Effects of different stabilization techniques on the shelf life of cold brew coffee: Chemical composition, flavor profile and microbiological analysis

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ARTICLE INFO

ABSTRACT

Cold brew coffee is a beverage prepared at low temperatures and over long brewing times resulting in particular physicochemical and sensory characteristics. This type of coffee is usually consumed immediately after preparation or after short storage in refrigerated conditions. However, in recent years many commercial coffee vendors started investing in ready-to-drink cold brew coffees packaged for extended storage. These products present the potential problem of microbial and sensorial alteration. In this study we evaluated five different stabilization techniques with the aim to extend the shelf life of the cold brew coffee while preserving its peculiar organoleptic properties, e.g. HPP (High Pressure Processing), microfiltration, UV irradiation, pasteurization and blast chilling process. The effects were investigated over four months, evaluating the caffeine and the chlorogenic acids contents by HPLC-DAD analysis and carrying out anevaluation of selected volatile compounds by HS-SPME-GC-MS. After four months of storage, the samples treated with pasteurization and HPP maintained a stable content of caffeine and chlorogenic acids, guaranteeing also the microbiological safety of the beverage. At the same shelf-time, the pasteurized samples showed an unaltered flavor profile, while a decrease of about 25% of the total content of volatile compounds was registered for the HPP sample.

1. Introduction

A large number of different coffee brewing and extraction methods have been introduced in recent years. Their distribution varies worldwide depending on traditions, personal preferences, social behavior and commercial factors. The brewing method affects the composition of the final coffee beverage, producing considerable differences in phenolic and caffeine content, physical parameters, antioxidant activity and volatile profile (Lopez-Galilea, De Pena, & Cid, 2007).

Caffeine is widely studied in coffee due to its stimulatory effects on the central nervous system and the positive effect on long-term memory (Heckman, Weil, & Gonzalez de Mejia, 2010; Ludwig et al., 2014), while chlorogenic acids (CGAs), the main phenolic compounds in coffee, have been recognized as antioxidants and responsible for several health benefits by a large number of studies (Brezova, Slebodova, & Stasko, 2009; Tajik, Tajik, Mack, & Enck, 2017; Yen, Wang, Chang, & Duh, 2005). These bioactive compounds are partially recovered during the

extraction process, and their content varies depending on several factors such as the brewing technique and the specific selected recipe (Angeloni, Masella, et al., 2019; Gloess et al., 2013; Lopez-Galilea et al., 2007; Parenti et al., 2014).

Generally, the most popular coffee beverages are prepared by hot brewing methods, but the cold brew coffee preparation techniques have grown in popularity in recent years. Cold brew coffee is prepared at room temperature (20 °C or colder) over a longer extraction time than traditional hot brewing methods, with typical steeping times ranging from 3 to 20 h resulting in a final coffee beverage with specific physicochemical and sensory characteristics (Angeloni, Guerrini, Masella, Innocenti, et al., 2019; Fuller & Rao, 2017).

Despite the growing consumption of cold brew coffee and the interest regarding this market, only few scientific studies have been published on its chemical composition, sensorial characteristics and flavor profile. Angeloni et al. (2019) compared two different cold extraction processes, the cold brew and cold drip methods, demonstrating the significant

effects of extraction method and temperature on caffeine and CGAs concentrations in the beverages, in addition to the flavor profiles in terms of bitterness, sweetness, sourness and global intensity. An additional study by Cordoba, Pataquiva, Osorio, Moreno, and Ruiz (2019), reported that the grinding level of coffee beans, the extraction time and the coffee type can affect some physicochemical and sensorial characteristics of cold brew coffee, leading to beverages with different flavor profiles. The bioactive compounds in cold brew coffee have been studied by Fuller and Rao (2017), who observed higher concentrations of 3-*O*-caffeoylquinic acid and caffeine in coffee made with medium-roasted beans, while the grind size did not significantly impact on the concentrations of these compounds.

Cold brew coffee is usually consumed immediately after preparation or after short storage in refrigerated conditions, even if in recent years many commercial coffee vendors have invested in ready-to-drink beverages that can be consumed after shelf storage. This practice presents the potential problem of microbial and sensorial alteration. An exhaustive literature search revealed very limited publications analyzing cold brew coffee shelf-life.

The aim of this study was to compare different stabilization techniques, applied in their standard process conditions, in order to extend the shelf life of cold brew beverages, trying also to preserve the peculiar organoleptic properties of this product.

For this purpose, five physical treatments were considered: HPP (High Pressure Processing), microfiltration, UV irradiation, pasteurization and blast chilling process. The effects of these different techniques were evaluated investigating over four months the caffeine and the CGAs contents by HPLC-DAD analysis. At the same time, microbiological analyses were performed to guarantee the absence of microorganisms like yeasts, bacteria and molds. Finally, an estimation of the flavor profile of the cold brew coffee by a quantitative determination of some selected volatile compounds by HS-SPME-GC-MS technique was carried out.

2. Materials and methods

2.1. Coffee samples and cold brew extraction

A single batch of 100% Arabica coffee (Ethiopian, Gidey) kindly provided by the D612 coffee roastery (Florence, Italy) was used for the cold brew extraction. The roasting process was carried out by the same coffee roastery for 12 min at 200 °C to give light-medium roasting degree. Beans were coarse-ground using a professional grinder (EK43 Mahlkönig AG, Switzerland). Cold brew coffee was prepared by steeping using a Cold Pro™ Brewing Systems, with 1.8 kg of coffee powder and 30 L of a commercial mineral water (Acqua Levissima; TDS 80 mg/L, calcium hardness 57 mg/L, alkalinity 32 mg/L, pH 7.8). Powder and water were in contact for 16 h at room temperature (20 °C); when extraction ended the beverage was filtered and immediately subjected to the different treatments.

2.2. Stabilization treatments

Five stabilization treatments were tested, applying for each one the standard conditions described below.

2.2.1. HPP (High Pressure Processing)
The cold brew coffee was packed in 250 mL transparent PET bottles and exposed to HPP treatment, as already described in a previous study (Guerrini et al., 2020). A JBT Avure™ HPP industrial plant (HPP Italia srl, Traversetolo, Parma, Italy) was used. A chamber was filled with water at controlled temperature (around 18–20 °C) throughout the HPP treatment, the pressure was increased from atmospheric pressure to a working pressure of 608 MPa in 200 s, the working pressure was maintained for 360 s and then the coffee samples were returned, almost instantaneously, to atmospheric pressure.

2.2.2. Filtration process
Microfiltration process was applied on the cold brew coffee sample using a disc filter equipped by a paper filter membrane. The technical specifications, which were provided by the filter producer, were as follows: weight, 1500 g/m^2; nominal cut-off filtration, 0.5 μm. The procedure was operated with a constant flow rate of 600 mL/min. After treatment the sample was bottled in 250 mL new PET bottles and immediately sealed with a plastic screw cap under sterile conditions.

2.2.3. UV irradiation
A low-pressure mercury tubular lamp (Philips TUV PL-S 11W 2P UV-C), mainly emitting light at 254 nm, was used for the UV irradiation experiments. The coffee sample was located under the lamp in a petri dish equipped with a magnetic stirrer. The coffee depth was 1 cm. The lamp was arranged with a collimating tube in such a way that parallel beams reached the petri dish, as described by Qualls, Flynn, and Johnson (1983). During the test runs, the UV intensity was kept constant. All samples were exposed at room temperature (25 ± 5 °C).

After treatment, the cold brew coffee sample was bottled in 250 mL new PET bottles and immediately sealed with a plastic screw cap under sterile conditions.

2.2.4. Pasteurization
The cold brew coffee was placed in 250 mL transparent PET bottles and then in a thermostatic bath (FA 90, Falc Instruments s.r.l) at 65 °C; the samples were left in this conditions for 30 min, according to the standard protocol reported in the EC Regulation 853/2004 (EC, 2004).

2.2.5. Blast chilling
Cold brew coffee sample was bottled in 250 mL new PET bottles and then exposed to blast chilling (−18 °C for 60 min) in a refrigerator (IRINOX, MF25.1).

Following treatment, each bottle of cold brew coffee was labelled and stored at room temperature (20 °C) for the shelf life study.

2.3. Experimental design

The experiment was designed to evaluate the effects of the five stabilization techniques over four months, carrying out microbiological analysis and evaluating changing in the chemical and flavor profile. For this aim a shelf life study was conducted keeping coffee at room temperature and taking samples at 0, 7, 30, 60 and 120 days, corresponding to t_0, t_1, t_2, t_3 and t_4 respectively.

