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Thermal stabilisation of cocoa fruit pulp — Effects on sensory properties, colour and microbiological stability

Thomas Bickel Haase [a,c], Susanne Naumann-Gola [a,*], Eva Ortner [a], Holger Zorn [c,d], Ute Schweiggert-Weisz [a,b]

[a] Fraunhofer Institute for Process Engineering and Packaging IVV, 85354, Freising, Germany
[b] Institute for Nutritional and Food Sciences, University of Bonn, 53115, Bonn, Germany
[c] Institute of Food Chemistry and Food Biotechnology, Justus-Liebig University, 35392, Giessen, Germany
[d] Fraunhofer Institute for Molecular Biology and Applied Ecology IME, 35392, Giessen, Germany

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ABSTRACT

To improve cocoa pulp's shelf-life, preservation processes are necessary while maintaining the quality of the pulp. We applied pasteurisation and UHT-treatment and investigated different quality parameters: dry matter content, water activity, total soluble solids, colour and peroxidase activity. Both technologies inactivated peroxidase successfully. The colour of the pasteurised pulp was similar to the fresh, while UHT-treated pulp was more brownish. The sensory properties were investigated in detail by descriptive analysis and the identification of aroma-active volatile organic compounds. Fresh pulp revealed the highest aroma intensity for attribute *unripe banana-like*, whereas UHT-treated pulp scored highest in the intensity of attribute *tropical fruit-like*. Pasteurised pulp showed strong similarities to the fresh pulp. Fresh cocoa pulp exhibited 74 aroma-active regions identified by GC-MS/O. UHT-treated and pasteurised pulp accounted for 66 and 60 aroma-active regions, respectively. Five identified substances were only found in the fresh and pasteurised pulp, namely: δ-carene, 1-pentanol, 3-(methylthio)propanol, phenol and δ-undecalactone. Similarly, fresh and UHT-treated pulp shared ten exclusive odorants, such as decanal, geraniol, and δ-nonalactone. The pasteurised and UHT-treated pulp shared two compounds, δ–decalactone and 5-(hydroxymethyl)furfural. Furthermore, the thermally treated pulps could be stored at 4 °C and 23 °C for 24 weeks without observing a significant growth of microorganisms. The rate of non-enzymatic browning was higher in samples stored at 23 °C compared to those stored at 4 °C, leading to higher browning indices. We demonstrated that pasteurisation and ultra-high temperature treatment are suitable technologies for the stabilisation of cocoa fruit pulp. These resulted in prolonged shelf-lifes and minimal changes in the sensory prorperties of the treated pulps, characterised by a reduction in the aroma diversities. This work provides important insights for the thermal stabilisation of further side-streams.

1. Introduction

The fruits of the cocoa tree (*Theobroma cacao* L.), are composed of the cocoa pod husk, the beans and the pulp, representing about 70%, 20% and 10% of the whole fruit, respectively. Up to now, the cocoa beans have the main commercial importance, while the husks and the pulp have been considered as by-products (Figueroa et al., 2020). To increase the sustainability along the cocoa supply chain and to create new sources of income for cocoa farmers, concepts for a complete valorisation of cocoa fruits should be developed. This paper explores opportunities to increase the shelf-life of cocoa pulp for its transport from cocoa producing countries and its use in food applications.

The cocoa pulp is the moist layer embedding the cocoa beans. It contains approximately 83–86% water, 11–13% sugars, 0.5–1.2% pectin, 0.2–3% hemicelluloses, 0.7–0.9% cellulose, 0.1–0.3% lignin and 0.3–1.3% citric acid. The presence of citric acid and other organic acids make the cocoa pulp acidic, so its pH-value usually ranges between 3.3 and 3.9 (Pettipher 1986; Santos et al., 2014; Figueroa et al., 2020). The pulp is important for the fermentation of the cocoa beans, as it serves as a substrate for yeasts and bacteria. During fermentation, cocoa pulp is liquefied by the enzymes released by the microorganisms and is lost as so-called cocoa sweatings (van Ho et al., 2014). Fermentation is an

* Corresponding author.
E-mail address: susanne.gola@ivv.fraunhofer.de (S. Naumann-Gola).

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important step for the aroma quality of the beans (Schwan and Fleet 2014), but previous studies suggest a partial de-pulping of the cocoa beans without negatively affecting the fermented beans' quality (Lopez 1979; Amanquah 2013). The separated pulp could then be used as a food ingredient, adding value to the cocoa fruit. Hitherto, some examples of foods produced with cocoa pulp exist in the cocoa-growing countries (dos Santos Filho et al., 2019; Firdaus et al., 2022).

A huge challenge in making cocoa fruit pulp available on the European market is its short shelf-life, caused by its high moisture and sugar content, making it prone to spontaneous fermentation (Vuyst and Weckx 2016). Thus, preserving the cocoa pulp through mild processing with minimal effect on the pulp's sensory quality needs to be thoroughly investigated. Pasteurisation can be used for products with a pH value of 4.5 or less (preferably below 4.0), in which the acidic conditions effectively prevent the growth of pathogens. Certain pathogenic bacteria (e.g. some *E. coli* strains) can still thrive in these conditions, hence some vegetable and freshly pressed juices are processed by ultra-high temperature (UHT) treatment (Ashurst et al., 2017). In a previous study, Endraiyani et al. (2017) studied the effect of single and double pasteurisation treatments on the total phenolic content of cocoa pulp. Throughout an 8-week storage at 4 °C, 25 °C, and 37 °C, single and double pasteurised pulps showed only minimal changes in phenolics when stored at 4 °C and 25 °C, suggesting colder storage temperatures are better suitable for maintaining the pulps' quality. The effects of thermal treatment on the organoleptic quality and aroma composition of cocoa pulp were disregarded in this study.

The pleasant flavour of cocoa pulp makes it highly attractive for the international food sector, especially for the development of beverages (Klis et al., 2023). Only a few studies focused on the aroma composition of cocoa pulp. However, they mainly investigated the role of cocoa pulp in the fermentation of cocoa beans and its influence on the chocolate quality (Chetschik et al., 2018; Hegmann et al., 2020; Schlüter et al., 2022). In our previous study, we identified the aroma-active regions of fresh cocoa pulp from Indonesia, Vietnam, Cameroon and Nicaragua. We reported substantial differences in the aroma compositions of the four pulps, providing key insights for future food applications (Bickel Haase et al., 2021). To the moment, the effect of processing on the sensory quality and microbial stability of cocoa pulp for food applications remains largely unexplored.

To obtain shelf-life-stable cocoa pulps with pleasant aroma attributes, it is crucial to understand how the stabilisation of cocoa pulp with well-established technologies such as pasteurisation and UHT-treatment may affect its sensory properties, colour and microbiological stability. This study focuses on the compositional changes of Cameroonian cocoa pulp after thermal treatment. Additionally, the microbiological and colour stabilities of thermally treated pulps were investigated in a 24-week storage test at 4 °C and 23 °C to predict the shelf-life of cocoa pulp. We describe for the first time the effects of pasteurisation and UHT-treatment on cocoa pulp, providing suitable processing protocols for future applications.

2. Material and methods

2.1. Separation of the fresh cocoa pulp

Fresh cocoa pods (*Theobroma cacao* L.), harvested in 2020, were imported from Cameroon to Germany in a cool shipment directly after harvest. Upon arrival, the fruits were washed, cut open and the cocoa pulp was immediately separated by means of a straining machine with a 2.8 mm sieve mesh (Fructmas P006, Karl Bockmeyer Kellereitechnik GmbH, Germany). An aliquot of the fresh pulp was vacuum-sealed in odorless plastic bags (PA/PE 90/130 × 280 mm, Dagema eG, Germany) and immediately frozen at −50 °C until analysis, while the residual fresh pulp was preserved by thermal treatment (2.2) immediately after de-pulping.

