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ORIGINAL PAPER

Microbiological characterization and sterilization-induced changes in the profile of the hydrophobic organic substances in Latvian balneological peat

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Abstract The abundance and predominant groups of bacteria, filamentous fungi and yeasts have been studied by culture-dependent microbiological methods in peat probes obtained in two Latvian balneotherapy spa sites, Kemeri and Baldone. Unsterilized peat samples from both the sites contained 5.7–8.1 log bacterial colony-forming units (CFU) and 3.0–5.3 log fungal CFU per gram of dry peat. Isolated species belonged to *Alpha*-, *Beta*-, and *Gamma-Proteobacteria*, *Actinobacteria*, *Clostridia*, *Bacilli* and *Flavobacteria* as well as to filamentous fungi and yeasts. The composition of microbial population of the peat from both sites shared just four micro-organism groups (*Bacillus mycoides*, *Burkholderia cepacia*, *Streptomyces* spp. and *Trichoderma* spp.) within totally 36 groups identified. No pathogenic bacteria or fungi and no faecal pollution indicators were recovered. Decimal reduction doses for micro-organisms in peat samples and radiation sterilization doses of peat for the gamma and electron beam radiation were determined. The highest radiation resistance was observed for *B. mycoides* and *Aureobasidium* sp. Gamma-sitosterol was the most abundant hydrophobic organic compound in both peats according to GC–MS data. All the sterilization procedures increased concentration of alkanes, alcohols, and ketones and decreased the amount of fatty acids. Heat sterilization proved to be more preserving for the peat sterols than the radiation sterilization. It is concluded that the heat and radiation sterilization methods induce different changes of the profile of hydrophobic organic compounds of balneological peats, what may lead to different therapeutic effects at their application.

Keywords Balneological peat · Micro-organisms · Gamma irradiation · Electron beam irradiation · Sterols

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Introduction

The peat from boggy deposits (peat mud, peat peloids and medicinal peat) is widely used in balneotherapy (Uosukainen and Pihlaja 2006). Peat extracts and isolated substances are of great interest for their possible applications in dermatology and cosmetology (Wollina 2009).

Balneotherapy has been introduced in Latvia since the end of the XIX century in the Kemeri and Baldone resorts using their own boggy peat deposits for the spa procedures. The Kemeri Moorland can be considered as a territory little affected by the human activities (Pakalne 2008). Balneological peat for the Kemeri resort is yielded by Slokas raised bog (ombrotrophic mire). The diseases of neural system, joints, bones and muscles are treated by mud baths and mud applications in Jaunkemeri Spa (Terentjeva and Fridenberga 2008). Baldone boggy peat is yielded in Pladu fen (minerotrophic mire). Its extracts are used in cosmetic and hygiene ointment formulas, as well as distributed to the cosmetic cabinets in the form of dried powder or NaCl-supplemented (preserved) suspension lots.

Although the quality standards for the balneological peat and other natural peloids are not officially approved in EU

countries, the possible contaminants should be carefully controlled. The main microbiological concern is the presence of the pathogenic bacteria, yeasts or moulds. Pathogens or opportunistic pathogens include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella* spp., *Candida albicans* and *Aspergillus niger*. Maximal acceptable titre for coliform bacteria is 10,000 per 100 ml and for *Escherichia coli* 2,000 per 100 ml (Quality Criteria of the European Spas Association 2006). Limited quantities of other micro-organisms, which are generally regarded as harmless are permitted in cosmetics. These standards may be extended also to the peat mud used in balneotherapy. For the Category 1 products of cosmetics intended for the children under 3 years of age or for the use in the area of eyes and on the mucous membranes, the total viable count of aerobic mesophilic micro-organisms should not exceed 100 colony-forming units (CFU) per 1 g or 1 ml. For the other products, the total viable count for aerobic mesophilic micro-organisms should not exceed 1,000 CFU per gram or millilitre (SCCS/1501/12 2012). Although molecular techniques are valuable to discover microbial community changes and potential pathogens (Feng et al. 2013), standards still use the culture-dependent methods.

Natural balneological peats are heavily inseminated with micro-organisms, and the traditional approach to reduce this contamination is the heat treatment. However, chemical substances, which may be important to ensure the health beneficial effects of the peat mud, may be degraded under harsh thermal treatment. The chemical structure of peat contains both hydrophilic and hydrophobic components, which will penetrate the skin surface during mud applications (Odabasi et al. 2007) and would be affected by heat treatment. Therefore, alternative methods, e.g. radiation sterilization, should be considered.

