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RESEARCH ARTICLE

The effect of spent bleaching earth ageing process on its physicochemical and microbial composition and its potential use as a source of fatty acids and triterpenes

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Abstract This study was aimed at investigating the physicochemical and microbiological changes that took place during the ageing process of spent bleaching earth in the presence of autochthonous microorganisms. Research material included fresh spent bleaching earth (SBE_0) and the same material after 3 years of storage at the constant temperature of 20 °C, without aeration and moistening (SBE_3). Changes in the chemical composition of analysed waste material were observed during its ageing process point to a spontaneous bioconversion of fat substance towards formation and/or release of free saturated fatty acids C16:0 and C18:0 (14.3 g 100 g^{-1} D.M.), triterpenes (8.48 g 100 g^{-1} D.M.), cholesterol (3.29 g 100 g^{-1} D.M.), small quantities of carbohydrates and esters (0.80 g 100 g^{-1} D.M.). This process was accompanied by other changes in physicochemical parameters of the waste material, such as colour, odour and viscosity, decrease in fat content from 28.27 to 24.6 % and that of soluble forms of metals (Mo, Cu, Fe, Zn, Ni, Cr and Mn), ranging from 25 to 75 %, and an increase in pH, from 3.85 to 4.2. At the same time, changes in the microbial consortium were observed.

Keywords Spent bleaching earth (SBE) · Lipolytic microorganisms · Bioconversion · Triterpenes · Saturated fatty acids

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Introduction

Waste generation is an inseparable part of economic activity, and therefore, it is reasonable to minimise both the quantity of generated waste materials as well as their negative environmental impact. In the waste management scheme being valid throughout the EU, there are to be found in the order of priority such sections as recovery of usable components and recycling of components which cannot be directly used Directive 2008/98/EC of the European Parliament and of the Council (EU) of 19 November 2008 (European Parliament 2008). However, waste disposal, particularly through landfilling, is still a disagreeable and undesirable necessity (European Council 1999).

Despite many years of research carried out on the management of spent bleaching earth (SBE), a waste material from fat industry is still serious and unsolved, economic and ecological problem.

The fat industry provides substantial quantities of spent bleaching earth (SBE) each year. For example, it is 40,000 t in Poland, 80,000 t in Japan and 1.0–2.0 million t worldwide (Huang and Chang 2010; Tippkötter et al. 2014). This waste material contains from 25 to 40 % oil and various substances being absorbed during bleaching (Beneke and Lagaly 2002;

Lara and Park 2003; Ming et al. 2003; Kojima et al. 2004; Park and Ming 2004; Krzyśko-Łupicka 2013). These waste materials are difficult to manage or even to dispose of due to the diversified composition, high quantities of water-insoluble substances, such as fatty acids, macro-elements (sodium, potassium, calcium, magnesium, phosphorus, sulphur and chlorine) and micro-elements (iron, manganese, zinc, copper, iodine, cobalt and selenium), plant pigments and heavy metals (cadmium, mercury, arsenic, lead, chromium and nickel) (Chandra and Sathiavelu 2009; Piotrowska-Cyplik et al. 2013).

Therefore, their decomposition in the environment is inhibited and very slow. Removing process of such waste materials through storage is thus inadvisable due to ecological reasons as these pollutants may penetrate into the ground water and even infiltrate water-bearing horizons (Murray 1989) and may be forbidden by law or significantly limited. Moreover, their open-air storage may cause spontaneous combustion (Kheang et al. 2006).

This type of properties and effects significantly impede waste management and environmental protection, which are warranted in the Polish legal provisions, in particular in the Environmental Protection Act of 24 April 2001 (Polish Parliament 2001) and the Waste Act of 14 December 2012 (Polish Parliament 2012). According to European legislation, SBE is classified as hazardous waste with code 070610* A (European Commission 2000). Due to its high organic content, which exceeds the 6 % limit of the waste acceptance criteria (WAC) for hazardous waste landfills under the EU Landfill Directive, rough SBE cannot be landfilled (European Council 1999) and must be disposed by other means. These may be both classic and modern processes. Anderson (1996) suggested that SBE could be used as an asphalt additive, replacement for plastic parts in refractories, in cement manufacturing, soil stabilisers and road foundation. The SBE also could be added at 0.5 to 2 % as an ingredient in broiler chicks feed (Blair et al. 1986; Jeon and Son 2007). To ensure safety of the animals and people, such use of SBE shall be governed by European Parliament and Council (2002). The recovered oil from SBE may by use for the production of surfactants, biodegradable polyesters, lubricants, anti-rust products, biofuels (Lara and Park 2004; Park et al. 2008; Huang and Chang 2010; Selvabala et al. 2011; Pourvosoghi et al. 2013) and other chemical compounds (Park and Ming 2004). Tippkötter and co-workers (2014) propose the use of spent bleaching earth as source of glycerol for the anaerobic production of acetone, butanol and ethanol. The possibilities of using the activated SBE as a sorbent of heavy metals are also tested (Seng et al. 2001; Wambu et al. 2009). In the Europe, incineration of SBE remains the main disposal method (Beshara and Cheeseman 2008).