2.2. Thermal treatment of cocoa pulp

The fresh pulp was thermally treated in an ultra-high temperature System (HT220 Lab UHT/HTST; OMVE Nederland B.V., Netherlands). In a first run, the pulp was heated to 80 °C core temperature and held for 30 s. The hot pulp was filled into sterile 1 L flasks (Schott AG, Germany). After 20 min, the flasks were submerged in ice water (0 °C) to decrease the temperature rapidly (= **pasteurised samples**). In a second experimental setup, the pulp was heated to 135 °C and held for 30 s. The hot pulp was also filled into the sterile flasks and immediately cooled down in a bath with ice water (0 °C) (= **UHT-treated samples**).

2.3. Characterisation of fresh, pasteurised and UHT-treated cocoa pulp

2.3.1. Dry matter content, total soluble solids and water activity

The dry matter content was determined gravimetrically by drying the sample at 105 °C with a moisture analyser MA100 (Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany) until constant weight was achieved. The total soluble solids (° Bx) were determined at room temperature (~21 °C) using a digital refractometer DR301-95 (A. Krüss Optronic GmbH, Hamburg, Germany) against distilled water. For this, approximately 1 mL of cocoa pulp was placed in the measuring cell for the reading. The water activity was determined by means of an AQUALAB 4 TE system (Meter Group Europe, Munich, Germany) by filling a sample cup (Aqualab, Meter Group, USA) until the cup's bottom was completely covered. Measurements were performed at room temperature (~21 °C). The equipment was calibrated using standards provided by the system producer with a_w-values of 0.984 (0.5 M KCl) and 0.760 (6 M NaCl), as well as distilled water (a_w-value of 1.0). All experiments were carried out in triplicate.

2.3.2. Colour measurement

The DigiEye colour imaging system (DigiEye V2.62, VeriVide, Leicester, UK) was used for colour measurements, comprising an illumination box with diffuse illuminant D65 and a Nikon D90 digital camera. Digitizer calibration charts were used to calibrate the system. For the colour measurements of the fresh, thermally treated and stored samples (2.5), pulp was evenly distributed in a white sample cup (Aqualab, Meter Group, USA) and the average surface colour was expressed as CIE L*a*b*-values. The browning index (BI) was calculated using the L*a*b* values and following the formula described by Bal et al. (2011):

$$BI = \frac{100(x - 0,31)}{0,17} \quad (1)$$

$$\text{Where x}: x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)} \quad (2)$$

Analyses were performed immediately after thermal treatment as well as after 2, 4, 6, 12 and 24 weeks of storage at 4 °C and 23 °C. Analyses were performed in triplicate to obtain three browning indices for each sample, which were used to obtain mean values and standard deviations (2.6).

2.3.3. Peroxidase activity

Peroxidase activity (POD) was determined in fresh, pasteurised and UHT-treated cocoa pulp adapted according to Ghamsari et al. (2007) using guaiacol as substrate. To extract the POD, 0.2 g of fresh pulp was weighed in a beaker and dispersed in 15 mL 0.1 M citrate-phosphate buffer (citric acid-$Na_2HPO_4 \cdot 12\ H_2O$) at pH 7.0. The mixture was stirred at 4 °C for 30 min and centrifuged at 2000 *g* at 4 °C for 10 min. The extract was kept in refrigeration until use. To determine the optimal pH for the enzymatic reaction, the enzymatic assay was carried out at different pH values (3.0–7.0). Therefore, the citrate-phosphate buffer solutions with 15 mM guaiacol (Merck KGaA, Darmstadt, Germany) were set to different pH values (Gomori 1955). The highest enzyme

activity was determined at pH 6 in the fresh pulp. Therefore, POD activity in the fresh and thermally treated samples was determined at pH 6. The reaction mixture contained 90 μL of the enzyme extract, 2.9 mL of a citrate-phosphate buffer with 15 mM guaiacol and 10 μL of 3.3 mM H_2O_2. Blanks of the reagents and of the sample were also prepared and analysed. The sample was placed in a 1 cm cuvette and measured immediately by means of a spectrophotometer (Specord 210 Plus, Analytik Jena GmbH, Germany) at 470 nm in intervals of 30 s for 10 min. The formula described by Chance and Maehly (1972) was used to calculate the enzymatic activity. Taking the samples' weight into consideration, the peroxidase activity was described as E_A (μmol s^{-1} g^{-1}).

2.4. Sensory evaluation

2.4.1. Panelists

Sensory evaluation was performed by seven trained experts (2 male, 5 female, aged 27 to 42, all non-smokers) from the Fraunhofer Institute for Process Engineering and Packaging IVV (Freising, Germany) sensory panel. Panellists were trained to correctly identify and name odors during weekly training sessions with selected in-house made odorant pens, corresponding to a total of over 250 different odorants. The correctness of the individual answers was evaluated following DIN EN ISO 8586:2014–05. Panellists were trained for at least six weeks and were required to achieve a minimum average correctness score of 65% prior to inclusion in the panel. Furthermore, the panelists were trained regularly to accurately perceive and describe the five basic taste qualities. They were selected based on their extensive experience in sensory evaluation of different fruit pulps. The panelists reported no known illnesses at the time of the examination.

2.4.2. Aroma profile analysis

For the sensory evaluation following DIN 10969:2001–05 (Sensory analysis - Descriptive analysis with following quality evaluation), an aliquot of 20 ± 1 g of the fresh and the thermally treated pulps, respectively, was presented in 140 mL covered glass vessels in a sensory room at 21 °C. Aroma qualities were determined in consensus by the expert panel (2.4.1). The quality attributes were systematically elaborated by the trained experts based on their long time sensory experience on fruit pulps and their training in describing different odor qualities based on single aroma-active compounds. It was ensured that all testers understood the terms in the same way. Afterwards, the intensity of the selected attributes was directly evaluated on a scale from 0 (no perception) to 5 (strong perception). The results were averaged and the means were plotted in a spider-web diagram. Results were evaluated by Tukey's multiple comparison tests (2.6).

2.4.3. Comparative aroma extract dilution analysis (cAEDA)

The isolation of the volatile organic compounds was carried out as described in detail by Bickel Haase et al. (2021). For this, 50 g pulp was vigorously stirred with 200 mL of dichloromethane (DCM) (Merck KgaA, Darmstadt, Germany) for 1 h at room temperature in a closed vessel. After decanting 150 mL of DCM, the volatile compounds were separated from the non-volatile components using the Solvent Assisted Flavour Evaporation (SAFE) technique (Engel et al., 1999) under high vacuum, while the SAFE apparatus was maintained at a temperature of 55 °C. The resulting distillates were dried using anhydrous sodium sulfate (Merck KgaA, Darmstadt, Germany), filtered, concentrated to approximately 3 mL at 50 °C utilizing a Vigreux column (50 cm × 1 cm i.d.), and further reduced to a volume of approximately 100 μL through microdistillation (Bemelmans 1978). The same workup procedure was used for the fresh, pasteurised and UHT-treated pulps. To avoid a potential overlooking of aroma-active areas, the original distillates were evaluated by three trained panellists of the Fraunhofer IVV sensory panel using GC-O. A blank was performed for each sample by applying the same work-up procedure omitting the pulp sample. The flavour dilution (FD) factors of the odorants were determined by diluting the distillate stepwise (1 + 1, *v* + *v*) with dichloromethane up to factor 1024 and analysing the dilutions with GC-O (Grosch 1993). GC-O was performed as described in subsection 2.4.4. For each aroma-active area, the respective FD factor was assigned, correlating to the highest dilution in which the compound was perceivable at the odor detection port for the last time.

