Activated natural zeolites for beer filtration: A pilot scale approach

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A B S T R A C T

A clinoptilolite-rich natural zeolite was tested as a substitute for kieselguhr as a filtering material to eliminate ingredients that cause beer haze formation. Two-grain sizes of micronized natural zeolite were thermally activated to 400 °C, to enhance its adsorption performance and remove the impurities adsorbed in the microporous system of zeolites, followed by their physicochemical characterization. The activated zeolites mixed with four commercial filter aids in different ratios were used for beer filtration at the pilot scale. Most of the physicochemical and sensory characteristics of beers filtered with commercial filter aids and with zeolites were similar. Using zeolite in filtering mixtures significantly reduces the number of microorganisms present in the filtered beer, which can eliminate the necessity of beer sterilization after filtration. The results evidenced that activated natural zeolites, which are cheap materials, are promising candidates as filter aids and can replace kieselguhr without producing any degradation of the beer filtration process.

1. Introduction

Beer is one of the most widespread drinks in the world, after water and tea, due to its enjoyable sensory potentials, dietary features and lower cost compared to other alcoholic drinks [1,2]. Light-to-moderate drinking of beer provides several positive effects on human health, *i.e.* dropping the hazard of cardiovascular illness, cholesterol level in blood, diabetes, dementia, osteoporosis, attributable to the presence of proteins, B vitamins, antioxidants, minerals, fibres, or prebiotic compounds [1,3,4].

Currently, ithere are over 12,000 operational breweries in Europe, about 75% of them functioning as microbreweries [5]. Accordingly, this sector has practiced prominent adjustments, the consumers becoming more interested and required in terms of their chosen value, consumption and purchasing behavior [6,7]. Commercial beer should be clear for good marketability and stability, except for unfiltered beers class (craft beers). Consumers regularly consider haziness an imperfection and a possible health threat, avoiding hazy beers [8,9]. The most common haze components are proteins, polysaccharides, polyphenols, melanoidins and carbohydrates (mainly β-glucans, arabinoxylans and starch) [10,11].

The beer clarification process has been effectively used for decades in beer filtration, the common filter aids being porous materials, primarily of kieselguhr and other materials such as perlite, cellulose or active carbon [8,9,11–14]. Generally, the particle elimination is

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contingent on the dosing rate and properties of the filter aid, beer brand, and size distribution of suspended solids in the raw beer [14]. However, handling kieselguhr requires assuring safe working conditions since the crystalline silica from kieselguhr causes lung disease [15]. Moreover, the highest expense of the filtration process is caused by diatomaceous earth expenditure and sludge discarding [9,11, 15].

Natural zeolites are among the most valuable microporous aluminosilicate substances that find extensive use in industrial, agricultural, environmental, and medical applications [16–20]. Zeolites are three-dimensional crystalline frames built up of SiO_4 and AlO_4 tetrahedra linked through oxygen atoms, looking like infinite interconnected cage-like structures [18,21]. About 232 zeolites, including more than 50 natural zeolites, have been identified by the Structure Commission of the International Zeolite Association, clinoptilolite, analcime, laumontite, mordenite, chabazite and phillipsite being the most predominant [16]. Though their characteristics differ on the mined geographical zone, natural zeolites exhibit increased porosity and bulk volume, well-developed specific surface, good sorption capacity, high ion exchange, chemical resistance, and remarkable thermal stability [16,20–22]. The properties of natural zeolites can be enhanced by individual or mixed treatment modification methods such as ion exchange, acid or base treatment, and surfactant functionalization [16,23,24]. Natural zeolites are potential candidates for common commercial filter aids with no necessary significant modifications in the brewing industry apparatus and manufacturing process [12,25–27].

This study aims to evaluate the filtration capability of a zeolite from a natural source in Northwest Romania in the form of two different grain sizes <40 and < 100 μm activated by thermal treatment at 400 °C and mixed with different commercial filter aids (kieselguhr) in different ratios at the pilot scale, by considering the main physicochemical, sensory and microbial characteristics of the unfiltered and filtered beer, at different filtration times.

2. Materials and methods

2.1. Chemicals

The concentrated acids (37% HCl, 65% HNO_3 and 40% HF), 30% (m/m) H_2O_2 and isooctane (99.5% purity) of analytical grade used to digest the zeolite samples were produced by Merck, Germany. Major elements (Na, K, Ca, Mg, Fe, Al) were analyzed using an inductively coupled plasma-optical emission spectrometer (ICP-OES) Optima 5300 DV (PerkinElmer, Canada), and calibration standards were made from traceable commercial multielement stock solution (Merck, Germany). The graphite furnace atomic absorption spectrometer (GFAAS PerkinElmer, PinAAcle 900 T, USA) used for measurement of trace elements (As, Cd, Cr, Cu, Ni, Pb, Zn) was calibrated using mono element standards (Merck, Germany). Ultrapure water was used to for all dilutions. For the quality control, BCS-CRM 375/1 soda feldspar (Bureau of Analyzed Samples, UK) CRM was tested, and accurate analytical results were achieved (recoveries in the range 94.2–107.5%).

2.2. Natural zeolite preparation and characterization

The natural zeolite was sampled from the Chilioara open pit sited in Salaj County, Romania. The extracted minerals were shredded using a grinder RS 200 (Retsch, Germany), put through a sieve to achieve a powder with particles below 1 mm, and dried at 105 °C. Afterwards, the resulted materials were micronized as two batches in particle sizes of <40 μm (NZ40) and <100 μm (NZ100) with the aid of a PilotMill-2 system (Italy) and activated at 400 °C for 4 h, with the scope to remove impurities and for sterilization.

The zeolite samples (NZ40 and NZ100) were analyzed for element concentrations, cation exchange capacity (CEC), surface area and porosity. Microwave-assisted digestion (HNO_3: HCl: HF, 3:9:2, v:v:v) using a digestion system (Speedwave Xpert, Berghof, Germany) was applied on zeolite samples [28]. An amount of 0.2 g zeolite was digested and the resulting solution was diluted to 100 mL. Afterwards, the concentration of Al, Fe, Na, K, Ca and Mg were converted to the oxides (Al_2O_3, Fe_2O_3, CaO, MgO, K_2O, Na_2O and MnO) considering the stoichiometry. The SiO_2 was measured by gravimetric method [28]. To quantify the possible leaching of toxic elements from the zeolite to the beer during the filtration process, an amount of 3 g zeolite with 100 mL ultrapure water acidified with 65% HNO_3 Suprapur (Merck, Germany) to pH = 4.2 (close to the beer pH) cooled down at 3.5 °C was filtered using a laboratory filtration unit (Sartorius, Germany) having a filtration area of 12.5 cm^2 coupled to vacuum with a pressure of 0.2 bar. The As, Cd, Co, Cr, Cu, Ni, Pb and Zn concentrations released into the solution were measured by GFAAS. The bulk density was determined according to ISO 23145–2:2012 [29], while the CEC value was calculated using the concentrations of Na^+, K^+, Mg^{2+} and Ca^{2+} extracted in 1 M ammonium acetate solution [30] and quantified by ICP-OES. Three determinations (n = 3) were performed in parallel for each sample/parameter. Total pore volume, surface area, and pore radius were measured considering N_2 adsorption-desorption isotherms. The X-ray diffraction (XRD) spectra were obtained using a diffractometer (Bruker D8 Advance, Germany), as described before [25].