One of the most important and most effective methods of disposal for such waste materials and their removal from the environment is biological decomposition by the microorganisms displaying lipolytic activity. Lipid reach waste is a high-value substrate for microorganisms, being a fully alternative source of carbon and energy.

Under natural conditions, their biodegradation is a spontaneous process taking place with autochthonous microorganisms showing a synergistic action. A factor determining this process is microorganisms' composition and their activity. It particularly depends on the presence of antagonistic microorganisms, properties of pollutants, waste "age", presence of different chemical compounds, temperature, availability of oxygen and nutrients content. Generally, it depends on microbial and physicochemical properties of the environment where this process is taking place. The biomass of microorganisms, in particular the activity of their lipolytic enzymes, should have a significant effect on changes in physicochemical parameters of the environment, inducing a modification of its composition and, in consequence, affecting the rate of biodegradation processes (http://www.ejpau.media.pl/volume7/issue1/biotechnology/art-01.html).

Therefore, it is necessary to know the transformations occurring in SBE, particularly fat bioconversion towards development of different chemical compounds and changes in the microbial consortium, In this context, research aiming at investigating the physicochemical and microbiological changes taking place in the process of ageing of spent bleaching earth in the presence of autochthonous microorganisms is reasonable from both economic and legal point of view.

Materials and methods

Research material obtained from Polish local rapeseed oil refinery was the diatomaceous spent bleaching earth waste (SBE_0), collected directly from filters after the refining of vegetable oils at fat processing plants, and the same waste being stored for 3 years (SBE_3) in two plastic screw top barrels, at a constant temperature of 20 °C without aeration and moistening. From each barrel, three samples were collected from a depth of 30 cm. The initial waste (SBE_0) had the form of a loose, grease mass of yellow colour and characteristic oily odour. The stored SBE (SBE_3) had the form of a loose grey-brown mass with a characteristic odour of rancid fat. Determinations were made from averaged samples. Each measurement was repeated two times. If the results were significantly different, a third measurement was performed. The obtained results were analysed statistically, using analysis of variance and testing the factors with the Duncan's test with the use of Satistica 5.5 software (StatSoft).

Research methods

1. Examination of the physicochemical properties of SBE_0 and SBE_3 included the following determinations:

    - Exchangeable acidity in a KCL solution using an ELPO N-512 pH meter (Ewing 1981),
    - Sulphate sulphur by the nephelometric method, phosphorus by the spectrophotometric method (Ewing 1981; Ostrowska et al. 1991), total nitrogen (Grace Analytical Lab 1994; Ostrowska et al. 1991), chlorides by the Mohr method according to the ISO standard 9297 (1989) and Skoog et al. (1996),
    - Metals (Fe, Cu, Mg, Mn, Zn, Cr, Co, Ni, Pb) by the method of atomic absorption spectroscopy (Rauert et al. 1999) using AAS PU-9100X Philips Unicam.

2. Chemical analysis of fatty extracts included:

    - Preparation of fatty extracts from the waste materials under analysis,
    - Performance of chromatographic and IR spectrophotometric analyses of fatty extracts.

A weighted portion of SBE (5 g) was extracted with n-hexane in a Soxhlet apparatus. Obtained solution has been concentrated in an evaporator until an oily substance was received, and its weight was determined.

The chromatographic analysis gas chromatography–mass spectrometry (GC/MS) (Sparkman et al. 2011) was conducted using a Hewlett Packard 5890 Series II gas chromatograph under the following conditions: HP-5 capillary column, injector temperature 60 °C, temperature gradient 10 °C/min to 280 °C, isothermal heating 20 min [60/10/280–20], detector temperature 280 °C. Samples were dosed by an on-column injection. Identification of fats, their bioconversion products and their quantitative determinations in the extract were made by using *HP Analytical NIST MS Search Program and Spectra Database* software and the results were given in g 100 g^{-1} D.M. SBE.

