in Fig. 2	CFU/g
Control	1.54 ± 0.06^{A}		D0	2.6×10^{6}
Treatment 1	1.48 ± 0.08^{A}	0	D1	1.0×10^{2}
Treatment 2	1.43 ± 0.07^{A}	0	D2	4.7×10^{5}
Treatment 3	1.54 ± 0.08^{A}	0	D3	1.3×10^{5}
Treatment 4	1.52 ± 0.02^{A}	0	D4	1.8×10^{6}

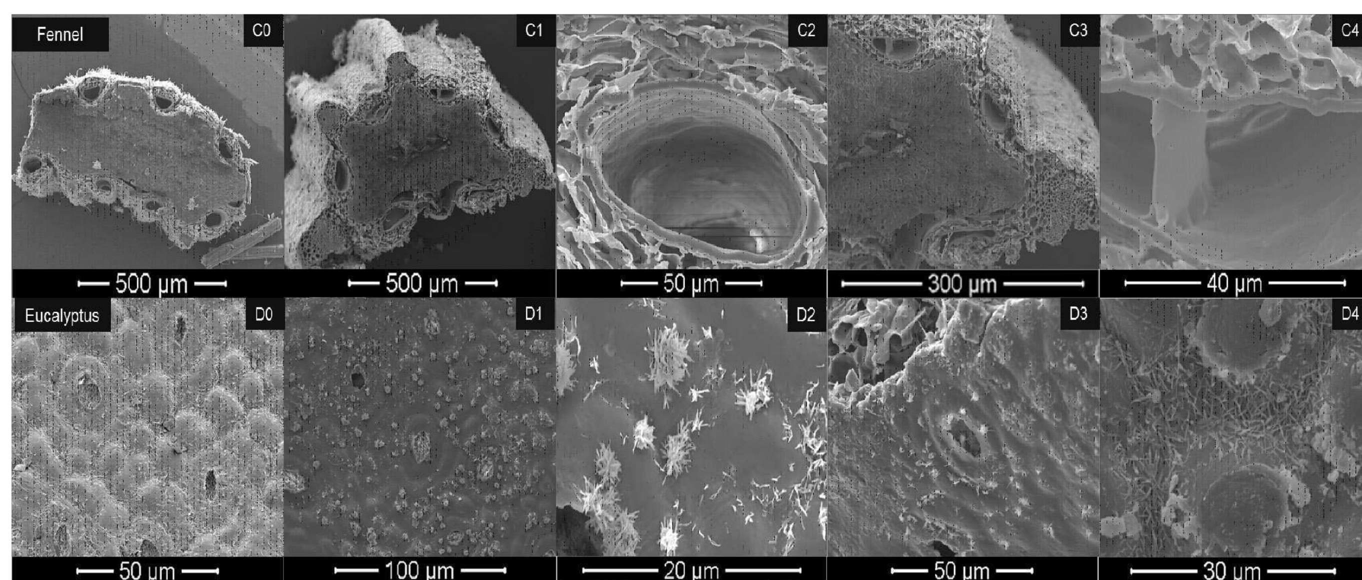

Fig. 3: Scanning electron microscopy of fennel and eucalyptus: C0 - C1= cross-sectional fennel fruit with oil channels, C3= cross-sectional fennel fruit with three oil channels, C2 - C4 = oil channel with thick channel wall and partition of fennel fruit / D0 = three oil gland vents with intact wax layer on the control sample of eucalyptus, D1 = one oil gland vent with cumulated wax layer around it, D2 = close cumulated wax layer of eucalyptus after treatment 2, D3 = cumulated wax layer, D4 = normal wax layer.

Discussion

Indeed the observed essential oil losses within species of the *Lamiaceae* family are larger than with those of investigated members from other plant families of *Myrtaceae* and *Apiaceae*. The obtained data on plant morphology provide clear evidence that structure and position of essential oil storage cells are mainly accountable for the observed differences in response to heat exposure. Herbal drugs which belong to the family of *Lamiaceae* store essential oils in peltate glandular hairs, where the storage compartment is protected by an elevated cuticle (Fig. 1) covered by a thin wax layer (GUENTHER et al., 1949; CROTEAU and WILDUNG, 2005). It is known that essential oils evaporate at a very low rate as long as the wax coated cuticle is intact. Less than 10 % essential oil loss per year has been observed in dried leaf material when stored at room temperature (BURBOTT and LOOMIS, 1969). Furthermore it has been demonstrated that peltate glandular hairs of peppermint kept their essential oil for several years when freeze-dried (Maffei, CHIALVA et al., 1989).

However, the wax-coated cuticle of *Lamiaceae* can be broken by mechanical forces or by high temperatures (GUENTHER, 1949). The holes visible in peltate glandular hairs of marjoram after exposure to 100 °C and 120 °C heated steam (Fig. 2, A0-A2) provide direct evidence that the wax-coated cuticle has been destroyed. We assume that also the severe shrinkage of peltate glandular hairs after such heat treatment (Fig. 2, B1-B3) resulted in distortion of the protective cell layer promoting release of essential oil. The temperature effects on deformation of thick wax layers were clearly illustrated on the eucalyptus leaf surface, a member of the *Myrtaceae* (Fig. 3, D1-D3). Eucalyptus, however, showed no essential oil loss. This is due to the different leaf structure harboring internal oil glands (JAMES and BELL, 1999) that are not modified during heat exposure (Fig. 4 C). This is also true for the tested member of *Apiaceae* fennel. The protective layers surrounding the oil storage cells within the fennel grain prevent major oil losses (Fig. 4 D).

Interestingly the degree of essential oil loss after steam decontamination was not equal among the members of *Lamiaceae*. This indicates that interactions between plant surfaces and steam during the decontamination process are rather complex.

Whereas in oregano more than half of essential oil content from the glandular hairs is preserved during the decontamination process, marjoram shows almost a complete loss of essential oils.

The anatomy of the peltate glandular hairs of the different *Lamiaceae* species alone does not provide a sufficient explanation for this phenomenon. It has been shown that the structure and size of the peltate glandular hairs of oregano (*Origanum vulgare*) and marjoram (*Majorana hortensis* L. In Fig. 1) are identical (BOSABALIDIS et al., 1997).

The analysis of leaf context surrounding the glandular scale and its positioning on the leaf revealed differences among the investigated *Lamiaceae* and those most likely contributed mainly to the broad range in observed losses. The function of epidermal cells that are arranged around the basal cell of the peltate glandular hairs of oregano is distinct (STAHL-BISKUP et al., 2002; BOSABALIDIS et al.,1997; BOSABALIDIS et al., 2002) from those in marjoram. Adjacent cells of oregano form an accessory of the peltate glandular hair, having a role in the volatile oil secretion. Depending on cell-distribution, -size, -shape, -vacuolization and -density they are involved in transport of photosynthesis products from the mesophyll to the basal cells of the peltate glandular hairs (BOSABALIDIS et al., 2002).

The positioning of peltate glandular hairs among the investigated *Lamiaceae* is distinct and thus the susceptibility to external forces. Whereas the peltate glandular hairs of marjoram are exposed on the leaf surface, they are immersed in the epidermal cell layer in case of oregano (Fig. 1). Therefore they provide less target space for the steam. In consequence fewer damage of the top cell layer occurs, illustrated by reduced essential oil loss. In Fig. 4 a model for the steam-plant surface interactions is exemplified for exposed and immersed scales. Due to the different positioning of the upper trichome cell with regard to the leaf surface the size of formed condensate layer during steam deposition phase is much larger in case of treated marjoram leaves compared to treated oregano leaves (Fig. 4 upper panel). Consequently the vapor-accessible surface in exposed scales is larger, allowing a higher number of thermal as well as physical interactions leading to additive effects. For example the essential oils within the marjoram scales may heat up faster and the wax layer is broken easier. The immersed peltate glandular hair of oregano (Fig. 4, B) is only superficially touched by the steam. At the moment we cannot exclude that, perhaps, differences in cell wall composition are also contributing to the noticed variability in sensitivity to applied incubations during decontamination.

Further investigations will have to address such interactions in greater detail, for example, the chemical composition of the wax layer or its deformation during saturated steam treatment. Furthermore, ultra structural studies of peltate glandular hairs as well as surrounding leaf tissue before and after treatments will help to identify the most sensitive parts. Such knowledge will be seminal for designing procedures that guarantee efficient decontamination by a minimum loss of valuable ingredients even for heat sensitive material.

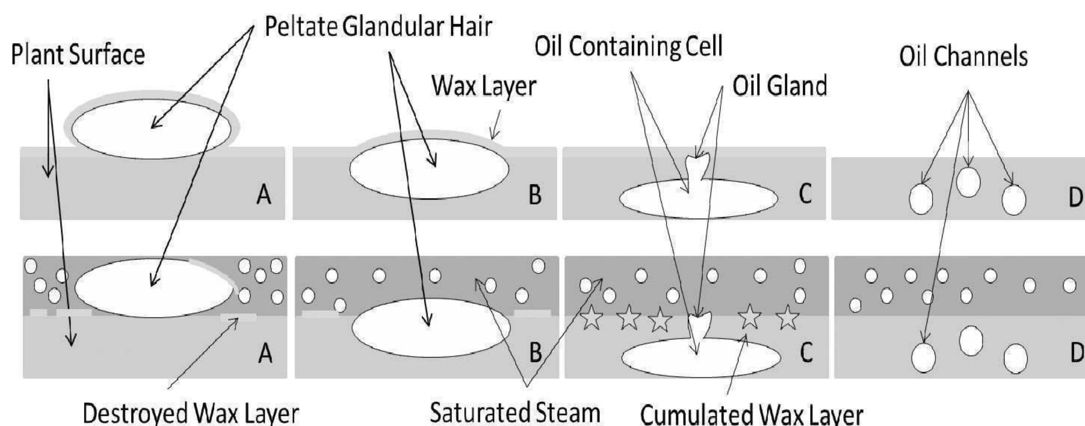

Fig. 4: Upper panel = Peltate glandular hair of marjoram with epidermis in a plane (A), sunken peltate glandular hair of oregano (B), oil containing cell and oil gland of eucalyptus (C) and oil channel of fennel (D) before the steam deposition phase in which the wax layer is fully functional. **Lower panel** = (A), (B), (C) and (D) during steam deposing phase when the condensate layer is formed.

Analysis of the total plate count indicated that efficient removal of microbes depended rather on physical forces applied than texture and shape of biological material (Tab. 2 - 5). Only joined application of vacuum and hot steam as provided in treatment 1 affected the total plate count negatively (increase of about 2 - 3 decades). The other three treatment variations decreased the total plate count only about a half decade. Most likely a temperature of 100 °C for 20 s and subsequently sudden vacuum (treatment 2) is too low for a flash effect which would rip the microorganisms off the plant surface and the application time is too short for a thermal killing of the germs. The latter is probably also true for applied parameters during treatment 3 (LILIE, 2009; KESSLER, 1996). As revealed in treatment 4 application of vacuum alone does not lead to removal of bacteria (Tab. 2 - 5).

In summary our studies provided clear evidence that in case of plants with peltate glandular hairs (marjoram, oregano) further improvement of the applied decontamination technology is necessary. Whereas the benefits regarding microbial decontamination are obvious the accompanied essential oil losses are intolerable and need to be minimized.