2.4.4. Gas chromatography-olfactometry (GC-O)

GC-O was carried out as described by Bickel Haase et al. (2021) using a Trace GC Ultra (Thermo Fisher Scientific GmbH, Dreieich, Germany) equipped with either a DB-FFAP or DB-5 capillary column (both 30 m × 0.32 mm, 0.25 μm film thickness) (J&W Scientific, Agilent Technologies GmbH, Waldbronn, Germany). The initial temperature of 40 °C was held for 2 min, raised at 6.0 °C/min to 235 °C (DB-FFAP) or 250 °C (DB-5), respectively, and held for 5 min. The effluent was split 1:1 leading to a flame ionization detector (FID) held at 250 °C and to an odor detection port (ODP) held at 235 °C. Linear retention indices (RI) were calculated using a homologous series of n-alkanes ranging from C_6 to C_{26} for the DB-FFAP and C_6 to C_{18} for the DB-5 column (van den Dool and Dec. Kratz, 1963).

2.4.5. Gas chromatography-mass spectrometry/olfactometry (GC-MS/O)

For identification of the volatile organic compounds (VOCs) present in the distillates, GC-MS/O was performed using a Trace GC Ultra and a Trace dual stage quadrupole (DSQ) mass spectrometer (both Thermo Fisher Scientific GmbH) equipped with a DB-FFAP column (30 m × 0.32 mm, 0.25 μm film thickness, J&W Scientific, Waldbronn, Germany). Measurements were performed following the method described in detail by Bickel Haase et al. (2021). Samples were evaluated by three trained panellists.

Further GC-MS/O determinations were performed using an Agilent GC-MSD (Agilent Technologies, Waldbronn DE). Aliquots (2 μL) of the samples were injected in pulsed splitless mode at 250 °C by means of an autosampler PAL RSI 85 (CTC Analytics AG, Zwingen, Switzerland). At the end of the column (DB-FFAP, 30 m × 0.25 mm, 0.25 μm film thickness, J&W Scientific), the effluent was split 1:1 by a Y-splitter. Two deactivated fused silica capillaries led either to an odor detection port (235 °C) or a mass spectrometer. The oven temperature was 40 °C at the beginning, raised at 6.0 °C/min after 2 min to 235 °C, and held for 5 min. The flow rate of the helium carrier gas was 2.0 mL/min in constant flow. Mass spectra were generated in the positive electron impact (EI) ionization mode at 70 eV (Scan 35–400 *m/z*).

2.4.6. Stir-bar sorptive extraction gas chromatography-olfactometry/mass spectrometry (SBSE-GC-MS/O)

SBSE was performed as described in detail by Bickel Haase et al. (2021) to confirm VOCs and to evaluate possible changes in the aroma composition of the pulp during the isolation step. Cocoa pulp samples (2.0 g ± 0.01) were diluted with distilled water in a 1:1 ratio (*w/w*) and placed in 20 mL headspace vials. The vials were sealed tightly, and the suspension was stirred at room temperature for 15 min using a pre-conditioned (280 °C, 5 h) SBSE Twister® (polydimethylsiloxane sorbent (PDMS), 20 mm length and 0.5 mm coating thickness, Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany). Following the extraction, the Twister® was automatically transferred to the Thermal Desorption Unit (TDU) at 40 °C (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany). Desorption commenced at 40 °C (initial time: 0.1 min) and ramped up to 250 °C at a rate of 12.0 °C/s, where it was held for 5 min. The desorbed volatile compounds were then transferred to the cold-injection system (CIS) (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany), cooled to −70 °C using liquid nitrogen, and subsequently introduced into the GC-MS/O system via thermal desorption. Mass spectra were generated in full scan mode (*m/z* range 35–400, EI 70 eV) on the same GC-MS system as described in 2.4.5. Samples were evaluated by two trained panellists.

2.4.7. Headspace solid-phase microextraction gas chromatography-olfactometry/mass spectrometry (HS-SPME GC-O/MS)

Highly volatile aroma-active compounds were identified by means of HS-SPME-GC-O/MS to exclude any losses during the concentration steps by liquid extraction (2.4.3). The same workup procedure as described by Bickel Haase et al. (2021) was followed. Cocoa pulp samples (1.0 g ± 0.01) were individually diluted 1:1 (*w/w*) with distilled water and sealed in 20 mL headspace glass vials. The vials were agitated using an orbital shaker (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) at 50 °C and 250 rpm for 10 min, with a change in direction every 90 s. Prior to use, a SPME fiber (PDMS, 100 μm, Supelco, USA) was conditioned at 250 °C for 5 min. The fiber was automatically introduced into the vial for volatile adsorption, and the cocoa pulp was exposed to it for 10 min at 50 °C during the headspace equilibration. The analysis was performed using the MPS autosampler and GC-O/MS system with a DB-FFAP capillary column, as described in section 2.4.5. The aroma-active regions of the fresh and thermally treated samples were evaluated by two trained panellists.

2.5. Storage test with the thermally treated cocoa pulps

Under a sterile bench, 30 g of thermally treated cocoa pulp was filled in sterile 50 mL tubes (PP, 30/115 mm, Greiner Bio-One GmbH, Germany). Samples were stored for 24 weeks at ambient temperature (23 °C) and at 4 °C. Three sample tubes each were taken after 0, 2, 4, 6, 12 and 24 weeks of storage and evaluated for their colour properties (2.3.2). The microbiological load was determined after 0, 6, 12 and 24 weeks of storage.

2.5.1. Microbiological load

Sample preparation was performed following the international guidance for preparation of test samples, initial suspension and decimal dilutions for the microbiological examination of the food chain (DIN EN ISO 6887–1:2017–07). The count of aerobic mesophilic bacteria [CfU/g] present in the fresh and thermally treated cocoa pulp samples was determined following DIN EN ISO 4833–2:2014–05. The total yeast and moulds' count [CfU/g] was determined following DIN 10186:2005–10. Analyses were performed after processing as well as after 0, 6, 12 and 24 weeks of storage at 4 °C and 23 °C. Analyses were performed in duplicate and the results were averaged.

2.6. Statistical analysis

The results of the pulp characterization were expressed as mean average ± standard deviation (SD). Statistical analysis was performed using Tukey's multiple comparison test ($p < 0.05$) to determine significant differences among two groups using Origin 2022b (OriginLab Corporation, Massachusetts, USA). Tukey's multiple comparisons tests were applied to following results: dry matter content, total soluble solids, pH-values, water activities, colour and browning index, peroxidase activity and descriptive sensory evaluation (taste and aroma profile).

3. Results and discussion

3.1. Characterization of fresh, pasteurised and UHT-treated cocoa fruit pulp

To understand how thermal processing affected the cocoa fruit pulp, fresh, pasteurised and UHT-treated cocoa pulps were investigated right after processing for their dry matter contents, total soluble solids (° Bx), pH, water activity (a_W), peroxidase activity, L*a*b* and the browning index (BI). Overall, the differences in the chemical composition of the fresh pulp were statistically significant from the pasteurised and the UHT-treated pulps, but the colour properties were similar (Table 1). The fresh material showed a dry matter content of 16.71%, a pH of 3.85 and total soluble solids of 16.1° Bx. The values correspond to those reported in the literature, accounting for a pH of 3.90 and total soluble solids of 16.2° Bx (Escalante et al., 2015). The dry matter content, pH and soluble solids of pasteurised pulp were 17.2%, 3.95 and 15.5° Bx, respectively. In the UHT-treated pulp, the dry matter content was 16.2%, the pH 3.88 and the soluble solids content corresponded to 14.30° Bx. Even though the dry matter contents, pH-values and soluble solids of the fresh, pasteurised and UHT-treated pulps were significantly different, all values ranged within common values for cocoa pulp. No clear effect of the treatments could be observed, so differences might be attributed to slight variations in the raw material.

Table 1
Characterisation of fresh, pasteurised and UHT-treated cocoa pulp.