Ionizing radiation induces direct and indirect, free-radical-mediated changes in the structure of biological macromolecules, leading to the inactivation of micro-organisms (review, Da Silva Aquino 2012). Decimal reduction dose (D_{10}) is the absorbed radiation dose required to reduce the number of viable micro-organisms to 10 % of its original number. An absorbed dose of 25 kGy is usually required to sterilize any substrates or instruments. This dose is useful for initial contamination levels of $\leq 10^3$ CFU per unit (ANSI/AAMI/ISO 2012). It is equivalent to sterilization with saturated steam at 121 °C for 15 min or dry heat at 160 °C for 2 h. Generally, at this dose, a sterility assurance level (SAL) of 10^{-6} is achieved what is required for medical products that are likely to come in contact with human tissues (IAEA 1990). However, higher doses of radiation may be required at higher initial microbial contamination rates if the radiation-resistant cells are present in the sample (Mukherjee 1974). Radiation resistances vary widely among different micro-organisms. Bacterial endospores are generally more resistant to radiation than vegetative bacteria (A Joint FAO/IAEA/WHO Study Group 1999).

The aim of this work was to characterize the microbiological profile of the boggy peat from two traditional Latvian spa centres located in Kemeri and Baldone (within 40 km distance from the capital of Latvia, Riga) and to evaluate the chemical changes in the profile of the peat hydrophobic organic substances induced by heat and radiation sterilization. The experiments reported in this paper were done in the time period from January 2012 till May 2013 at the University of Latvia in Riga.

Materials and methods

Peat

Peat samples were obtained from two traditional Latvian spa centres located in the vicinity of the capital city Riga. Kemeri Spa peat was supplied by "Rider-Ta" Ltd. (Latvia). Content of organic matter was 98 %. Value of pH was 3.06. Moisture content was 95 %. Baldone Spa peat was received from ELIER International Ltd. (Latvia). Two kinds of peat were analysed: (1) dry and (2) conserved, with moisture content 98 %. Manufacturers declared content of the conserved peat: aqua, peloid and sodium chloride. Dry peat had a pH value of 6.16, while conserved peat peloid had pH value of 5.34.

Three lots of Kemeri peat and six lots in total of Baldone dry and conserved peat were analysed. In laboratory, peat was stored in original formulae at 4 °C and analysed during 2 weeks.

Chromatographical analyses

Hydrophobic compounds in the Kemeri and Baldone peat, both untreated or sterilized by gamma irradiation, electron beam irradiation and heat treatment, were subjected to gas chromatography/mass spectroscopy. Analyses were carried out in duplicate. Samples were dried over NaOH in the desiccator. Four grams of dry peat was extracted with 30 ml of acetone: *n*-hexane (1:1) in an ultrasound bath for 15 min and kept at room temperature for 24 h. The liquid phase was separated, and sediment was rinsed twice with 20 ml of extraction solution. All solutions were combined, evaporated under flux of nitrogen until final volume 2.0 ml and filtered through 0.45-μm PTFE filter.

The extracts were analysed using Shimadzu QP2010 GC–MS system operating at 70 eV with a mass range of *m/z* 50–500 in the scan modus. A capillary column Restek Rtx1MS (30 m × 0.25 mm, thickness 0.25 μm) was used with helium as the carrier gas at a flow rate 1 ml min^{-1}.

The oven temperature was programmed as follows: 60 °C for 1 min, then 310 °C at a rate of 10 °C per minute, with a hold time of 1 min, 320 °C at 3 °C min^{-1}, with a hold time of 4 min. One microlitre of the sample was split injected with split ratio 30.0. Injection temperature was 300 °C. Ion source temperature was 250 °C. The eluent from the column was transferred, via an interface line heated to 250 °C. Components were identified by comparison of their mass spectra and retention times with synthetic standards or published data.