Process of chemical transformations of fatty substances during the fat ageing was observed using the IR spectroscopy (Stuart 2004). The IR spectrophotometric analyses were performed using a Philips Analytical PU 9800 FT-IR Spectrophotometer with a resolution of 2 cm^{-1}. Samples, in the form of a film have been applied on a sodium chloride crystal and studied using the wave number range of 4,000–750 cm^{-1}.

Microbiological analysis of SBE_0 and SBE_3 included determinations of the quantitative and qualitative microbial composition by the culture method based on the following mediums:

- Czapek's agar with glucose for fungi (Difco Laboratories 1984),
- YPG agar with chloramphenicol 0.1 g/dm^3 for yeast (Difco Laboratories 1984),
- Nutrient LAB-Agar™ (BIOCORP) for bacteria,
- Starch Casein Nitrate Agar (Difco) for actinomycetes,
- Tributyrin Agar (Fluka); neutral Tributyrin (Fluka) for microorganisms displaying lipolytic activity (Difco Laboratories 1984),
- Waksman's agar for microorganisms displaying amylolytic activity (Difco Laboratories 1984),
- Fraser broth for microorganisms displaying proteolytic activity (Difco Laboratories 1984).

After 5 days of incubation at 25 °C, the total number of bacteria and yeast was determined, while that of other microorganism groups was after 10 days (Kelley 2001). The results were given in colony-forming units per gram dry matter of the waste material. The isolated, macroscopically different bacteria colonies were subject to biochemical identification using analytical profile index (API) tests: API 20 NE and ID32GN for Gram-negative rods, API 50 CHB for Gram-positive bacilli, ID32 STAPH for Gram-positive cocci and API 20 Aux and ID32C for yeast. Filamentous fungi were identified based on their morphological features using the diagnostic keys (Pitt and Hocking 1985; Hoog and Guarro 1995; Pitt 1988; Barnett et al. 2000).

Results and discussion

The initial waste (SBE_0) had the form of a loose, grease mass of yellow colour with acid reaction (pH 3.85), characteristic oily odour and a fat content of 28.27 % *w/w*. After 3 years of storage, its physicochemical and organoleptic characteristics underwent distinct alterations. The stored SBE (SBE_3) had the form of a loose grey-brown mass with pH 4.2, with a characteristic odour of rancid fat and lower fat content (24.6 % *w/w*). Clearly, lower contents of the extractable forms of metals (Fe, Cu, Zn, Ni, Cr and Mn) were observed in SBE_3, within a range from 25 to 75 % (Table 1), and twofold lower contents of sulphates and total phosphorus (Table 2). The content of total nitrogen drastically increased, that of chlorides increased almost twofold (Table 2), whereas the lead content increased sixfold when compared to SBE_0 (Table 1). Also, the content of cadmium increased, while cobalt content has not clearly changed.

Changes observed in both, the physical characteristics of the waste material and the content of respective inorganic components, suggest the chemical transformation leading to the conversion of the bond forms of elements during ageing waste. It resulted in an apparent increase or decrease in the content of chemical elements.

Table 1 The results of chemical analysis of mobile forms of metals in SBE_0 and SBE_3

	The content of fatty substances (%) mass	The concentration of metals (mg kg^{-1} D.M. SBE)									
		Fe	Cu	Zn	Ni	Cr	Pb	Cd	Co	Mn	Hg
SBE_0	28.27	10,089	6.0	19.5	12.0	6.0	2.0	0.0	2.5	269	0.004
SBE_3	24.6	6,000	3.0	15.0	3.0	2.5	11.0	0.5	2.0	85.5	<0.004

Results of the instrumental analyses (IR, GC/MS) of fatty extracts from the waste materials being examined also point to the process of chemical transformations during the fat ageing. Based on the IR spectra, it was found that the chemical composition of fatty extracts was diversified (Fig. 1), while the identifiable components of the waste samples under analysis were carboxylic acids, aldehydes, mono-cyclic hydrocarbons, esters and other aliphatic and aromatic compounds containing characteristic atom formations being specified in Table 3.