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9 Appendix 3

Original contributions

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Chemical, physical and sensory changes of herbal drugs after saturated steam decontamination

Introduction

The five medicinal plants *Linum usitatissimum* L. (linseed), *Foeniculum vulgare* Mill. (fennel), *Origanum majorana* L. (marjoram), *Tropaeolum majus* L. (nasturtium) and *Allium cepa* L. (onion) have been used for many years as herbal drugs (2). These plants were selected as model plants for decontamination because they present a range of morphologies (grain: fennel and linseed, leaf: marjoram and nasturtium, root: onion) and ingredients. This range of morphologies and ingredients can demonstrate which plants are basically suited for mechanical decontamination, known as the Lemgo process.

The European Pharmacopoeia sets boundaries (total viable count ≤ 10^5) for the maximum allowed microbial load (3, 2.6.12) of herbal material to be processed to phytopharmaceuticals. For meeting the specification of this regulatory framework, decontamination methods often have to be applied. In this context, it is of particular importance that the dry herbal material is mainly contaminated with heat-resistant bacterial spores and less with easily destroyable vegetative germs (6). Generally, the application of short-wave radiation is unacceptable in Germany (1). Various decontamination methods have been developed, but few have been approved for use in the pharmaceutical field (23). There are many variations of saturated steam decontamination. In general, these are methods which use moist heat for germ destruction. Continuous und batch processes are known (18). Due to the intensive heat transfer and the moisturization of the herbal surfaces, these processes lead to an efficient and well applicable thermal decontamination of spore containing material. A treatment time of several minutes is needed for this purpose (9).

In contrast, the Lemgo process developed at the Hochschule Ostwestfalen-Lippe in Lemgo uses only a short period of vaporisation (≤ 20 s) to moisten the surface of the plant material and to reduce the bonding force between the microorganisms and the surface (15), followed by a flash evaporation to remove the germs (17). This so-called flash-effect is caused by an extremely rapid evacuation of the treatment chamber and requires special technical conditions (15).

Decontamination studies of inoculated model systems (15) as well as naturally contaminated plant material such as pepper (16), chamomile (21) and nasturtium (14) were conducted in 0.7 L-laboratory equipment. To approach an industrial level, a 5 L ploughshare mixer was installed at the Hochschule Ostwestfalen-Lippe. In a detailed study, the quality of five herbal drugs (linseed, fennel, marjoram, nastur-

tium and onion) was analysed. Particular attention was given to the reduction of spores. With fennel, linseed and marjoram, an average reduction of 2 powers of magnitude of germs was achieved. Nasturtium and onion, however, even reached an average reduction of 3 to 4 powers of magnitude with respect to total plate count. Under optimised conditions, a spore reduction below the detection limit of 100 colony forming units (CFU)/g was possible for all drugs (14, 20). Thus the regulatory framework of the European Pharmacopoeia was observed easily.

In this comprehensive study, the focus is on the quality changes of the drugs caused by the Lemgo process. The value-giving ingredients including the essential oil of fennel and marjoram, the crude fat of linseed, the glucotropaeoline of nasturtium as well as the cysteine sulfoxide of onion were analysed. Changes in colour were investigated as well. In the case of linseed, the change of sensory and swelling index was also investigated.

Materials and methods

Plant materials

Linseed (*Linum usitatissimum* L.) was supplied by SALUS Haus GmbH & Co. KG (Bruckmühl, Germany) with no pre-treatment having been done. The fennel (*Foeniculum vulgare* Mill.) fruits and the dry leaves and flower buds of marjoram (*Origanum majorana* L.) were provided by MAWEA Majoranwerk Aschersleben GmbH (Aschersleben, Germany). REPHA GmbH Biologische Arzneimittel GmbH (Langenhagen, Germany) supplied dry chopped nasturtium (*Tropaeolum majus* L.) leaves and stems. The air-dried onion (*Allium cepa* L.) pieces (1 cm^2) were reaped and delivered by the company Kräutermix GmbH (Abtswind, Germany).

Saturated steam decontamination

As decontamination reactor, a solid state ploughshare mixer (Lödige Labormischer Typ M5, Lödige Maschienenbau GmbH, Paderborn) was used to provide an adequate disaggregation of the plant material. The stirrer had a volume of 5 L and was equipped with a double wall jacket allowing temperature control of the reactor which provided control of the condensate film, available for the flash evaporation. An electric steam generator was attached to the mixer, delivering saturated steam with 8 bar absolute pressure at about 180 °C. Steam pressure was throttled to the desired pressure according to the temperature level via a pressure reduction valve. Steam was introduced into the mixer for vaporisation. For establishing a pre- or post-vacuum, the equipment also included a vacuum unit. A 100 L heat exchanger was used as a vacuum buffer to minimize evacuation time. To condense the steam it was cooled to -20 °C (15, 16).

Decontamination parameters

The five herbal drugs were treated in the 5 L ploughshare mixer described above with a filling degree of 40 % using steam temperatures of 110 °C and 125 °C, respectively, and an exposure time of 20 s. The double jacket was heated to 20 °C, 40 °C, and 80 °C. For the optimization of the germ reduction in some treatments two cycles of steam addition and vacuum („double treatment“) were applied. For details concerning the treatment see *Tab. 1*. After the single and double treatment the herbal drugs were dried to the initial water activity. These treatment parameters were chosen because in preliminary tests, they were described as the correct choice between effective and gentle removal of microorganisms.

Chemical analysis

After the decontamination all drugs were air dried at 35 °C for 24 h. The essential oil content in mL/100 g of marjoram and fennel is defined as the total value of the specific individual components determined by gas chromatography (11). This analytic procedure was selected because previous investigations were done in a small decontamination room so that insufficient plant material was available for a typical essential oil analysis. The cysteine sulfoxide of the industrially chopped onions was measured by HPLC/MS (24). Methiine, alliine and propiine were analysed separately and stated as entities of cysteine sulfoxides. The crude fat of linseed was analysed employing Soxhlet extraction according to the German Food and Feed Code (5). The glucotropaeoline of nasturtium was measured by HPLC (22).

Colour measurement

The Hunter´s colour values L (degree of whiteness, white +100, black 0), a (degree of redness, red +100, green -80), and b (degree of yellowness, yellow +70, blue -80) were determined using a colourimeter (Minolta CR-200). The total colour difference E ($\Delta E=\sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)}$) was calculated from the Hunter`s values obtained. Photos

Abstract

The novel saturated steam decontamination method called Lemgo process is based on a short steam treatment, followed by an extremely rapid evacuation of the treatment chamber. This results in the reduction of the bonding forces between the microorganisms and the surface and in a sudden evaporation of the condensate film on the herbal material, and therefore in a mechanical removal of the superficial microorganisms. 2 L of drugs were treated in a ploughshare stirrer for 20 s with saturated steam of 110 °C and 125 °C, respectively, followed by the evacuation of the stirrer. A double treatment, consisting of a twofold vaporization and evacuation, was examined as well. The process was able to reduce the total plate count and aerobic spore count of the five investigated herbal drugs *Linum usitatissimum* L. (linseed), *Foeniculum vulgare* Mill. (fennel), *Origanum majorana* L. (marjoram), *Tropaeolum majus* L. (nasturtium) and *Allium cepa* L. (onion) by up to 4 powers of magnitude, reaching the microbiological detection limit of 100 CFU/g. The steam and vacuum treatments of linseed caused neither a loss of its value-adding ingredient crude fat nor a change of colour. Furthermore, the swelling index and flavour of linseed, determined for assessing the quality, showed no significant changes. In the case of fennel, only 7 % of the essential oil was lost, and the treated herbal material did not show remarkable differences in appearance. Marjoram was the only material with a high loss of ingredients (at least 86 % of the essential oil). Glucotropaeoline and cysteine sulfoxide as value-added ingredients of nasturtium and onion are usually prone to fast enzymatic degradation. However, due to the decontamination treatment and probably the short heat treatment, there was only a loss of 16 % glucotropaeoline and 18 % cysteine sulfoxide. Changes in colour of nasturtium were moderate but onion suffers a noticeable embrowning.

Keywords:
Allium cepa, decontamination, *Foeniculum vulgare*, *Linum usitatissimum*, mechanical decontamination, medicinal plants, *Origanum majorana,* saturated steam, spice plants, *Tropaeolum majus*

Original contributions

from all drugs were taken with an SLR camera (Canon EOS 1000D) with fixed lighting condition and angle for comparability.

Sensory evaluation

As control for rancidity, linseed was evaluated with respect to colour, odour, pungent taste and overall acceptability by 10 trained judges six months after the decontamination treatment. Storage conditions in the room were dark, dry with a temperature of 18 °C. The panelists were trained according to the DIN 10950 (4) and instructed to record their rating using a five-point hedonic scale (5 like extremely and 0 dislike extremely for colour, odour and overall acceptability; and 5 extremely strong and 0 extremely weak for pungent taste). Approximately 2 g of linseed in a coded disposable Petri dish were given to each panelist.

Swelling index

Swelling index of linseed was determined according to the European Pharmacopoeia 7th edition, 2.8.4. (3).

Statistical analysis

All saturated steam treatments were carried out three times and each treatment was analysed twice. For testing significance, a one-way ANOVA was carried out with Sigmaplot 11 (Systat Software Inc). For group comparison a Tukey-Test was performed. Values represent means ± Standard Deviation, n = 3. Means followed by the same superscript are not significantly different from one another.

Results

Linseed

Linseed was decontaminated with 110 °C (single treatment) and 125 °C (single and double treatment) saturated steam, followed by rapid evacuation. The results of the quality characteristics are shown in *Tab. 2*. The valuable ingredient of linseed, crude fat, is embedded in the grain. Thus, it is protected from the influence of the short saturated steam exposure. As expected, no loss of crude fat could be detected. Moreover, most of the other measured quality parameters are statistically identical. No change in colour was visually apparent, but some loss of glossiness was detectable (*Fig. 1*). This was confirmed by Lab colour space measurement, where slight changes became apparent.

