Journal of Applied Botany and Food Quality 85, 34 - 40 (2012)

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

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(Received March 16, 2012)

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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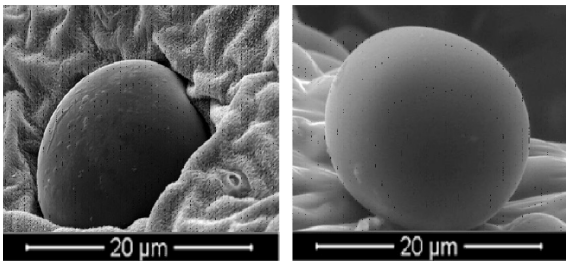

Fig. 1: Scanning electron microscopy (SEM) images of peltate glandular hairs of oregano left (*Origanum vulgare*) and marjoram right (*Majorana hortensis*).

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-

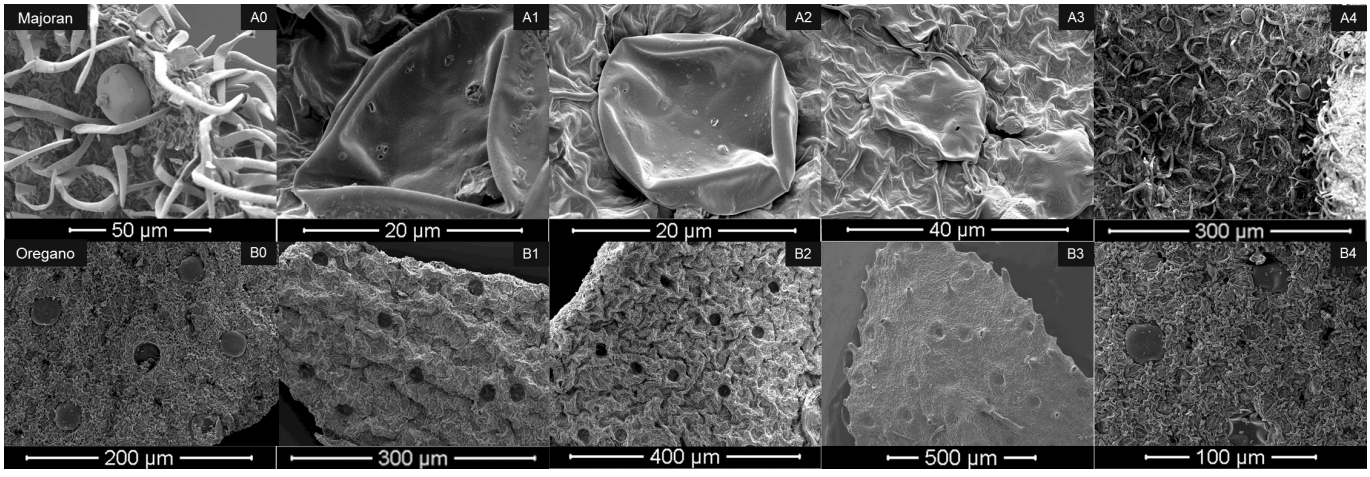

Fig. 2: ***Scanning electron microscopy of marjoram and oregano.*** A0 = on the surface-fitting intact peltate glandular hair, A1 = collapsed peltate glandular hair with large holes in cuticle after, A2 = collapsed peltate glandular hair with minor holes, A3 = collapsed peltate glandular hair with large 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