The chromatographic analysis by the GC/MS method showed, much like the IR spectroscopy, quantitative and qualitative differentiation of components in fatty extracts from SBE_0 and SBE_3 samples (Fig. 2). Its quantitative results are presented in Table 4. The identification of components was made based on the similarity of mass spectra.

In the fatty acid of the initial waste (SBE_0), mainly oleic acid (C18:1 ω9) was identified, and small quantities of cyclopentaundecanoic acid. On the other hand, in the stored waste (SBE_3), saturated fatty acids, such as hexadecanoic acid (C16:0), octadecanoic acid (C18:0) and cyclopentaundecanoic acid (C16:0), were found. They could arise in the process of oxidation; the result of which was complete disappearance of oleic acid. But the appearance of cyclic compounds, such as cyclopentaundecanoic acid, triterpenes and cholesterol (Table 4), may be the result of stereomutation with oxidation and formation of cyclic compounds, such as cyclopentaundecanoic acid, triterpenes and cholesterol (Table 4).

The changes observed in the qualitative and quantitative composition of bleaching earth as a consequence of ageing could be both the result of chemical and biochemical transformations of the components being adsorbed on bleaching earth and, at least in part, the result of transformations occurring in the matrix, in this case in spent bleaching earth, leading to the release of chemical elements and organic compounds being the pollutants of bleached oil, strongly adsorbed during that process. In this way, substances previously immobilized on the matrix become available and detectable by using standard analytical procedures.

The resulting intermediate products of decomposition of fat, such as free fatty acids, aldehydes, triterpenes, as well as changes in the concentration of metal forms and other elements available on the biological transformation can determine the quantitative and qualitative changes in the microbial population. The microbial consortium inhabiting spent bleached earth, the fresh one and that after ageing, was comprised of both bacteria, yeast and fungi not displaying lipolytic activities as well as the microorganisms showing lipolytic activity.

In SBE_0, no yeasts were found; the reason of which could be a high concentration of oleic acid (Smith and Alford 1966). During the storage of the waste, a new microbial system was developed as a result of succession. The bacterial count decreased hundredfold, as well as that of the microorganisms displaying lipolytic activity decreased tenfold, whereas the number of filamentous fungi increased tenfold (Fig. 3). Microorganisms displaying amylolytic and proteolytic disappeared totally; however, the amount of yeast increased (100 cfu g^{-1}) (Fig. 3).

Higher biodiversity of the microorganisms not displaying lipolytic activity was found in SBE_0 30 species (12 bacteria species and 18 filamentous fungi species), comparing to SBE_3, where 17 species (nine bacteria species, four yeast species and four filamentous fungi species) were found (Table 5).

Higher diversity was also characteristic for the microorganisms displaying lipolytic activity in SBE_0 (32 species—of which 12 bacteria species) than in SBE_3 (23 species—of which 11 bacteria species) (Table 6).

Table 2 The inorganic compound content in SBE_0 and SBE_3

	pH_{KCL}	The inorganic compounds (mg kg^{-1} D.M. SBE)				Dry mass (%)
		Total nitrogen (N)	Total phosphorus (P)	Chlorides (Cl^-)	Sulphates (SO_4^{-2})	
SBE_0	3.85	0.05	1.64	452	1,650	98.4
SBE_3	4.2	2,200	0.862	753.4	930	95.9

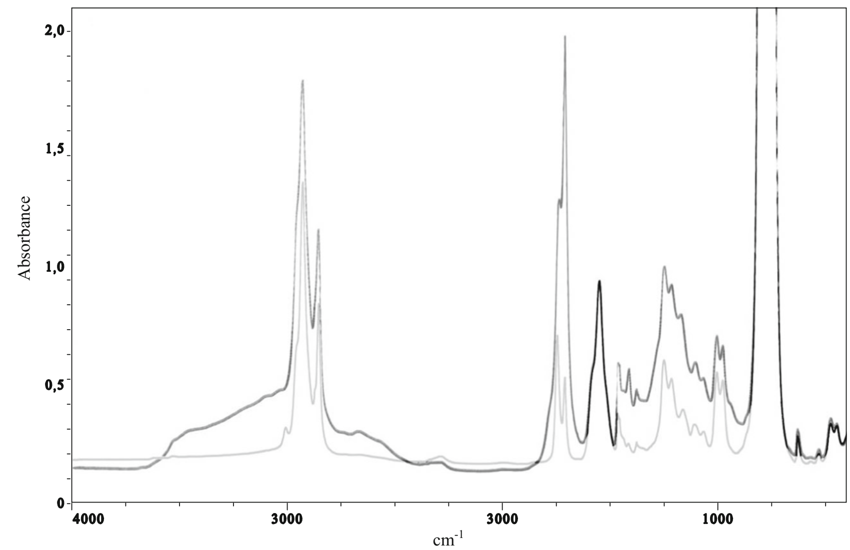

Fig. 1 The IR absorption spectra of fatty extracts in the initial waste SBE_0 (*black*) and the stored waste SBE_3 (*grey*)