2.3. Beer production

The beer was yielded in a microbrewery (pilot scale, 300 L capacity) based on one of our previously published protocols [31] with few modifications. The lager beer from 100% all grain malted barley (Weyermann Specialty Malting Company, Germany) was produced on the pilot scale. Hop pellets 90 type (Magnum and Perle cultivars) crop 2021, were provided from Moragroind hops farm (Romania). Yeast starter culture (*Saccharomyces cerevisiae*) was obtained from a local brewery. After the grounding with a motorized roller mill MAV3 (Tehnofavorit, Romania), malt was removed to a controlled saccharification kettle (Centra, Romania). When saccharification ended, the mash was transported to the lauter tun for filtration, then to the boiling kettle. Boiling with hops took 90 min and the wort was directed for clarification in the rotapool. Prior to yeast pitching, the wort was cooled to 8 °C. The primary

fermentation (in a Pierre Guerin, France, fermentation tank) lasted for 7 days at 12 °C, and the secondary fermentation lasted 14 days at 3 °C, and the process was permanently monitored, controlled and recorded by Neptune™ software (National Instruments, USA). The ethanol and real extract were monitored periodically using an analyzer (FermentoStar type 3572, Germany). Afterwards, the obtained beer was subjected to filtration operations and chemical and microbiological analytical experiments. All conduct experiments were performed in triplicate.

2.4. Pilot-scale beer filtration experiments

For beer filtration, a pilot-scale filtration installation (Velo, Italy) with the following components/characteristics was used: (i) filter chamber with 14 horizontal discs with a total surface area of 1.76 m^2), (ii) dosing pump, (iii) mixing tank of 40 L, (iv) exit sight for monitoring the clarity of beer, (v) beer temperature: 3.5 °C, (vi) pressure during the formation of the filter layer: 1.5 bar; (vi) beer supply pressure: 1.5 bar and (vii) filtration flow rate: 35 L min^{-1}. Filter aids and amounts utilized as filter cake and filtration dosage for beer filtration, and beer samples resulted in the clarification trials on pilot-scale installation (n = 3 parallel samples) are presented in Table 1.

The total filter surface obtained using 14 filter discs (φ = 40 cm) was 1.76 m^2. To form the primary (base) layers on the filter supports, 0.1 g filter aids per cm^2 (1 kg per m^2) were used. On the total surface of 1.76 m^2, the primary layer contained 1760 g filter aids in order to obtain a layer thickness of approximately 1.5 mm. In order to achieve a high-performance filtration operation, three layers of filter aids were formed. The primary (base) layer was obtained by dosing a coarse filter material in at least 50–70% of the total material to form the base layer. The secondary (safety) layer was obtained by dosing a mixture of filter materials that ensure the filtration, with a medium and fine granulation, in quantities to complete the difference up to 1760 g/1.76 m^2. The dosing materials mixed with the beer were appropriately added in order to maintain the pressure difference growth up to 2 bar/h (when using too low dosage, the pressure does not increase linearly and the yeast progressively plugs the dosing layer - "yeast strike", while a too high dosage results in a slow increase of pressure difference and early filling of filter working volume with dosage material). The amount of filtration dosage used for dosing was 150 g. Used mixtures of NZ100 and NZ40 zeolites in different ratios marketable filter aids, specifically CBR3 (rough-size kieselguhr D80 ≤ 170 μm), CBR (middle-size kieselguhr D80 ≤ 150 μm), CBL (middle-size kieselguhr D80 ≤ 100 μm) and CBL3 (fine-size kieselguhr, D80 ≤ 60 μm) from CLARCEL (France) are presented in Table 1. The zeolites (NZ40 + NZ100) amounts related to the total amount of filter aids and filter cake and filtration dosage amounts (in brackets) are indicated in Table 1. Beer samples were taken at different times of filtration process from each filtration experiment at the pilot-scale, as presented in Table 1. All experiments were carried out in triplicate.

The evaluation of activated natural zeolite as a potential filtration aid for the colloidal and microbiological stabilization of beer was assessed by comparing the characteristics of filtered beer using the traditional method with diatomite earth with filtered beer with zeolite-based filter material.

2.5. Unfiltered and filtered beer analysis

Unfiltered and filtered beer were analyzed for parameters such as pH, alcoholic concentration, original extract, real and apparent extract, bitterness, turbidity, color, pH, concentration of major cations, trace elements, sensory and microbiological (the total number

Table 1
Experimental design of beer filtration experiments at pilot scale (n = 3 parallel samples).

Experiment	Filter cake		Filtration dosage	Zeolites amount (%)	Sample code	Sampling time
	Layer 1	Layer 2				
V0	–	–	–	–	V0	Unfiltered beer
V1	880 g CBR3	700 g CBR + 180 g CBL	120 g CBR + 30 g CBL3	0%	V1-0[a]	0 min
					V1-5[a]	5 min
					V1-10[a]	10 min
V2	1230 g NZ100	530 g CBR	75 g NZ40 + 75 g CBL3	68% (64% filter cake, 4% dosage)	V2-0	0 min
					V2-5	5 min
					V2-10	10 min
V3	1180 g NZ100	580 g CBR3	37 g NZ40 + 38 g CBR + 75 g CBL3	64% (62% filter cake, 2% dosage)	V3-0	0 min
					V3-5	5 min
					V3-10	10 min
V4	590 g NZ100 + 290 CBR3	590 g NZ40 + 290 CBR	115 g NZ40 + 22 g CBR + 13 g CBL3	68% (62% filter cake, 8% dosage)	V4-0	0 min
					V4-5	5 min
					V4-10	10 min
V5	616 g NZ100 + 264 g CBR	440 g NZ40 + 440 CBR	150 g CBL3	55% (55% filter cake, 0% dosage)	V5-0	0 min
					V5-5	5 min
					V5-10	10 min
V6	880 g NZ100	880 g NZ40	150 g CBL3	92% (92% filter cake, 0% dosage)	V6-0	0 min
					V6-5	5 min
					V6-10	10 min