Because linseed is known to deteriorate, 6 months after the treatment, a tasting study was carried out. The results showed constant values for co-

Tab. 1: Steam temperature, double jacket temperature and sequences of steam and vacuum in "single" and "double treatments" to study chemical and sensory effects of steam decontamination on herbal drugs (20v = 20 s vacuum, 20 s = 20 s steam)
Tab. 1: Parameter Dampf- und Doppelmanteltemperatur und Dampf- und Vakuumsequenzen der angewendeten „Einfach-" und „Zweifachbehandlung" zur Untersuchung der chemischen und sensorischen Auswirkungen einer Sattdampfentkeimung an Drogen (20v= 20 s Vakuum, 20 s= 20 s Dampf)

Treatments Behandlungen	Steam temperature (°C) Dampftemperatur (°C)	Double jacket temperature (°C) Manteltemperatur (°C)	Steam and vacuum sequence Dampf- und Vakuumsequenzen
Control Kontrolle	–	–	–
Single treatments / Einfachbehandlungen			
1	110	20	20v – 20s – 20v
2	110	40	20v – 20s – 20v
3	110	80	20v – 20s – 20v
4	125	20	20v – 20s – 20v
5	125	40	20v – 20s – 20v
6	125	80	20v – 20s – 20v
Double treatments / Zweifachbehandlungen			
11	110	20	20v – 20s - 20v – 20s – 20v
12	110	40	20v – 20s - 20v – 20s – 20v
13	110	80	20v – 20s - 20v – 20s – 20v
14	125	20	20v – 20s - 20v – 20s – 20v
15	125	40	20v – 20s - 20v – 20s – 20v
16	125	80	20v – 20s - 20v – 20s – 20v

Chemische, physikalische und sensorische Veränderungen in pflanzlichen Drogen durch Sattdampfentkeimung

Zusammenfassung

Alle in dieser Studie analysierten Arzneipflanzen wurden mit einem neuartigen Sattdampfentkeimungsverfahren namens Lemgoer Verfahren behandelt. Das Verfahren basiert auf einer kurzen Behandlung mit Dampf, welcher eine extrem schnelle Evakuierung der Behandlungskammer folgt. Dies führt erst zu einer Reduzierung der Haftkraft zwischen den bakteriellen Keimen und der Pflanzenoberfläche und dann zu einer schlagartigen Verdampfung des Kondensatfilms auf dem pflanzlichen Material und somit zu einer mechanischen Entfernung der Mikroorganismen von der Oberfläche. 2 l der Arzneidrogen wurden mit einem Pflugscharmischer für 20 s mit Sattdampf bei 110 °C bzw. 125 °C behandelt, gefolgt von der Evakuierung des Mischers. Es wurde auch eine doppelte Behandlung untersucht, bei der zweifach bedampft und anschließend evakuiert wurde. Das Lemgoer Verfahren war in der Lage, sowohl die Gesamtkeimzahl als auch die Zahl der aeroben Sporen auf den fünf untersuchten pflanzlichen Drogen *Linum usitatissimum* L. (Leinsamen), *Foeniculum vulgare* Mill. (Fenchel), *Origanum majorana* L. (Majoran), *Tropaeolum majus* L. (Kapuzinerkresse) und *Allium cepa* L. (Zwiebel) um bis zu vier Zehnerpotenzen unter Erreichen der mikrobiologischen Nachweisgrenze von 100 KBE/g zu reduzieren. Die unterschiedlichen Behandlungsbedingungen verursachten keine Verluste an dem wertgebenden Rohfett von Leinsamen. Auch die Farbe, der Quellwert und der Geschmack zeigten keine signifikanten Veränderungen. Fenchel zeigte eine Reduktion des ätherischen Öls von 7 %, und es kam zu geringen farblichen Abweichungen. Majoran wies als einzige Droge einen hohen Verlust an wertgebenden Inhaltsstoffen (wenigstens 86 % des ätherischen Öls) auf. Glucotropaeolin und Cysteinsulfoxide als wertgebende Inhaltsstoffe von Kapuzinerkresse und Zwiebel sind normalerweise anfällig für einen enzymatischen Abbau. Wahrscheinlich war jedoch aufgrund der Entkeimungsbehandlung und der kurzen Hitzebehandlung der Verlust von 16% Glucotropaeolin und 18 % Cysteinsolfoxide gering. Die farbliche Veränderung von Kapuzinerkresse war sehr gering, die Zwiebelstücke bekamen nach der Behandlung eine deutlich rotbraune Farbe.

Schlagwörter:

Allium cepa, Arzneipflanzen, Entkeimung, *Foeniculum vulgare*, Gewürzpflanzen, *Linum usitatissimum*, mechanische Entkeimung, *Origanum majorana*, Sattdampf, *Tropaeolum majus*

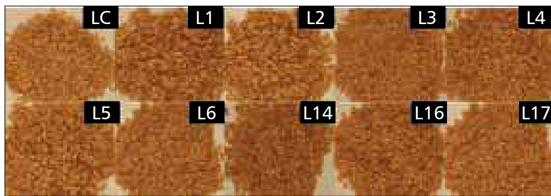

Fig. 1: Linseed before and after mechanical saturated steam decontamination. LC = Control, L1 – L6 = single treatment with saturated steam, L14 – L16 = double treatment with saturated steam. For details of the treatment see *Tab. 1*.
Abb. 1: Leinsamen vor und nach der mechanischen Sattdampfentkeimung. LC = Kontrolle, L1 – L6 = Einfachbehandlung mit Sattdampf, L14 – L16 = Doppelbehandlung mit Sattdampf. Für Einzelheiten der Behandlungen siehe *Tab. 1*.

Fig. 2: Fennel before and after mechanical saturated steam decontamination. FC = Control, F1 – F6 = single treatment with saturated steam, F11 – F16 = double treatment with saturated steam. For details of the treatment see *Tab. 1*.
Abb. 2: Fenchel vor und nach der mechanischen Sattdampfentkeimung. FC = Kontrolle, F1 – F6 = Einfachbehandlung mit Sattdampf, F11 – F16 = Doppelbehandlung mit Sattdampf. Für Einzelheiten der Behandlungen siehe *Tab. 1*.

lour, odour and pungent taste. Thus rotting could be excluded.
One of the pharmaceutically relevant properties of linseed is swelling, described by the swelling index. Despite some swelling took place during the decontamination process and in consequence some agglutination after the drying process, there was only a slight reduction in the swelling index. The index of swelling fell within the range of 4 as postulated by the European Pharmacopoeia (3, 2.8.4.).

Fennel

The essential oil of fennel is included in the grain and thus protected from short superficial heating. As shown in *Tab. 3*, there is almost no reduction in the essential oil content after a single treatment with saturated steam. Only under the most intense test conditions of 125 °C, a significant reduction in essential oil content of 7 % could be detected. Using a double treatment, a significant difference of 8 % was detectable. Additionally no change in the colour was visually apparent (*Fig. 2*). Only by the Lab measurements of samples treated with saturated steam of 125 °C, significant changes in the L- and b-values (*Tab. 3*) became evident.

Tab. 2: Crude fat content, "Lab" colour space values, sensory parameter and swelling index before and after "single" and "double treatment" of saturated steam decontamination of linseed
Tab. 2: Gehalte an fettem Öl, "Lab"-Farbwert, sensorische Eigenschaften sowie Quellwert vor und nach „Einfach-" und „Zweifachbehandlung" einer Sattdampfentkeimung von Leinsamen

Linseed single and double treatment parameters / Einfach- und Zweifachbehandlung von Leinsamen										
Treatments Behandlungen	Control Kontrolle	1	2	3	4	5	6	14	15	16
Ingredients analysis/Inhaltsstoffanalyse										
Crude fat % * fettes Öl	42.48 ± 0.95^{1}	43.74 ± 1.39^{1}	42.83 ± 1.30^{1}	42.76 ± 0.61^{1}	43.43 ± 0.65^{1}	42.98 ± 0.53^{1}	43.55 ± 1.25^{1}	43.34 ± 0.65^{1}	43.44± 1.53^{1}	43.19± 1.25^{1}
„Lab" colour space values/„Lab"-Farbwert										
L *	36.59 ± 0.57^{1}	32.25 ± 0.48^{2}	34.09 ± 0.52^{3}	34.94 ± 0.29^{3}	33.36 ± 0.63^{3}	33.95 ± 0.54^{3}	34.22 ± 0.39^{3}	32.67 ± 0.28^{2}	32.98 ± 0.45^{2}	34.21 ± 0.52^{3}
a *	4.93 ± 0.73^{1}	4.94 ± 0.58^{1}	4.80 ± 0.72^{1}	4.95 ± 0.67^{1}	4.75 ± 0.45^{1}	4.96 ± 0.56^{1}	5.01 ± 0.39^{1}	4.60 ± 0.18^{1}	4.66 ± 0.28^{1}	5.01 ± 0.34^{1}
b *	19.49 ± 0.82^{1}	17.45 ± 0.45^{2}	17.86 ± 0.67^{2}	18.38 ± 0,49^{2}	17.69 ± 0.19^{2}	17.95 ± 0.68^{2}	17.97 ± 0.57^{2}	16.84 ± 0.37^{2}	16.96 ± 0.56^{2}	17.97 ± 0.38^{2}
ΔE	–	4.8	2.99	1.99	3.7	3.06	2.82	4.74	4.42	2.83
Sensory Analysis/sensorische Analyse										
Colour * Farbe	3.6 ± 0.5^{1}	3.9 ± 0.6^{1}	3.3 ± 0.6^{1}	3.6 ± 0.7^{1}	3.8 ± 0.7^{1}	3.5 ± 0.6^{1}	3.7 ± 0.5^{1}	3.5 ± 0.4^{1}	3.9 ± 0.5^{1}	3.3 ± 0.6^{1}
Odour * Geruch	3.2 ± 0.4^{1}	3.7 ± 0.5^{1}	3.6 ± 0.5^{1}	3.8 ± 0.4^{1}	3.9 ± 0.6^{1}	3.7 ± 0.4^{1}	3.6 ± 0.3^{1}	3.2 ± 0.4^{1}	3.5 ± 0.6^{1}	3.4 ± 0.5^{1}
Pungent taste * Scharfer Geschmack	3.1 ± 0.4^{1}	3.2 ± 0.5^{1}	3.4 ± 0.5^{1}	3.9 ± 0.5^{1}	3.3 ± 0.5^{1}	3.8 ± 0.5^{1}	3.1 ± 0.4^{1}	3.3 ± 0.4^{1}	3.8 ± 0.6^{1}	3.2 ± 0.6^{1}
Overall acceptability* allgemeine Akzeptanz	3.3 ± 0.3^{1}	3.8 ± 0.3^{1}	3.5 ± 0.4^{1}	3.4 ± 0.4^{1}	3.5 ± 0.5^{1}	3.2 ± 0.5^{1}	3.3 ± 0.5^{1}	3.4 ± 0.5^{1}	3.9 ± 0.4^{1}	3.1 ± 0.4^{1}
Swelling Index/Quellwert										
Swelling Index (mL)* Quellwert (mL)	6.33 ± 0.29^{1}	6.83 ± 1.15^{1}	6.67 ± 0.58^{1}	6.83 ± 1.15^{1}	6.83 ± 1.15^{1}	6.33 ± 1.32^{1}	6.75 ± 0.58^{1}	5.67 ± 0.29^{2}	5.67 ± 0.29^{2}	5.17 ± 0.28^{2}

*** Same superscript means no significant difference between groups.**
* Bei gleichen hochgestellten Buchstaben gibt es keine signifikanten Unterschiede zwischen den Gruppen.