It is characteristic (Table 6) that yeast species displaying lipolytic activity, such as *Sporobolomyces salmonicolor*, *Rhodotorula glutinis*, *Candida colliculosa* (syn. *Torulaspora delbrueckii*) and *Candida* sp., were identified only in SBE_3.

Discussion

The examined spent bleaching earth was a system composed of diatomaceous earth, adsorbed fats, metals and microorganisms, including those being able to grow in the presence of fat or using it in life processes. During ageing, such systems are subject to dynamic changes, both microbiological and chemical ones (Krzyśko-Łupicka 2009). The processes taking place in SBE were slow and extremely complex as a result of high concentration of fat being adsorbed on the carrier, its hampered availability for microorganisms (Wakelin and Foster 1997; Lara and Park 2003; Kojima et al. 2004) and also due to interactions taking place between the metabolites of different populations and the factors activating their growth and activity (Ambachtsheer 2000). The role of mobile metal forms is also important which, depending on environmental conditions, are adsorbed (immobilised) on diatomaceous earth or released from SBE. A decrease in the content of metals being observed in this study could be a result of their binding by fungi and bacteria during biosorption (Chang and Hong 1994; Mullen et al. 1989; White et al. 1997; Delgado et al. 1998; Yin et al. 1999; Park et al. 2003; Al-Kadeeb 2007), intracellular accumulation or precipitation (formation of chelate complexes) due to which they became non-determinable under analytical conditions.

Observed decrease in sulphate content may be very likely associated with a formation of volatile sulphur compounds, such as hydrogen sulphide, organic sulphides and others, under mild reducing conditions prevailing in the waste material being stored. On the other hand, an increase in the content of total nitrogen is most likely related to the mentioned above reversible sorption–desorption processes of substances containing given chemical elements in the bleaching earth.

As a result of the waste ageing, an increase in the weight of fatty extracts was observed (from 28.27 g 100 g^{-1} D.M. in SBE_0 to 34.08 g 100 g^{-1} D.M. in SBE_3), being an evidence for the course of fat substance bioconversion towards

Table 3 The IR absorption bands of fatty extracts and corresponding structural elements

Wave number (cm^{-1})	Absorption band	Structural elements
3008	Stretching=CH	-CH=CH-, CR_1R_2=CHR_3, aromatic system
2928, 2856	Stretching C-H (sym. and asym.)	Ar-CH_3,-CH_2- of cycloalkanes
1746, 1711	Stretching C=O	Aldehydes, carboxylic acids, esters
1467	Deformation asym. (def) C-H shear C-H	-CH_3, -CH_2–CH_2-O-, esters
1417	Deformation C-H	CH_3, CH_2, C=CH-
1378	Def. sym. C-H	-CH_3
1252	Stretching C-O	Carboxylic acid esters
978	Def. C-H and C=H	C=C-H
727	Def. out of plane C-H	R_1CH=CHR_2 (cis), $(CH_2)_n$ $n>4$ of aliphatic compounds or aromatic system