Microbiological analyses

The amount of cultivable micro-organisms was analysed in three lots of Kemeri peat, three lots of Baldone dry peat and three lots of Baldone conserved peat. The tests were performed in triplicate. Ten grams of peat samples was suspended in 90 ml of sterile water in 500-ml Erlenmeyer flasks by shaking at room temperature at 150 rpm for 30 min (Pepper et al. 1995; Alef and Nannipieri 1998). In total, 0.1 ml from the serial dilutions of the suspensions was plated on or inoculated in the following media: R2A (Becton & Dickinson, France) for aerobic bacterial plate count; malt extract agar (MEA, Becton & Dickinson, France) for fungal count; Endo agar (Becton & Dickinson, France) for most gram-negative bacteria, thioglycollate broth (Bio-Rad, France) for anaerobic bacteria count and medium for sulphate-reducing bacteria (peptone 10.0 g l^{-1}, NaCl 5.0 g l^{-1}, yeast extract 1.0 g l^{-1}, trisodium citrate 0.2 g l^{-1} and agar 1.5 g l^{-1}). Endo agar plates were incubated at 37 °C for 24 h. R2A and MEA plates and thioglycollate and sulphate-reducing bacteria broth tubes were incubated at 20 °C for 7 days. The detection limit was ten CFU g^{-1}.

The number of micro-organisms was expressed as logarithms of CFU per gram of sample. The total number of cultivable bacteria was estimated as the number of CFU of the micro-organisms per gram of dry peat on R2A after 120 h at 20 ± 2 °C (Vanderzant and Splittstoesser 1992). The mean and standard deviation (SD) were calculated. The significance of the difference in values was determined by ANOVA analysis at a significance level of 0.05.

Based on colony and cell morphology, predominant micro-organisms were isolated from the highest dilutions of peat samples and purified using streaking method. Identification of bacterial pure cultures was performed with BBL® Crystal™ Gram-Positive ID kit, Enteric/Nonfermenter ID kit and Anaerobe ID kit (Becton & Dickinson, USA). Genera of the isolated filamentous fungi were identified using macroscopic and microscopic appearance and keys (Barnett 1957; Kiffer and Morelet 2000). Yeasts were identified using Auxacolor™ (Bio-Rad, France).

Electron beam and gamma irradiation, heat treatment of peat samples

Radiation sterilization of the peat samples was performed in cylindrical polypropylene vials (Sarstedt, Germany; diameter 30 mm, height 100 mm). Experiments were carried out in duplicate.

The 5 MeV electron beam with 0.10 μA cm^{-2} s^{-1} current and up to 30 kGy h^{-1} power was obtained from the linear accelerator ELU-4 (Thoriy Ltd., former Soviet Union) at solenoid current 44 A and the magnetron current 0.16 A. The beam divergence angle was 30°; at 10 cm from the centre of the beam; the radial distribution of the dose was up to ± 15 %. Sample vials were placed at 100 cm distance from the electron window for 10, 30, 45 or 90 s. Temperature of the samples after irradiation was in the range of 30–40 °C. The electron beam was converted into gamma radiation at 3 % efficiency by targeting water-cooled 1.0-mm tungsten converter. The obtained gamma radiation had continuous spectrum with the maximum energy of 5 MeV and the mean energy of 1.5 MeV at up to 25–30 kGy h^{-1} power. Fricke dosimeter was used to determine the absorbed gamma ray dose. Irradiation time 80, 40, 20, 8 min. for absorbed dose 30, 15, 7.5 and 3 kGy, respectively, distance of a sample from electron beam window 50 cm.

Decimal reduction dose (D_{10}) was calculated from the equation: $D_{10} = D/(\log N_o - \log N)$, where D is the applied radiation dose, kGy; N_o the initial bacterial number, and N the bacterial number after the irradiation (Patterson and Loaharanu 2000). The radiation sterilization dose (RSD) required for 10^{-6} SAL was calculated from the equation: $RSD = D_{10}$ (Log Bioburden–Log SAL), where Log Bioburden is logarithm of number of viable micro-organisms (Yusof 1999; Hilmy et al. 2003).

Heat sterilization of the peat samples was carried out in a steam autoclave at 121 °C for 15 min.