	Fresh	Pasteurised	UHT-treated
Dry matter [%]	16.7 ± 0.1 ^{b}	17.2 ± 0.1 ^{a}	16.2 ± 0.2 ^{c}
pH [-]	3.85 ± 0.01 ^{b}	3.95 ± 0.01 ^{a}	3.88 ± 0.01 ^{b}
Total soluble solids [°Bx]	16.1 ± 0.1^{a}	15.5 ± 0.1 ^{b}	14.3 ± 0.1 ^{b}
a_w [-]	0.980 ± 0.02 ^{c}	0.981 ± 0.00 ^{b}	0.984 ± 0.00 ^{a}
L* [-]	68.25 ± 0.12 ^{c}	70.30 ± 0.19 ^{b}	71.25 ± 0.09 ^{a}
a* [-]	2.02 ± 0.12 ^{b}	1.65 ± 0,11 ^{c}	2.63 ± 0.17 ^{a}
b* [-]	11.03 ± 0.32 ^{b}	12.01 ± 0.67 ^{b}	14.37 ± 0.61 ^{a}
Browning index [-]	19.43 ± 0.61 ^{b}	20.07 ± 1.17 ^{b}	24.79 ± 1.20 ^{a}
Peroxidase activity [μmol/s g]	24.7 ± 0.3	n.d.	n.d.

Values are expressed as mean ± standard deviation of three experiments. Means in the same row with different letters indicate significant differences. n.d. not detected.

The water activity of fresh cocoa pulp accounted for 0.980. Pasteurised and UHT-treated samples showed similar values, with 0.981 and 0.984, respectively (Table 1). Due to the fresh cocoa pulp's high water activity, a stabilisation step is recommendable to diminish possible spoiling reactions and increase the shelf-life of the pulp.

We investigated the activity of guaiacol peroxidase (POD) as an indicator for the success of the thermal treatments in inactivating enzymes (Table 1). The POD activity of fresh cocoa pulp accounted for 24.7 ± 0.3 μmol $s^{-1}g^{-1}$. After pasteurisation and UHT-treatment, no POD activities could be determined. As POD is a relatively thermostable enzyme, it can be assumed that both thermal processing technologies were sufficient to inactivate other enzymes in the samples as well.

The L*a*b* values of the fresh and the thermally treated pulps were determined (Table 1). The fresh pulp showed L*, a* and b* values of 68.25, 2.02 and 11.03, respectively. In pasteurised pulp, the L*a*b* values were 70.30, 1.65 and 12.01. The b* value of pasteurised pulp presented no statistical difference compared with the fresh pulp's b* value. In contrast, the L*a*b* values of UHT-treated pulp were significant different from those of the fresh material. The L* value accounted for 71.25, while the a*value was 2.63 and the b* value corresponded to 14.37. Furthermore, the browning index was calculated (Table 1). The browning index of pasteurised pulp (20.07) was not statistically different from fresh pulp (19.43), whereas UHT-treated pulp (24.79) differed significantly from the fresh and the pasteurised materials. The higher browning index of the UHT-treated material may be explained by a higher processing temperature (135 °C compared to 80 °C), increasing the rate of non-enzymatic browning reactions.

3.2. Sensory evaluation and aroma composition of fresh and thermally treated cocoa pulp

3.2.1. Descriptive sensory analysis

The orthonasal aroma and the taste characteristics of the fresh, pasteurised and UHT-treated cocoa pulps were assessed according to simple descriptive testing (DIN 10969:2001–05) by rating various

impressions on a linear scale from 0 (not present) to 5 (strongly present). Fig. 1 displays the taste profiles (A) and the orthonasal aroma profile (B) of the three pulps. The means and standard deviations are listed in the supplementary material (Supplementary Table 1 and Supplementary Table 2). Even though the differences in the various taste perceptions (Fig. 1 A) between the pulps were not statistically significant, the pulps were rated differently to some extent. The fresh cocoa pulp scored highest in the attribute sour (3.7), followed by the pasteurised (3.2) and UHT-treated samples (3.1). The lower score in the sour attribute of the UHT-treated sample was also accompanied by a lower astringency, perceived with an intensity of 0.9. In contrast, this attribute scored similarly in the fresh and the pasteurised samples with 2.0 and 2.1, respectively. The UHT-treated sample was rated higher in the attribute sweet (3.0) compared to the pasteurised (2.4) and the fresh (2.8) pulps, possibly because the sample was perceived as less sour and less astringent than the other ones. As the differences were not significant, it can be assumed that both thermal processes do not alter the taste quality of the treated pulps compared to the fresh pulp. Therefore, we describe the effects of thermal processing on the aroma profiles and aroma composition of the treated cocoa pulps in the following.

The following orthonasal aroma properties were described for the fresh cocoa pulp (Fig. 1 B): the *malty* impression was rated with the highest intensity (2.8), followed by *unripe banana-like* and *lactic* (both with 2.6), *metallic* (2.4), *pungent* (2.3), *apple/pear-like* (2.1) and the *tropical fruit-like* (1.3) impressions. The attribute *unripe banana* was chosen by the panel as the note reminded of green banana peels. On the other hand, the panel agreed on the attribute *tropical fruit-like*, which encompassed notes perceived as *passionfruit-* and *peach-like*. In comparison, the pasteurised pulp scored higher in the impressions *apple/pear*-like (3.1), *lactic* (3.1) and *tropical fruit-like* (2.6). Interestingly, the intensity of the *tropical fruit-like* attribute was significantly higher in the UHT-treated sample (4.4) compared with the other samples, suggesting the higher thermal input during the UHT process promoted the formation of *tropical fruit-like* notes. Furthermore, the intensity of the *unripe banana-like* impression, present in the fresh sample, tended to decrease with an increase of the treatment temperature and accounted for 1.3 in the pasteurised pulp and for 0.7 in the UHT-treated material. The differences in the aroma profiles of the fresh and thermally treated samples could be described in more detail by identifying the aroma-active substances.

3.2.2. Identification of aroma-active volatiles in fresh, pasteurised and UHT-treated cocoa pulp

The cAEDA showed a total of 82 aroma-active regions within a FD factor range of 1 and ≥ 1024, of which 79 were successfully identified and corroborated by SBSE- and HS-SPME-GC-MS/O (Table 2). The identified aroma-active regions comprised substances from various chemical groups. Aldehydes predominated and were followed by carboxylic acids, ketones, alcohols, lactones and terpenes. Additionally, the distillates exhibited esters, aliphatic compounds, furans, thiazolines, pyrrolidines and pyrazines. The presence of these chemical groups in cocoa pulp was previously described (Chetschik et al., 2018; Bickel Haase et al., 2021). The fresh and thermally treated pulps shared 50 common aroma-active substances (No. 3–5, 10, 13–26, 29–33, 35, 36, 39, 40, 42, 45, 46, 48, 49, 51, 53, 55, 56, 58, 60, 61, 63, 64, 67, 71–74, 76, 78, 79 and 82). Of all aroma-active regions determined, 48 substances (No. 2–6, 10, 11, 13, 15, 16, 18–21, 23–26, 28–30, 32, 34–37, 39, 40, 42, 47–50, 52, 54, 55, 58, 61, 63, 65–67, 69–71, 76, 80 and 82) were previously reported in cocoa pulp by means of AEDA (Chetschik et al., 2018; Bickel Haase et al., 2021). In Bickel Haase et al. (2021), we identified 43 aroma-active regions in Cameroonian cocoa pulp with FD factors above 2. In this study, we also included aroma-active regions with FD factors of 1, resulting in 74 aroma-active regions in fresh, 66 in UHT-treated and 60 in the pasteurised cocoa pulp.