[a] Vn-0 - beer sampled at 0 min after the formation filtration layer (during 15 min); Vn-5 - beer sampled at 5 min after the formation filtration layer, Vn-10 - beer sampled at 10 min after the formation filtration layer.

of germs (NTG), total yeast and mold count (TYMC) and *Enterobacteriaceae*) analysis. pH of beer was recorded using a pH-meter from Mettler Toledo (Switzerland); the elimination of carbon dioxide was performed by shaking the beer samples at room temperature. The turbidity (haze) was determined by the nephelometric method with a turbidity meter (Turb 555 IR, Germany) according to the EBC method 9.29. Turbidity was measured 470 nm in nephelometric turbidity units (NTU) and expressed in EBC units (1 EBC = 0.25 NTU) [32]. The color was measured using a UV-VIS spectrophotometer (Lambda 25, PerkinElmer, USA) at 430 nm and expressed in EBC units according to the EBC method 9.6, while the bitterness was determined following EBC method 9.8 using a spectrophotometer UV-1600PC (VWR, USA) at 275 nm [32]. Sensory analysis (appearance, taste, mouthfeel, and aftertaste and finish) was completed by a triangle test corresponding to the EBC method no. 13.7 [32]. Total polyphenols content was measured spectrophotometrically as described in Analytica EBC Method 9.11 at 600 nm [32]. The element concentrations in beer were determined after microwave wet acid digestion (HNO_3: H_2O_2, 3:1, *v:v*) [25]. The concentrations of major minerals were analyzed by ICP-OES, while trace elements in beer were measured by GFAAS [33]. Alcoholic concentration, original extract, real and apparent extract were measured with the FermentoStar analyzer type 3572 (Germany). Beer bitterness was determined based on EBC Analytica 9.8 [32]. The total number of germs (NTG) were analyzed according to ISO 4833–1:2013 standard [34], yeasts and molds were accounted according to ISO 21527–1:2008 standard [35], and the detection and enumeration of *Enterobacteriaceae* was carried out considering the ISO 21528–1:2017 standard [36].

2.6. Statistical analysis

Analytical data were provided as average ± standard deviation. One-way analysis of variance followed by Tukey's test using Origin 2022 software (OriginLab) was applied to assess statistically significant differences between data at $p < 0.05$.

3. Results and discussion

3.1. Monitoring of fermentation process

The management of the fermentation process was accomplished by a fermentation diagram designed by the Neptune™ software. The data were recorded in the software database (data not shown). The samples were collected from fermentation tank and characterized periodically (Table 2). The ethanol percent and original extract of the obtained beer were 11.61% and 5.23% vol., respectively. The original beer extract represents the quantity of material taken from the mash (before fermentation) [37]. During fermentation, the sugar content is transformed to CO_2 and alcohol, leading to a decrease in the specific mass of the mixture. By evaporating a beer to one-third of its initial size, the content of CO2 and alcohol is removed, resulting in the real beer extract [37]. The apparent beer extract is the amount of dissolved solids in a fermenting fluid without rectification for ethanol content [38].

3.2. Activated natural zeolites characteristics

The total surface area of the NZ100 and NZ40 samples was 72 $m^2\,g^{-1}$, whereas the total pore volume was 0.173 $cm^3\,g^{-1}$ for NZ100, and 0.164 $cm^3\,g^{-1}$ for NZ40. The pore radius of 27 Å for both samples indicated a mesoporous assembly, rendering to the International Union of Pure and Applied Chemistry (IUPAC) [39]. The bulk density was 0.73 $g\,cm^{-3}$ for NZ100 and 0.63 $g\,cm^{-3}$ for NZ40, respectively. The particle dimensions do not meaningly impact the chemical characteristics, while the cation exchange capacity (CEC) calculated considering the major extractable cations was lower (75.4 mEq 100 g^{-1}) for the NZ100 sample compared to NZ40 (81.2 mEq 100 g^{-1}) sample. The physicochemical characteristics demonstrate the suitability of activated natural zeolites as filter aids for beer filtration.

The chemical characteristics in terms of pH of the aqueous extract, major oxides, and total and leachable trace elements concentrations of the natural zeolites thermally activated at 400 °C are shown in Table 3. Trace elements were determined in terms of total concentrations (T-M) in the solutions resulted from acid microwave digestion and in the solutions obtained from the leaching tests (L-M) and expressed in mg kg^{-1}. The different values of limits of quantification (LOQs) for the same element are given by the different amounts of zeolites used for digestion (0.5 g) and leaching (3 g), respectively. For food processing, the materials used for beverage filtration must not hold soluble As and Pb contents higher than 10 mg kg^{-1} [40,41].

Natural zeolites are considered highly inert and safe for human ingestion, being listed among the compounds that can be safely used as direct food ingredients and filter aids [18]. The concentrations of As, Cd and Co were below LOQs, while the concentrations of other

Table 2

Monitoring of the fermentation process (primary alcoholic fermentation - days 2–7 and secondary fermentation - days 7–20).

Day of fermentation	Temperature (°C)	Alc. conc. (% vol.)	Original extract (%)	Real extract (%)	Apparent extract (%)
2	12.0	2.03	11.46	5.85	4.25
3	12.0	4.94	11.37	3.76	1.97
5	12.5	4.95	11.41	3.78	1.98
7	12.5	4.98	11.43	3.78	1.98
12	3.0	5.09	11.50	3.62	1.84
20	3.0	5.23	11.61	3.66	1.86

Table 3
Chemical characteristics of thermally activated zeolites (average $\pm$ s of three parallel measurements).

Parameter	Measurement unit	NZ100	NZ40
pH	pH units	8.11 ± 0.18	8.14 ± 0.18
SiO_2	wt.%	67.5 ± 1.9	67.6 ± 2.5
Al_2O_3		11.0 ± 0.71	11.4 ± 0.88
Fe_2O_3		1.34 ± 0.26	1.42 ± 0.21
CaO		1.75 ± 0.31	1.88 ± 0.27
MgO		0.76 ± 0.08	0.74 ± 0.08
K_2O		2.51 ± 0.26	2.33 ± 0.29
Na_2O		0.34 ± 0.04	0.32 ± 0.04
MnO		0.04 ± 0.01	0.04 ± 0.01
T-As	mg kg^{-1}	<0.50	<0.50
T-Cd		<0.10	<0.10
T-Cr		3.43 ± 0.28	3.09 ± 0.26
T-Cu		9.47 ± 0.82	9.23 ± 0.74
T-Co		<0.20	<0.20
T-Ni		3.31 ± 0.32	3.45 ± 0.28
T-Pb		7.11 ± 0.50	6.36 ± 0.62
T-Zn		20.4 ± 2.02	22.9 ± 2.33
L-As	mg kg^{-1}	0.12 ± 0.03	0.15 ± 0.03
L-Cd		0.01 ± 0.003	0.02 ± 0.004
L-Cr		0.10 ± 0.02	0.11 ± 0.03
L-Cu		0.36 ± 0.08	0.41 ± 0.07
L-Co		<0.013	<0.013
L-Ni		<0.017	<0.017
L-Pb		0.24 ± 0.05	0.28 ± 0.06
L-Zn		0.52 ± 0.07	0.57 ± 0.08