Results and discussion

Micro-organisms in peat

Native peat samples from the analysed spa locations contained versatile populations of micro-organisms with different dominating cultivable species and species composition. Pathogenic bacteria and bacterial indicators of faecal pollution, *E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans* or *A. niger* were not recovered from the studied peat samples. We cannot totally rule out the presence of pathogens and indicators of faecal pollution because culture-dependent methods with the detection limit of 10 CFU g^{-1} were used. However, the concentration of

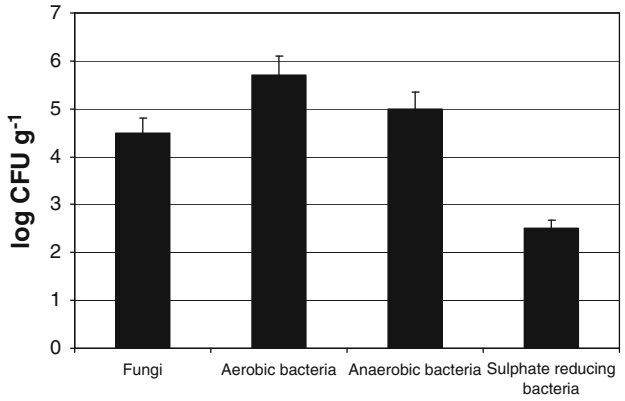

Fig. 1 Number of CFU of micro-organisms per gram of Kemeri peat. The tests were performed in triplicate, and the final results were presented as the arithmetic average ± SD

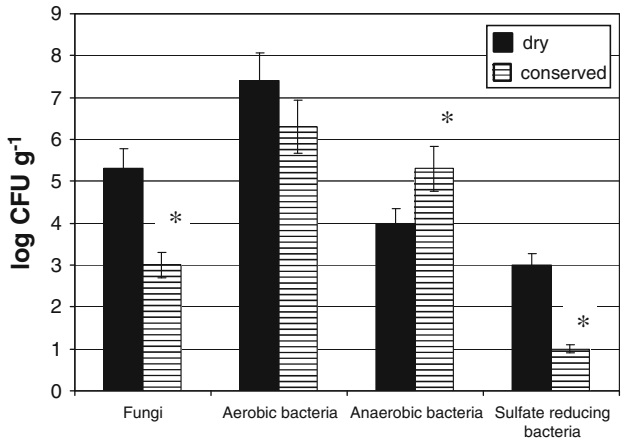

Fig. 2 Number of CFU of micro-organisms per gram of Baldone dry and conserved peat. The tests were performed in triplicate, and the final results were presented as the arithmetic average. $*P < 0.05$ log CFU g^{-1} in dry versus conserved peat

eventual pathogens in the studied substrates was well below minimal infectious dose needed to cause illness.

The counts of aerobic bacteria per gram of dry Kemeri peat averaged at 5.7 log CFU, anaerobic bacteria at 5.0 log CFU, sulphate-reducing bacteria at 2.5 log CFU and fungi at 4.5 log CFU (Fig. 1). The counts of aerobic bacteria per gram of dry Baldone peat averaged at 7.3 log CFU, 4.0 log CFU of the anaerobic bacteria, 3.0 log CFU of the sulphate-reducing bacteria, as well as 5.3 log CFU of the fungi. In NaCl-preserved Baldone peat the abundance of aerobic bacteria, sulphate-reducing bacteria and filamentous fungi was substantially reduced, while the abundance of anaerobic bacteria was increased (Fig. 2).

The micro-organisms, which were obtained in pure cultures and identified at least at the level of genus, are listed in Table 1. The bacteria isolated from Baldone peat belonged to *Beta-* and *Gamma-Proteobacteria*, to the *Actinobacteria* and *Bacilli*. The bacteria isolated from Kemeri peat belonged also to *Alpha-Proteobacteria*, *Clostridia* and *Flavobacteria* (Table 1). The predominant species was cosmopolitan widespread *Burkholderia cepacia* belonging to the *Beta-Proteobacteria*. Bacteria of the genus *Burkholderia* constitute a substantial portion of the aerobic chemoorganotrophic isolates in sphagnum peat bogs (Belova et al. 2006). Some of the *B. cepacia* strains express the proteins, e.g. adhesins, siderophores, extracellular proteases, lipases and haemolysins (Leitao et al. 2010), which are needed to develop pathogenic potential (Luigi 2006). Despite the absence of pathogenic organisms and indicators of faecal pollution notable microbial count and ubiquity of *B. cepacia* suggest the necessity of sterilization before the employment of the peat in balneologic procedures. The predominant bacterial species in Baldone peat were *Bacillus mycoides*, *B. licheniformis*, *Leifsonia aquatica* and *Moraxella* sp. (Table 1).