3.2.3. Aroma-active volatiles with high FD factors in fresh and thermally treated pulps

In this study, the following odorants showed the highest intensities in fresh Cameroonian pulp: 1-pentanol (*pungent, cheesy,* FD 512), linalool (*flowery,* FD 512), (*E,E*)-2,4-decadienal (*deep fried, fatty,* FD 512), *trans*-4,5-epoxy-(*E*)-2-decenal (*metallic*, FD 512) and 2,5-dimethylphenol (*smoked ham-like, medical*, FD 512). Pasteurised pulp also exhibited the *deep fried-like* and *fatty* odorant (*E,E*)-2,4-decadienal. This, together with (*E,E*)-2,4-nonadienal, were determined with FD ≥ 1024. The aroma-active regions 3-(methylthio)propanal (*cooked potato-like*, FD 256), 2-methylphenol (*medical, ink-like, phenolic*, FD 256), 4-vinylphenol (*phenolic,* FD 256), 5-(hydroxymethyl)furfural (*butter-like, caramel-like, fatty*, FD 256) and phenylacetic acid (*honey-like,* FD 256) had lower FD factors. The aroma-active compound (*E,E*)-2,4-decadienal scored also high in the UHT-treated sample (FD ≥ 1024). (*E,E*)-2,4-Decadienal can be formed when decanal is dehydrogenized and *trans*-double bonds are introduced at the 2–3 and 4–5 positions. This aroma-active compound is also a product of lipid degradation (Belitz et al., 2004). (*E,E*)-2,4-Decadienal imparts a characteristic *fried* note, although some describe the same note as *lemon-* or *citrus-like* (Parker et al., 2015). This odorant is more occurrent in frying oils and oil fumes (Boskou et al., 2006) but it has been found naturally in several food products like orange and mandarin juices (Feng et al., 2018). Further aroma-active compounds perceived with high intensities in the UHT-treated pulp were 2,3-pentanedione (*butter-like,* FD 256), 2-methylbutanol (*solvent-like, fruity,* FD

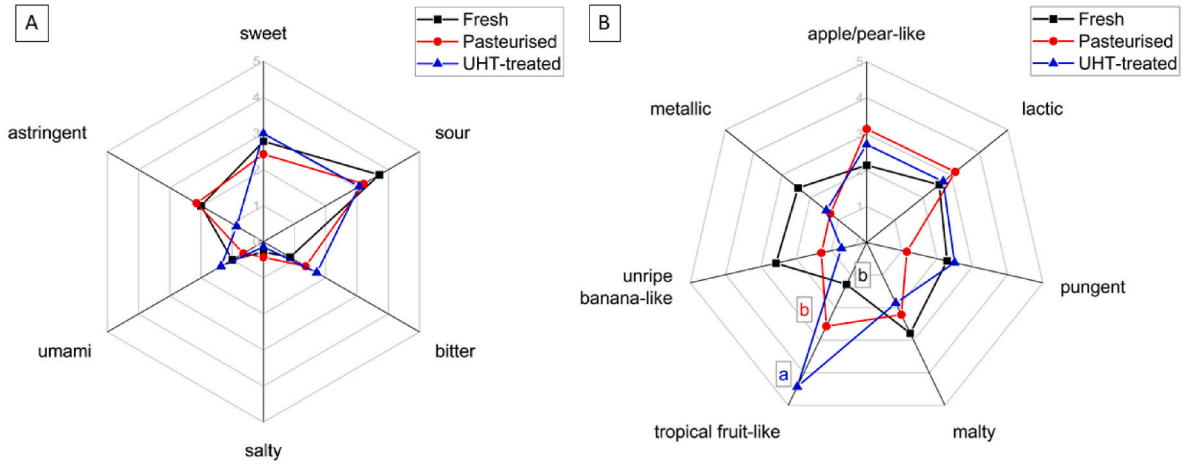

Fig. 1. Taste (A) and aroma profile analyses (B) by simple descriptive analysis of fresh, pasteurised and UHT-treated cocoa pulp. Different letters (c.f. attribute tropical fruit-like) indicate significant differences ($p < 0.05$) between the samples.

Table 2
Aroma-active regions identified in distillates obtained from fresh, pasteurised and UHT-treated cocoa pulps.

No.[a]	Odorant [b]	Odor Quality [c]	Retention Index on		FD Factor [d]		
			DB-FFAP	DB-5	Fresh	Past	UHT
1	α-pinene [f]	green, woody	1008	n.d.	16	<1	<1
2	methyl 2-methylbutanoate	fruity, banana-like	1017	776	<1	<1	64
3	2,3-pentanedione	butter-like	1056	706	64	128	256
4	hexanal	green	1089	802	32	32	16
5	3-methylbutyl acetate	fruity	1118	880	4	8	128
6	δ-carene	green	1140	1014	8	1	<1
7	1-penten-3-ol	pungent	1153	695	64	<1	4
8	myrcene	earthy, metallic, geranium-like	1158	991	8	<1	4
9	2-methylbutanol	solvent-like, fruity	1201	738	<1	<1	256
10	3-methylbutanol	malty, roasty	1206	745	1	32	2
11	2-heptanone	fruity, flowery	1207	891	<1	32	<1
12	1-pentanol	pungent, cheesy	1235	769	512	4	<1
13	(*Z*)-4-heptenal	fishy	1245	894	8	1	1
14	hexyl acetate	fruity, green apple-like	1275	1014	16	8	2
15	octanal	citrus-like, green	1280	1002	64	8	64
16	1-octen-3-one	mushroom-like	1285	978	16	32	4
17	2-methyl-3-furanthiol [f]	broth-like	1304	n.d.	128	128	4
18	(*E*)-2-heptenal	green, flowery	1311	951	32	32	64
19	2-acetyl-1-pyrroline [f]	popcorn-like	1342	930	32	32	64
20	(*Z*)-3-hexen-1-ol	green, grassy	1376	861	64	32	64
21	nonanal	citrus-like, soapy	1386	1106	32	4	64
22	2-nonanone	cheesy, fatty, green	1398	1094	32	16	64
23	(*E*)-2-octenal	fatty, grassy, green	1417	1055	64	32	16
24	acetic acid	vinegar-like	1430	619	256	64	16
25	3-(methylthio)propanal	cooked potato-like	1455	903	64	256	256
26	(*E,E*)-2,4-heptadienal	fatty, roasty	1480	1020	128	64	64
27	decanal	soapy	1489	1203	64	<1	64
28	2-isobutyl-3-methoxypyrazine	bell pepper-like, earthy	1510	1090	32	<1	2
29	benzaldehyde	almond-like	1515	965	2	4	1
30	(*E*)-2-nonenal	fatty, cardboard-like	1524	1164	128	128	16
31	propanoic acid	pungent, sweaty, fruity	1535	924	256	16	2
32	linalool	flowery	1539	1103	512	128	256
33	1-octanol	soapy, citrus-like, fatty	1550	1077	2	32	64
34	2-methylpropanoic acid	cheesy	1562	782	32	<1	<1
35	(*E,Z*)-2,6-nonadienal	cucumber-like, fatty	1574	1158	128	32	16
36	butanoic acid	cheesy	1626	804	1	1	4
37	(*E*)-2-decenal	green, fatty	1627	1252	64	<1	<1
38	acetophenone	almond-like, solvent-like	1634	n.d.	1	<1	4
39	phenylacetaldehyde	flowery, honey-like	1634	1040	4	1	256
40	3-methylbutanoic acid	cheesy, sweaty, banana-like	1650	859	128	32	256
41	unknown	musty, smoky	1665	n.d.	4	8	<1
42	(*E,E*)-2,4-nonadienal	deep fried, fatty	1696	1216	32	≥1024	64
43	3-(methylthio)propanol	cooked potato-like	1707	982	64	4	<1
44	farnesene	earthy, flowery	1712	1459	16	<1	2
45	pentanoic acid	fruity, sweaty, pungent	1725	892	16	16	16
46	(*E*)-b-farnesene	pumpkin-like, cucumber-like	1740	1508	64	16	16
47	2-acetyl-2-thiazoline [f]	popcorn-like, roasty	1747	n.d.	64	<1	<1
48	(*E,E*)-2,4-decadienal	deep fried, fatty	1800	1325	512	≥1024	≥1024
49	β-damascenone [f]	fruity, grape-like	1808	1374	128	8	16
50	geraniol	flowery, earthy	1841	1428	32	<1	64
51	1-undecanol	soapy, green	1851	1370	64	2	2
52	2-methoxyphenol	smoky, ham-like, vanilla-like	1853	1096	256	<1	4
53	butyl benzoate	fruity	1862	n.d.	4	4	32
54	2-phenylethanol	rose-like, flowery	1897	1110	128	<1	<1
55	δ-octalactone	fruity, coconut-like	1908	1152	2	8	16
56	5-methylguaiacol	smoky, vanilla-like	1920	1191	256	64	128
57	unknown	metallic	1947	1543	128	<1	<1
58	*trans*-4,5-epoxy-(*E*)-2-decenal [f]	metallic	1994	1379	512	64	64
59	2-methylphenol	medical, ink-like, phenolic	1997	n.d.	<1	256	<1
60	methyl cyclopentenolone	maggi-like, spicy	2003	1030	2	64	4
61	γ-nonalactone	fruity, flowery	2014	1360	32	16	32
62	phenol	phenolic	2017	959	16	16	<1
63	octanoic acid	green, soapy	2043	118	4	16	64
64	2,5-dimethylphenol	smoked ham-like, medical	2072	n.d.	512	32	64
65	4-methylphenol	horse stable-like, faecal	2078	1089	4	<1	<1
66	δ-nonalactone	fruity, coconut-like	2084	1380	256	<1	1
67	γ-decalactone	fruity, peach-like	2134	1474	128	8	1
68	unknown	honey-like, flowery	2158	n.d.	<1	<1	32
69	2-methoxy-4-vinylphenol	clove-like, smoked ham-like, vanilla-like	2182	1326	1	<1	1
70	δ-decalactone	coconut-like	2188	1498	<1	4	2
71	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	maggi-like, celery-like	2194	1002	256	128	256
72	3,4-dimethylphenol	horse stable-like, leather-like	2212	1216	16	8	16
73	decanoic acid	coriander-like, plastic-like, soapy	2254	1374	128	32	32

