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Toxicology

Preliminary applications of Ultra High Pressure (UHP) in deactivation of microflora contaminating cosmetics

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Summary:

Introduction. The aim of the study was a preliminary assessment of potential UHP application in deactivating environmental bacterial and fungal microflora, as well as bacteria commonly inhabiting human skin in contaminated aqueous extracts of mint and camomile.
Material and methods. The assays were conducted using *Pseudomonas aeruginosa*, S*taphylococcus aureus* and *Staphylococcus epidermidis* at 10^6 CFU/ml, as well as environmental bacterial and fungal microflora contaminating the examined herbal extracts. Microbial samples were exposed to the pressure of 500 MPa for 30 min at 20°C and 600 MPa for 30 min at 50°C. The effect of pressure ranging from 200 to 400 MPa at 50°C was also assessed using a 15-minute exposure of aqueous mint extracts contaminated with *P. aeruginosa* and *S. epidermidis*.
Results. The obtained results indicate that a complete deactivation of mesophilic aerobic spore-forming bacteria, both in mint and camomile extracts, occurs under the pressure of 600 MPa at 50°C after a 30-minute exposure. Complete deactivation of the *P. aeruginosa* and *S. epidermidis* strains, whose suspensions were added to the herbal extracts, was observed after a 15-minute exposure to the pressure of approx. 400 MPa. Lower pressure (approx. 300 MPa) applied for the same amount of time caused a radical but not complete decrease in the number of live bacterial cells. The lowest pressure applied in our study (200MPa) had no effect on the survival of the examined bacterial strains.
Conclusion. Based on the obtained results we may presume that the UHP method of liberating cosmetic herbal extracts from bacterial contamination may be the method of choice in the production of cosmetics.

Key words: cosmetic products, microbiological contamination, UHP.

Introduction

Chemical agents used for conservation of cosmetics have bacteriostatic and fungistatic, or even bactericidal and fungicidal effect. Due to those properties, the agents may not be neutral for the skin of the user. This is one of the reasons for searching other nonchemical methods of preserving the microbiological durability of cosmetic products. In recent years, much attention has been given to the microbiological purification of alimentary products by

subjecting them to Ultra High Pressure (UHP). UHP destroys opportunistic microflora and, according to current knowledge, remains neutral for the physicochemical structure and sensoric properties of exposed products [1-5].

Therefore it seems interesting to elucidate the potential of UHP application in the liberation of cosmetic raw materials and intermediate products from unwanted microflora. No reports on that topic have been found in the available literature.

The aim of the study was a preliminary assessment of potential UHP application in deactivating environmental bacterial and fungal flora, as well as bacteria commonly inhabiting human skin in the simplest but representative cosmetic products.

1. Material and methods

1.1. Examined cosmetic products

The simplest, but at the same time the most popular cosmetic products were used in the assays, namely aqueous extracts of mint and camomile.

To prepare them, 100 g of each of the commercially available herbs were weighed and poured over with 1 L of distilled water boiled shortly beforehand and cooled down to 60°C. The mixtures were then left for 3 h at room temperature. Subsequently, the extracts were filtered and subjected to UHP.

The study was conducted using unidentified strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from bacteriological study samples[1]. The bacteria, cultured for 24 h in nutritive broth, were used to quantitatively inoculate the prepared herbal extracts, previously subjected to autoclave sterilization at 120°C for ½ h to deactivate the contaminating environmental microflora. Once UHP treatment was applied, the number of live bacteria cells was determined.

1.2. Examination of the effect of UHP treatment on environmental bacterial and fungal microflora contaminating mint and camomile extracts

High-pressure assays were conducted on the U11 Compressor device at the Institute of High Pressure Physics, Polish Academy of Sciences in Warsaw, Poland.

Procedure

The examined extract samples were transferred into dedicated 3 ml polypropylene ampoules, placed in welded plastic wraps from which air was removed, and then subjected to high pressure. A 1:1 mix of polypropylene glycol and distilled water was used as the medium for direct pressure transfer.

Pressure methods

1) Application of 50 MPa for 10 min at 20°C.
2) Double reiteration of the above treatment after 10 – and 40-minute pauses. Steps 1 and 2 were aimed to stimulate the vegetation of bacteria and fungi (including mould) contaminating the hoods.
3) Application of 500 MPa for 30 min.
4) Application of 600 MPa for 30 min.