Two groups of filamentous fungi, *Fusarium* and *Gliocladiopsis*, and two groups of yeasts, *Lipomyces* and *Rhodotorula*, were isolated at log 1 CFU level only from the NaCl-preserved Baldone peat. On the other hand, preservation resulted in the loss of *Brevibacillus brevis* and *Streptomyces* sp. *B. brevis* is known as an antagonistic and plant-growth promoting rhizobacterium (Seddon et al. 2000), while *Streptomyces* play a role in the degradation of cellulose (Pankratov and Dedysh 2009) and lignin (Zeng et al. 2013). All the samples contained *Trichoderma* spp.

Our study provides insight into the microbial composition of the commercial peat products for balneotherapy spa. The phylogenetic diversity of complex microbial communities in peatlands, including fens and bogs, which may be stratified along an ecohydrological gradient, has been studied by Juottonen et al. (2005), Morales et al. (2006) etc. Careful handling, special sampling and treatment methods are required to maintain the anoxic conditions of the natural peat despite of the capability of bacteria to create microenvironment niches (Hunting and Kampfraath 2013). Commercial products are collected, stored and processed under aerobic conditions. Therefore, most of the micro-organisms isolated in our study, with exception of strict anaerobic *Actinomyces*, *Clostridium*, *Lactobacillus* and *Tissierella*, belong to the aerobic or facultative anaerobic groups (Table 1).

Table 1 Pure cultures of micro-organisms isolated from Kemeri and Baldone peat

Group	Class	Name	Amount, log CFU g^{-1}		
			Kemeri	Baldone	
				Dry	Preserved
Gram-positive bacteria	*Actinobacteria*	*Actinomyces bovis*	3	–	–
		Corynebacterium propinquum	–	5	5
		Corynebacterium tuberculostearicum	4	–	–
		Leifsonia aquatica	–	7	6
		Streptomyces spp.	4	6	–
	Bacilli	*Bacillus licheniformis*	–	7	5
		Bacillus megaterium	4	–	–
		Bacillus mycoides	3	7	5
		Brevibacillus brevis	–	6	–
		Lactobacillus sp.	3	–	–
	Clostridia	*Clostridium paraputrificum*	3	–	–
		Clostridium sp.	3	–	–
		Tissierella praeacuta	4	–	–
Gram-negative bacteria	*Alphaproteobacteria*	*Sphingomonas paucimobilis*	4	–	–
	Betaproteobacteria	*Burkholderia cepacia*	5	5	5
	Gammaproteobacteria	*Acinetobacter* sp.	4	–	–
		Aeromonas sp.	3	–	–
		Enterobacter aerogenes	–	5	4
		Enterobacter cancerogenes	3	–	–
		Enterobacter cloacae	3	–	–
		Enterobacter gergoviae	–	5	5
		Kluyvera ascorbata	3	–	–
		Moraxella sp.	–	6	6
		Pseudomonas putida	–	5	5
		Serratia liquefaciens	4	–	–
	Flavobacteria	*Chryseobacterium meningosepticum*	4	–	–
Filamentous fungi	*Eurotiomycetes*	*Aspergillus* sp.	3	–	–
		Penicillium spp.	–	5	3
	Sordariomycetes	*Acremonium* sp.	4	–	–
		Fusarium sp.	–	–	1
		Gliocladiopsis sp.	–	–	1
		Trichoderma spp.	4	4	1
	Zygomycetes	*Mucor* spp.	–	3	1
Yeasts	*Saccharomycetes*	*Eutorulopsis sake*	4	–	–
		Lipomyces sp.	–	–	1
	Urediniomycetes	*Rhodotorula* sp.	–	–	1

– not detected

Hydrophobic substances in non-sterilized, heat-treated and irradiated peat

The balneological peat contains various hydrophilic and hydrophobic substances (Odabasi et al. 2007). Humic acids and fulvic acids belong to the most studied ones (Beer et al. 2000; Orru et al. 2011). Our attention has been directed mainly to the predominant hydrophobic components, sterols, whose presence in the peat samples may be associated with its health-improving effects. Extraction with solution of acetone/*n*-hexane removed 0.27 % of the dry matter from Kemeri peat (Table 2)

Table 2 Concentration of main organic compounds in natural, heat treated (121 °C 15 min.) and with gamma rays or electron beam (30 kGy) irradiated Kemeri peat. Extraction was carried out in acetone/*n*-hexane (1:1) solution. Results were obtained from GC–MS analyses