Marjoram

The leaf-fraction (leaves and flower buds) of marjoram contains essential oil as valuable ingredient. The essential oil is produced in peltate glandular trichomes at the leaf surface and thereby is very sensitive to a superficial, humid heat treatment (13). In this investigation even with the mildest test condition a loss of 86 % of the essential oil occurred. With higher sleeve or steam temperatures the loss increased up to 95 % (*Tab. 4*). Due to the already high losses of essential oil after a single treatment a double treatment was not completed for marjoram. The superficial peltate glandular trichomes of marjoram were

Tab. 3: Content of valuable ingredients of fennel (essential oil), nasturtium (glucotropaeoline) and onion (cysteine sulfoxide) as well as the „Lab" colour space values before and after „single" and „double treatment" of saturated steam decontamination

Tab. 3: Wertgebende Inhaltstoffe von Fenchel (ätherisches Öl), Kapuzinerkresse (Glucotropaeolin) und Zwiebel (Cysteinsulfoxide) sowie „Lab"-Farbwert gemessen vor und nach „Einfach-" und „Zweifachbehandlung" einer Sattdampfentkeimung

Treatment Behandlungen	Control Kontrolle	1	2	3	4	5	6	11	12	13	14	15	16
Fennel single and double treatment parameters/Einfach- und Zweifachbestimmung von Fenchel													
Ingredients analysis/Inhaltsstoffanalyse													
Essential Oil (mL/100g) * Ätherisches Öl (mL/100g) *	8.79 ± 0.201	8.85 ± 0.221	8.39 ± 0.201	8.46 ± 0.311	8.44 ± 0.351	8.64 ± 0.231	8.19 ± 0.152	8.53 ± 0.231	8.51 ± 0.191	8.16 ± 0.122	8.17 ± 0.152	8.04 ± 0.132	8.02 ± 0.212
„Lab" colour space values/„Lab"-Farbwert													
L*	33.91 ± 0.651	31.17 ± 0.763	31.90 ± 0.353	32.83 ± 0.412	31.50 ± 0.933	31.16 ± 1.023	31.05 ± 0.593	41.43 ± 0.654	40.69 ± 0.814	41.47 ± 0.324	40.72 ± 0.454	41.38 ± 0.654	40.15 ± 0.394
a*	1.74 ± 0.281	2.13 ± 0.111	2.18 ± 0.241	1.73 ± 0.291	2.31 ± 0.321	1.83 ± 0.141	2.08 ± 0.271	4.95 ± 0.812	4.79 ± 0.322	4.93 ± 0.412	4.76 ± 0.482	4.82 ± 0.722	4.94 ± 0.532
b*	14.63 ± 0.421	16.80 ± 0.342	15.60 ± 0.192	16.42 ± 0.492	16.05 ± 0.622	16.89 ± 0.252	15.65 ± 0.282	14.39 ± 0.631	13.75 ± 0.291	14.39 ± 0.451	14.04 ± 0.681	14.49 ± 0.551	13.75 ± 0.281
ΔE	–	3.52	2.27	2.09	2.85	3.56	3.06	3.7	4.5	3.65	4.42	3.73	5.04
Nasturtium single and double treatment parameters/Einfach- und Zweifachbestimmung von Kapuzinerkresse													
Ingredients analysis/Inhaltsstoffanalyse													
Glucotropaeoline (mg/g) * Glucotropaeolin (mg/g) *	12.01 ± 0.361	11.05 ± 0.382	10.57 ± 0.532	10.63 ± 0.312	10.04 ± 0.352	10.36 ± 0.452	10.06 ± 0.172	12.45 ± 0.402	10.35 ± 0.452	10.07 ± 0.282	9.83 ± 0.292	10.22 ± 0.332	9.27 ± 0.373
„Lab" colour space values/„Lab"-Farbwert													
L*	38.21 ± 0.831	36.25 ± 0.752	36.24 ± 0.372	35.99 ± 0.952	37.86 ± 0.562	37.36 ± 0.892	36.40 ± 1.092	41.33 ± 0.743	37.06 ± 0.892	37.37 ± 0.482	36.65 ± 1.082	36.65 ± 0.932	35.36 ± 0.842
a*	-1.56 ± 0.251	0.10 ± 0.062	0.06 ± 0.122	0.05 ± 0.082	0.03 ± 0.112	0.12 ± 0.092	0.12 ± 0.092	-2.27 ± 0.103	-0.50 ± 0.053	-0.29 ± 0.034	-0.29 ± 0.024	-0.29 ± 0.024	0.07 ± 0.012
b*	6.32 ± 1.111	5.07 ± 0.981	5.10 ± 0.921	5.08 ± 0.891	6.29 ± 1.041	5.85 ± 0.501	5.09 ± 0.691	9.34 ± 0.342	6.74 ± 0.291	6.90 ± 0.281	6.74 ± 0.301	6.74 ± 0.231	6.03 ± 0.281
ΔE	–	2.86	2.83	3.01	1.63	1.94	2.76	–	5.30	5.06	5.71	5.71	7.22
Onion single and double treatment parameters / Einfach- und Zweifachbestimmung von Zwiebel													
Ingredients analysis/Inhaltsstoffanalyse													
Cysteine sulfoxide (µg/g) * Cysteinsulfoxide (µg/g)	51.05 ± 2.091	50.67 ± 3.121	50.53 ± 2.871	49.59 ± 2.591	42.48 ± 2.111	43.94 ± 2.521	44.22 ± 2.391	51.05 ± 3.101	49.98 ± 2.551	48.54 ± 3.831	50.91 ± 2.891	41.67 ± 2.921	42.19 ± 2.051
„Lab" colour space values/„Lab"-Farbwert													
L*	45.16 ± 0.891	35.84 ± 1.292	38.03 ± 0.973	25.59 ± 1.104	16.83 ± 1.914	16.93 ± 0.954	15.27 ± 1.434	45.16 ± 0.981	36.69 ± 0.832	30.08 ± 0.375	26.98 ± 0.734	15.86 ± 0.824	14.73 ± 0.344
a*	0.89 ± 0.011	3.57 ± 0.212	3.78 ± 0.132	3.42 ± 0.232	8.59 ± 0.473	9.25 ± 0.373	9.75 ± 0.283	0.89 ± 0.011	3.66 ± 0.392	3.74 ± 0.422	4.76 ± 0.842	8.45 ± 0.563	8.32 ± 0.873
b*	14.70 ± 0.581	17.77 ± 0.452	18.94 ± 0.492	18.72 ± 0.732	30.01 ± 0.493	31.30 ± 0.383	31.50 ± 0.863	14.70 ± 0.281	13.15 ± 0.311	13.45 ± 0.821	14.13 ± 0.151	20.45 ± 0.624	22.89 ± 0.925
ΔE	–	10.17	8.78	20.14	33.11	33.80	35.41	–	9.05	15.40	18.60	30.80	32.38

*** Same superscript means no significant difference between groups.**

* Bei gleichen hochgestellten Buchstaben gibt es keine signifikanten Unterschiede zwischen den Gruppen.

investigated via scanning electron microscopy. These analyses revealed that the high loss of oil can be traced back to holes (*Fig. 3, left*) in the trichomes after treatment with the Lemgo process (*Fig. 3, right*). Marjoram leaves of natural green colour turned to pale green or grey after saturated steam treatment. The Lab-values with a maximal ΔE of 4.4 show still only insignificant changes of L (*Tab. 4, Fig. 4*).

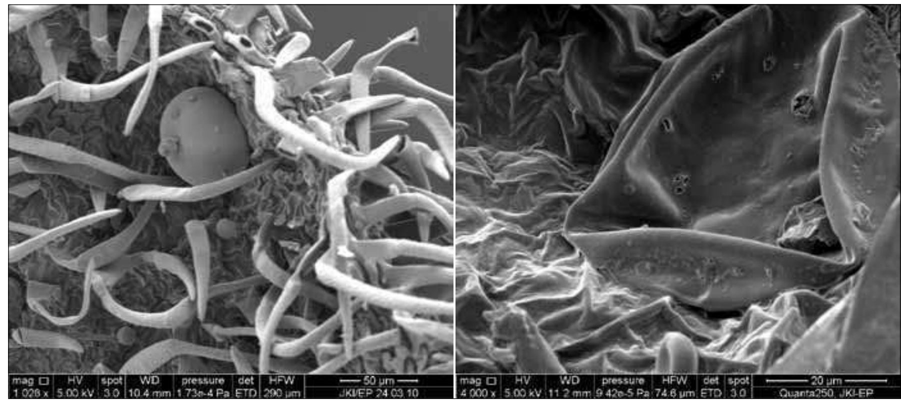

Fig. 3: Scanning electron microscopy image of marjoram: left = intact peltate glandular trichomes, right = damaged peltate glandular trichomes after treatment with saturated steam
Abb. 3: Rasterelektronenmikroskopisches Bild von Majoran: links = intakte Drüsenschuppe, rechts = zerstörte Drüsenschuppen nach Sattdampfbehandlung

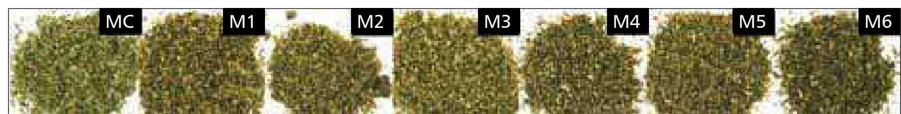

Fig. 4: Marjoram before and after mechanical saturated steam decontamination. MC = Control, M1 – M6 = Single treatment with saturated steam. For details of the treatment see *Tab. 1*.
Abb. 4: Majoran vor und nach der mechanischen Sattdampfentkeimung. MC = Kontrolle, M1 – M6 = Einfachbehandlung mit Sattdampf. Für Einzelheiten der Behandlungen siehe *Tab. 1*.

Nasturtium

After treatment with a single cycle of saturated steam decontamination and successive thermal (35 °C) air drying, the loss of glucotropaeoline as the valuable ingredient of nasturtium amounted to 8–16 % depending on the particular test conditions. With double treatment the loss increased up to 28 % (*Tab. 3*). Colour changes after a single treatment were small with ΔE 3.0 at the most, considering only the Lab values (*Tab. 3*). Upon investigating the samples, it was visibly apparent that stems were brighter and leaves are darker in comparison with the control (*Fig. 5*). With double treatment the changes became more obvious. Stems lost their green colour significantly and leaves became dark green (*Fig. 5*). This resulted in ΔE-values between 5.1 and 7.2, marking obvious changes (*Tab. 3*).

Onion

Onions contain cysteine sulfoxides as valuable ingredients, which are decomposed enzymatically during cell destruction accompanied by high heat inputs (12). With the Lemgo process there were no significant cysteine sulfoxide losses after treatment with 110 °C steam, followed by rapid evacuation (*Tab. 3*). With steam of 125 °C losses of up to 18 % of cysteine sulfoxide occurred. While the cysteine sulfoxide content was little affected by the Lemgo process, there were rather large changes in colour. As shown in *Fig. 6*, even relative mild conditions led to a distinguishable darkening with a reddish-brown colour. Even after a single treatment not only in the L

Tab. 4: Essential oil content and „Lab" colour space values before and after „single treatment" of saturated steam decontamination of marjoram
Tab. 4: Gehalt an ätherischen Ölen und „Lab"-Farbwert vor und nach „Einfachbehandlung" einer Sattdampfentkeimung von Majoran

Marjoram single treatment parameters / Einfachbehandlung von Majoran							
Treatments Behandlung	Control Kontrolle	1	2	3	4	5	6
Ingredients analysis / Inhaltsstoffanalyse							
Essential oil (mL/100g) * Ätherisches Öl (mL/100g)	1.64 ± 0.07[1]	0.23 ± 0.02[2]	0.16 ± 0.03[3]	0.12 ± 0.02[3]	0.15 ± 0.04[3]	0.11 ± 0.02[3]	0.09 ± 0.01[3]
„Lab" colour space values/„Lab"-Farbwert							
L*	40.02 ± 0.82[1]	37.61 ± 0.79[2]	37.55 ± 0.65[2]	37.65 ± 0.72[2]	36.10 ± 0.91[2]	36.79 ± 0.90[2]	36.80 ± 0.86[2]
a*	0.42 ± 0.22[1]	1.18 ± 0.43[1]	1.27 ± 0.32[1]	1.24 ± 0.38[1]	1.07 ± 0.42[1]	1.13 ± 0.45[1]	1.09 ± 0.39[1]
b*	7.26 ± 0.81[1]	6.19 ± 0.75[1]	6.02 ± 0.93[1]	6.10 ± 0.91[1]	5.34 ± 0.85	5.60 ± 0.79[1]	5.57 ± 0.65[1]
ΔE	–	2.74	2.89	2.76	4.41	3.70	3.70

*** Same superscript means no significant difference between groups.**
* Bei gleichen hochgestellten Buchstaben gibt es keine signifikanten Unterschiede zwischen den Gruppen.