At t_0 and t_1 all the samples were subjected to the specified analysis; after 30, 60 and 120 days of storage only the treated samples which did not exhibit a microbiological contamination were investigated. For food safety reasons, the sensory evaluation was performed only for the sample at t_0. Three replicates were carried-out for each treatment.

2.4. pH and titratable acidity

The pH was measured in triplicate at 25 °C using a digital pH meter (GLP 21, Crison Instruments, Spain). The titratable acidity (TA) was measured using 40 mL of each sample; this volume was titrated with 0.1 mol/L NaOH up to pH 6.0 and 8.0, recording the volumes of NaOH; the TA was expressed as difference between the volumes of NaOH registered at the two pH.

2.5. Microbiological analysis

The microbiological tests were carried out by FoodMicroTeam s.r.l., Academic Spin-Off of the University of Florence.

WL nutrient agar (Oxoid Ltd, Basingstoke, Hampshire, UK) with the addition of sodium propionate (2 g/L) and streptomycin (0.3 g/L) (Alfa Aesar, Thermo Fisher, Kandel, Germany) was used for the determination of the yeasts. Sabouraud agar (glucose 40 g/L, peptone 10 g/L, agar 20

g/L, all from Oxoid Ltd) was used for the determination of molds; for total bacterial count, PCA agar (Oxoid Ltd) with the addition of Pimaricin (0.05 g/L) (VGP, SL, Barcelona, Spain) was used. The enumeration of yeasts and molds population and total bacterial count in cold brew coffee were determined by serial dilutions of the sample and subsequent surface sowing on selective culture media.

The plates were counted after incubation for 3 days at 30 °C under aerobic conditions. The microbiological limits of acceptability have been set according to Rev. 00/2013 (Regione Piemonte D.D. n. 780, Rev. 00/2013).

2.6. Analyses of caffeine and CGAs

Cold brew coffee samples were diluted 1:10 with distilled water and then centrifuged at 16,900×g for 5 min before HPLC-DAD analysis.

HPLC was carried out using an Agilent HP 1100 system equipped with an autosampler, column heater module and quaternary pump, coupled to a diode array detector (DAD) all from Agilent Technologies (Palo Alto, CA, USA). A 150 mm × 3 mm i.d., 2.7 μm Poroshell 120, ECC18 column (Agilent Technologies) was used, equipped with a pre-column of the same phase, and maintained at room temperature. The analysis conditions were the same reported in our previous works (Angeloni, Guerrini, Masella, Bellumori, et al., 2019; Angeloni, Guerrini, Masella, Innocenti, et al., 2019); the elution method was performed at a flow rate of 0.4 mL/min using water at pH 3.2 by formic acid and acetonitrile, applying a multistep linear solvent gradient.

Chromatograms were registered at 278 and 330 nm for caffeine and CGAs, respectively. Caffeine and CGAs were identified by comparing their retention times and UV–Vis spectra to those of the respective standards, when it was possible, or with our previous data. CGAs were evaluated by HPLC-DAD using a six-point calibration curve of 5-O-caffeoylquinic acid (purity 99%) (Extrasynthèse, Genay, France) at 330 nm (0–1.509 μg; $R^2 = 0.9991$) and caffeine content using a six-point calibration curve from Extrasynthèse (purity 95%) at 278 nm (0–0.7575 μg; $R^2 = 0.9993$). Quantitative data were expressed as mg/mL of cold brew coffee.

2.7. HS-SPME-GC-MS analysis

The concentration of selected volatile organic compound in the beverages was determined by HS-SPME-GC-MS. An Agilent 7820 gas chromatograph, equipped with a 5977 MSD detector with electron ionization ion source (Agilent, Santa Clara, CA, USA) was used for the analyses. A DVB/CAR/PDMS 50/30 μm-2 cm fiber (Supelco, Sigma, Darmstadt, Germany) was used for head space sampling, as indicated in literature (Akiyama et al., 2007; Mondello et al., 2005). The system was equipped with a Gerstel MPS2 XT (Gerstel GmbH & Co, KG, Germany) autosampler for automated SPME analysis. The analyses were carried out by pipetting 5 mL of each specimen and 50 μL of an internal standard mixture (ISTD MIX) and 2 g of sodium chloride into 20 mL screw-cap vials fitted with a PTFE/silicone septum. After 5 min of equilibrium at 60 °C, the SPME fiber was exposed in the vial headspace for 15 min under orbital shaking (750 rpm). Then, the fiber was immediately desorbed for 20 min in the GC/MS injection port operating in splitless mode at 260 °C. The ISTD MIX consisted of ethyl acetate-d8 (isotopic purity 99.5 atom % D, 99% CP), o-xylene-d10 (isotopic purity 99 atom % D, 99% CP), 1-butanol-d10 (isotopic purity 99 atom % D, 99% CP), 3, 4-dimethylphenol (purity 98%), naphthalene-d8 (isotopic purity 99 atom % D, ≥98% CP) and 5-methylhexanol (purity 97%). All these standards were from Sigma-Aldrich (Steinheim, Germany).

A 60 m × 0.20 mm ID, 0.5 μm DF Innowax capillary column (J&W, USA) was used for the analysis. The initial column temperature was held at 40 °C for 1 min, then increased to 220 °C at 5 °C/min, then to 260 °C at 10 °C/min, and finally to 250 °C at 10 °C/min, with a hold time of 2 min. Helium was used as the carrier gas at a constant flow of 1.2 mL/min. The temperature of the ion source was 230 °C and the transfer line was held at 250 °C. The mass detector was operated in scan mode within a 30–330 Th mass range at 1500 Th/s, with an IE energy of 70 eV.

The identification of the volatile compounds was performed by comparison of retention times and mass spectra with those of authentic standard injected for calibration. For constructing the calibration lines, a standard solution mix (STD MIX) was prepared containing the 17 selected analytes as follows: each pure compound was initially diluted in a water/acetone solution 50/50 to a concentration of 10,000 mg/L (1:100 dilution). Aliquots of these diluted solution were added in a 50 mL volumetric flask and brought to volume with distilled water such to obtain the highest concentration of the calibration scales. All the other calibration levels were obtained by dilution (2, 4, 8, 16, 32 times) of this STD MIX solution. Finally, 5 mL of each calibration level was pipetted into 20 mL headspace vials and analyzed as for the specimens.

The complete list of the 17 quantitated compounds, their calibration equations, the R^2 and the ISTD used for the quantification of each compound are reported in Table 1S of supplementary materials section.

2.8. Sensory analysis

Sensory evaluation was conducted by 12 trained panelists with Projective Mapping (Napping) methods (Nestrud & Lawless, 2010). The respondents gave their written informed consent at the beginning of the test according to the principles of the Declaration of Helsinki. All panelists were trained in sensory evaluation and had already participated in previous sensory studies. Judges were asked to try the samples and to locate them on an A3 white sheet (42 × 30 cm), according to their similarities or dissimilarities. Judges were explained that they had to complete the task according to their own criteria. They were also explained that two samples close together on the sheet would correspond to very similar samples and that if they perceived two samples as very different, they had to locate them very distant from each other. After positioning the samples on the evaluation sheet, consumers were asked to provide a brief description of them. For each consumer map, the X and Y coordinates of each sample were determined, considering the left bottom corner of the sheet as the origin of coordinates. The X and Y coordinates for each session and sample set were analyzed using multiple factor analysis (MFA), as recommended by Pagès (2005).

2.9. Statistical analyses

Conventional analysis of variance (ANOVA) was used to compare means and standard deviation determined for the different samples. The tested factors were considered significantly different at $P < 0.05$. All statistical analyses were performed using R software (version 3.4.0 for Windows). Concerning the sensory analysis, data were analyzed by Multiple Factor Analysis (MFA) using R software, with the addition of FactoMineR packages for multivariate data processing.

3. Results and discussion

The interest around cold brew coffee increased in the recent years, particularly on ready-to-drink (RTD) beverages which can be consumed at a later date. In this context, the safety of the product is mandatory and the potential problem of microbial contamination is a crucial point on which depend further chemical and sensory investigations. For the purposes of this study, the shelf life of cold brew coffee was examined by evaluating the effects of the five physical treatments over four months, and particularly after 7, 30, 60 and 120 days.