Compound	Similarity, %	Concentration, mg kg^{-1} (% from total organic extracted)			
		Natural	Heat-treated	Electron beam	Gamma rays
Total organic extracted		2,700	2,700	2,700	2,100
Compound classes					
Alkanes		170 (6.3)	250 (9.3)	180 (6.7)	160 (7.6)
Alcohols		100 (3.7)	110 (4.1)	200 (7.4)	170 (8.1)
Ketones		160 (5.9)	200 (7.4)	240 (8.9)	220 (10.5)
Sterols		1,700 (63.0)	1,800 (66.7)	1,300 (48.1)	1,100 (52.4)
Fatty acids		130 (4.8)	20 (0.7)	60 (2.2)	40 (1.9)
Individual compounds					
Beta-tocopherol	91	22 (0.8)	25 (0.9)	26 (1.0)	25 (1.2)
A-friedoolean-6-ene	75	180 (6.7)	220 (8.1)	110 (4.1)	100 (4.8)
n-dotriacontane	96	61 (2.2)	110 (4.1)	80 (3.0)	63 (3.0)
Alpha-tocopherol	86	45 (1.7)	50 (1.9)	54 (2.0)	47 (2.2)
D-friedoolean-14-en-3-one	88	130 (4.8)	160 (5.9)	77 (2.9)	70 (3.3)
Gamma-sitosterol	91	610 (22.6)	660 (24.4)	430 (15.9)	320 (15.2)
Stigmastanol	89	120 (4.4)	100 (3.7)	77 (2.9)	75 (3.6)
Lupeol	89	53 (2.0)	40 (1.5)	30 (1.1)	24 (1.1)
13,27-cycloursan-3-one	85	57 (2.1)	80 (3.0)	42 (1.6)	38 (1.8)
Cholestan-3-one	84	20 (0.7)	30 (1.1)	26 (1.0)	23 (1.1)
Stigmasta-3,5-dien-7-one	78	30 (1.1)	50 (1.9)	55 (2.0)	28 (1.3)
Sitostenone	87	70 (2.6)	100 (3.7)	54 (2.0)	58 (2.8)
Friedooleanan-3-ol,3-alpha	90	110 (4.1)	160 (5.9)	95 (3.5)	60 (2.9)
Friedelan-3-one	93	100 (3.7)	170 (6.3)	95 (3.5)	85 (4.0)

and 0.05 % of the dry matter from Baldone dry peat. GC–MS chromatography revealed presence of various hydrophobic organic compounds (Table 2). Sterols constituted 63 % of the organic matter of Kemeri natural peat extracted in acetone/*n*-hexane. Detailed analysis of the sterols is shown in Fig. 3. Gamma-sitosterol (clionasterol) was found to be the main sterol compound in Kemeri peat (Table 2) as well in Baldone peat (data not shown). Gamma-sitosterol has generic relation to bryophytes (Chiu et al. 1985) and algae (Khalio-Uz-Zaman et al. 1998). In Kemeri peat extracts, the second predominant compound was A-friedoolean-6-ene and the third was D-friedoolean-14-en-3-one. In Baldone peat, the second predominant compound was stigmastanol.

It is known that alpha-tocopherol and beta-tocopherol are synthesized by different plants (review, Lagarda et al. 2006). Triterpenoids with ursane, oleanane and lupane carbon skeletons are common in peatlands, especially towards the bottom of the bog. These compounds are particularly abundant in the higher plants (López-Días et al. 2010) and prove the presence of dicotyledonous angiosperm-derived organic matter (Otto and Simoneit 2001). Our findings confirm that Kemeri and Baldone peat lipids originate from mosses and higher plants. The differences in the concentration of main hydrophobic organic compounds indicate that the Kemeri peat is more humificated than Baldone peat because the content of lipidic matter increases with increasing humification (Lehtonen and Ketola 1993).

It should be considered that irradiation causes free radical formation, the reactions of their recombination and consequent changes in the chemical composition. Irradiation of Kemeri peat with gamma rays caused a 22 % decrease in the amount of the total extracted organic compounds, the decrease of the total con-