Immediately after the exposure, quantitative cultures were plated on nutritive agar and Sabouraud agar (Biomed). Determination of live bacterial and fungal cells, not deactivated by high pressure, was performed according to the PN-ISO 4833:2004 [6] and PN-ISO 7954:1999 [7] standards[2].

1.3. Examination of the effect of UHP treatment on the *S. epidermidis* and *P. aeruginosa* suspensions in mint and camomile extracts

The studied bacterial strains were cultured in nutritive broth for 24 hours at 37°C. Fully grown cultures were centrifuged for 30 min at 6000 rpm. Thus created cell pellet was washed three times with distilled water with subsequent centrifugation as above. The washed pellet was then resuspended in water and cell density of the suspension was determined by counting colonies grown from plated serial logarithmic dilutions. A working suspension of 10^6 CFU/ml was then prepared from the initial suspension and 0.1 ml of the former was added to the previously prepared samples of mint and camomile extracts. Thus created suspensions were treated as in the aforementioned quantitative analysis of environmental

1. The strains were kindly granted by the Bacteriological Analyses Laboratory of the Military Institute of Hygiene and Epidemiology in Pulawy, Poland.

2. Quantitative analyses of microflora survival were conducted at Department of Food and Consumer Articles Research, National Institute of Hygiene in Warsaw, Poland.

microflora contamination. Immediately after the exposure to high pressure, the samples were quantitatively plated on nutritive agar for subsequent colony count.

2. Results

Table 1 presents the results of the survival studies of the environmental bacteria contaminating aqueous extracts of mint and camomile exposed to the pressure of 500 MPa for 30 min at 20°C.

Table 1: Survival of environmental bacterial flora in mint and camomile extracts exposed to the pressure of 500 MPa for 30 min at 20°C.

Sample type	Type of isolated bacteria	Pre-exposure cell count [CFU/ml]	Post-exposure cell count [CFU/ml]	% deactivation
Mint extract	Mesophilic aerobic	4.4 x l04	4.2 x 102	>99
Camomile extract	Mesophilic aerobic	2.4 x 105	2.9 x 102	>95
Mint extract	Bacillus cereus	1.2 x l02	0	100
Camomile extract	Bacillus cereus	2.2 x 102	0	100
Mint extract	Other mesophilic spore-forming	1.1 x 103	4.0 x l01	>99
Camomile extract	Other mesophilic spore-forming	2.3 x 103	4.0 x 101	>99

Data presented in Table 1 indicate that in the conditions applied during UHP exposure of mint and camomile extracts, under the pressure of 500 MPa, a radical decrease in the number of live mesophilic aerobic bacteria was obtained in both extracts with a complete deactivation of *B. cereus* and almost complete deactivation of other spore-forming bacteria. Since in the applied conditions we did not obtain a complete deactivation of all examined mesophilic aerobic bacteria and spore-forming bacteria (apart from *B. cereus*), in the second step of the study the applied pressure was increased to 600 MPa and the temperature was increased to 50°C, while the 30-minute exposure time was retained (Tab. 2). Samples subjected to this assay were only examined for the presence of aerobic spore-forming bacterial flora, as it was presumed a priori that the non-spore-forming microflora would be completely deactivated.

Table 2: Effect of the exposure to the pressure of 600 MPa for 30 min at 50°C on the survival of mesophilic aerobic spore-forming bacteria in mint and camomile extracts.

Sample type	Pre-exposure cell count [CFU/ml]	Post-exposure cell count [CFU/ml]	% deactivation
Mint extract	6×10^2	0	100
Camomile extract	8.8×10^3	0	100

Data presented in Table 2 indicate that under pressure increased to 600 MPa all mesophilic aerobic spore-forming bacteria were completely deactivated, both in mint and camomile extracts. Table 3 presents the results of the survival studies of the microscopic fungi in aqueous extracts of mint and camomile, exposed to the pressure of 600 MPa at 50°C for 30 min, i.e. the conditions in which all bacteria were deactivated in both extracts.

Table 3: Effect of the exposure to the pressure of 600 MPa for 30 min at 50°C on the survival of microscopic fungi in mint and camomile extracts.