(*continued on next page*)

Table 2 (*continued*)

No.[a]	Odorant [b]	Odor Quality [c]	Retention Index on		FD Factor [d]		
			DB-FFAP	DB-5	Fresh	Past	UHT
74	3-phenyl-2-propen-1-ol	honey-like, vanilla-like	2269	1313	64	64	32
75	δ-undecalactone	fruity, peach-like	2278	n.d.	64	4	<1
76	4-vinylphenol [f]	phenolic	2388	1231	16	256	32
77	δ -dodecalactone	fruity, peach-like	2395	1700	<1	<1	4
78	2-(methylthio)benzothiazole	medical, smoky	2420	n.d.	128	64	16
79	benzophenone	flowery	2477	n.d.	16	128	32
80	indole	faecal	2485	1320	16	<1	<1
81	5-(hydroxymethyl)furfural	butter, caramel-like, fatty	2511	1228	<1	256	16
82	phenylacetic acid	honey-like	2545	1256	128	258	64

[e] n.d. not detected.
[a] Consecutive numbering of odorants according to their retention indices on capillary column DB-FFAP.
[b] Odorant was identified by comparison of its odor quality and intensity and retention indices on capillaries DB-FFAP and DB-5 as well as mass spectra (EI mode) with data of reference compounds.
[c] Odor quality perceived at the odor detection port by four trained panellists.
[d] Flavour dilution factor determined on DB-FFAP.
[f] No unequivocal mass spectrum was obtained; identification is based on the remaining criteria given in footnote b.

256), 3-(methylthio)propanal (*cooked potato-like,* FD 256), linalool (*flowery,* FD 256), phenylacetaldehyde (*flowery, honey-like*, FD 256), 3-methylbutanoic acid (*cheesy, sweaty, banana-like*, FD 256) and 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (*maggi-like, celery-like*, FD 256).

3.2.4. Aroma-active volatiles found exclusively in the fresh and the thermally treated pulps

Fresh pulp. The aroma-active compounds α-pinene (*green, woody,* FD 16), 2-methylpropanoic acid (*cheesy,* FD 32), (*E*)-2-decenal, (*green, fatty,* FD 64), 2-acetyl-2-thiazoline (*popcorn-like, roasty,* FD 64), odorant no. 57 (*metallic,* FD 128), 4-methylphenol (*horse stable-like, faecal*, FD 4), 2-phenylethanol (*rose-like, flowery,* FD 128) and indole (*faecal,* FD 16) were only perceived in the fresh pulp (FD > 1). As a flavour molecule, 2-phenylethanol is of high commercial importance (Welsh et al., 1989). It is often added to ice cream, candy, non-alcoholic beverages, gelatines, puddings and chewing gum (Furia and Bellanca 1971). Many authors reported the presence of 2-phenylethanol in cocoa pulp from different origins (Pino et al., 2010; Chetschik et al., 2018; Bickel Haase et al., 2021). Several thermal degradation processes were proposed for this substance, yet further research is needed (Sakai et al., 2013). Moreover, indole was only perceived in the fresh material. Indole is often formed by bacteria (Lee and Lee 2010) and it imparts a floral taint with a faecal note to foods and beverages (Parker et al., 2015). Due to its off-flavour character, its absence in the thermally treated samples may be advantageous for the overall cocoa pulp's aroma profile.

Pasteurised pulp. The aroma-active compounds 2-heptanone (*fruity, flowery,* FD 32) and 2-methylphenol (*medical, ink-like, phenolic,* FD 256) were exclusively detected in pasteurised pulp. Methyl ketones such as 2-heptanone, 2-nonanone and 2-undecanone are important aroma constituents of blue cheese and are formed from triglycerides by thermally induced β-oxidation followed by a decarboxylation reaction (Belitz et al., 2004). Pino et al. (2010) identified the methylketone 2-heptanone in pulp of an undefined cocoa genotype from Colombia, while Rottiers et al. (2019) described this compound to be abundant in raw cocoa. Accordingly, Akoa et al. (2023) described the presence of 2-heptanone in cocoa beans from different varieties grown in Cameroon. Furthermore, in the degradation of polyphenols and lignin, enzymes and microorganisms release phenols (Zaprometov 1989). Phenols and their derivatives can be strong aroma-active substances. Methylphenols are often described as *phenolic* and *smoky*, whereas methoxyphenols can have a broad range of odor qualities reaching from *smoky* to *vanilla-like* (Lentz 2018; Genovese et al., 2018; Issa-Issa et al., 2020).

UHT-treated pulp. The exclusive substances perceived in UHT-treated pulp were methyl 2-methylbutanoate (*fruity, banana-like,* FD 64), 2-methylbutanol (*solvent-like, fruity,* FD 256), an unidentified compound with *honey-like* and *flowery* attributes (no. 68, FD 32) and δ–dodecalactone (*fruity, peach-like,* FD 4). It is to note that these compounds impart *fruity* and *flowery* odor qualities. Additioanlly, the UHT-treated pulp was considered more *tropical fruit-like* than the fresh and the pasteurised cocoa pulps during the sensory evaluation (Fig. 1). Even though these substances do not directly fit the description *tropical fruit-like,* they may have contributed to the UHT-treated pulp to be perceived as such. Due to the intricate interplay of multiple aroma-active compounds, it is often difficult to draw definitive conclusions regarding the correlation between a single volatile compound and olfactory perception (Brattoli et al., 2013). For instance, Strube et al. (2009) showed the interaction between several odorants, predominantly *fatty-smelling* aldehydes, resulting in a *plastic-like* off-odor in mineral water. Moreover, AEDA serves as a screening method, but it does not provide an absolute indication of which compounds have the greatest impact on the overall aroma of food. This limitation arises partly because the odor compounds in dilution extracts are volatilized and evaluated, whereas in actual foods, the volatility of aroma compounds depends on their solubility, vapour pressure and specific thresholds in the food matrix as well as their interactions with non-volatile constituents. Additionally, the FD factors ascribed in AEDA do not take into account the physiological impressions that influence our perception of odor character or the sensory thresholds of odor-active compounds in the food matrix (Marsili 2020). New studies indicate that odorants can undergo diverse biotransformation processes in the olfactory and/or respiratory nasal epithelium and mucus, influencing overall odor perception if their concentrationis lie above the detection thresholds (Kornbausch et al., 2022). In this sense, cAEDA, as applied in this study, only enables to determine qualitative changes in the cocoa pulp with thermal processing. To fully understand the influence of these *fruity* and *floral* odorants to the total aroma profile of thermally treated cocoa pulp a calculation of odor activity values and the implementation of recombination and omission experiments are required.