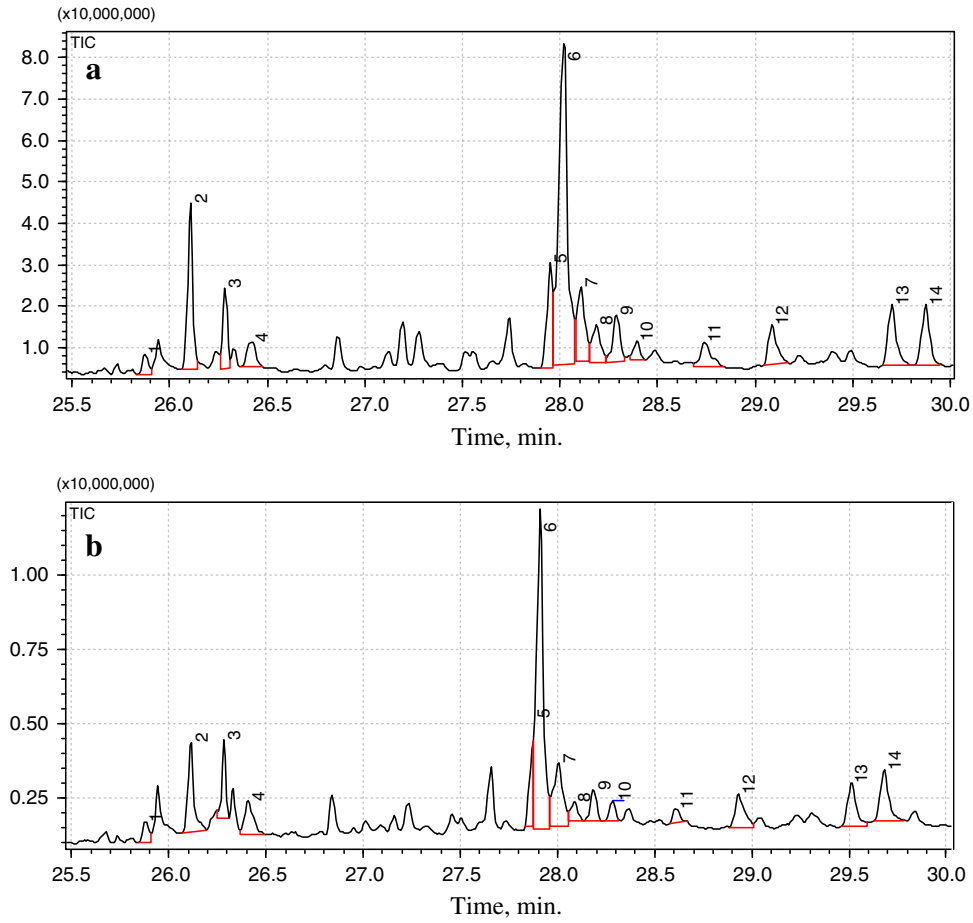

Fig. 3 GC–MS sterol group chromatogram of acetone/*n*-hexane (1:1) extract of Kemeri natural peat (**a**) and peat after gamma irradiation (**b**) with a dose of 30 kGy. The numbered peaks are as follows: *1* Beta-tocopherol; *2* A-friedoolean-6-ene; *3* *n*-dotriacontane; *4* Alpha-tocopherol; *5* D-friedoolean-14-en-3-one; *6* Gamma-sitosterol; *7* Stigmastanol; *8* Lupeol; *9* 13,27-cycloursan-3-one; *10* Cholestan-3-one; *11* Stigmasta-3,5-dien-7-one; *12* Sitostenone; *13* A-friedooleanan-3-ol,3-alpha; and *14* Friedelan-3-one

centration of sterols and fatty acids as well as the decrease of the concentration of seven individual compounds: A-friedoolean-6-ene, D-friedoolean-14-en-3-one, gamma-sitosterol, stigmastanol, lupeol, 13,27-cycloursan-3-one and friedooleanan-3-ol 3-alpha (Fig. 3), while the increase in the concentration of alkanes, alcohols and ketones was detected (Table 2). Irradiation with electron beam did not change the concentration of total extracted organic compounds. The decrease of the concentration of sterols and fatty acids, whose concentration was decreased also by the gamma rays, was compensated by the increase in the concentration of alkanes, alcohols and ketones (Table 2).

Heat-treated Kemeri peat contained increased amount of alkanes, alcohols, ketones and sterols, and decreased amount of fatty acids (Table 2). Unlike after the irradiation sterilization, there was an increased concentration of sterols after the heat treatment.