3.1. Effects of stabilization treatments (t_0)

3.1.1. Microbiological analysis

Molds and yeasts counting and the total bacterial count (TBC) from the non-treated and treated cold brew coffee samples are presented in Table 1.

Table 1
Populations of yeasts and molds and total bacterial count (TBC) expressed as CFU/mL detected in cold brew coffee samples at t_0, t_1, t_2, t_3 and t_4.

Samples	t_0			7 days			30 days			60 days			120 days		
	Yeasts	Molds	TBC	Yeasts	Molds	TBC	Yeasts	Molds	TBC	Yeasts	Molds	TBC	Yeasts	Molds	TBC
Non-treated	<5	<5	10^3	10^4	20	10^7	-	-	-	-	-	-	-	-	-
Blast chilling	<5	<5	118.3	10^4	398.3	10^4	-	-	-	-	-	-	-	-	-
UV irradiation	<5	<5	109.3	10^5	78	10^7	-	-	-	-	-	-	-	-	-
Filtration	<5	<5	10.0	43.3	270	<5	<5	10^3	<5	-	-	-	-	-	-
Pasteurization	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
HPP	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5

No yeasts and molds (<5 CFU/mL) were detected in all the samples. The non-treated sample showed a TBC of 10^3 CFU/mL, below the limits of acceptability set for this type of beverage (<10^6 CFU/mL). However, all treated samples registered a decrease in the TBC and particularly, HPP and pasteurization processes showed at these operating conditions the highest efficacy with values below 5 CFU/mL.

3.1.2. pH and titratable acidity (TA)

The pH value and the TA were measured in the non-treated and treated cold brew coffee sample and are reported in Fig. 1.

The measurement of pH quantifies the concentration of aqueous hydrogen ions at the time of analysis, providing a metric for the quantity of deprotonated acid molecules in a sample. The acidity of the brew is an important criterion for a good cup of coffee, because a well-balanced acidity is often associated to a good coffee flavor (Gloess et al., 2013).

The cold brew coffee samples at day 0 were found to have pH values ranging from 4.90 to 4.98, in line with those reported in literature for this type of coffee (Rao & Fuller, 2018). Only the UV-irradiated sample showed statistically significant differences compared to the non-treated one, showing to be the most acidic sample with a pH of 4.98 ± 0.02.

Total titratable acidity (TA) is a measure of all acidic protons in a sample, including non-dissociated protons, that can be neutralized through the addition of a strong base. The TA was expressed as the difference in mL of 0.1 mol/L NaOH required to titrate 40 mL of coffee to a pH of 6.0 and a pH of 8.0. These two endpoints were chosen in accordance with other studies (Gloess et al., 2013; Rao & Fuller, 2018) in order to provide some insights about the acid contents in coffee. As reported in Fig. 1, no significant differences were observed between the

non-treated and the treated samples. The UV-irradiated sample showed the lowest value of TA and therefore the lowest concentration of total titratable acids at both pH of 6 and 8 (2.67 ± 0.12 and 5.07 ± 0.12, respectively) highlighting a correlation between pH and TA.

3.1.3. Caffeine and CGAs evaluation

The caffeine and the chlorogenic acids (CGAs) are the main bioactive compounds in coffee beverages. Considering the biological importance and the influence on the aroma and the flavor of these compounds, the study of the effect of the five technologies on the concentration of these molecules was carried out.

The HPLC/DAD profiles at 278 and 330 nm for monitoring caffeine and CGAs respectively, showed the presence of the same bioactive molecules in all the cold brew samples (Fig. 1S and Table 2S of supplementary material); a total of 15 CGAs were detected and identified based on retention time, UV spectra and mass spectrometric data, in accordance with our previous studies (Angeloni, Guerrini, Masella, Bellumori, et al., 2019; Angeloni, Guerrini, Masella, Innocenti, et al., 2019). In particular, six caffeoylquinic acid, one feruloylquinic acid, one p-coumaroylquinic acid, four caffeoylquinic acid lactones and three dicaffeoylquinic acids were identified. The most abundant CGA was 5-O-caffeoylquinic acid (5-CQA) followed by its isomers 3- and 4-CQA, as already reported in previous studies (Angeloni, Guerrini, Masella, Innocenti, et al., 2019; Fujioka & Shibamoto, 2008).

Fig. 2 showed the concentration (mg/mL) of these molecules at t_0, after the stabilization treatment. Regarding the caffeine content, none of the five treatments showed significant effects, including heating processes such as pasteurization. In fact, the stability of this alkaloid at the
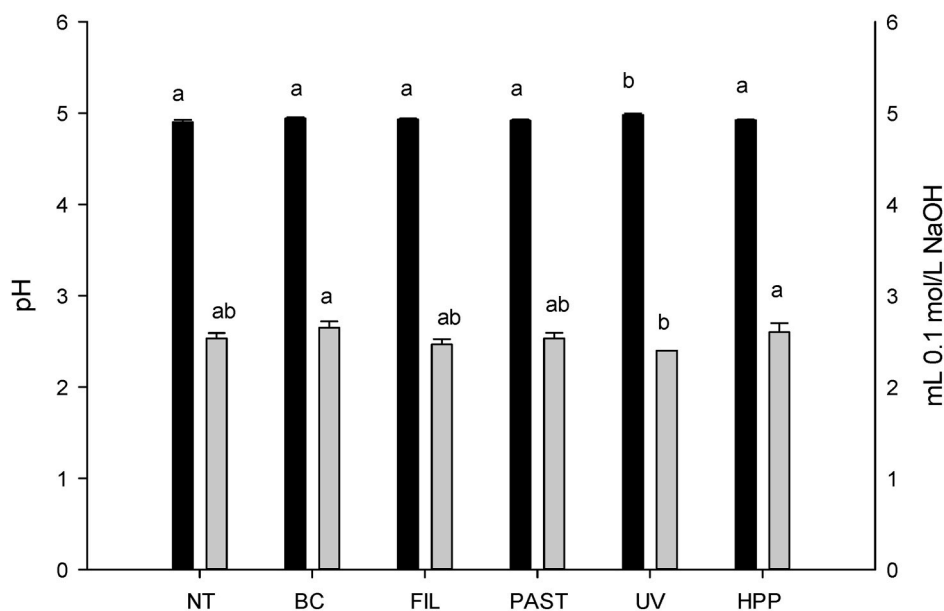

Fig. 1. pH and titratable acidity (TA) of each sample at day 0 (t_0). TA was expressed as difference between the mL of 0.1 mol/L NaOH registered at pH 6 and 8. NT: Non-treated sample, BC: blast chilling, FIL: filtration, PAST: pasteurization, UV: UV irradiation, HPP: HPP process. Data are expressed as mean ± standard deviation of triplicates. Different letters indicate significant differences among treatments ($P < 0.05$). pH: black bars, TA: grey bars.
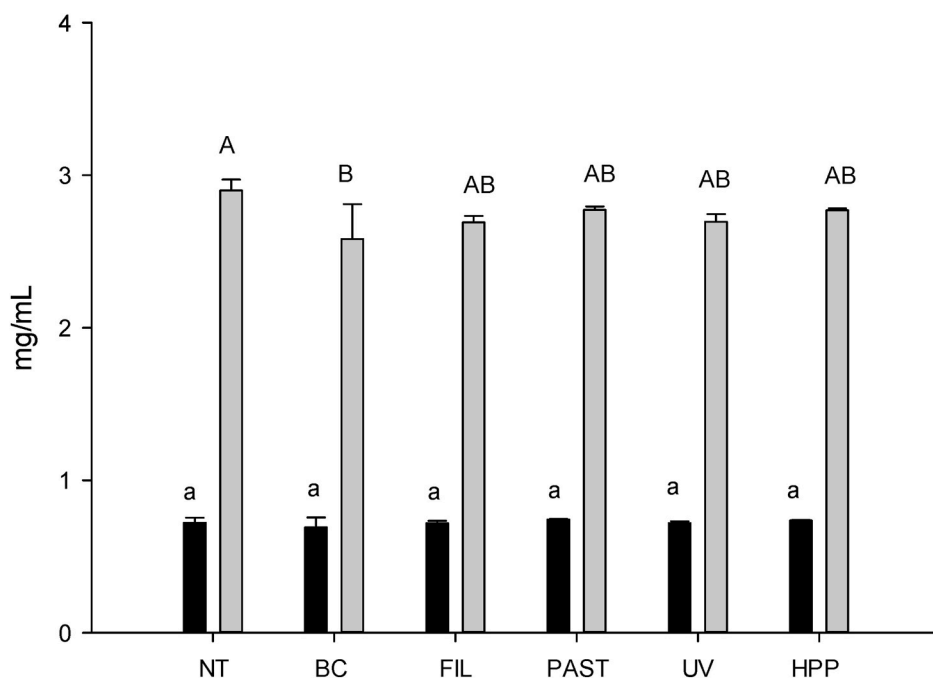
Fig. 2. Concentration (mg/mL) of caffeine and total chlorogenic acids (CGAs) at t_0.
NT: Non-treated sample, BC: blast chilling, FIL: filtration, PAST: pasteurization, UV: UV irradiation, HPP: HPP process. Data are expressed as mean ± standard deviation of triplicates. Different letters indicate significant differences among treatments ($P < 0.05$) for the caffeine content (lowercase letters) and for the CGAs content (uppercase letters). Caffeine: black bars, CGAs: grey bars.