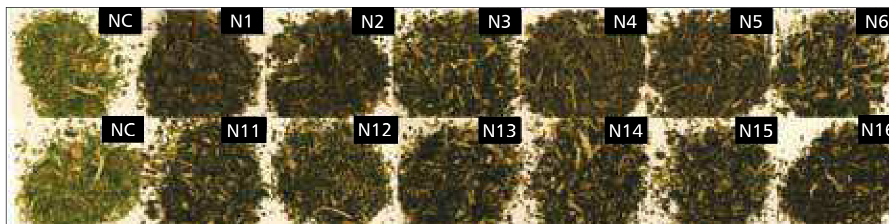

Fig. 5: Nasturtium before and after mechanical saturated steam decontamination. NC = Control, N1 – N6 = Single treatment with saturated steam, N11 – N16 = Double treatment with saturated steam. For details of the treatment see *Tab. 1*.
Abb. 5: Kapuzinerkresse vor und nach der mechanischen Sattdampfentkeimung. NC = Kontrolle, N1 – N6 = Einfachbehandlung mit Sattdampf, N11 – N16 = Doppelbehandlung mit Sattdampf. Für Einzelheiten der Behandlungen siehe *Tab. 1*.

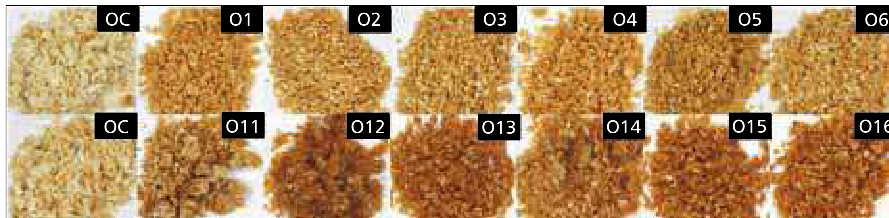

Fig. 6: Onion before and after mechanical saturated steam decontamination. OC = Control, O1 – O6 = single treatment with saturated steam, O11 – O16 = double treatment with saturated steam. For details of the treatment see *Tab. 1*.
Abb. 6: Zwiebelwürfel vor und nach der mechanischen Sattdampfentkeimung. OC = Kontrolle, O1 – O6 = Einfachbehandlung mit Sattdampf, O11 – O16 = Doppelbehandlung mit Sattdampf. Für Einzelheiten der Behandlungen siehe *Tab. 1*.

value but also in a- and b-value considerable changes could be found, leading to a ΔE between 8.78 and 35.41 (*Tab. 3*).

Discussion

This investigation shows that the Lemgo process was not only effective in decontaminating herbal drugs but also that the value adding ingredients mostly were not impaired. For four (linseed, fennel, nasturtium and onion) out of five drugs there was no or only a small loss of the analysed value adding ingredients. Only marjoram lost almost all of the essential oil.

Linseed, with its crude fat inside the seeds, is unproblematic and, as expected, no loss of the fat was observed. With other processes linseed is often found deteriorated after short time storage (19). In case of the Lemgo process, however, no deteriorated grains of linseed could be detected.

The essential oil of fennel is a volatile constituent, but as it is stored inside the seeds almost no loss due to the decontamination process was stated. The mild decontamination conditions were sufficient to reduce the germs by at least 2 powers of magnitude or to arrive at a contamination level even below the detection limit of 100 CFU/g. Thus the Lemgo process could be shown as an effective and mild technique for the decontamination of fennel.

Even more delicate is the treatment of nasturtium with wet heat. Its valuable ingredient glucotropaeoline is known to easily react enzymatically when heated with saturated steam (7). As expected some of the glucotropaeoline is lost even by the short heating of the Lemgo process. On the other hand, the surface of the leaves of nasturtium shows the lotus effect (10). This seems to reduce the adhesive force of the germs. Therefore a single treatment of nasturtium with a jacket temperature of 80 °C was sufficient for a reduction of the total viable count and aerobic spores below the detection limit. No double treatment was necessary for an effective decontamination (14). Under these conditions the loss of glucotropaeoline of about 10 % was quite low.

In onion, no loss of cysteine sulfoxides was detected when using steam with a temperature of 110 °C and rapid evacuation for decontamination. With this steam temperature a reduction of the microbial load of up to 3 powers of magnitude was possible.

Thus, an efficient sterilisation of four of the tested drugs could be carried out using the Lemgo process, though in some cases small losses of the respective valuable ingredients occurred. Merely marjoram shows an extensive loss of its essential oil. It is located in the peltate glandular hairs where it is protected only by a wax layer and the underlying cuticle (8). As visible in *Fig. 3*, the loss was the consequence of the mechanical destruction of these peltate glandular hairs. It is unclear whether the heat, the vacuum, the flash evaporation or the fast evacuation brought about the destruction. The combination of these factors could as well play a key role. In addition, the Lemgo process exerted high mechanical stress on the surface of the drugs. This could lead to the destruction of surface structures and leakage of the ingredients, as observed in marjoram.

Besides the valuable ingredients, other criteria are as well important for the marketability of herbal drugs.

First of all, the colour should be fresh and unchanged. The green leaves as well as the seeds tested had significant changes in colour. The diced bulbs of onion showed a non-enzymatic browning whereby the dices were significantly darker (*Fig. 6*). This reaction is temperature dependent and the reaction rate varies as a quadratic of water activity (20). Thus only 20 sec of steam atmosphere sufficed to induce colour changes in such extent to exclude the product from direct marketing. Usage for formulas or encapsulated will be no problem because the valuable ingredients were not affected by this reaction (*Tab. 3*). For direct marketing further reduction of the steam temperature may lead to the desired result.

The situation with linseed is similar. While the chemical and physical parameters show only small significant changes, the surface lost some glossiness (*Fig. 1*) and the swelling index was reduced. This could be explained by the swelling of the polysaccharide layer during the steam exposition and drying.

Both problems should be manageable by exact process control. The optimal

steam and sleeve temperature should give rise to a minimal amount of condensation to guarantee the sterilization effect, but no more.

The results of this study demonstrate that the decontamination method Lemgo process is qualified for industrial application to fennel, nasturtium, linseed and onion.

Acknowledgements

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10 Appendix 4

K. Schwarzer, A. Dammann, H. Lange, H. Krüger, U. Müller

Mechanische Sattdampfentkeimung von Drogen mit unterschiedlichen Oberflächeneigenschaften

Zusammenfassung

Pflanzliche Drogen besitzen häufig eine höhere natürliche Verkeimung als die gesetzlichen Vorgaben es erlauben. Eine Keimreduzierung erfolgt zumeist durch hitzethermische Abtötung, die in verschiedenen Sattdampfverfahren umgesetzt wird. Die vorliegenden Untersuchungen wurden nach dem sogenannten Lemgoer Verfahren durchgeführt, einem Sattdampfverfahren, bei dem neben der hitzethermischen Keimreduzierung vor allem eine weitgehende Abtrennung der Sporen durchgeführt werden kann. Nachdem die Behandlungen bisher in einer 1 L-Kammer durchgeführt wurden, wurde untersucht, ob der Entkeimungserfolg auch bei einem Scale up in den halbtechnischen Maßstab erhalten bleibt. Fünf Arzneidrogen und Gewürzee (Kapuzinerkresse, Majoran, Leinsamen, Fenchelfrüchte und Zwiebel) wurden in einem 5 L-Pflugscharmischer entkeimt. Dabei wurde Sattdampf von 110 bzw. 125°C für 20 s oder 2 mal 20 s aufgebracht. Bei allen Drogen konnten die Sporen um mindestens 2 Dekaden, zumeist auf unter 10^2 Koloniebildende Einheiten (KbE), reduziert werden. Dies traf auch auf Drogen mit problematischen Oberflächen wie Leinsamen (Polysaccharide auf der Oberfläche) oder Zwiebelwürfel (viele angeschnittene Zellen) zu. Schimmelpilze, soweit vorhanden, wurden ebenfalls bis unter die Nachweisgrenze reduziert. Um Wiedereinfangeffekte beim Scale up zu vermeiden, war es nötig, vom Festbett zum Bewegtbett überzugehen. Die in diesem Zusammenhang notwendige Partikelzurückhaltung wurde durch ein zusätzliches Sieb gewährleistet.
Mit dem Lemgoer Verfahren zur mechanischen Sattdampfentkeimung steht ein schonendes und effizientes Verfahren zur Sporenreduktion vieler Arznei- und Gewürzdrogen zur Verfügung.

Einleitung

In der freien Natur gewachsene Kräuter, weisen häufig eine hohe natürliche Verkeimung auf. Hinzu können weitere Keimbelastungen kommen, die während der Weiterverarbeitung und Lagerung auf die Materialien gelangen [1]. Insbesondere für die Verwendung als Arzneidrogen sind aber strikte Grenzwerte bezüglich der mikrobiellen Belastung einzuhalten. Je nach Anwendung dürfen 10^7 bis 10^4 KbE/g Gesamtkeime beziehungsweise 10^5 bis 10^2 KbE/g Gesamt-Hefen und Pilze nicht überschritten werden [2].

Zur Reduzierung der Keimbelastung auf Pflanzen sind verschiedenste Methoden entwickelt, jedoch nur wenige sind für die Anwendung im pharmazeutischen Bereich zugelassen worden. Am häufigsten wird eine Behandlung mit Sattdampf durchgeführt [3]. Es gibt viele Varianten der Sattdampfentkeimung. In der Regel handelt es sich um eine hitzethermische Abtötung der Keime mittels feuchter Hitze. Es sind sowohl kontinuierliche, als auch Batchverfahren bekannt [4]. Da der überwiegende Teil der Mikroorganismen aufgrund der Trockenheit des Pflanzenmaterials in Form von hitzeresistenten Sporen vorliegt, muss die hitzethermische Belastung entsprechend groß sein. Die meisten Verfahren bedampfen für mehrere Minuten bei über 120 °C [4]. Der Dampf schlägt sich auf den Oberflächen nieder und führt angesichts der hohen Temperaturen zu einer schnellen Aktivierung der Sporen. Anschließend erfolgt die hitzethermische Abtötung.