elements in NZ100 and NZ40 samples were 3.43 and 3.09 mg kg^{-1} Cr, 9.47 and 9.23 mg kg^{-1} Cu, 3.31 and 3.45 mg kg^{-1} Ni, 7.11 and 6.36 mg kg^{-1} Pb, and 20.4 and 22.9 mg kg^{-1} Zn (Table 3). Metal concentrations in the same order of magnitude were reported for Romanian natural zeolites from Racosu de Jos deposit (7.31–8.70 mg kg^{-1} Cr, 1.65–2.03 mg kg^{-1} Cu, 3.21–4.35 mg kg^{-1} Ni, 4.69–6.78 mg kg^{-1} Pb, 4.08 and 4.41 mg kg^{-1} Zn, and <1 mg kg^{-1} Cd [42] and different deposits (<1–11.1 mg kg^{-1} Cr, 7.9–27.9 mg kg^{-1} Cu, 2.1 and 10.7 mg kg^{-1} Ni, <1–28.5 mg kg^{-1} Pb, and 14 and 64.1 mg kg^{-1} Zn [43]. However, Tomasevic-Canovic [44] reported 29–38 mg kg^{-1} Pb, 240–305 mg kg^{-1} Mn, 40–305 mg kg^{-1} Zn for natural zeolites from Serbia and Montenegro and highlighted the necessity of testing heavy metals bioavailability in zeolites when used for human. Due to their unique adsorption characteristics, zeolites have been widely studied in reducing metal contents from various solutions [45]. However, despite their usability in food processing, no previous studies reported the leachability of heavy metals from zeolites to foods. The leachable concentrations of trace elements (L-M) represent about 3% for Cr, 4% for Cu, 4% for Pb and 2.5% for Zn of the total concentration, while less than 0.5% of the total Ni is leachable. The leachable As and Pb concentrations were well below the maximum admitted concentration for soluble As and Pb (10 mg kg^{-1}) for food processing [41].

The XRD patterns of both natural zeolites thermally activated at 400 °C (Fig. 1) showed clinoptilolite (PDF card No. 01-080-1557) as the major constituent, displaying the typical diffraction peaks related to the clinoptilolite zeolite structure [46]. The diffraction peaks belonging to albite (PDF card No. 00-020-0548), montmorillonite (PDF card No. 00-058-2038), muscovite (PDF card No.
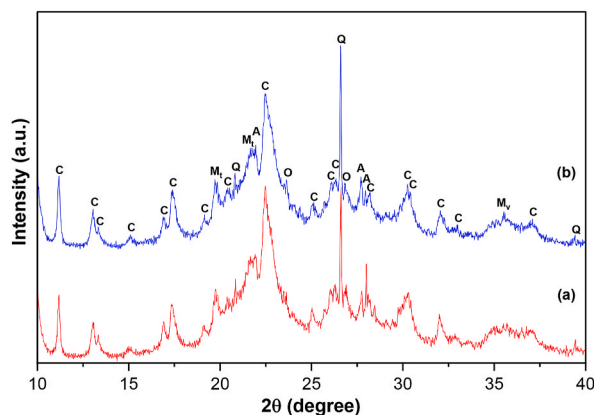

Fig. 1. X-ray diffraction patterns of the natural zeolites thermally activated at 400 °C: (a) NZ100 and (b) NZ40 (A – albite, C – clinoptilolite, M_t - montmorillonite, M_v – muscovite, O - orthoclase and Q – quartz).

00-060-1516), orthoclase (PDF card No. 00-031-0966) and quartz (PDF card No. 01-070-7344) were also remarked. The reference intensity ratio (RIR) method indicated a clinoptilolite content of around 65%. The degree of crystallinity was approximatively 55% for both samples with noncrystalline minerals (or amorphous volcanic glass) denoted by the broad hump at around $2\theta = 25°$ [47].

3.3. Physicochemical properties of filtered beer from pilot-scale filtration experiments

Ideally, beer filtration should remove unwanted components that can cause turbidity without affecting its main characteristics, such as specific flavor, color and nutritional value. Classic pre-coat filtration with kieselguhr includes using a triple-layer filter cake containing a first and second pre-coating and subsequent, continuous product filtration dosing. In this regard, we used activated zeolites mixed with four commercial filter aids in different ratios for beer filtration at the pilot scale.

The effect of filtering aids mixtures on the filtered beer's physicochemical properties is shown in Table 4. The obtained results were compared with the criteria for beer according to the standard SR 4230:2004 [48]. The microbiological results of the filtered beer were compared with the microbiological criteria for foodstuffs according to EC Regulation 2073:2020 [49].

The pH value (4.0) of unfiltered beer slightly increased for the filtered beer, probably due to the interaction with the filter aids. The highest pH value (4.6) corresponded to the filtered beer from experiment V6, in which only zeolite was used to build the filter cake. A possible explanation for the slight pH increase could be the discharge of alkaline (Na^+, K^+) ions from the natural zeolite to the filtered beer. However, the beer pH generally remained at 4.0–4.6 pH units, similar to previously reported values for commercial beer analysis [50–52].

According to the granulometry, three types of filtering aids were used for beer clarification, namely CBR3, CBR and CBL, NZ100, CBL3 and NZ40. The permeability of these materials decreased gradually as the gran size decreased. A high silica content characterizes the kieselguhr materials and zeolites, but the main difference is their density. Natural zeolites have a higher density of 0.73 g cm^{-3} for NZ100 and 0.63 g cm^{-3} for NZ40, respectively, while CBL3 and CBL have a lower density of 0.40 g cm^{-3}, CBR and CBR3 have a density

Table 4

Main physicochemical properties of filtered beer resulted from pilot-scale experiments (average $\pm$ s, three parallel measurements; different letters indicate statistically significant differences, $p < 0.05$).