The effect of irradiation and heat treatment on the viability of micro-organisms

The possibilities of the sterilization of balneological peat by heat, electron beam and gamma radiation treatment were studied. After 30 kGy electron or gamma irradiation, no viable micro-organisms were detected in Baldone dry peat. In Kemeri peat samples, gamma radiation doses starting from 3 kGy eliminated all the viable micro-organisms. The calculated D_{10} value was $\leq$0.53 kGy, and corresponding RSD value was 6.20 kGy. Peat samples were sterile after 30 kGy and 15 kGy electron beam irradiation. Electrons at 3 kGy and 7 kGy doses resulted in significant ($P < 0.05$), but incomplete effect of sterilization, the count of micro-organisms was reduced by 1.4 and 3.6 log units, respectively. The calculated D_{10} for electron beam radiation was 2.05 kGy, and RSD value was 23.99 kGy.

The radiation-resistant endospore-forming bacterium *B. mycoides* and yeast-like fungus *Aureobasidium* sp. were

isolated from the 3 and 7 kGy electron beam irradiated Kemeri peat. The CFU number of *B. mycoides* was in the range of log 3 per gram of the dry peat. *Aureobasidium* sp. obviously was presented in low numbers (Table 1) and was detected only after irradiation. It is known endospores display significantly higher D_{10} values than vegetative bacteria. The average D_{10} for spores was estimated to be 2.11 kGy of gamma radiation, while for vegetative bacteria, this average was 0.42 kGy (Van Gerwen et al. 1999). Also the vegetative bacterial cells are more susceptible to high-speed electrons than are the bacterial spores (Hayashi et al. 1994; Elliott et al. 2005). Our estimated D_{10} value, 2.05 kGy, for electron beam treatment of endospore-forming bacteria is similar to the one calculated by Van Gerwen et al. (1999).

The more complex is the medium, the greater is the competition by its components for the radicals formed by irradiation (A Joint FAO/IAEA/WHO Study Group 1999). Peat is a complex medium; to achieve complete effect of sterilization the electron beam had to be used at a dose that is 30 % higher than the gamma rays. The fact that the radiation sensitivity of the endospores to the electron beam treatment is slightly lower than to the gamma rays has been reported also by the other investigators (Ito et al. 1993). Negatively charged high-energy electron beam has a limited penetrating power; it can be efficiently used only for the samples, which are not more than 5–10 cm thick (Earle and Earle 2003). We suggest that RSD value of 6.2 kGy of gamma irradiation or RSD of 24.0 kGy of electron irradiation is effective for sufficient full sterilization of the Kemeri peat. Complete sterilization of the peat samples was achieved at relatively low doses of gamma radiation $D_{10} \leq 0.53$ kGy. For the electron beam irradiation, D_{10} value was significantly higher, 2.05 kGy.

Heat treatment of peat samples at 121 °C for 15 min eliminated all the viable micro-organisms. The steam under pressure in an autoclave kills microbes by denaturing their proteins. Full sterilization, encompassing inactivation of bacterial endospores requires direct contact of the treated item with the steam at 121 °C and 103.4 kPa during about 15 min (Sattar 2011). Heat treatment is non-toxic, has broad-spectrum microbicidal activity and good penetrating ability. Therefore, heat is a reliable sterilant and steam treatment under pressure is the most reliable method of sterilization, although it may cause also versatile unwanted side effects by denaturing or decomposing not only the proteins, but also the substances, which may be needed for the biological activity of the peat suspensions in spa applications. Nevertheless, our data show that the sterilization of peat in autoclave do not induce more substantial changes in the chemical composition of hydrophobic compounds of its extracts than the "cold" sterilization methods employing ionizing radiation. Thus, we have demonstrated that the treatment with the steam under pressure is suitable for peat sterilization.

Conclusion

Although peat is traditionally used in balneology, and since recently also in cosmetics, there still is a lack of scientifically proven information on the microbiological and chemical constituents of the peat. Our study provides the insight into the microbiology and composition of hydrophobic compounds in the balneological peat from two traditional spa centres in Latvia: Kemeri and Baldone. High microbial counts in the peat samples and the presence of the bacterial strains possessing some pathogenic potential justify the need of the sterilization of the peat before its applications in spa procedures. Irradiation with gamma rays or electron beam as well as standard heat treatment may be used for sterilization. All the sterilization procedures increased concentration of alkanes, alcohols and ketones and decreased the amount of fatty acids. Heat sterilization proved to be more preserving for the peat sterols than the radiation sterilization. Further research is needed to evaluate the therapeutic significance of the changes in the concentration of peat hydrophobic substances after sterilization and hence to decide on the most appropriate method of sterilization.

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