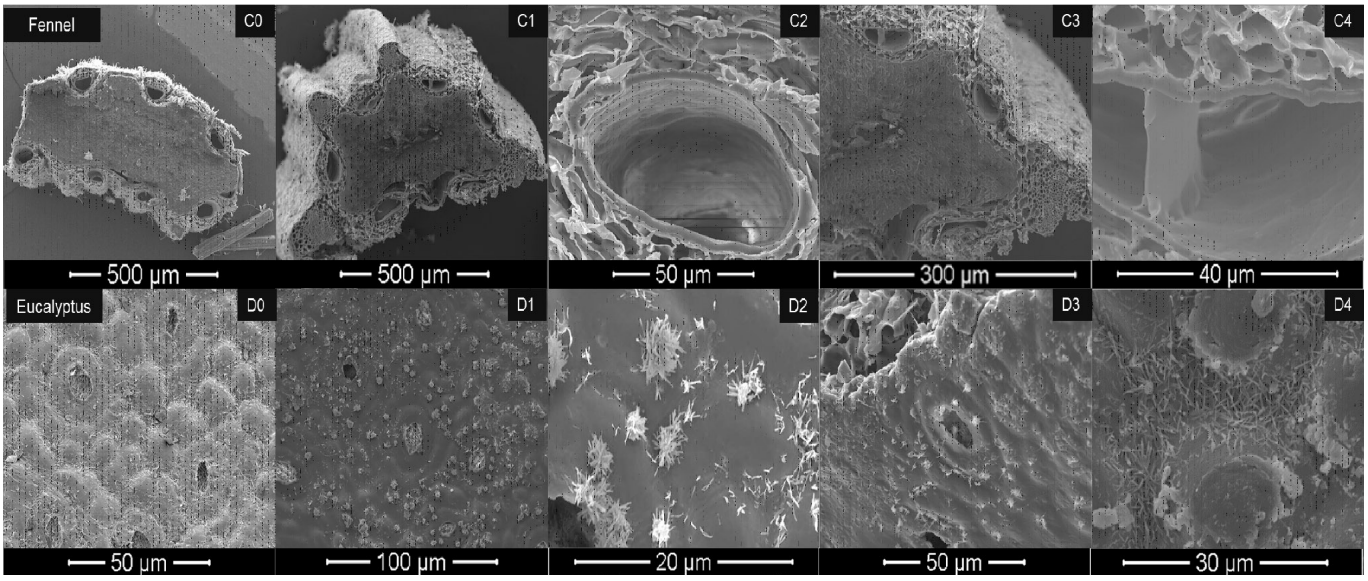

Fig. 3: Scanning electron microscopy of fennel and eucalyptus: C0 - C1= cross-sectional fennel fruit with oil channels, C3= cross-sectional fennel fruit with three oil channels, C2 - C4 = oil channel with thick channel wall and partition of fennel fruit / D0 = three oil gland vents with intact wax layer 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.

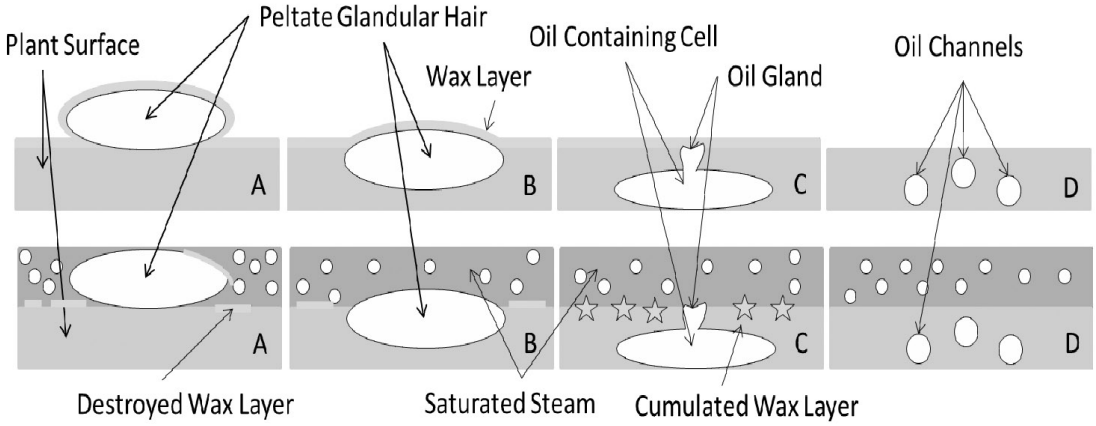

Fig. 4: **Upper panel** = Peltate glandular hair of marjoram with epidermis in a plane (A), sunken peltate glandular hair of oregano (B), oil containing cell and oil gland of eucalyptus (C) and oil channel of fennel (D) before the steam deposition phase in which the wax layer is fully functional. **Lower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

Acknowledgements
The research project (AiF-RP-No. 15547 BG) was supported from the budget of the Federal Ministry of Economic Affairs through the Arbeitsgemeinschaft industrieller Forschungsvereinigungen "Otto von Guericke" e.V. (AiF) (Association of Industrial Research Organisations). We would like to thank all funding organizations. We are very grateful to Elke Zimmermann for excellent technical assistance in sample preparation for SEM analysis and to Christine Langanke as well as Bärbel Zeiger for great reprocessing and analytic measurement of the plant materials.

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