Sample	pH	Beer color (EBC)	Beer turbidity (EBC)	Alc. conc. (% vol)	Polyphenols (mg/L)	Original extract value (%)	Real extract (%)	Apparent extract (%)	Bitterness (EBC)
V0	4.0 ± 0.2[a]	7.60 ± 0.26[a]	19.4 ± 0.39[a]	5.23 ± 0.13[a]	127 ± 10.4[a]	11.7 ± 0.29 [abcd]	3.45 ± 0.21[a]	2.29 ± 0.17[a]	26.0 ± 0.58[a]
V1-0	4.3 ± 0.2[a]	7.33 ± 0.33 [abc]	0.63 ± 0.10 [bc]	5.10 ± 0.10[a]	113 ± 9.4[a]	11.5 ± 0.32 [abcde]	3.16 ± 0.23[a]	2.18 ± 0.20[a]	22.1 ± 0.48[h]
V1-5	4.2 ± 0.2[a]	7.40 ± 0.16 [ab]	0.50 ± 0.07[c]	5.13 ± 0.16[a]	114 ± 9.9[a]	11.5 ± 0.28 [abcde]	3.17 ± 0.10[a]	2.17 ± 0.12[a]	22.3 ± 0.52[h]
V1-10	4.2 ± 0.2[a]	7.42 ± 0.20 [ab]	0.49 ± 0.07[c]	5.18 ± 0.22[a]	117 ± 10.1[a]	11.6 ± 0.28 [abcd]	3.16 ± 0.18[a]	2.17 ± 0.15[a]	22.0 ± 0.34[h]
V2-0	4.4 ± 0.2[a]	7.03 ± 0.31 [abcd]	0.72 ± 0.11 [bc]	5.10 ± 0.17[a]	119 ± 9.8[a]	11.5 ± 0.28 [abcde]	3.15 ± 0.27[a]	2.11 ± 0.10[a]	25.0 ± 0.53 [abc]
V2-5	4.4 ± 0.2[a]	6.83 ± 0.28 [bcde]	0.62 ± 0.09 [bc]	5.18 ± 0.18[a]	115 ± 9.5[a]	11.3 ± 0.41 [abcde]	3.32 ± 0.26[a]	2.16 ± 0.23[a]	24.3 ± 0.44 [cde]
V2-10	4.5 ± 0.3[a]	6.77 ± 0.30 [bcdef]	0.58 ± 0.09 [bc]	5.13 ± 0.23[a]	115 ± 9.7[a]	11.8 ± 0.26 [ab]	3.15 ± 0.30[a]	2.19 ± 0.17[a]	23.9 ± 0.29 [cdef]
V3-0	4.4 ± 0.2[a]	7.28 ± 0.24 [abc]	0.84 ± 0.08 [bc]	5.20 ± 0.18[a]	116 ± 9.6[a]	10.9 ± 0.16 [def]	3.33 ± 0.21[a]	2.28 ± 0.22[a]	24.4 ± 0.17 [cde]
V3-5	4.5 ± 0.2[a]	7.09 ± 0.20 [abc]	0.68 ± 0.07 [bc]	5.07 ± 0.23[a]	114 ± 9.3[a]	11.8 ± 0.27[a]	3.16 ± 0.11[a]	2.17 ± 0.15[a]	23.5 ± 0.35 [efg]
V3-10	4.6 ± 0.3[a]	6.78 ± 0.29 [bcde]	0.65 ± 0.08 [bc]	5.20 ± 0.42[a]	111 ± 9.4[a]	11.5 ± 0.36 [abcde]	3.10 ± 0.09[a]	2.30 ± 0.16[a]	23.0 ± 0.25 [fgh]
V4-0	4.3 ± 0.2[a]	6.65 ± 0.30 [cdef]	0.87 ± 0.10 [b]	5.10 ± 0.23[a]	116 ± 10.1[a]	11.0 ± 0.25 [bcdef]	3.16 ± 0.22[a]	2.18 ± 0.15[a]	24.8 ± 0.41 [bcd]
V4-5	4.3 ± 0.2[a]	6.32 ± 0.24 [def]	0.75 ± 0.09 [bc]	4.89 ± 0.32[a]	115 ± 9.9[a]	10.4 ± 0.20[f]	3.04 ± 0.24[a]	2.01 ± 0.10[a]	23.5 ± 0.36 [efg]
V4-10	4.4 ± 0.3[a]	6.30 ± 0.25 [ef]	0.70 ± 0.09 [bc]	4.93 ± 0.30[a]	114 ± 9.7[a]	11.0 ± 0.22 [cdef]	3.11 ± 0.12[a]	2.08 ± 0.12[a]	23.7 ± 0.21 [defg]
V5-0	4.2 ± 0.2[a]	7.23 ± 0.29 [abc]	0.61 ± 0.09 [bc]	5.03 ± 0.21[a]	116 ± 10.0[a]	11.5 ± 0.18 [abcde]	3.16 ± 0.15[a]	2.17 ± 0.11[a]	24.6 ± 0.36 [bcde]
V5-5	4.2 ± 0.2[a]	7.18 ± 0.21 [abc]	0.58 ± 0.08 [bc]	5.11 ± 0.18[a]	112 ± 9.9[a]	11.4 ± 0.22 [abcde]	3.10 ± 0.20[a]	2.01 ± 0.20[a]	22.5 ± 0.43 [gh]
V5-10	4.4 ± 0.3[a]	7.03 ± 0.16 [abcd]	0.56 ± 0.09 [bc]	5.22 ± 0.25[a]	114 ± 8.8[a]	11.7 ± 0.25 [abc]	3.41 ± 0.36[a]	2.26 ± 0.19[a]	23.7 ± 0.37 [defg]
V6-0	4.6 ± 0.3[a]	6.23 ± 0.11 [ef]	0.80 ± 0.10 [bc]	5.13 ± 0.19[a]	119 ± 10.3[a]	11.6 ± 0.16 [abcd]	3.33 ± 0.26[a]	2.30 ± 0.11[a]	25.0 ± 0.51 [abc]
V6-5	4.5 ± 0.2[a]	6.11 ± 0.22 [ef]	0.73 ± 0.09 [bc]	4.79 ± 0.33[a]	122 ± 11.1[a]	10.8 ± 0.19 [ef]	3.26 ± 0.19[a]	2.16 ± 0.09[a]	25.8 ± 0.44 [ab]
V6-10	4.6 ± 0.3[a]	6.06 ± 0.17[f]	0.61 ± 0.08 [bc]	4.81 ± 0.27[a]	117 ± 10.9[a]	11.0 ± 0.15 [cdef]	3.17 ± 0.16[a]	2.20 ± 0.14[a]	25.0 ± 0.31 [abc]

of 0.41 g cm^{-3}. Accordingly, for the same amount of filtering material (1760 g), the thicknesses of the formed bed decreased with the increase in material density. The flow through any filter bed is directly related to the bed permeability, *i.e.* a higher percentage of smaller particles undergoes a reduction in filter bed permeability for beer flow and, consequently, lower turbidity [53].