high temperatures, such as those applied during the roasting process, is well known (Ludwig et al., 2014). The caffeine concentration (mean value of 0.72 mg/mL) was similar to that reported by Angeloni et al. (2019), who showed values of 0.97 ± 0.12 mg/mL in cold brew coffee obtained at room temperature after about 6 h of extraction time. On the other hand, in another previous study a higher concentration of caffeine (1.25 ± 0.12 mg/mL) was found in cold brew coffee extracted in similar conditions (Angeloni, Guerrini, Masella, Bellumori, et al., 2019). It is well known that during the brewing process, the extraction of coffee compounds, such as caffeine, can be affected by several factors; in this latter study, the powder-to-water ratio was lower to that used in the previous ones and, in addition, Arabica coffees of different geographical origin and roasting degree were used, contributing to the variations observed in the caffeine contents among these three studies.

As regards CGAs, the non-treated cold brew sample showed a content of 2.90 ± 0.07 mg/mL (Fig. 2). The concentration of these compounds was not affected by treatments, except for the blast chilling process which led to a 11% reduction. The blast chilling process showed also the highest variability and this may be due to an irregular freezing phase during the cooling of the samples.

Unlike caffeine, CGAs are heat-sensitive compounds and their content can strongly decline during roasting; Ludwig et al. (2014) reported losses of 90% in the dark roasted beans compared to the unroasted green beans. Our data did not show a decrease in the content of these compounds in the sample after pasteurization at 65 °C for 30 min, probably due to the lower temperatures applied.

Comparing the concentration of these compounds with those reported in previous studies, our data showed intermediate values; particularly, a lower content of total CGAs (about 1.12 mg/mL) was registered in Angeloni et al. (2019), while an higher amount (about 3.9 mg/mL) was observed in another of our previous work (Angeloni, Guerrini, Masella, Bellumori, et al., 2019). This variability could be due to the different degree of roasting of coffee beans, which affected the concentration of these thermolabile compounds.

3.1.4. Volatile organic compounds

Coffee is mostly consumed for its flavour and its aroma that are key attributes defining the quality of the product. Only a few studies regarding cold brew coffee and its flavour characteristics have been published so far.

In this study we monitored the changes in the concentration of some key aromatic compounds selected within the most representative classes in coffee, e.g. pyrazines, furans, aldehydes, ketones and phenols; particularly, 17 volatile compounds were selected and quantitated with a quantitation method in HS-SPME-GC-MS using the multiple internal standard normalization and calibration curves constructed with pure authentic standards (Table 2). The total ion current profile of a cold brew coffee extract and the extract ion chromatograms for each quantitated compound are reported in Figures 2S and 3S of supplementary materials.

The highest number of volatile compounds in the cold brew coffee samples were furans (Cordoba et al., 2019), which exhibit malty and sweet roasted aromas with relatively high sensory thresholds compared to other volatile groups found in coffee (Sunarharum, Williams, & Smyth, 2014). Table 2 reported that furfuryl alcohol was the most abundant volatile compound among the selected ones, with values of 4.94 mg/kg in non-treated sample. Furfuryl alcohol is present in many fruits, tea, coffee and cocoa, and its flavor characteristics is related to sweet, bread-like and caramel flavors (Amanpour & Selli, 2016). No significant differences were observed between the non-treated sample and the treated ones for the concentration of this compound at t_0. Regarding the other furans, the only treatment that showed a significant decrease in the content of a compound compared to non-treated cold brew was the blast chilling process in the case of furfuryl acetate, showing a variation from 0.80 to 0.60 mg/kg.

Ketones and aldehydes were the second highest number of volatile compounds in cold brew coffee samples. According to López-Galilea (2006), ketones are one of the most abundant compounds in filtered coffee brews and have been described as having buttery, caramel-like, musty, mushroom-like or fruity odor notes. Three ketones were monitored in our study (2,3-pentanedione, β-damascenone and 2-butanone) and no difference was observed in the concentration of these compounds among the different stabilization treatments. Three aldehydes were also considered in our samples; these compounds have been related to chocolate and malty odours in coffee (Lopez, Wellinger, Gloess, Zimmermann, & Yeretzian, 2016). Among these, 2-methylbutanal showed the highest concentration (3.75 mg/kg) and no statistically significant differences were observed for this class of compounds

Table 2

Concentration of the 17 volatile compounds monitored in cold brew coffee samples at t_0. Data are expressed in mg/kg as mean ± standard deviation of triplicates.

Compounds	Non-treated	Blast chilling	Filtration	Pasteurization	UV irradiation	HPP	OT (mg/kg)	Sensory descriptors
2-methylfuran	0.58 ± 0.17	0.43 ± 0.11	0.7 ± 0.09	0.9 ± 0.37	0.79 ± 0.34	0.67 ± 0.13	0.0035	Spicy smokey*
	a	a	a	a	a	a		
2,5-dimethylfuran	0.03 ± 0.02	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	-	Spicy smokey
	a	a	a	a	a	a		
Furfuryl methyl ether	0.40 ± 0.05	0.33 ± 0.03	0.37 ± 0.05	0.41 ± 0.04	0.43 ± 0.02	0.46 ± 0.01	-	Roasted coffee
	ab	b	ab	ab	ab	a		
Furfuryl methyl sulphide	0.12 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.1 ± 0.02	0.11 ± 0.01	0.12 ± 0.00	-	Roast Burnt
	a	a	a	a	a	a		
2-furanmethanol acetate (furfuryl acetate)	0.80 ± 0.11	0.60 ± 0.07	0.67 ± 0.03	0.64 ± 0.02	0.74 ± 0.05	0.73 ± 0.02	0.1	Fruity*
	a	b	ab	ab	ab	ab		
2-furanmethanol (furfuryl alcohol)	4.94 ± 0.22	4.35 ± 0.96	5.05 ± 0.61	4.98 ± 0.33	4.82 ± 0.25	4.11 ± 0.32	2	Bread-like, caramel*
	a	a	a	a	a	a		
2-methylbutanal	3.75 ± 0.17	3.08 ± 0.22	3.00 ± 0.92	3.13 ± 0.84	3.31 ± 0.50	4.69 ± 0.60	0.0019	Malty*
	ab	ab	b	ab	ab	a		
3-methylbutanal	0.73 ± 0.18	0.75 ± 0.05	0.70 ± 0.18	0.73 ± 0.16	0.77 ± 0.11	1.06 ± 0.12	0.0004	Malty*
	a	a	a	a	a	a		
Hexanal	1.44 ± 0.13	1.05 ± 0.47	1.34 ± 0.22	1.46 ± 0.16	1.60 ± 0.15	1.77 ± 0.09	0.0007	Vegetable, green*
	ab	b	ab	ab	ab	a		
2,3-pentanedione	3.84 ± 1.01	2.82 ± 0.29	3.22 ± 1.69	2.77 ± 1.26	3.82 ± 1.41	3.55 ± 0.43	0.030	Buttery, caramel*
	a	a	a	a	a	a		
β-damascenone	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.00000075	Fruity*
	a	a	a	a	a	a		
2-butanone	3,3 ± 0.27	3.41 ± 0.1	2.5 ± 1.3	3.71 ± 0.13	2.92 ± 1.57	3.76 ± 0.21	50	Chemical, fruity
	a	a	a	a	a	a		
2,5-dimethylpyrazine	1.89 ± 0.41	1.06 ± 0.68	1.57 ± 0.18	1.74 ± 0.14	1.79 ± 0.26	1.59 ± 0.31	1.6	Cocoa, nutty*
	a	a	a	a	a	a		
2,3,5-trimethylpyrazine	2.16 ± 0.28	1.58 ± 0.29	1.50 ± 0.72	2.06 ± 0.07	1.57 ± 0.76	1.96 ± 0.20	0.6	Earthy *
	a	a	a	a	a	a		
1-furfurylpyrrole	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.00	0.1	Vegetable, green*
	a	a	a	a	a	a		
2-acetyl-1-methylpyrrole	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	2	Nutty
	a	a	a	a	a	a		
Guaiacol	0.48 ± 0.07	0.39 ± 0.16	0.46 ± 0.06	0.46 ± 0.04	0.44 ± 0.01	0.39 ± 0.03	0.007	Smoky, Phenolic *
	a	a	a	a	a	a		
Total volatile compounds	24.6 ± 1.61	20.06 ± 1.55	21.27 ± 1.34	23.23 ± 2.6	23.25 ± 1.23	24.98 ± 0.06		
	a	b	ab	ab	ab	a		