Sample type	Pre-exposure cell count [CFU/ml]	Post-exposure cell count [CFU/ml]	% deactivation
Mint extract	5.1×10^2	0	100
Camomile extract	1.4×10^2	0	100

Data presented in Table 3 indicate that the exposure to the pressure of 600 MPa for 30 minutes led to a complete deactivation of the microscopic fungi contaminating the examined mint and camomile extracts. Table 4 presents the results of the second part of the UHP effect study on *P. aeruginosa* and *S. epidermidis* suspensions. Since no difference in the results obtained with the mint and camomile

extracts was observed, this part of the study was conducted using only the former extract.

Table 4: Results of the P. aeruginosa and S. epidermidis suspension deactivation in mint extract exposed to various UHP values at 50°C during a 15-minute exposure.

Sample type	Pressure (MPa)	Pre-exposure cell count [CFU/ml]	Post-exposure cell count [CFU/ml]	% deactivation
Suspension of *P. aeruginosa* in mint extract	200	1.0 x l0^5	1.0 x 10^5	0
Suspension of *P. aeruginosa* in mint extract	300	1.0 x 10^5	1.3 x l0^2	>97
Suspension of *P. aeruginosa* in mint extract	400	1.0 x 10^5	0	100
Suspension of *S. epidermidis* in mint extract	200	1.0 x 10^5	1.0 x 10^5	0
Suspension of *S. epidermidis* in mint extract	300	1.0 x 10^5	1.9 x 10^2	>98
Suspension of *S. epidermidis* in mint extract	400	1.0 x 10^5	0	100

Data presented in Table 4 indicate that a complete deactivation of the *P. aeruginosa* and *S. epidermidis* strain suspensions was obtained after a 15-minute exposure to the pressure of 400 MPa at 50°C. On the other hand, the applied pressure of 300 MPa at the same exposure time resulted only in a decrease in the number of live bacteria cells, while the lowest applied pressure of 200 MPa did not reduce the populations of the examined bacterial strains.

3. Discussion

To search nonchemical methods of preserving the microbiological durability of cosmetic products, a preliminary assessment of potential high pressure application for that purpose was conducted. The obtained results indicate that some bacteria may be resistant to the deactivating action of a pressure as high as 500 MPa. However, the combination of 600 MPa and the temperature increased to 50°C with appropriate duration of the exposure to both of these factors resulted in a complete deactivation of the entire environmental microflora occurring in mint and camomile extracts. Encouraging may be the relatively high sensitivity to UHP observed for the *Aeromonas hydrophila* strain related to the aqueous environment, whose reduction in cheese may already be obtained by applying the pressure of 300 MPa for 14.58 min (8). This information may be interesting if we consider that many cosmetics have ingredients derived from algae and other aquatic organisms.

By examining the effect of UHP on the most common bacteria, which are *S. aureus* and *P. aeruginosa*, we obtained results which justify further studies, as both bacterial strains were found to be relatively sensitive to UHP. It is a significant finding, since those species constitute both environmental microflora and potential pathogens.

The *Pseudomonas* group may be found in soil, water, sewage and air. The bacteria usually colonize new locations as the first organisms, as long as minerals and organic acids or saccharides are available. Similarly to the examined *S. aureus*, the *Pseudomonas* group contains pathogenic strains producing toxins. They are the cause of abscess formation. *Pseudomonas* also produce endotoxins during their growth on improperly stored food (9). Considering fungal contamination, the obtained results provided a significant finding that this type of eukaryotic microflora is more sensitive to UHP that bacteria.

4. Conclusions

1) Exposing cosmetic herbal extracts to the UHP of 500 MPa for 30 minutes at 20°C causes a radical decrease in the number of live cells of mesophilic aerobic bacteria, complete deactivation of *B. cereus* and almost complete deactivation of other spore-forming bacteria.

2) Complete deactivation of mesophilic aerobic spore-forming bacteria and microscopic fungi, both in the mint and camomile extracts, occurs under pressure increased to 600 MPa and the temperature of 50°C during a 30-minute exposure.

3) Complete deactivation of the *P. aeruginosa* and *S. epidermidis* strains, inoculated into herbal extracts from a 10^6 CFU/ml bacterial suspension, occurs after a 15-minute exposure to the pressure of 400 MPa. A lower pressure of 300 MPa with the same exposure time results in a radical but incomplete decrease in the number of live bacterial cells. The lowest applied pressure of 200 MPa does not reduce the populations of both examined bacterial strains.

4) Based on the obtained results we may presume that this method of liberating cosmetic herbal extracts from bacterial contamination may be the method of choice in the production of cosmetics.

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