The turbidity of unfiltered beer (19.4 EBC), corresponding to a very hazy beer (EBC >8.0), decreased sharply by about 95% of the total decrease for all filtered beers [9]. The turbidity of all filtered beers is not statistically significant at the p < 0.05 level from that of the unfiltered beer. According to the European Brewery Convention, the recommended turbidity is less than 0.6 EBC units for beer filtrated with kieselguhr [32]. In our study, in the first filtration experiment, V1, in which only kieselguhr is used for filtration (rough-size for layer 1, middle-size for layer 2, and a mixture of middle-size and fine-size kieselguhr for filtration dosage), the turbidity value of beer was 0.63 EBC and decreased at 0.49 EBC after 10 min. In the filtration experiment V2, in which the first layer was zeolite NZ100, layer 2 middle-size kieselguhr and filtration dosage a mixture of fine-size kieselguhr and fine-size zeolite, the turbidity was 0.72 EBC and decreased after at 0.58 EBC after 10 min. The higher turbidity in V2 than in V1 is probably due to the shorter pathlength through the thinner layer formed by zeolite. The filtration experiment V3, in which the first layer was also zeolite NZ100 and layer 2 was rough-size kieselguhr, resulted in a higher turbidity value of 0.84 EBC that decreased to 0.65 EBC after 10 min. The shorter pathlength could explain this higher turbidity through the thinner layer 1 formed by zeolite, corroborated with the more permeable layer 2 formed from the rough-size kieselguhr. In the filtration experiment V4, the first layer consisted of a mixture of NZ100 and rough-size kieselguhr CBR3, layer 2 contained a mixture of fine-size zeolite NZ40 and middle-size kieselguhr CBR, while the filtration dosage was a mixture of NZ40, CBR and CBL3. The turbidity was also higher than the EBC standard value of 0.6 units but slightly decreased with the filtration time. When the rough-size kieselguhr CBR3 was replaced in layer 1 with middle-size kieselguhr CBR, in the filtration experiment V5, the turbidity value was 0.61 EBC units and decreased at 0.56 EBC units after 10 min. In the case of the filtration experiment V6, when only zeolite NZ100 and fine-size zeolite NZ40 were used to build the filter cake, the thinnest layer was obtained. Moreover, the turbidity of 0.80 EBC units decreased to 0.61 EBC units during a more extended filtration. In all filtration experiments, the filtered beers had turbidity lower than 1.0 EBC, stating a nearly brilliant to brilliant beer. Accordingly, it can be concluded that a mixture of zeolite NZ100 with middle-size kieselguhr is adequate to form a suitable thick filter layer to ensure satisfactory beer clarity.

Beer is regarded as an excellent source of polyphenols due to a wide range of phenolic compounds from barley and hops, but the final composition and levels of the phenolic compounds are contingent on the used raw materials, brewing process, quality and type of beer [53]. Besides their positive impact on avoiding oxidation, the polyphenols can affect the colloidal stability of beer, hence shortening its shelf life. However, it is difficult to assess the effect of polyphenols on foam stability, considering that the production industry of beer foam endorses additives to improve head retention [53]. A lower bitterness resulted in lower total polyphenolic content, similar results being reported by Jardim et al. [54]. The statistical evaluation using ANOVA indicated that the filtration does not affect the alcoholic concentration, polyphenols content, real extract or apparent extract of beer (Table 4). However, small variations in the original extract values were observed.

Colloidal particles sized around 0.5–3 μm give the specific color of beer [55] and should be maintained following the filtration. As seen in Table 4, the color of beer slowly decreased from 7.60 EBC to values in the range of 6.06–7.42 EBC, but, in all cases, it remained yellow color (6–8 EBC). In the filtration experiment V6, in which only zeolite (NZ100 and NZ40) samples were used to form the filter cake, a decrease of about 1 EBC unit in color was registered. The statistical evaluation showed that compared with the color of unfiltered beer, the color of beers filtered in the experiments using over 60% zeolite as filtering material is significantly different (at < 0.05 significance level), mainly when the filtering time increases, the zeolites owing the ability to slowly reduce the intensity of beer color with increasing contact time.

Table 5
Microbiological analysis of beer samples resulted from pilot-scale experiments (average $\pm$ s, three parallel measurements).

Sample	TPC (CFU mL^{-1})	Yeasts and mold (CFU mL^{-1})
V0	$(410 \pm 20) \times 10^2$	$(49 \pm 5) \times 10^2$
V1-0	$(60 \pm 9) \times 10^2$	$(19 \pm 2) \times 10^2$
V1-5	$(60 \pm 8) \times 10^2$	$(19 \pm 2) \times 10^2$
V1-10	$(60 \pm 10) \times 10^2$	$(19 \pm 3) \times 10^2$
V2-0	$(6 \pm 1) \times 10^2$	$(8 \pm 1) \times 10^2$
V2-5	$(6 \pm 1) \times 10^2$	$(8 \pm 1) \times 10^2$
V2-10	$(6 \pm 1) \times 10^2$	$(8 \pm 1) \times 10^2$
V3-0	$(8 \pm 1) \times 10^2$	$(14 \pm 1) \times 10^2$
V3-5	$(7 \pm 1) \times 10^2$	$(14 \pm 1) \times 10^2$
V3-10	$(7 \pm 1) \times 10^2$	$(13 \pm 1) \times 10^2$
V4-0	$(12 \pm 1) \times 10^2$	$(11 \pm 1) \times 10^2$
V4-5	$(12 \pm 2) \times 10^2$	$(11 \pm 1) \times 10^2$
V4-10	$(12 \pm 2) \times 10^2$	$(10 \pm 1) \times 10^2$
V5-0	$(12 \pm 2) \times 10^2$	$(7 \pm 0.6) \times 10^2$
V5-5	$(11 \pm 1) \times 10^2$	$(6 \pm 0.7) \times 10^2$
V5-10	$(11 \pm 1) \times 10^2$	$(6 \pm 0.6) \times 10^2$
V6-0	$(2 \pm 0.2) \times 10^2$	$(9 \pm 0.8) \times 10^2$
V6-5	$(2 \pm 0.3) \times 10^2$	$(8 \pm 0.9) \times 10^2$
V6-10	$(2 \pm 0.2) \times 10^2$	$(8 \pm 0.8) \times 10^2$

For the brewing industry, bitterness is an important quality parameter that arises when the α-acids from hops are isomerized into their corresponding iso-α-acids during beer brewing [37]. In the filtration experiment V1, which uses kieselguhr exclusively as a filtering aid, the highest decrease in bitterness value was observed, significantly different, due to the filter material's retention of bitter hop compounds (iso-α-acids). Oppositely, in the filtration experiment V6 using only natural zeolite to form the filter cake, the bitterness value is not significantly different in filtered beers (at < 0.05 significance level), indicating that the filtration with zeolite does not affect beer bitterness.