Die mechanische Sattdampfentkeimung, auch Lemgoer Verfahren genannt, unterscheidet sich jedoch grundlegend von einer rein hitzethermischen Abtötung. Bei diesem Verfahren wird der Dampf in erster Linie verwendet, um einen heißen Wasserfilm auf den Oberflächen zu erzeugen. Hierfür reichen bereits wenige Sekunden Bedampfungszeit aus. Durch den Wasserfilm kommt es zu einer erheblichen Reduktion der Haftkräfte der Mikroorganismen [5]. Anschließend wird extrem schnell evakuiert. Dadurch verdampft das Wassers explosionsartig (Flashverdampfung) und reißt die Mikroorganismen von der Oberfläche [6]. Da die Keime nicht in erster Linie abgetötet werden, spielt die Hitzeresistenz der Sporen hier nur eine untergeordnete Rolle. Die Wirksamkeit des Verfahrens wurde im Labormaßstab eingehend untersucht [5]. Dabei wurde sowohl die Haftkraftverringerung, als auch das Entfernen verschiedener Bacillussporen nachgewiesen. Außerdem war die Entkeimungswirkung deutlich größer, als durch rein hitzethermische Effekte erklärt werden konnte. Auf verschiedenen Materialien, welche teils künstlich, teils natürlich verkeimt waren, konnte die Sporenzahl durch Flashverdampfung um 3 und mehr Dekaden reduziert werden [6; 7], nachdem zuvor Dampf von 115 bzw. 130 °C fünf bis 30 s eingewirkt hatte. Um die Wirkung zu verstärken, kann, falls notwendig, an die Nachvakuumphase eine zweite Bedampfung und eine zweite Flashverdampfung angeschlossen werden [8].

Voruntersuchungen im Labormaßstab wurden in einem 1 L-Behandlungsraum durchgeführt. Neben Modelloberflächen wurden Blatt- und Körnerdrogen verwendet, die in einem speziellen Drahtkäfig von ca. 100 mL festgehalten wurden. Es zeigte sich, dass trotz der Sporenentfernung um mehrere Dekaden vereinzelt noch vegetative, pathogene Keime (Salmonellen, Enterobakterien) nachweisbar waren [9]. Dies wurde auf eine ‚Nesterbildung' der Keime oder einen, trotz des Vorvakuums, unzureichenden Dampfzutritt ins Innere der Produktschicht zurückgeführt. Vereinzelte trockene Bereiche nach der Entkeimung sprechen für letztere Annahme. Bei der Maßstabsvergrößerung auf den kleintechnischen Maßstab kam es daher zum einen darauf an, dem Dampf einen ausreichenden Zutritt zu allen Oberflächen zu gewähren und durch eine gute Durchmischung des Produktes Nester aufzulösen. Darüber hinaus galt es zu verhindern, dass bei zu großen Schichtdicken die abgelösten Keime von anderen Produktoberflächen wieder eingefangen werden. Als Behandlungsraum wurde daher ein 5 L-Pflugscharmischer verwendet um das Probenmaterial (Körner, Blätter, Zwiebelstücke)in einer Wirbelschicht zu halten und so die Partikelabstände zu vergrößern. Ein zusätzliches Sieb verhinderte den Austrag des Produktes während der Flashverdampfung.

Primäres Ziel war der Nachweis einer hinreichenden Keimreduktion auch im halbtechnischen Maßstab. Darüber hinaus sollte untersucht werden, wie sich unterschiedliche Oberflächeneigenschaften auf den Entkeimungserfolg auswirken. Daher wurden Modelldrogen mit sehr unterschiedlichen Oberflächenstrukturen ausgewählt. Majoran und Fenchel sind zwei Drogen mit sehr unterschiedlicher Stabilität der ätherischen Ölbehälter. Leinsamen enthält fette Öle und eine Schicht aus Polysacchariden an der Oberfläche. Geschnittene Zwiebeln enthalten ebenfalls Zucker und weisen viele nicht mehr intakte Zellen auf. Kapuzinerkresse besitzt eine Wachsoberfläche [10], die sich durch einen sogenannten Lotuseffekt günstig auf die Ablösung von Sporen und Keimen auswirken sollte.

Über die parallel durch das Julius Kühn-Institut Quedlinburg durchgeführten Wirkstoffanalysen wurde bereits an anderer Stelle berichtet [11].

Material und Methoden

Pflanzenmaterial

Es wurden handelsübliche Industriedrogen untersucht. Die beiden Blattdrogen Kapuzinerkresse (*Tropaeolum majus* L., REPHA GmbH Biologische Arzneimittel, Langenhagen) und Majoran (*Majorana hortensis* Moench., MAWEA Majoranwerk Aschersleben GmbH, Aschersleben) lagen gerebelt und getrocknet vor. Leinsamen (*Linum usitatissimum* L.,SALUS Haus GmbH & Co. KG, Bruckmühl) und Fenchel (*Foeniculum vulgare* Mill.,MAWEA Majoranwerk Aschersleben GmbH, Aschersleben) wurden ohne Vorbehandlung verwendet. Die Zwiebeln (*Allium cepa* L.,Kräutermix GmbH, Abtswind) waren geschnitten (1 cm-Würfel) und getrocknet. Alle Drogen waren bewusst mikrobiologisch unbehandelt, lagen also mit natürlicher Verkeimung vor.

Mechanische Sattdampfentkeimung nach dem Lemgoer Verfahren (Aufbau und Durchführung)

Als Reaktionsraum für die Entkeimung der Medizinaldrogen fand ein 5 L-Pflugscharmischer (Lödige Labormischer Typ M5, Lödige Maschinenbau GmbH, Paderborn) Verwendung (Abbildung 1). Der Mischer ist mit einem temperierbaren Doppelmantel versehen, um die Kondensationsrate des Dampfes beeinflussen zu können. Die Manteltemperatur wurde auf 20 °C, 40 °C oder 80 °C eingestellt. Ein Dampfgenerator (Beckmesser) lieferte Sattdampf mit einer Temperatur von 180 °C (ca. 8 bar). Dieser wird durch einen Druckreduzierer auf die gewünschte Temperatur-Druck-Kombination reduziert und über Stahlleitungen in die Mischkammer des Mischers geleitet. Das notwendige Vakuum wird von einer Ölschiebervakuumpumpe (Trivac, Levbolo Vacuum GmbH, Köln) erzeugt. Ein Eiskondensator (ca. 100 L Nutzvolumen, -20 °C Betriebstemperatur) dient als Vakuumpuffer und dem Abscheiden des Wasserdampfes zum Schutz der Vakuumpumpe. Da die Drogenpartikel zum Teil mit dem Gasstrom aus dem Mischer ausgetragen wurden, war eine Partikelabscheidung notwendig. Zwei Systeme wurden hier getestet, zum einen ein Zyklon und zum anderen ein Sieb am Mischerausgang. Ein piezoresistiver Druck- und zwei Temperatursensoren (Pt 100) im Mischerraum dienten der Überwachung des Vorgangs. Im Kopfraum wurde die Dampftemperatur gemessen und am tiefsten Punkt des Mischers die Oberflächentemperatur der Drogen. Abbildung 2 zeigt ein Fließbild der Anlage.

<Hier Abb. 1>

<Hier Abb. 2>

Die Bedampfung und Evakuierung der Behandlungskammer erfolgte mittels Handventilen. Dabei erfolgte nach 20 s Vorvakuum eine Bedampfung für 20 s mit 110 °C oder 125 °C heißem Dampf und anschließend 20 s Nachvakuum. Für eine Doppelbehandlung wurde an das Nachvakuum direkt eine zweite Bedampfung und ein zweites Nachvakuum mit den gleichen Parametern angeschlossen. Die Be- und Entladung des Mischers erfolgte sehr zeitnah. Die Gesamtchargendauer überschritt nie 3 Minuten. Je Parameterkombination wurden 3 Versuche durchgeführt.

Keimzahlbestimmung

Die Bestimmung der Keimzahl erfolgte zeitnah im Mikrobiologischen Labor der Hochschule Ostwestfalen-Lippe. Bestimmt wurden die Gesamtkeimzahl, die Gesamtsporenzahl, Schimmelpilze sowie Enterobakteriaceaen, Salmonellen und E. coli. Dabei wurden die Analysen nach dem Europäischen Arzneibuch [12] durchgeführt.

Ergebnisse und Diskussion

Reduktion der aeroben Keime

Die fünf exemplarischen Arznei- und Gewürzdrogen wurden mit verschiedenen Dampfdrücken, Manteltemperaturen und mit oder ohne Wiederholungsbehandlung entkeimt. Für 4 Drogen konnten geeignete Parameterkombinationen gefunden werden, ab denen die Konzentration der aeroben Keime auf weniger als 100 KbE/g reduziert wurde. Nur für Majoran waren die Ölverluste so groß [11], dass nur eine einfache Behandlung durchgeführt wurde. In Tabelle 1 sind die maximal nachgewiesenen Reduktionsraten für Sporen und vegetative Keime aufgeführt. Begrenzt wurden diese nicht vom Verfahren, sondern von der Ausgangsverkeimung und der Nachweisgrenze bei 100 KbE/g. Es wurde in den Versuchen bewusst darauf verzichtet, die Ausgangsverkeimung künstlich zu erhöhen, da nicht sicher ist, dass künstlich aufgebrachte Sporen bezüglich der Haftkräfte auf der Drogenoberfläche der natürlichen Verkeimungen hinreichend ähnlich sind.

<Hier Tabelle 1>

Während in der 1 L-Laboranlage noch vereinzelt vegetative Pathogene auf den Drogen zurückblieben (Enterobakterien, Salmonellen) [9], wurden in der hier untersuchten kleintechnischen Anlage nur in einer Probe Spuren von Enterobakterien ($<10^2$ KbE/g) auf Kapuzinerkresse nachgewiesen. Die Ergebnisse zeigen, dass die Wirbelschicht zu einer Verbesserung der Entkeimungswirkung führt. Nicht nur wird dadurch der Zutritt des Dampfes zu allen Oberflächen verbessert, es erfolgt auch eine weitgehende Auflösung von sogenannten Nestern. Darüber hinaus wird der Abstand zwischen den Drogenpartikeln vergrößert, wodurch die Dampfströme mit den abgetrennten Keimen besser abgeführt werden, d.h. es besteht ein geringeres Risiko der Rekontamination. Die erfolgreiche Eliminierung der pathogenen Keime zeigt den Erfolg dieser Strategie. Die Spuren von Enterobakterien, die auf einer Kapuzinerkresseprobe gefunden wurden, sind auf die besonders hohe Ausgangsverkeimung von 10^6 KbE/g zurück zu führen. In weiteren Versuchen konnte selbst diese Kontamination unter die qualitative Nachweisgrenze reduziert werden.

Reduktion von Schimmelpilzen und Hefen

Die Belastung mit Schimmel und Hefen war bereits in den verwendeten Proben sehr gering. Bei Zwiebeln, Kresse und Leinsamen konnten bereits in den unbehandelten Proben keine oder nur vereinzelt Schimmelpilze nachgewiesen werden. Lediglich bei Fenchel und Majoran fanden sich ca. 10^3 KbE/g. Diese konnten durch die mechanischen Sattdampfentkeimung bis unter die Nachweisgrenze von 10^2 KbE/g reduziert werden.

Temperaturverlauf

Die Temperatur des Dampfes und der Drogenoberfläche wurde durch zwei Messfühler überwacht. Abbildung 3 zeigt typische Temperaturverläufe bei unterschiedlichen Versuchsparametern. Gut zu sehen ist, dass insbesondere die Temperatur im Sumpf des Mischers, die der Oberflächentemperatur des zu entkeimenden Gutes entspricht, nur wenige Sekunden in einem für Sporen letalen Bereich liegt. Hitzebedingte chemische Veränderungen wie Farbveränderungen durch Maillard-Reaktionen sind in dieser kurzen Zeit kaum zu erwarten. Danach sinkt die Temperatur wieder durch die vom Vakuum ausgelöste Flashverdampfung. Auch bei einer doppelten Behandlung sinkt die Temperatur zwischen und nach der zweiten Behandlung schnell auf unkritische Werte. Insbesondere im Vergleich

mit klassischen Sattdampfentkeimungen, bei denen hohe Temperaturen für mehrere Minuten benötigt werden, stellt dies eine deutliche Verbesserung dar.