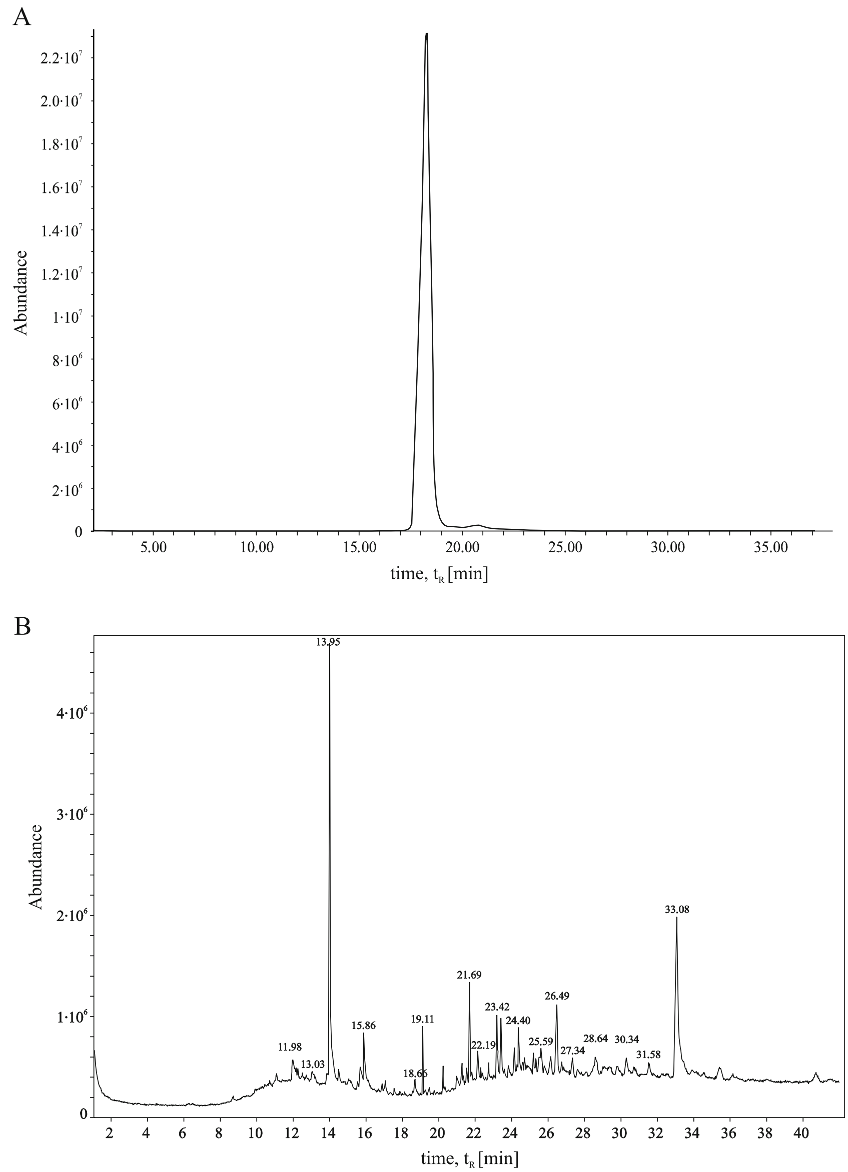

Fig. 2 The GC/MS chromatograms of fatty extracts from SBE_0 (**a**) and SBE_3 (**b**)

development of new chemical compounds, better soluble in hexane used during extraction. Considering the high acidity of the examined waste material (pH 3.80–4.20), it is most probable that bioconversion took place with the presence of microflora displaying lipolytic activity and is characterised by low living requirements and high adaptability (Gunstone 1967, 1999; Wakelin and Forster 1997; Edwards 1990; Atlas and Bartha 1998; Brune et al. 2000; Kottler et al. 2001; Das and Dangar 2008; Xiang et al. 2008; Krzyśko-Łupicka 2009; Krzysko-Łupicka and Robak 2011).

The emergence of new microorganism species in the analysed SBE_3 may be a result of their activation in the presence of newly developed products of fat substance biodegradation (Millet and Lonvaud-Funel 2000; Oliver 2005).

Under these conditions, the products of fat substance degradation and oxidation connected with its transformations were straight-chain saturated fatty acids (mainly C16:0 and C18:0) and a cyclic fatty acid—cyclopentaundecanoic acid (C16:0), found in substantial quantities in SBE_3. It can therefore be stated that β-oxidation, hydrogenation, isomerisation and cyclisation processes took place during the storage. On the other hand, oxygen compounds (e.g. aldehydes, ketones) developed probably by hydroperoxide formation and degradation (Gunstone 1967; Park and Ming 2004).

The mechanism resulting in the emergence of other final products, such as triterpenes, in SBE_3 is difficult to explain. However, due to their quantities and biological properties, the waste material being examined may be a new source for obtaining these compounds.