Sensory evaluation was conducted by trained assessors, which permits a consumer to blindly identify any differences between beer samples. Three samples were presented for each test, one different and two similar samples. The evaluated sensory characteristics included appearance, taste, mouthfeel, and aftertaste & finish. For all filtered beers, all the assessors appreciated that the characteristics of beer appearance (including color, clarity and foam) and taste (including sweetness, bitterness and sourness) were appropriate. Predominantly, for the beers filtered using individual zeolites (V6), six of the nine assessors noticed that reduced wort flavor of the beer. A possible explanation could be the elimination of some aldehydes by zeolites that acted as molecular sieves for these compounds [56,57]. The mouthfeel (alcohol, carbonation, body) and the aftertaste were also considered appropriate for all the filtered beers.

Natural zeolites are promising materials for the efficient immobilizing some microorganisms because of their high surface area and porosity. Metal-loaded zeolites considerably improve the antibacterial activity against *E. coli* and *S. Aureus* [58,59]. The microbiological analysis of beer samples resulting from pilot-scale experiments is presented in Table 5. No *Enterobacteriaceae* were detected in unfiltered beer or filtered beer, indicating that the natural zeolite does not contribute to the contamination of beer with this type of bacteria due to its sterilization at 400 °C during the thermal activation. The total plate count (TPC), including the total number of aerobic microorganisms in the unfiltered beer, was 410×10^2 CFU mL^{-1}. The beer filtration process had a positive impact on reducing TPC levels. Accordingly, when only kieselguhr was used for filtration (V1), TPC decreased to 60×10^2 CFU mL^{-1}, while introducing zeolites in filter mixtures significantly reduced the number of microorganisms in the filtered beer. The highest reduction of TCP, more than 200 times higher, was remarked when using only zeolites to form the filter cake (V6) confirming their high capacity for immobilization of microorganisms because of the zeolite's favorable characteristics (*i.e.* large surface area of 72 m^2 g^{-1} and total pore volume of about 0.16–0.17 cm^3 g^{-1}). Yeasts are chemoorganotrophic microfungi from processing organic matter, while mold presence is mainly associated with mycotoxins [26]. Considering the severe chronic and acute toxicity on human health, the mycotoxins beer contamination should be restricted as a priority for consumer health [60]. The microbiological analysis indicated that using natural zeolites as filtration aids in meaningfully reducing the yeasts and mold present in the filtered beer. The highest reduction of yeast and mold content, more than 5 times higher, was observed when using only zeolites and CBR to form the filter cake, and zeolites and CBL3 for filtration dosage (V2, V5 and V6).

Beer filtration with the activated natural zeolites presented here permitted the removal of beer microbiota and attained similar results to those achieved by other innovative non-thermal techniques, *i.e.* pulsed electric fields as an alternative to the traditional pasteurization process [61]. The filtered beer achieved adequate microbial reduction for studied microorganisms, delivering the minimum requirements for microbiological safe, non-thermal pasteurized beer. Moreover, while the traditional thermal pasteurization (60 °C for 15–20 min) disturbs the beer characteristics, alternative natural zeolites and non-thermal treatments could overcome these disadvantages.

Table 6

Concentration of major (Na, K, Ca, Mg, Fe) and trace (As, Cd, Co, Cr, Cu, Ni, Pb, Zn) elements in the beer samples resulted from pilot-scale experiments.

Sample	Na	K	Ca	Mg	Fe	As	Cd	Cr	Cu	Ni	Pb	Zn
	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	µg L	µg L	µg L	µg L	µg L	µg L	µg L
V0	3.26	176	14.0	40.0	1.22	<0.50	<0.20	<0.50	40.3	19.8	2.25	5.85
V1-0	4.91	218	15.7	40.9	0.52	1.36	<0.20	0.62	51.0	15.2	3.53	16.6
V1-5	4.38	202	17.4	40.3	0.44	1.53	<0.20	0.59	51.7	15.5	3.56	16.7
V1-10	4.12	218	16.2	40.1	0.51	1.42	<0.20	0.58	51.5	14.6	3.57	16.7
V2-0	4.24	190	24.0	33.4	0.47	2.44	<0.20	6.20	25.2	13.9	8.59	8.46
V2-5	4.71	201	23.0	34.6	0.46	2.35	<0.20	7.80	23.5	10.1	7.15	6.02
V2-10	3.99	229	23.8	34.0	0.51	2.76	<0.20	9.74	22.9	18.4	9.33	5.62
V3-0	4.67	252	27.0	39.2	0.65	1.77	<0.20	1.44	31.0	15.8	4.82	14.9
V3-5	4.86	260	25.5	39.0	0.71	1.93	<0.20	1.45	30.1	14.1	4.86	14.4
V3-10	4.99	265	25.7	39.4	0.89	2.06	<0.20	1.40	29.2	12.5	4.71	13.9
V4-0	4.77	204	25.3	37.8	0.94	1.12	0.35	1.43	38.0	18.0	6.60	16.3
V4-5	4.61	239	29.1	37.0	1.05	2.01	0.33	1.33	36.8	16.5	6.31	15.7
V4-10	4.47	237	30.6	37.3	1.07	2.34	<0.20	1.26	35.5	15.1	6.13	15.2
V5-0	3.46	216	29.5	32.5	0.78	2.68	0.41	2.30	22.1	10.0	6.10	11.4
V5-5	3.43	212	31.9	32.1	0.61	2.75	<0.20	2.24	20.4	9.91	5.82	10.8
V5-10	3.26	214	34.4	33.3	0.66	1.91	<0.20	2.20	19.2	8.06	5.65	10.3
V6-0	4.55	270	35.6	35.4	0.54	3.34	0.28	2.36	17.8	8.07	5.12	11.8
V6-5	5.08	280	36.2	36.6	0.62	3.26	0.42	2.33	17.4	7.77	5.04	11.6
V6-10	4.41	296	37.0	37.1	0.72	3.04	0.30	2.31	17.0	7.34	5.03	11.4

3.4. Effect of filtering materials on metals content in beer

Some elements play an important role in various biochemical and physiological functions and are required to maintain the human body's metabolism functioning efficiently. Fe, Cu, Cr, Ni, Co, and Zn are biologically essential micronutrients to ensure the catalytic activity of some enzyme reactions [62]. Other elements, such as As, Cd, and Pb are non-essential for biological processes and are hazardous even in low quantities, being a major public health problem [42,63].