<Hier Abbildung 3>

Parallel zu den hier vorgestellten mikrobiologischen Untersuchungen hat das Julius Kühn-Institut Quedlinburg Untersuchungen zu chemischen und farblichen Parametern durchgeführt [11]. Es wurden nur bei Zwiebeln starke Verfärbungen festgestellt. Inhaltsstoffe wurden praktisch nicht chemisch verändert, allerdings zeigte es sich, dass die oberflächlich liegenden Ölzellen des Majorans bei dem Verfahren platzen und so das Öl weitgehend verloren geht.

Einzelergebnisse

Kapuzinerkresse

Bei der Entkeimung der Kresse fällt auf, dass bereits bei einer einfachen Behandlung eine sehr gute Entkeimung erreicht werden konnte. Bei einer Ausgangsverkeimung von bis zu $7*10^6$ KbE/g Gesamtkeimen konnte mit 20 sec Behandlungsdauer und 125°C heißem Dampf die Keimbelastung bis unter die quantitative Nachweisgrenze von $1*10^2$ KbE/g reduziert werden. Das gleiche galt für $1*10^5$ KbE/g Sporen. Auf der anderen Seite führt eine nochmalige Bedampfung und Flashverdampfung, im Gegensatz zu den Ergebnissen aller anderen getesteten Pflanzen, nicht zu einer weiteren Verbesserung der Entkeimungswirkung. Beide Ergebnisse lassen sich mit der speziellen Oberflächenstruktur der Kapuzinerkresseblätter erklären. Die Oberfläche ist mit Clustern von Wachsschuppen bedeckt, die eine Benetzung der Oberfläche erschwert (Siehe Abbildung 4) [10]. Zdenek konnte in seiner Arbeit zeigen, dass diese Oberflächenstruktur die Entfernung von Mikroorganismen vereinfacht [13]. Allerdings kondensiert Dampf durchaus zwischen den Wachstubuli und kann so dem Lotuseffekt entgegenwirken [13]. Wie genau sich dies bei der mechanischen Sattdampfentkeimung auswirkt, ist noch zu klären. Eine Erklärung für die gute Entkeimungswirkung wäre, dass Wasser sogar direkt unter den Mikroorganismen kondensieren kann und so bei der Flashverdampfung die Keime, die nur auf den Wachstubuli aufsitzen, leicht entfernt werden können.

< Hier Abbildung 4>

Zwiebel

Abbildung 5 zeigt die Keimbelastung von Zwiebelwürfeln vor und nach der mechanischen Sattdampfentkeimung mit unterschiedlichen Prozessparametern. Sowohl die Gesamtkeimzahl als auch die Sporen konnten bereits bei einer Dampftemperatur von 110 °C um 3 Dekaden reduziert werden. Dies ist insofern bemerkenswert, als das in den Vorversuchen in der Laboranlage keine signifikante Keimreduzierung nachzuweisen war [9]. Die Zwiebelstücke neigen während der Bedampfungsphase zu einer besonders starken Wasseraufnahme verbunden mit einer Quellung. In der Laboranlage führte diese Quellung im Siebsystem zu einer Reduktion der Räume zwischen den einzelnen Zwiebelwürfeln. Die abgetrennten Sporen konnten nicht mit der Dampfphase aus dem System entfernt werden, da Wiedereinfangeffekte dies verhinderten. In der kleintechnischen Anlage wurden durch das Wirbelbett die Partikel vereinzelt und so ausreichend große Zwischenräume für den Abtransport der Keime ohne erhebliche Wiedereinfangeffekte geschaffen.

<Hier Abbildung 5>

Betrachtet man die verschiedenen Versuchsparameter, so ist festzustellen, dass sich die Entkeimung mit zunehmender Manteltemperatur verbessert. Dies geht, wie in Abbildung 3 für Kresse zu sehen, mit einer Erhöhung der Oberflächentemperatur des zu entkeimenden Gutes einher. Dennoch spielt die thermische Abtötung wohl nur eine untergeordnete Rolle. Die Verbesserung der Entkeimungswirkung erfolgt bei vegetativen Keimen und Sporen im gleichen Maß. Bei der thermischen Abtötung müssten vegetative Keime deutlich stärker betroffen sein. Aber, während an dem wärmeren Mantel weniger Kondensat niederschlägt erhöht sich gleichzeitig die Energie für die Wiederverdampfung. Die Folge ist eine Verstärkung des Flasheffektes und damit eine Verbesserung der Keimablösung. Entsprechend verstärkt auch eine Erhöhung der Dampftemperatur den Flasheffekt. Darüber hinaus führt eine zweite Bedampfung zu einer erneuten Keimreduktion. Dass diese doppelte Flashverdampfung wirkungsvoller ist, als eine einfache doppelt so lange Bedampfung mit einer Flashverdampfung wurde bereits früher in der Laboranlage nachgewiesen [8].

Leinsamen

Leinsamen wurde wegen der quellfähigen Polysaccharidschicht an der Kornoberfläche in das Untersuchungsprogramm aufgenommen. Es sollte speziell untersucht werden, ob die Keime auch von dieser Oberflächenmatrix mittels Flashverdampfung abgelöst werden können. Bereits in der Laboranlage war aufgefallen, dass es durch die Wasserzufuhr zu starken Quellungsvorgängen und einem Verkleben der Körner kam [9]. Das Quellen der Körner fand auch in diesen Versuchen wieder statt. Bei einigen Parameterkombinationen, bei denen viel Kondensat anfiel, erfolgte ein starkes Verkleben der Körner. Dennoch reichte bereits eine einfache Behandlung mit 110°C heißem Dampf aus, um die Gesamtkeimzahl um drei Dekaden zu reduzieren. Bei 125°C heißem Dampf konnte sowohl die Gesamtkeimzahl von $9*10^5$ KbE/g, als auch die Sporenzahl von $3*10^4$ KbE/g unter die quantitative Nachweisgrenze von $1*10^2$ KbE/g reduziert werden. Trotz der Polysaccharide auf der Oberfläche gelang es also, eine gute Keimreduktion durchzuführen.

Die Quellung der Polysaccharide führte zu einer gewissen Klebrigkeit des Produktes. Jedoch konnte durch die Steuerung der Manteltemperatur die Menge an Kondensat so verändert werden, dass nach der Flashverdampfung und der notwendigen Nachtrocknung ein streufähiges Produkt entsteht. Wie die Untersuchungen von Lange et al. gezeigt haben ist die Quellung nahezu vollständig reversibel, so dass auch die medizinisch wichtige Quellzahl nur wenig beeinflusst wird [11].

Produktrückhaltung

Das Produkt wurde bei der schnellen Evakuierung nach dem Bedampfungsschritt in erheblichen Mengen mit dem Wasserdampf ausgetragen. Um hier die Produktverluste zu minimieren, wurden zwei verschiedene Rückhaltesysteme getestet. Zunächst wurde ein Zyklon (Abbildung 1, Nr. 5) getestet. Dieses System wurde jedoch aufgrund verschiedener Probleme verworfen. Zum einen führt der instationäre Zustand des Dampfstroms zu einer unvollständigen Abtrennung und zum anderen führt das am Zyklon permanent anliegende Vakuum zusammen mit der Restfeuchte des Produkts zum Einfrieren der Proben, was nicht nur zu einem Verblocken des Zyklons, sondern auch zu Produktschädigungen führt.

Als Alternative wurde ein Sieb am Ausgang des Pflugscharmischers (Abbildung 1, Nr. 4) getestet. Dieses führte zu einer guten Rückhaltung des Produktes. Einer möglichen Verblockung des Siebes wurde dadurch entgegen gewirkt, dass sowohl die Dampf-, als auch die Frischluftzufuhr zur Belüftung bei Prozessende zur Freispülung des Siebes genutzt wurde. Dennoch kommt es gerade während der Flashverdampfung zu einer Verdichtung von Partikeln gerade an dem Punkt, an dem der mit Keimen

belastete Dampf ausströmt. Dies könnte zu einer verlangsamten Evakuierung des Reaktionsraums führen, was wiederum zu einer geringeren Flashverdampfung und damit geringerem Entkeimungserfolg führen kann. Messungen haben ergeben, dass die für die Flashverdampfung maßgebliche Änderung des Drucks über die Zeit sich nur geringfügig ändert und somit auch die Entkeimungswirkung nicht nachlässt. Vergleiche der Keimzahlen bestätigen dieses Ergebnis. Um sicher zu gehen, dass keine Wiedereinfangeffekte am Sieb zu einer nachträglichen Wiederverkeimung führen, wurden Proben von am Sieb haftendem Produkt auf ihre Keimbelastung geprüft und mit den Proben aus dem Mischerraum verglichen (Tabelle 2). Es konnten keine erhöhten Keimbelastungen auf den Drogen nachgewiesen werden, die im Bereich des Siebes hafteten. Auch im Vergleich mit Entkeimungsversuchen ohne Sieb trat keine Verschlechterung ein. Da sich das Sieb noch innerhalb des Bereiches befindet, an dem nur zeitweilig Vakuum herrscht tritt auch kein Einfrieren der Probe statt. Die Siebrückhaltung ist also eine geeignete Methode der Partikelrückhaltung ohne störende Wiedereinfangeffekte, Verblockung oder zusätzlicher Produktschädigung.

<Hier Tabelle 2>

Ausblick

Mit der hier vorliegenden Arbeit konnte auch gezeigt werden, dass ein Scale up in einer kleintechnischen Anlage die Effektivität des Lemgoer Entkeimungsverfahrens steigert. Durch Arbeiten im Bewegtbett wurden Wiedereinfangeffekte verhindert und Nester mit pathogenen Keimen aufgelöst und effektiv entkeimt. Scale up Berechnungen haben ergeben, dass mit dieser Methodenanpassung Behandlungsraumgrößen bis 150 L denkbar sind. Durch die kurzen Chargenzeiten sind aber bereits im 5 L-Maßstab für den pharmazeutischen Bereich hohe stündliche Durchsatzraten erzielbar.

Obwohl die Bedampfung sehr kurz ist und die anschließende Flashverdampfung einen Großteil des kondensierten Wassers wieder abführt, verbleibt eine gewisse Auffeuchtung des Produkts nach Ende der Entkeimung. Um eine Vermehrung der verbliebenen Keime zu verhindern, muss eine Nachtrocknung bis zu einem a_w-Wert von 0,6 erfolgen. Diese kann je nach Anwendung extern, zum Beispiel auf einem Bandtrockner, oder auch intern, direkt im Mischer, erfolgen.