3.2.5. Comparison of aroma-active volatiles in the fresh and thermally treated pulps

Fresh and pasteurised pulp. Both cocoa pulps shared six common aroma-active compounds not perceived in the UHT-treated pulp. These aroma-active VOCs were δ-carene (*green*, RI 1140), 1-pentanol (*pungent, cheesy*, RI 1235), odorant no. 41 (*musty, smoky*, RI 1665), 3-(methylthio) propanol (*cooked potato-like*, RI 1707), phenol (*phenolic,* RI, 2017) and δ-undecalactone (*fruity, peach-like*, RI 2278). In the pasteurised material, δ-undecalactone showed a FD factor of 4 compared to FD 64 in fresh pulp. Furthermore, its absence in UHT-treated pulp hints at increased losses with higher processing temperatures. Moreover, while 3-(methylthio)propanol showed the highest FD factor in fresh pulp, its oxidised form 3-(methylthio)propanal exhibited a higher FD factor after thermal

treatment. It changed from 64 in the fresh pulp to FD 256 in the pasteurised and UHT-treated samples. 3-(Methylthio)propanal is a thermally induced volatile flavour compound and the result of the Maillard reactions of α-dicarbonyl compounds with the amino acid methionine, followed by Strecker degradation reactions (Havkin-Frenkel and Belanger 2008). Various compounds of the fresh pulp such as 2-methyl-3-furanthiol (*broth-like,* RI 1304), acetic acid (*vinegar-like*, RI 1430) and (*E*)-2-nonenal (*fatty, cardboard-like, green*, RI 1524) were better preserved with pasteurisation, showing lower reductions in the FD factors compared with the UHT-treatment. An explanation could be a lower stability of these aroma-active compounds to higher temperatures (135 °C), and/or a higher stability to longer heat exposures (approximately 80 °C for 20 min).

Fresh and UHT-treated pulp. They shared a larger number of exclusive aroma-active regions (n = 10) than the fresh and pasteurised pulp. These were myrcene (*earthy, metallic geranium-like*, RI 1158), 1-penten-3-ol (*pungent,* RI 113), decanal (*soapy,* RI 1489), 2-isobutyl-3-methoxypyrazine (*bell pepper-like, earthy,* R 1510), acetophenone (*almond, solvent-like,* RI 1634), farnesene (*earthy, flowery,* RI 1712), geraniol (*flowery, earthy*, RI, 1841), 2-methoxyphenol (*smoky, ham-like, vanilla-like*, RI, 1853), δ-nonalactone (*fruity, coconut-like*, RI, 2084) and 2-methoxy-4-vinylphenol (*clove-like, smoked ham-like, vanilla-like*, RI 2182). The FD factor of decanal remained the same after UHT-treatment. Likewise, UHT-treatment affected geraniol's FD factor minimally, changing it from FD 32 in the fresh sample to FD 64 in the UHT-treated. Contrarily, the FD factors of the other substances decreased strongly with ultra-high temperature treatment.

Our descriptive testing of the orthonasal characteristics (3.2.1) showed a significant difference for the attribute *tropical fruit-like* between the fresh and UHT-treated pulp. The perception of highly volatile compounds, methyl 2-methylbutanoate (*fruity, banana-like,* FD < 1 in fresh and FD 64 in UHT-treated pulp), 2,3-pentanedione (*butter-like*, FD 64 and FD 256), hexanal (*green,* FD 32 and FD 16) and 3-methylbutyl acetate (*fruity*, FD 4 and FD 128) may have been contributed for the difference in this attribute. As aforementioned, their quantification and OAV calculation in cocoa pulp may help comprehend better the changes taking place during thermal treatment.

Pasteurised and UHT-treated pulp. The thermally treated pulps exhibited two compounds not perceived in the fresh material, δ–decalactone (*coconut-like*, RI 2188) and 5-(hydroxymethyl)furfural (*butter, caramel-like, fatty,* RI 2511). The substance 5-(hydroxymethyl) furfural showed FD factors of 256 in pasteurised and 16 in UHT-treated pulp. The heating of monosaccharides under acidic conditions, e.g. in juice preservation, leads to the formation of a large number of furanoid and pyranoid compounds. These arise from enolizations and dehydrating reactions of carbohydrates (Belitz et al., 2004). Murkovic and Bornik (2007) described the formation of 5-(hydroxymethyl)furfural (HMF) during coffee roasting, which peaked at 240 °C in the first 3 min of roasting. In cocoa pulp, the higher FD factor in pasteurised pulp may be attributed to the pulp's longer exposure to heat. While UHT-treatment was short, with only 30 s exposure to 135 °C and immediate cooling in an ice bath, pasteurised pulp was kept hot for 20 min, possibly giving rise to increased HMF formation.

3.3. Storage stability of thermally treated cocoa pulp

3.3.1. Microbiological analysis

In order to assess the shelf-life of the pulps, microbiological analyses (yeasts, moulds and aerobic mesophilic bacteria) of the fresh pulp and the thermally-treated pulps were carried out directly after processing. In addition, the thermally-treated pulps were also investigated after 6, 12 and 24 weeks of storage (Table 3). The colony forming units per gram of pulp [CfU/g] of yeasts and moulds were below 100 prior and after thermal treatment. Independent from the storage temperature and the sampling time point, the total mould and yeast count remained unchanged (<100 CfU/g). Furthermore, we investigated the presence of viable bacterial cells. The aerobic mesophilic bacteria in the fresh pulp accounted for 4.6×10^3 CfU/g. After pasteurisation, 6.3×10^2 CfU/g were found, indicating a reduction of approximately one logarithmic unit compared to the fresh material. The inactivation of the aerobic mesophilic bacteria was more effective with the ultra-high temperature treatment. The bacterial count after UHT-treatment was below 100 CfU/g, representing an inactivation of over two logarithmic units or 99% of the initial bacterial count. In addition, the total aerobic mesophilic bacterial count of the pasteurised and the UHT samples remained constant throughout storage, indicating the remaining viable bacterial cells were not able to grow and proliferate. Accordingly, differences between the two storing temperatures in either the pasteurised and the UHT-treated pulps could not be observed. The constancy in the total yeast and mould count as well as in the aerobic mesophilic bacterial count might be explained by the low pH-value of cocoa pulp, which conceivably acted as a hindering factor for the growth of microorganisms (Doyle and Buchanan 2013). Some approaches for the microbial inactivation of cocoa pulp for various products have been previously described (Escalante et al., 2015; Puerari et al., 2012). However, the microbial load of the stabilised cocoa pulp over a longer storage was first described in this study. Additionally, in a recent study, Firdaus et al. (2022) produced cocoa pulp syrups using different sugar types from West Sumatra. The syrups were not subject to any thermal treatment for preservation. The microbiological tests for fungi and salmonella showed that the syrup kept at 5 °C could only be consumed safely during the first five days, highlighting the importance of preserving the cocoa pulp to reduce possible health hazards to the consumers.