So far, triterpenes, such as lupeol, betulin, demecolcine and cucurbitacin B, have been obtained from many plant species, e.g. from the bark of trees of the family

Table 4 The quantity and quality composition of free fat in SBE_0 and SBE_3 (g/kg D.M. SBE)

Retention time (min)	Compound	Content of the component in the fatty extract (g 100 g^{-1} D. M.)	
		SBE_0	SBE_3
Unsaturated fatty acids			
18.3	Oleic acid (C18:1 ω9)	27.14	
Saturated fatty acids			
20.81	Cyclopentaneundecanoic acid (C16:0)	1.13	6.48
13.96	Hexadecanoic acid (C16:0)		5.89
15.86	Octadecanoic acid (C18:0)		1.61
19.11	Cyclopropanenonanoic acid (C12:0)		0.32
	Summary		14.30
Triterpenes			
33.09	Betulin ($C_{30}H_{50}O_2$)		4.23
26.50	Lupeol ($C_{30}H_{50}O$)		2.16
27.34	Demecolcine ($C_{21}H_{25}NO_5$)		0.87
13.02	Cucurbitacin B		1.22
	Summary		8.48
Steroid alcohols			
23.43	Cholesterol		3.29
Hydrocarbons			
21.70	1-Docosene		2.11
Ester			
22.18	Tetradecanoic acid, tetradecyl ester		0.80
	Rest compounds		5.10
	Summary	28.27	34.08

Betulaceae in which they occur naturally at the concentration of about 10–30 % (Cole et al. 1991). They exhibit anti-microbial, anti-inflammatory and anti-neoplastic effects and have emulsifying properties. Triterpene compounds (betulinic acid and its derivatives) inhibit proliferation of influenza virus, HSV-1, ECH06 enterovirus or SARS coronavirus (Clinatl et al. 2003; Flekhter et al. 2003; Kanokmedhakul et al. 2003). Until now, the main source of triterpenes has been first of all the liquorice roots and the birch bark (Clinatl et al. 2003) but bioactive substances of that type are also produced by fungi *Scleroderma citrinum* (Kanokmedhakul et al. 2003).

The emulsifying properties of betulin and its synthetic ester derivatives (tartrate, phthalates, tetrachlorophthalate) are being mainly used in the chemical industry, using them as emulsifiers and stabilisers of the oil/water systems or plasticisers in PCV production.

Conclusion

Analysing the n-hexane esters from spent bleaching earth (SBE_0 and SBE_3), changes were observed in the chemical composition, accompanying the process of its ageing,

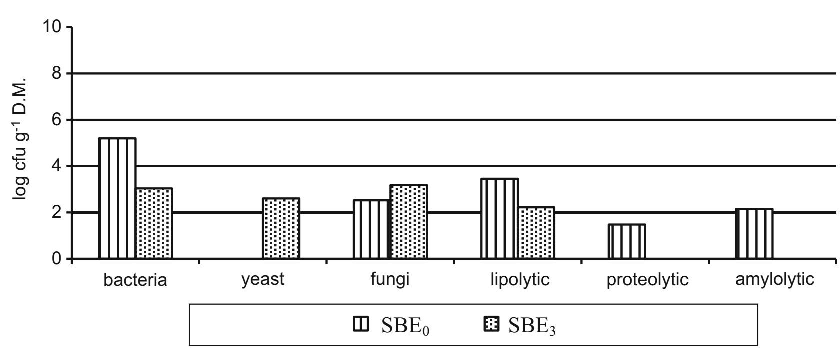

Fig. 3 The effect of waste ageing on quantitative differentiation of microorganisms in SBE_0 and SBE_3 (log cfu g^{-1} D.M.) $LSD_{0.05}$ bacteria=79307.7, yeast=108.5, fungi=503.3, lipolytic=1982.4, proteolytic=18.0 and amylolytic=82.7

Table 5 Qualitative composition of the microorganisms not displaying lipolytic activity in the fresh waste (SBE_0) and after ageing (SBE_3)