The contents of major and trace elements in filtered and unfiltered beers are presented in Table 6. The contents of major elements decreased as follows: K > Mg > Ca > Na > Fe. The concentrations of Na and Mg remained almost unaffected following the filtration, while the contents of K and Ca increased in the filtered beers compared with the unfiltered beer, displaying the highest value in V6 filtered beer with only zeolite as a filter cake. A possible explanation could be the highest Ca and K contents in the used natural zeolite. The concentrations of Fe generally decreased in the filtered beers, zeolite being a well-known adsorbent for Fe from aqueous solutions [42]. Similar Fe concentrations were reported for commercial beer from Turkey's market content [64].

Generally, the concentrations of Cd were below the quantification limit (LOQ = 0.20 µg L^{-1}), with the highest Cd concentration of 0.42 µg L^{-1} being observed in V6. However, in all beer samples, the concentrations of Cd were below the maximum admitted level (MAL) for Cd in drinking water of 5.0 µg L^{-1} [65]. The concentrations of Ni and Cu generally lessened following the filtration and were below the corresponding MAL in drinking water of 2.00 µg L^{-1} for Cu and, respectively 20 µg L^{-1} in the case of Ni [65].

Oppositely, the concentrations of other trace elements (As, Cr, Pb and Zn) slightly increased in filtered beers because of contact with the filter materials. In the initial beer, the content of As was <0.50 µg L^{-1} and augmented to contents in the range of 1.12–3.34 µg L^{-1} after filtration but remained lower than the MAL for drinking water of 10 µg L^{-1} [65]. In a report on the beers from Italy Donadini et al. indicated an average higher concentration of As of 10.82 µg L^{-1}. The MAL for total Cr in drinking water is 50 µg L^{-1} [66]. Even though Cr concentration in filtered beers reached the highest concentration of 9.74 µg L^{-1} these were much lower than MAL. The lowermost Pb content was determined in the unfiltered beer (2.25 µg L^{-1}). The concentrations of Pb in the filtered beers fluctuated amongst 3.53–9.33 µg L^{-1} and did not exceed the MAL for drinking water of 10 µg L^{-1}. Zn is implied in enzymatic activity in the human body, being an essential element, but it becomes toxic in elevated concentrations. In general, the concentrations of Zn augmented in filtered beers but remained well below the MAL for drinking water [65].

The concentrations of toxic elements extracted in acidified ultrapure water (pH = 4.2) from NZ100 and NZ40 samples were 3.56 and 4.50 µg L^{-1} As, 0.40 and 0.30 µg L^{-1} Cd, 2.89 and 3.15 µg L^{-1} Cr, 10.8 and 12.4 µg L^{-1} Cu, 0.50 and 0.50 µg L^{-1} Ni, 7.20 and 8.29 µg L^{-1} Pb and 15.6 and 17.1 µg L^{-1} Zn, respectively. These values were generally in same order of magnitude as trace elements in filtered beer. However, it should be noticed that the increase of these elements is also observed when only kieselguhr is used for filtration (V1); thus, the increase of trace elements occurs generally following contact with mineral filter aids. On the other hand, the decrease of Cu concentration in beer following the filtration is observed only in the experiments in which natural zeolites are used. At the same time, the decrease of Ni content in filtered beer is more evident in the filtration experiments implying zeolites, which is explained by the high affinity of natural zeolites for these two metals [45]. Moreover, Cu is recognized to form complexes with the organic ligands present in beer, which seems to be better eliminated by zeolite than kieselguhr. This aspect is essential because Cu is one of the transitional elements associated with beer aging throughout oxidation reactions leading to the formation of highly reactive oxygen species (ROS), which alter the quality and stability of beer flavor; thus, its removal is critical [67].

The filtering materials obtained in this study could substitute or supplement the traditional filtering process used in the brewing industry to clarify and stabilize beer. However, before being applied in a real situation, the retention capability against spoiling and pathogenic microorganisms and the impact of the proposed treatment process on beer properties should be investigated.

The spent filtration aids from the brewing industry are a significant challenge considering its environmental and economic consequences. Therefore, the opportunities to recycle and reuse the brewery byproducts are crucial in improving the efficacy of production and consumption patterns, considerable progress towards sustainable development goals. A favorable way to recycle and reuse the brewery spent diatomite sludge is by applying it to croplands as a soil amendment or organic fertilizer [68,69]. Consequently, since natural zeolites are currently utilized to enhance the quality of agricultural soils [70], the spent zeolites resulting from the beer clarification process could also be a promising alternative in this field due to their high nutrient content. However, future studies to investigate the value of brewery spent zeolites as fertilizer are required in order to improve their efficient use and management as a high-quality alternative fertilizer in agriculture.

4. Conclusions

Commercial beer should be clear for good marketability and stability, excep the unfiltered beer class (craft beer). In this regard, the filtration capability of a fine- and middle-size natural zeolite, thermally activated at 400 °C, in different mixtures with commercial rough-, middle- and fine-size kieselguhr at pilot scale, by considering the main physicochemical, sensory and microbial characteristics. The pH value (4.0) of unfiltered beer slightly increased for the filtered beer, while the turbidity of unfiltered beer (19.4 EBC), consistent with a very hazy beer, decreased suddenly by about 95% of the total decrease for all filtered beers. The color of filtered beer slowly decreased from 7.60 EBC but remained in the domain of yellow color (6–8 EBC). The main physicochemical properties of filtered beer (alcoholic concentration, original, real extract, apparent extract, bitterness) displayed a very low variability. The As, Cd, Cr, Cu, Ni, Pb and Zn concentrations in all filtered beers were below the MAL in drinking water. All filtered beers' appearance (color, clarity and foam) and taste (sweetness, bitterness and sourness) were appropriate. The microbiological analysis indicated that using natural zeolites as filtration aids considerably reduced the number of existent microorganisms, yeasts and mold in the filtered beer. Our results offer valuable information that can be successfully used as preliminary information for using activated natural zeolites in the filtration

process for small and minibreweries and even for the large-scale beer industry. Moreover, using activated natural zeolites as filtering aids could maintain the properties of craft beer and expand its shelf life better than traditional preservation practices.

Ethics statement

The experiments were conducted according to established ethical guidelines, and informed consent obtained from the participants. The study complies with all regulations and confirmation that informed consent was obtained.

Author contribution statement

Marin Senila, Oana Cadar: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Teodora Emilia Coldea, Lacrimioara Senila: Performed the experiments; Analyzed and interpreted the data. Elena Mudura: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20031.

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