3.3.2. Colour properties

Despite inactivation of the peroxidase activity and probably also of the more thermolabile enzymes (3.1), a strong browning was observed during storage. The L*a*b*-values of pasteurised and UHT-treated pulps stored at 4 °C and 23 °C for 24 weeks are shown in Supplementary Table 3. Over time, the L* value of the thermally treated samples decreased, indicating the samples became darker. However, the decrease in the L* values was more distinct at 23 °C. The a* values increased over time, while the b* values decreased when the samples were kept at 23 °C, indicating a yellowing. At 4 °C, the a* values

Table 3
Microbiological analysis of fresh, pasteurised and UHT-treated cocoa pulp at different time points.

Sample	Total yeast and mould count [CfU/g]						
	t_0	4 °C /6 W	23 °C/6 W	4 °C/12 W	23 °C/12 W	4 °C/24 W	23 °C/24 W
Fresh	<100						
Pasteurised	<100	<100	<100	<100	<100	<100	<100
UHT-treated	<100	<100	<100	<100	<100	<100	<100
Sample	Aerobic mesophilic bacterial count [CfU/g]						
	t_0	4 °C /6 W	4 °C/12 W	4 °C/24 W	23 °C/4 W	23 °C/12 W	23 °C/24 W
Fresh	$4.6 * 10^3$						
Pasteurised	$6.3 * 10^2$	$8.0 * 10^2$	$1.1 * 10^3$	$5.2 * 10^2$	$5.9 * 10^2$	$5.6 * 10^2$	$4.9 * 10^2$
UHT-treated	<100	<100	<100	<100	<100	<100	<100

W=weeks in storage, t_0 *= time point zero, sample taken directly after stabilisation.*

increased from week 2 to week 12, then dropped after 24 weeks; the b* values decreased slightly from week 12 to week 24. The BI of the stored pulps was calculated and plotted over time (Fig. 2). The browning effect was more pronounced in samples stored at 23 °C than in the samples stored at 4 °C, being very similar to the fresh ones (Table 1). Various authors attribute the browning of thermally processed fruit juices and fruit juice concentrates to non-enzymatic reactions, such as Maillard reactions or the oxidative degradation of ascorbic acid (Ibarz 1990; Selen Burdurlu and Karadeniz 2003). In our case, both reactions could be responsible for the browning, as free amino acids and reducing sugars as well as vitamin C can be present in the pulp (Pettipher 1986). However, the reason for the browning needs to be investigated in further studies.

4. Conclusions

The valorisation of the complete cocoa fruit pulp could help to increase the sustainability along the cocoa bean supply chain, improve the livelihoods of many cocoa farmers and promote the development of tasty new food products. Due to the fresh pulp's high water activity and sugar content, a stabilisation step is recommendable to slow down spoiling reactions and increase its shelf-life for transport and food applications. Pasteurisation and UHT-treatment were shown to be effective technologies for the preservation of cocoa pulp, as microorganisms and enzymes were successfully inactivated. Sensory evaluations suggested substantial changes of the pulps' aroma-active substances, which were confirmed by identification experiments by means of cAEDA, GC-MS/O, SBSE-GC-MS/O and HS-SPME-GC-MS/O. For a more thorough characterisation of the pulps' aroma profiles, quantitative analyses with subsequent calculation of odor activity values (OAV) as well as recombination and omission experiments should be carried out in future studies. Considering the higher degree of microbiological inactivation, the retention of a larger number of aroma-active regions as well as the higher score in the intensity of attribute *tropical fruit-like*, a UHT-treatment followed by a cold storage is the recommended technological approach for the preservation of cocoa pulp.

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CRediT authorship contribution statement

Thomas Bickel Haase: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Project administration. **Susanne Naumann-Gola:** Conceptualization, Writing – review & editing, Supervision, Project administration. **Eva Ortner:** Methodology, Data curation, Writing – review & editing, Supervision. **Holger Zorn:** Writing – review & editing, Supervision, All authors have read and agreed to the published version of the manuscript. **Ute Schweiggert-Weisz:** Conceptualization, Funding acquisition, Writing – review & editing, Supervision.

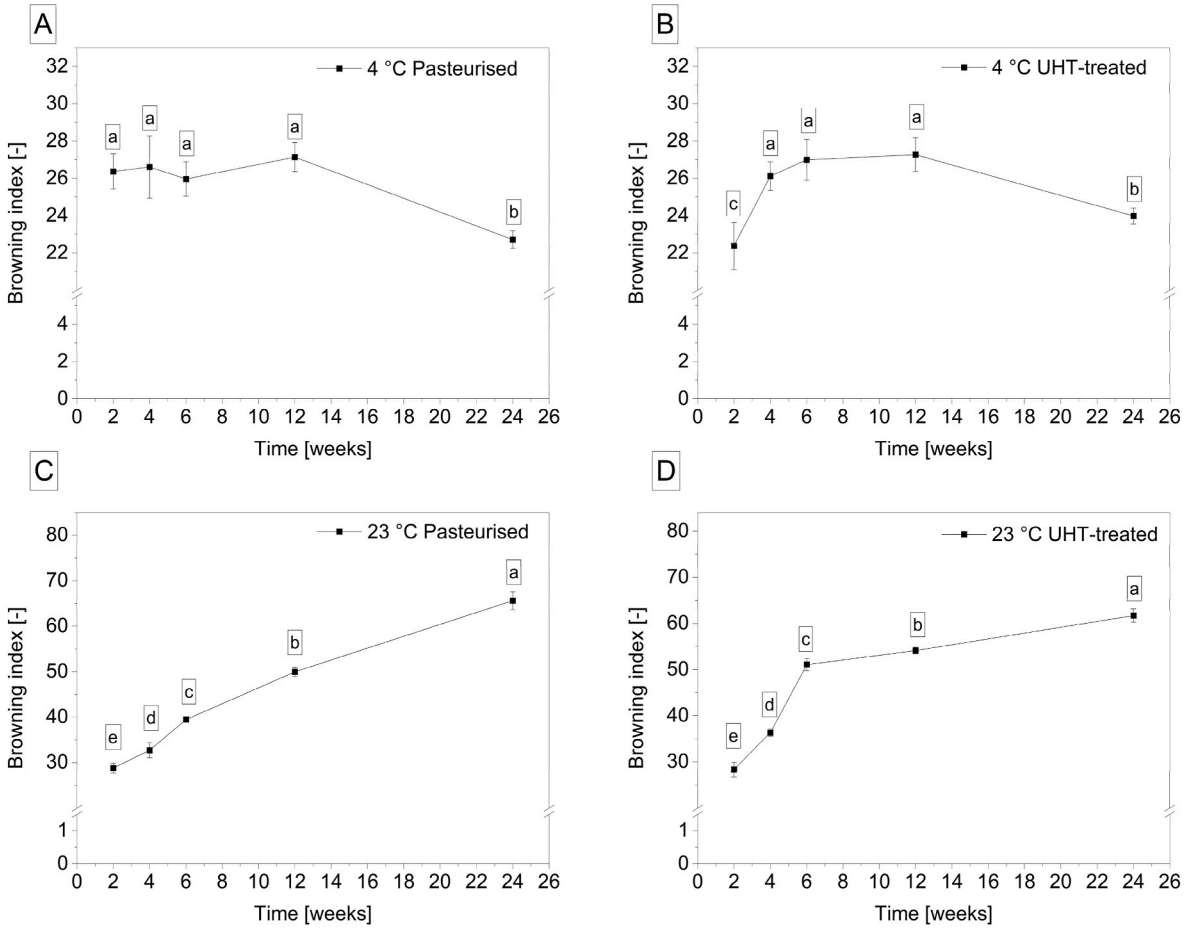

Fig. 2. Browning index of pasteurised and UHT-treated cocoa pulp stored at 4 °C and 23 °C for 24 weeks. Different letters indicate significant differences ($p < 0.05$) between the time points.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100549.

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