Type of waste	The species composition of microorganisms	
	Bacteria	Fungi
SBE_0	*Burkholderia cepacia, Stenotrophomonas maltophilia, Acinetobacter lwoffii, Aeromonas salmonicida, Bacillus* (*B. subtilis*, *B. cereus*, *B. firmus*), *Staphylococcus (S. arlettae*, *S. sciuri*), *Rothia mucilaginosa*, *Xanthobacter* sp. *Streptomyces* sp.	*Mucor hiemalis*, *Rhizopus* sp., *Mortierella* sp., *Penicillium* (*P. citrinum*, *P. frequentans*, *P. notatum*), *Aspergillus* (*A. nidulans*, *A. niger*), *Acremonium murorum*, *Trichoderma viride*, *Scopulariopsis brevicaulis*, *Cladosporium* (*C. cladosporioides*, *C. fulvum*), *Alternaria alternata*, *Fusarium* (*F. solani*, *F. culmorum*, *F. sporotrichioides*, *F. oxysporum*)
SBE_3	*Sphingomonas paucimobilis, Bacillus* (*B. subtilis*, *B. cereus*, *B. firmus*, *B. lentus*), *Staphylococcus* (*S. sciuri*, *S. haemolyticus*), *Rothia mucilaginosa*, *Streptomyces* sp.	*Rhodotorula glutinis*, *Sporobolomyces salmonicolor*, *Pichia* sp., *Candida* sp., *Mucor circinelloides*, *Penicillium citrinum*, *Aspergillus niger*, *Cladosporium resinae*, *Fusarium* (*F. aqueductum*, *F. solani*)

pointing to a spontaneous bioconversion of fat substance towards formation and/or release of saturated fatty acids C16:0 and C18:0 (14.3 g 100 g^{-1} D.M.), triterpenes (8.48 g 100 g^{-1} D.M.), cholesterol (3.29 g 100 g^{-1} D.M.), small quantities of carbohydrates and esters (0.80 g 100 g^{-1} D.M.). This process was accompanied by physicochemical

Table 6 Qualitative composition of the microorganisms displaying lipolytic activity in the fresh waste (SBE_0) and after ageing (SBE_3)

The presence of lipolytic activity of microorganisms								
Bacteria			Yeast			Fungi		
	SBE_0	SBE_3		SBE_0	SBE_3		SBE_0	SBE_3
Sphingomonas paucimobilis	+	–	*Rhodotorula glutinis*	–	+	*Mucor hiemalis*	+	–
Burkholderia cepacia	+	–	*Sporobolomyces salmonicolor*	–	+	*Mucor circinelloides*	–	+
Ochrobactrum anthropi	+	–	*Candida colliculosa*	–	+	*Mortierella* sp.	+	–
Pseudomonas alcaligenes	–	+	*Candida* sp.	–	+	*Penicillium citrinum*	+	–
Pseudomonas putida	+	–				*Penicillium frequentans*	+	–
Acinetobacter lwoffii	+	–				*Penicillium notatum*	+	–
Chryseomonas luteola	–	+				*Penicillium albidum*	+	–
Bacillus subtilis	+	–				*Penicillium* sp.	+	–
Bacillus cereus	+	+				*Paecilomyces variotii*	–	+
Bacillus firmus	+	–				*Aspergillus nidulans*	+	–
Bacillus lentus	–	+				*Aspergillus niger*	+	+
Geobacillus stearothermophilus	+	+				*Aspergillus terreus*	–	+
Bacillus mycoides	+	–				*Trichoderma viride*	+	–
Bacillus amyloliquefaciens	–	+				*Acremonium murorum*	+	–
Brevibacillus laterosporus	+	+				*Scopulariopsis brevicaulis*	+	–
Staphylococcus. warneri	+	–				*Cladosporium cladosporioides*	+	+
Staphylococcus. haemolyticus	–	+				*Cladosporium fulvum*	+	–
Staphylococcus. chromogenes	–	+				*Cladosporium resinae*	–	+
Rothia mucilaginosa	–	+				*Alternaria alternata*	+	–
Micrococcus luteus	–	+				*Fusarium solani*	–	+
Kocuria varians	–	+						
Dermacoccus nishinomiyaensis	+	–						
Streptomyces sp.	+	–						
Flavobacterium sp.	+	–						
Myroides sp.	+	–						
Xanthobacter sp.	+	–						

+ present

– not found

changes in the fat substance (colour, odour and viscosity), a decrease in the fat content (from 28.27 to 24.6 %) and that of soluble forms of metals (Mo, Cu, Fe, Zn, Ni, Cr and Mn), ranging from 25 to 75 %, and an increase in pH (from 3.85 to 4.2). At the same time, changes in the microbial consortium were observed.

The extraction of chemical compounds from the aged spent bleaching earth, occurring in it in substantial quantities, would have been certainly justified from the economic point of view. They might be used in both pharmaceutical and chemical industries and cosmetology (betulin esters with saturated fatty acids) after their previous extraction and separation. In the light of the results being obtained, it appears that spent bleaching earth may be a potential source of chemical compounds of the industrial use.

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