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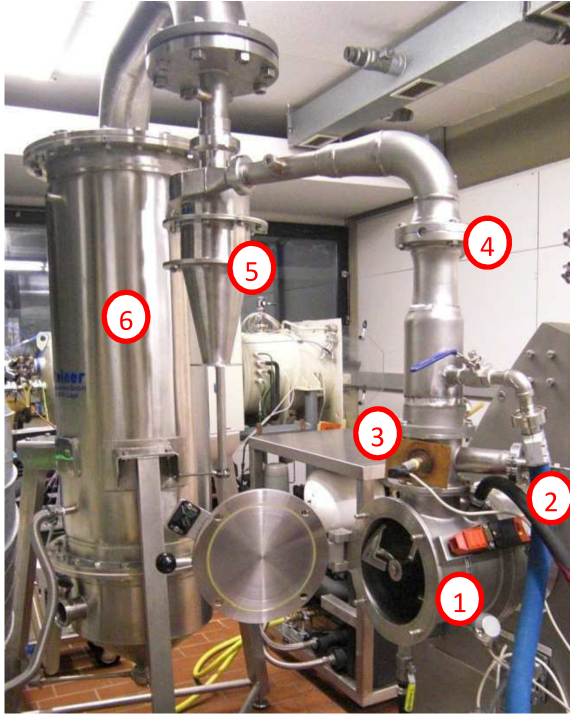

Abbildung 1 Ausschnitt der verwendeten halbtechnischen Anlage. 1: beheizbarer 5 L-Pflugscharmischer. 2: Dampfzuleitung. 3: Siebeisatz (optional). 4: Vakuumklappe 5: Zyklon. 6: Eiskondensator zur Kondensation des Dampfes und als Vakuumpuffer.
Fig. 1: Part of the semi-industrial plant. 1: heatable 5 L ploughshare mixer. 2: steam inlet pipe. 3: strainer insert. 4: vacuum valve. 5: cyclone. 6: ice condenser for steam condensation an as vacuum buffer

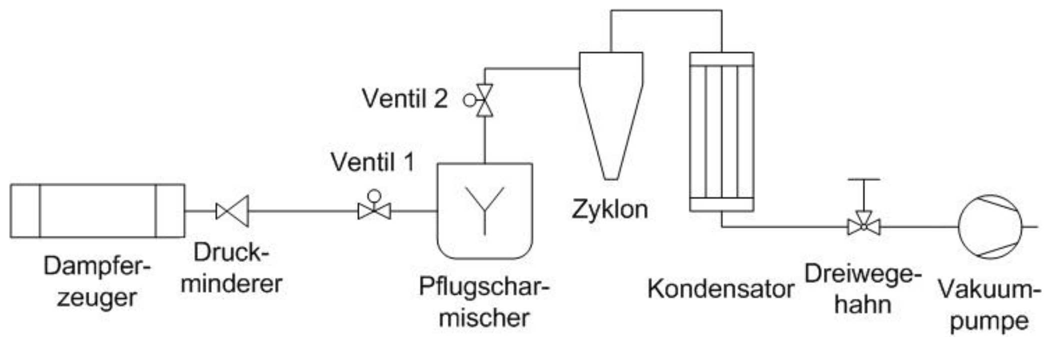

Abbildung 2: *R&I-Fließbild der verwendeten halbtechnischen Entkeimungsanlage mit einem beheizbaren 5 L-Feststoffmischer. Der (Eis-)Kondensator ist gleichzeitig Vakuumpuffer.*
***Fig. 2: Flow chart of the semi-industrial plant with* heatable 5 L ploughshare mixer. The ice condenser is used as vacuum buffer as well.**

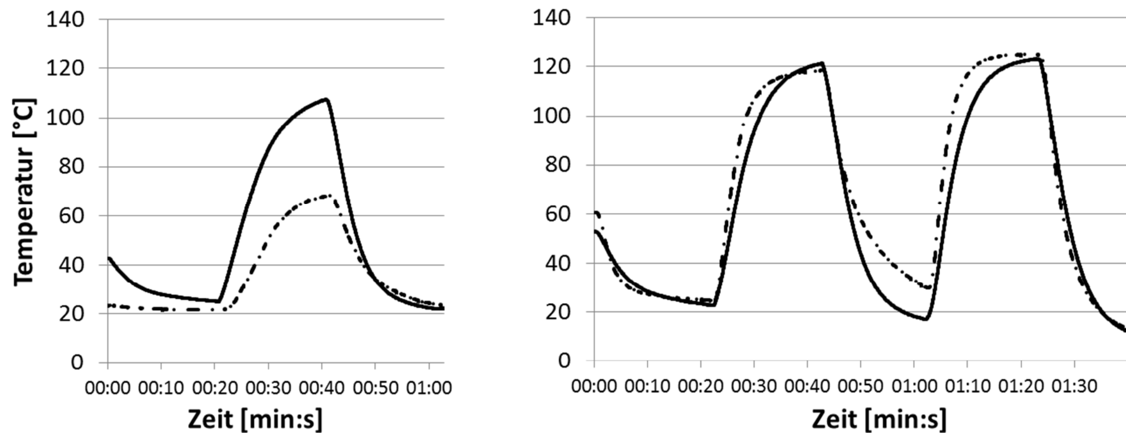

Abbildung 3: Zwei Beispiele eines typischen Temperaturverlaufs bei der mechanischen Sattdampfentkeimung. Entkeimt wurde Kapuzinerkresse. Links: Einfach bei 20°C Manteltemperatur und 110°C Dampf. Rechts: Doppelte Bedampfung bei 80°C Manteltemperatur und 125°C Dampf. Durchgezogene Linie: Kopfraumtemperatur, also die Temperatur des Dampfes. Gestrichelte Linie: Temperatur gemessen im Sumpf des Mischers, also etwa die Oberflächentemperatur der Kresse.
Fig. 3: Two examples of typical temperature sequence during the mechanical saturated steam decontamination. Nasturtium was decontaminated. Left: one time decontamination with 20°C jacket and 110°C steam. right double decontamination with 80°C jacket and 125°C steam. Solid line: temperature of headspace Dashed line: temperature at the bottom of the mixer and thus the surface temperature of the nasturtium

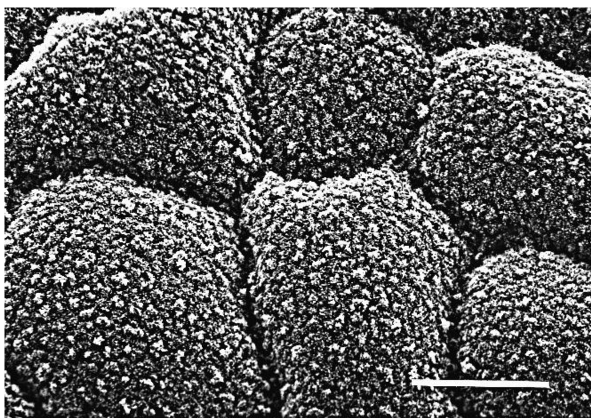

Abbildung 4 Oberfläche von Kapuzinerkresse. Wachsschuppen bilden eine wasserabstoßende Oberfläche (Lotus-Effekt). Balken = 20 µm. Übernommen aus [10].
Fig. 4 Surface of nasturtium. Wax scales build a hydrophobic layer.(lotus effect). Bar is 20 µm. From [10]

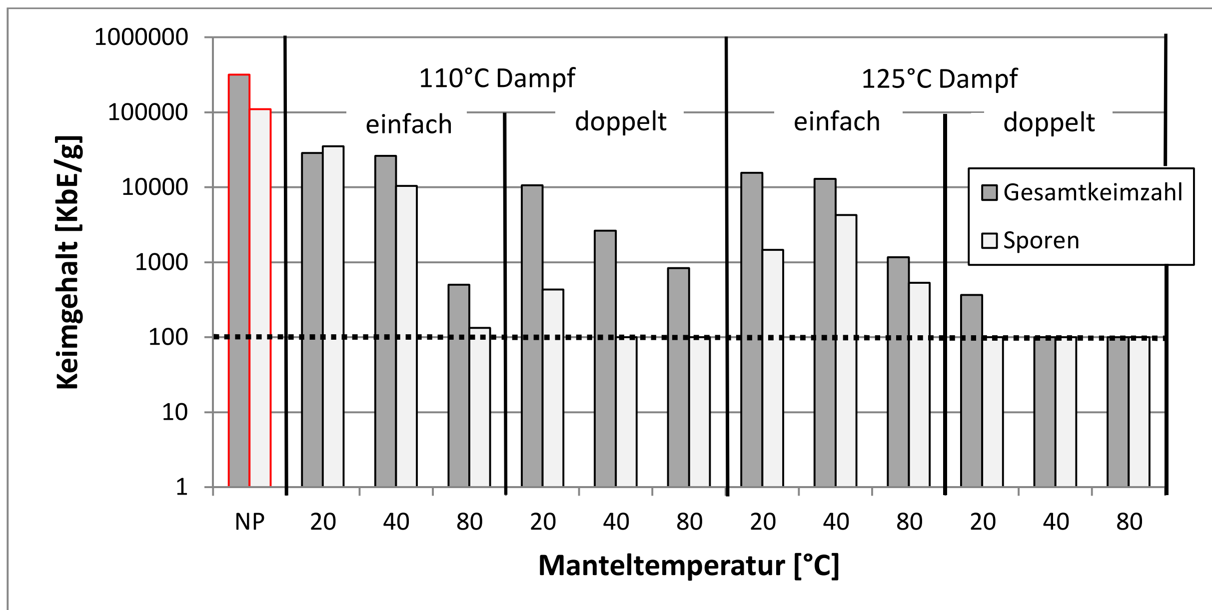

Abbildung 5 Keimreduktion bei Zwiebeln durch die mechanische Sattdampfentkeimung unter Variation der Dampftemperatur, der Manteltemperatur sowie der Behandlungswiederholung.
Fig. 5: Germ reduction of onion by the mechanical saturated steam decontamination by varying steam temperature, jacket temperature and number of treatment.

Tabelle 1: Reduktion der aeroben Keime (vegetativ und Sporen) bei der mechanischen Sattdampfentkeimung. Bei allen Medizinaldrogen wurden bei optimierten Bedingungen 100 KbE/g Sporen erreicht. Tab. 1: Reduction of aerobic germs (vegetative and spores) by the mechanical saturated steam decontamination. Spores on all drugs could be reduced to less than 100 cfu/g.

Produkt	Reduktion Gesamtkeimzahl [Dekaden]	Sporenreduktion [Dekaden]
Zwiebel (Knolle, gewürfelt)	3	3
Leinsamen (Körner)	3-4	2
Fenchel (Körner)	4	2
Majoran (Blätter)	1	2
Kapuzinerkresse (Blätter)	4	3-4

Tabelle 2: Keimreduktion von Kapuzinerkresse bei der mechanischen Sattdampfentkeimung (Dampf 110°C, 20 s, Mantel 80°C). Vergleich von Proben im Mischer und vor dem Rückhaltesieb. *: Je zwei von drei Messwerten unter der Nachweisgrenze von 100 KbE/g Tab. 2: Germ reduction of nasturtium by the mechanical saturated steam decontamination (Steam 110°C, 20 s, Jacket 80°C) Comparison of material within the mixer and at the strainer. *two of three experiments showed spores below the detection limit of 100 cfu/g.

	Nachgewiesene Keime [KbE]	
	Gesamtkeime	Sporen
Ausgangsverkeimung (N_0)	$6,9*10^6$	$5*10^4$
Entkeimt im Mischer	$0,9*10^3$	$1,4*10^2$ *
Entkeimt am Sieb	$4,2*10^3$	$2,4*10^2$ *

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