OT: Odor threshold; these values are taken from bibliographical sources (Amanpour & Selli, 2016; Grosch, 1998; Piccino, Boulanger, Descroix, & Sing, 2014; Steen, Waehrens, Petersen, Munchow, & Bredie, 2017). *Significant odor active values (OAV) > 1; this parameter was calculated by dividing the concentration by the odor threshold. Sensory descriptors are taken from the literature (Amanpour & Selli, 2016; Grosch, 1998; Piccino et al., 2014; Semmelroch & Grosch, 1996; Steen et al., 2017). Different letters indicate significant differences among treatments ($P < 0.05$).

between the non-treated and treated-samples.

Pyrazines were also detected in cold brew coffee samples. These compounds have been described as having nutty, earthy, roasty and green aromas (Cordoba et al., 2019) and to be most noticeable at higher serving temperatures (Lopez-Galilea, Fournier, Cid, & Guichard, 2006). The samples analyzed in this study showed a total content of these compounds of about 4.05 mg/kg (for non-treated sample) and their concentration did not show significant differences among the various treatments (Table 2). Another class of volatile compounds that can be found in coffee beverages are pyrroles. Flament (2002) describes these compounds as furan degradation products and amino acid derivatives. Our results showed that pyrroles have been detected only in low

amounts in cold brew coffee samples and their content did not show significant differences among the samples.

Only one phenol, i.e. guaiacol, was considered in cold brew coffee samples, with concentration of 0.48 ± 0.07 mg/kg for non-treated sample. Phenols are reported to be responsible for the smoky phenolic odor notes in roasted coffee (Semmelroch & Grosch, 1996). Guaiacol content did not show significant differences between the non-treated and the treated-samples.

Summarizing our results, the stabilization techniques showed an effect on the volatile composition of the cold brew coffee only for one compound of the seventeen selected.

On the other hand, considering the total volatile compounds content,

statistically significant differences were observed among the treatments ($P = 0.012$). Particularly, the blast-chilled sample showed the lowest value of total volatile compounds (20.1 mg/kg) and significant differences were observed between this sample and the non-treated and the HPP-treated ones (24.6 and 25.0 mg/kg, respectively).

Finally, some differences were observed among the treated samples for three compounds i.e. furfuryl methyl ether, 2-methylbutanal and hexanal. As reported in Table 2, HPP technique showed the highest content of these molecules; furfuryl methyl ether and hexanal showed significant differences between HPP and blast chilling processes, while 2-methylbutanal showed significantly lower values for filtration process.

3.1.5. Sensory analysis

Projective mapping or Napping® is a holistic method based on assessor's individual perception of overall similarities and dissimilarities among products. Assessors are asked to provide a two-dimensional representation of a group of samples, according to their own criteria (Risvik, McEwan, Colwill, Rogers, & Lyon, 1994). In this representation, the Euclidean distance between each pair of samples is a measure of their dissimilarity.

The first two dimensions of the Projective mapping explained 64.2% of the variance. As shown in Fig. 3, the disposition of samples evaluated at t_0 reflected the results obtained by GC-MS analysis. Indeed, the samples subjected to blast chilling (BC) and filtration (F) processes have been perceived as different by the assessors, compared to the others. The blast chilled sample showed strong sour and salty attributes, while the filtered sample was characterized by a strong bitter taste. The other samples showed a high degree of similarity in the two-dimensional representation, and the sensory descriptors used for describing them were fruity, flowery, low bitter, sour and toasted.

In the scientific literature, there are limited data regarding the flavor profile of cold brew coffee. Kim and Kim (2014) found differences in flavor in terms of chocolate, acidity, sweetness, bitterness, body, and aftertaste attributes when steeping and dripping methods were used. Recently, Angeloni and coworkers (2019) reported that the flavor can vary depending on the brewing method (dripping or steeping), and that cold brew coffee can exhibit higher intensities of toasty, sweetness and sourness compared to the hot extraction. In this study, the UV irradiated sample did not show differences in perceived acidity in flavor compared
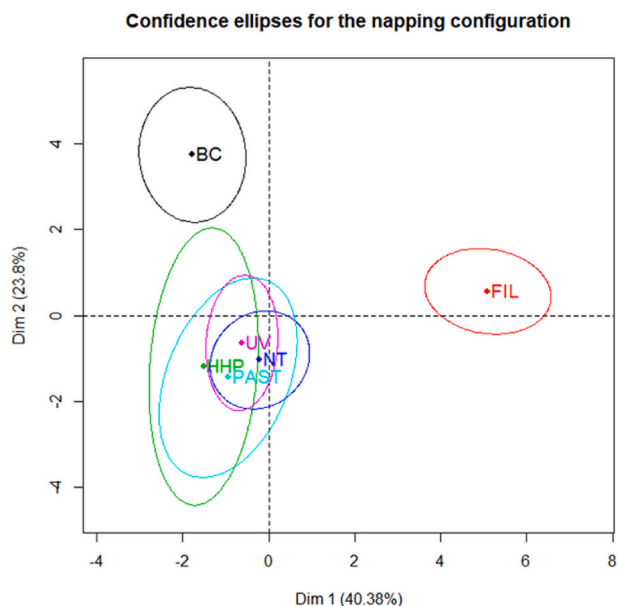

Fig. 3. Individual configurations for all performed treatments showing each sample's 95% confidence interval. NT: Non-treated sample, BC: blast chilling, FIL: filtration, PAST: pasteurization, UV: UV irradiation, HPP: HPP process.

to the other samples, not highlighting a correlation between this attribute and pH or titratable acidity, as previously reported by Gloess et al. (2013). On the other hand, the lack of correlation between these attributes in the sensory evaluation could be due to the small differences observed in pH and TA among the different samples.

3.2. Shelf-life evaluation

The coffee samples were monitored over four months, particularly after 7, 30, 60 and 120 days. As further described in the following paragraph, the non-treated, the blast-chilled and the UV irradiated samples showed a microbiological contamination after 7 days of storage, and the evaluation of the shelf-life was conducted up to t_1. Analogously, the shelf life of the filtered sample was monitored up to 30 days, when it showed the presence of molds. Pasteurization and HPP treatments were studied until t_4, since they observed the set microbiological limits of acceptability.

In this section the microbiological results for all the evaluated shelf life times and data obtained at t_1 and t_4 (7 and 120 days) by the other performed analyses were discussed. Data obtained at t_2 and t_3 are shown in the Supplementary Material section (Figures 4S, 5S and 6S).

3.2.1. Microbiological analysis

Table 1 reported the mold and yeast counting and the total bacterial count (TBC) of the non-treated and treated cold brew coffee samples after 7, 30, 60 and 120 days of storage at room temperature.

The non-treated sample showed a microbiological contamination after 7 days of storage with values of TBC of 10^7 CFU/mL, above the limits of acceptability set for this beverage. Furthermore, the presence of yeasts outside the setting levels was detected in this sample (10^4 CFU/mL), while a low concentration of molds were found. Regarding the treated samples, also for the blast chilling and the UV irradiation processes a contamination after 7 days has been registered with values of TBC of 10^4 and 10^7 CFU/mL, respectively. Moreover, a contamination by yeasts has been observed in the same samples at t_1 (10^4 and 10^5 CFU/mL). After 7 days of shelf life, the samples subjected to HPP, pasteurization and filtration processes showed a TBC <5 CFU/mL and a content of yeasts and molds below the limits of acceptability; only for these three samples the shelf life study was carried out for the later times.

After 30 days of storage (t_2), the filtered sample registered the presence of molds above the set microbiological limits of acceptability (10^3 CFU/mL), although showing appropriate values for the other microbiological parameters; also for this sample the evaluation of the shelf life was not carried out at t_3 and t_4.

As reported in Table 1, only HPP and pasteurization process showed values of TBC within the set limits of acceptability and appropriate levels of yeasts and molds (<5 CFU/mL) were registered after 60 and 120 days of storage. This result confirmed the efficacy of these two treatments, which have been able to guarantee the safety of the beverage up to four months.

3.2.2. pH and titratable acidity

The influence of the five treatments to preserve the shelf life of the cold brew coffee was evaluated over four months also in terms of acidity of the brews. Fig. 4 reported the pH and TA values of each sample at t_0 and after 7 and 120 days; in Figure 3S of Supplementary Material was also reported the results after 30 and 60 days.

The mean pH values slightly decreased over time; a statistically significant decrement was observed for non-treated, filtered, UV-irradiated, pasteurized and HPP-treated samples.

Particularly, the non-treated and the UV-irradiated samples showed a decrease from t_0 to t_1 of 1.4% and 3.2%, respectively. Also, the HPP sample registered a significant decrement after 7 days of storage ranging from 4.92 ± 0.01 to 4.83 ± 0.02 and a further decrease between t_2 and t_3. After four months of storage this sample showed an overall decrease of 4.3%, similarly to the pasteurized sample which registered pH values

pH

TA
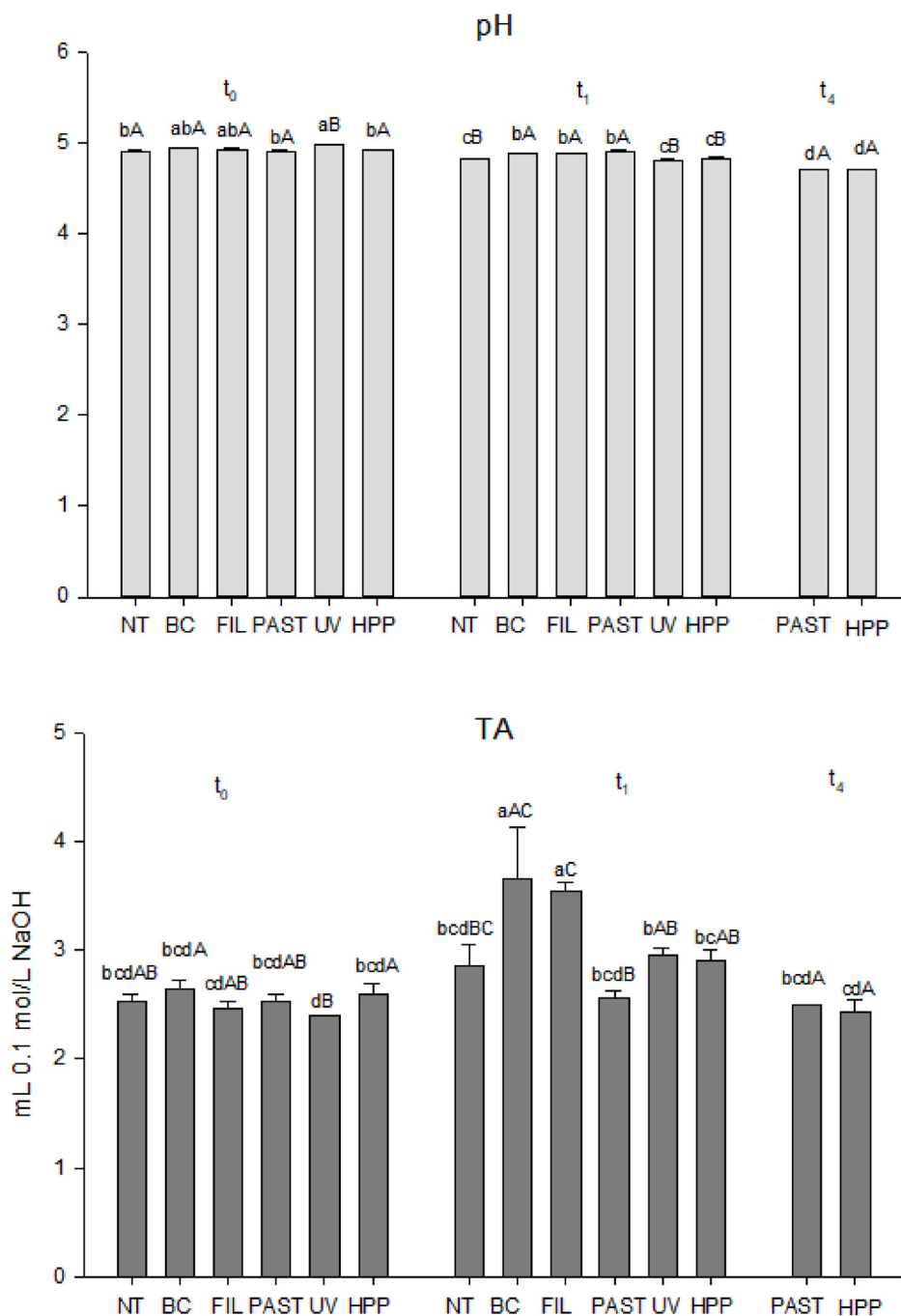
Fig. 4. pH and titratable acidity (TA) of each sample at t_0, t_1 and t_4. TA was expressed as difference between the mL of 0.1 mol/L NaOH registered at pH 6 and 8.
NT: Non-treated sample, BC: blast chilling, FIL: filtration, PAST: pasteurization, UV: UV irradiation, HPP: HPP process. Data are expressed as mean ± standard deviation of triplicates. The uppercase letters indicate significant differences among treatments ($P < 0.05$) for the same time of shelf-life; the lowercase letters indicate differences among treatments over time.

ranging from 4.92 ± 0.01 to 4.71 ± 0.01. The filtered samples showed a decrease in the mean pH of 2.4% after 30 days of storage; only for the blast chilled sample no significant decrement was observed over time.

Statistically significant differences were observed over time also for the mean TA. After 7 days, a significant increment of 37.7%, 43.7% and 22.9% was registered for blast chilled, filtered and UV-irradiated samples, respectively. On the other hand, the other samples did not show significant differences over time and the mean TA did not increase even for the pasteurized and HPP samples monitored for the 120-days period. This trend agrees only partially with the decrease in pH observed over four months.

3.2.3. Caffeine and CGAs evaluation

The effects of the five different techniques were evaluated investigating the caffeine and the CGAs contents over four months.

Fig. 5 showed the content of these molecules at t_0 and after 7 days for all the samples, while after 120 days the concentration of these compounds was reported only for the pasteurized and HPP-treated samples, which did not show microbial contamination after four months of storage. Data relating to the samplings at t_2 and t_3 are reported in the Supplementary materials section.

Regarding the caffeine content, no variations were observed among treatments over time (Fig. 5, lowercase letters). The blast chilled sample was the only one that showed at t_1 significantly lower values compared to the other analyzed samples (Fig. 5, capital letters).

The same trend was observed for the CGAs content, which remained stable over time, since no samples showed significant variations in the concentration of these compounds from t_0 to t_4 (Fig. 5, lowercase letters). After 7 days of storage, the blast chilled sample showed the lowest content of CGAs compared to the other treatments; however, the
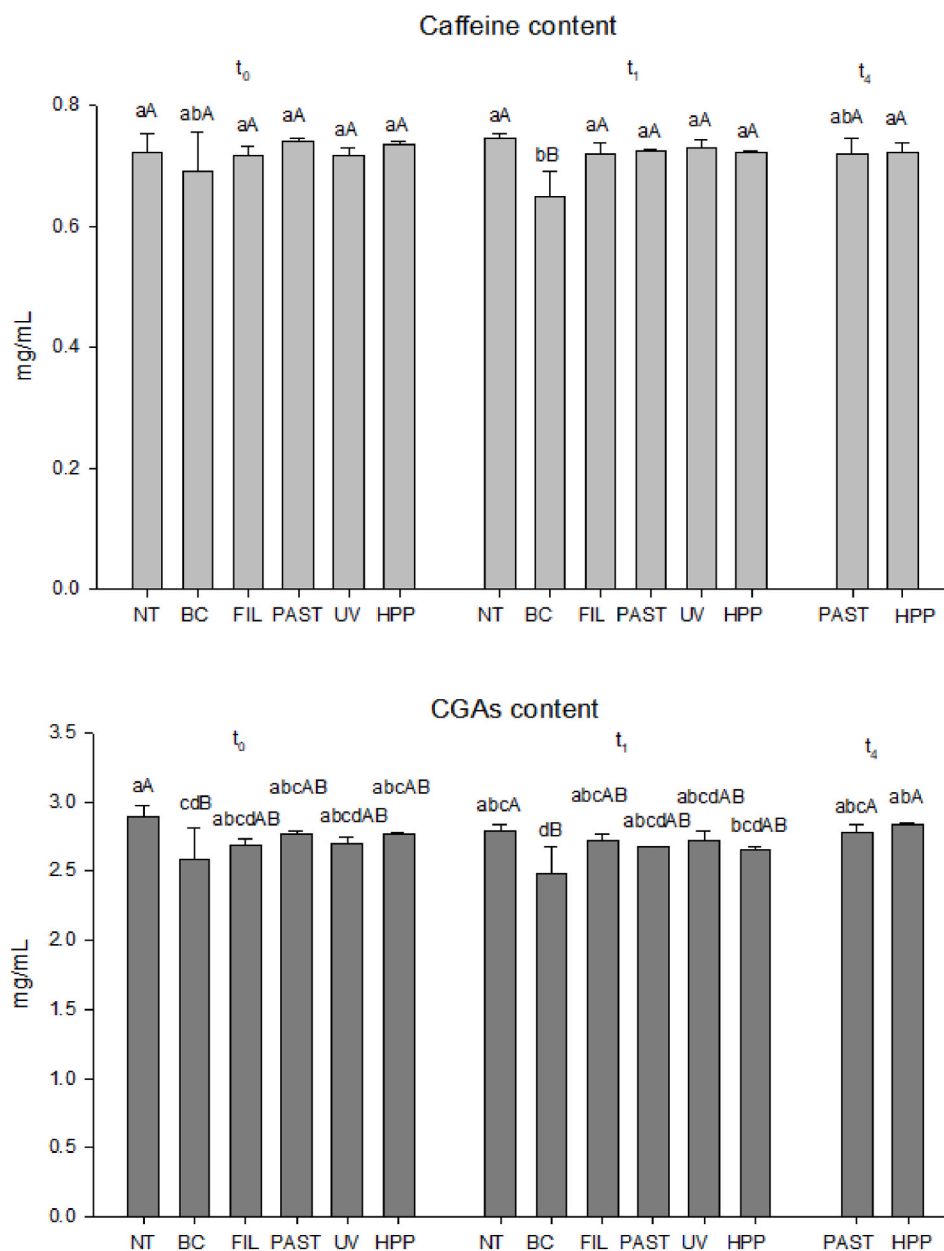
Fig. 5. Concentration (mg/mL) of caffeine and total chlorogenic acids (CGAs) at t_0, t_1 and t_4. NT: Non-treated sample, BC: blast chilling, FIL: filtration, PAST: pasteurization, UV: UV irradiation, HPP: HPP process. Data are expressed as mean ± standard deviation of triplicates. The uppercase letters indicate significant differences among treatments ($P < 0.05$) for the same time of shelf-life; the lowercase letters indicate differences among treatments over time.

differences were statistically significant only comparing it with the non-treated sample (Fig. 5, capital letters). The blast chilling process confirmed to be the treatment producing a different trend compared to the other techniques and showed also the highest standard deviation.

3.2.4. Volatile organic compounds

The volatile compounds in cold brew coffee samples were evaluated over four months of storage and Fig. 6 showed the total volatile content at t_0 and after 7 and 120 days.

After 7 days of storage, the concentration of the organic volatile compounds was significantly reduced ($P = 0.025$) for the non-treated sample, varying from 24.67 ± 1.6 to 16.15 ± 0.6 mg/kg (Fig. 6, lowercase letters), while the treated samples did not show significant differences over time maintaining a stable volatiles content. At this time of shelf life, the blast chilling and UV irradiation processes showed the highest standard deviation.

Analogously to the phenolic compounds, the analyses of the volatile compounds were performed at t_4 only for the pasteurized and HPP-treated samples. After 120 days of storage, no significant differences were found between the two treated samples (Fig. 6, uppercase letters), showing values of 19.9 mg/kg and 18.7 mg/kg for pasteurized and for HPP samples, respectively.

Comparing the results obtained over time, the HPP sample showed a decrease of 24.8% in the total content of volatile compounds after 120 days of storage while no statistically significant differences were observed for the pasteurized sample over time, even though higher standard deviation have been registered for this latter sample.

4. Conclusions

Cold brew coffee is usually produced and consumed in few hours, even though the interest about the possibility to drink this beverage at a
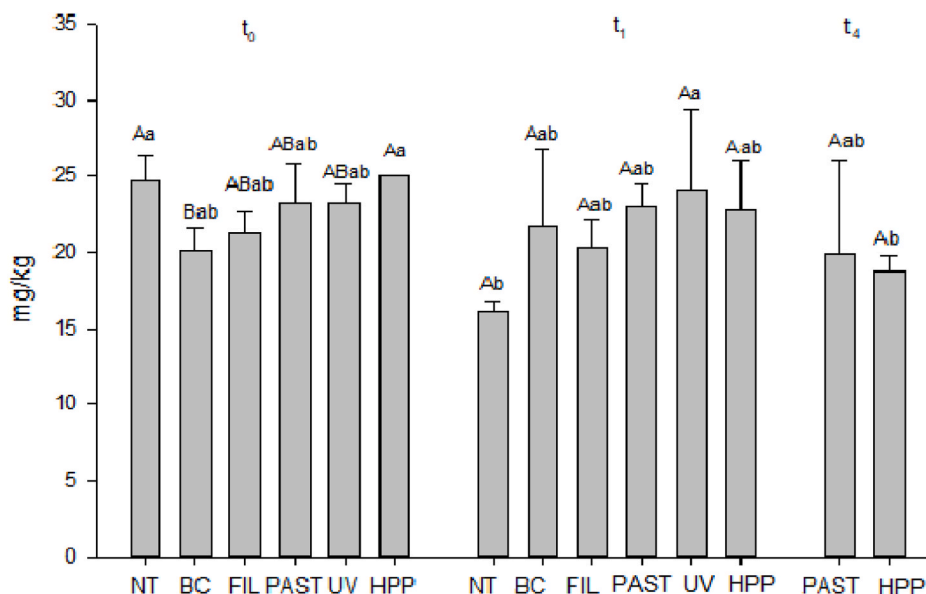
Fig. 6. Total content of volatile compounds in cold brew coffee samples at t_0 and after 7 (t_1) and 120 days (t_4) of storage. Data are expressed in mg/kg as mean ± standard deviation of triplicates. The uppercase letters indicate significant differences among treatments ($P < 0.05$) for the same time of shelf-life the lowercase letters indicate difference among treatments over time.

later date has increased in recent years. Our results showed that the most common physical treatments applied for food storage allowed to extend the shelf life of cold brew coffee maintaining its chemical and sensory characteristics.

Indeed, all the treated samples registered a decrease in the total bacterial count at t_0 andjust slight variations in the bioactive compounds content were observed.

After four months of storage, only the pasteurization and HPP processes registered microbiological values within the set limits of acceptability. The blast-chilled and the UV irradiated samples showed a microbiological contamination after 7 days, while the presence of molds in the filtered samples was observed after 30 days of storage.

The efficacy of pasteurization and HPP process was confirmed by the chemical analysis, which showed a stable content of caffeine and CGAs over time. After 120 days of storage, the pasteurized sample showed also an unaltered flavor profile.

Evaluating the effects of these techniques could contribute to the commercial development of this product, that today is mainly consumed immediately after its preparation. Further studies would be required to evaluate different processing parameters with the aim to improve the efficacy of these technologies, in particular regarding the worst-performing ones (filtration, UV irradiation and blast chilling process).

CRediT authorship contribution statement

Maria Bellumori: Conceptualization, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Giulia Angeloni:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft. **Lorenzo Guerrini:** Investigation, Data curation. **Piernicola Masella:** Investigation, Data curation. **Luca Calamai:** Methodology, Data curation, Writing - review & editing. **Nadia Mulinacci:** Resources, Writing - review & editing. **Alessandro Parenti:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration. **Marzia Innocenti:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111043.

References

Akiyama, M., Murakami, K., Ikeda, M., Iwatsuki, K., Wada, A., & Tokuno. (2007). Analysis of the headspace volatiles of freshly brewed arabica coffee using solidphase microextraction. *Journal of Food Science, 72*(77), 388–396.

Amanpour, A., & Selli, S. (2016). Differentiation of volatile profiles and odor activity values of Turkish coffee and French press coffee. *Journal of Food Processing and Preservation, 40*(5), 1116–1124. https://doi.org/10.1111/jfpp.12692

Angeloni, G., Guerrini, L., Masella, P., Bellumori, M., Daluiso, S., Parenti, A., et al. (2019a). What kind of coffee do you drink? An investigation on effects of eight different extraction methods. *Food Research International, 116*, 1327–1335. https://doi.org/10.1016/j.foodres.2018.10.022

Angeloni, G., Guerrini, L., Masella, P., Innocenti, M., Bellumori, M., & Parenti, A. (2019b). Characterization and comparison of cold brew and cold drip coffee extraction methods. *Journal of the Science of Food and Agriculture, 99*(1), 391–399. https://doi.org/10.1002/jsfa.9200

Angeloni, G., Masella, P., Guerrini, L., Innocenti, M., Bellumori, M., & Parenti, A. (2019c). Application of a screening design to recover phytochemicals from spent coffee grounds. *Food and Bioproducts Processing, 118*, 50–57. https://doi.org/10.1016/j.fbp.2019.08.017

Brezova, V., Slebodova, A., & Stasko, A. (2009). Coffee as a source of antioxidants: An EPR study. *Food Chemistry, 114*(3), 859–868. https://doi.org/10.1016/j.foodchem.2008.10.025

Cordoba, N., Pataquiva, L., Osorio, C., Moreno, F. L. M., & Ruiz, R. Y. (2019). Effect of grinding, extraction time and type of coffee on the physicochemical and flavour characteristics of cold brew coffee. *Scientific Reports, 9*. ARTN 844010.1038/s41598-019-44886-w.

Ec, European Commission. (2004). *Commission Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs*.

Flament, I. (2002). *Coffee flavor chemistry*. West Sussex, England: J. Wiley & Sons.

Fujioka, K., & Shibamoto, T. (2008). Chlorogenic acid and caffeine contents in various commercial brewed coffees. *Food Chemistry, 106*(1), 217–221. https://doi.org/10.1016/j.foodchem.2007.05.091

Fuller, M., & Rao, N. Z. (2017). The effect of time, roasting temperature, and grind size on caffeine and chlorogenic acid concentrations in cold brew coffee. *Scientific Reports, 7*(1), 17979. https://doi.org/10.1038/s41598-017-18247-4

Gloess, A. N., Schonbachler, B., Klopprogge, B., D'Ambrosio, L., Chatelain, K., Bongartz, A., et al. (2013). Comparison of nine common coffee extraction methods: Instrumental and sensory analysis. *European Food Research and Technology, 236*(4), 607–627. https://doi.org/10.1007/s00217-013-1917-x

Grosch, W. (1998). Flavour of coffee. A review. *Nahrung-Food, 42*(6), 344–350.

Guerrini, L., Zanoni, B., Breschi, C., Angeloni, G., Masella, P., Calamai, L., et al. (2020). Understanding olive oil stability using filtration and high hydrostatic pressure. *Molecules, 25*(2). ARTN 42010.3390/molecules25020420.

Heckman, M. A., Weil, J., & Gonzalez de Mejia, E. (2010). Caffeine (1,3,7-trimethylxanthine) in foods: A comprehensive review on consumption, functionality, safety, and regulatory matters. *Journal of Food Science, 75*(3), 77–87.

Kim, A. R., & Kim, J. S. (2014). Flavor contributing non-volatile chemical and sensory characterization of cold water extraction-based coffee by different extraction methods (dripping vs steeping) and time. *Journal of The Korea Society for Coffee Industry, 3*, 1–9.

Lopez-Galilea, I., De Pena, M. P., & Cid, C. (2007). Correlation of selected constituents with the total antioxidant capacity of coffee beverages: Influence of the brewing procedure. *Journal of Agricultural and Food Chemistry, 55*(15), 6110–6117. https://doi.org/10.1021/jf070779x

Lopez-Galilea, I., Fournier, N., Cid, C., & Guichard, E. (2006). Changes in headspace volatile concentrations of coffee brews caused by the roasting process and the brewing procedure. *Journal of Agricultural and Food Chemistry, 54*(22), 8560–8566. https://doi.org/10.1021/jf061178t

Lopez, J. A. S., Wellinger, M., Gloess, A. N., Zimmermann, R., & Yeretzian, C. (2016). Extraction kinetics of coffee aroma compounds using a semi-automatic machine: On-line analysis by PTR-ToF-MS. *International Journal of Mass Spectrometry, 401*, 22–30. https://doi.org/10.1016/j.ijms.2016.02.015

Ludwig, I. A., Mena, P., Calani, L., Cid, C., Del Rio, D., Lean, M. E., et al. (2014). Variations in caffeine and chlorogenic acid contents of coffees: What are we drinking? *Food Funct, 5*(8), 1718–1726. https://doi.org/10.1039/c4fo00290c

Mondello, L., Costa, R., Tranchida, P. Q., Dugo, P., Lo Presti, M., Festa, S., et al. (2005). Reliable characterization of coffee bean aroma profiles by automated headspace solid phase microextraction-gas chromatography-mass spectrometry with the support of a dual-filter mass spectra library. *Journal of Separation Science, 28*(9–10), 1101–1109. https://doi.org/10.1002/jssc.200500026

Nestrud, M. A., & Lawless, H. T. (2010). Perceptual mapping of apples and cheeses using projective mapping and sorting. *Journal of Sensory Studies, 25*(3), 390–405. https://doi.org/10.1111/j.1745-459X.2009.00266.x

Pagès, J. (2005). Collection and analysis of perceived product inter-distances using multiple factor analysis: Application to the study of 10 white wines from the Loire Valley. *Food Quality and Preference, 16*(7), 642–649.

Parenti, A., Guerrini, L., Masella, P., Spinelli, S., Calamai, L., & Spugnoli, P. (2014). Comparison of espresso coffee brewing techniques. *Journal of Food Engineering, 121*, 112–117. https://doi.org/10.1016/j.jfoodeng.2013.08.031

Piccino, S., Boulanger, R., Descroix, F., & Sing, A. S. C. (2014). Aromatic composition and potent odorants of the "specialty coffee" brew "Bourbon Pointu" correlated to its three trade classifications. *Food Research International, 61*, 264–271. https://doi.org/10.1016/j.foodres.2013.07.034

Qualls, R. G., Flynn, M. P., & Johnson, D. J. (1983). The role of suspended particles in ultraviolet disinfection. *Water Pollution Control Federation, 55*(10), 1280–1285.

Rao, N. Z., & Fuller, M. (2018). Acidity and antioxidant activity of cold brew coffee. *Scientific Reports, 8*. ARTN 1603010.1038/s41598-018-34392-w.

Risvik, E., McEwan, J. A., Colwill, J. S., Rogers, R., & Lyon, D. H. (1994). Projective mapping: A tool for sensory analysis and consumer research. *Food Quality and Preference, 5*(4), 263–269.

Semmelroch, P., & Grosch, W. (1996). Studies on character impact odorants of coffee brews. *Journal of Agricultural and Food Chemistry, 44*(2), 537–543. https://doi.org/10.1021/jf9505988

Steen, I., Waehrens, S. S., Petersen, M. A., Munchow, M., & Bredie, W. L. P. (2017). Influence of serving temperature on flavour perception and release of Bourbon Caturra coffee. *Food Chemistry, 219*, 61–68. https://doi.org/10.1016/j.foodchem.2016.09.113

Sunarharum, W. B., Williams, D. J., & Smyth, H. E. (2014). Complexity of coffee flavor: A compositional and sensory perspective. *Food Research International, 62*, 315–325. https://doi.org/10.1016/j.foodres.2014.02.030

Tajik, N., Tajik, M., Mack, I., & Enck, P. (2017). The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: A comprehensive review of the literature. *European Journal of Nutrition, 56*(7), 2215–2244. https://doi.org/10.1007/s00394-017-1379-1

Yen, W. J., Wang, B. S., Chang, L. W., & Duh, P. D. (2005). Antioxidant properties of roasted coffee residues. *Journal of Agricultural and Food Chemistry, 53*(7), 2658–2663. https://doi.org/10.1021/jf0402429