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A lab-scale model system for cocoa bean fermentation

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Abstract

Lab-scale systems modelling the spontaneous cocoa bean fermentation process are useful tools to research the influence of process parameters on the fermentation and the final bean quality. In this study in Honduras, a 1-kg lab-scale fermentation (LS-F) was compared to a 300-kg on-farm fermentation (OF-F) in a multiphasic approach, analysing microbial counts, microbial species diversity, physico-chemical parameters, and final dried bean quality. Yeast and total aerobic counts of up to 8 log CFU/g during the LS-F were comparable to the OF-F, while counts for lactic acid bacteria and acetic acid bacteria were up to 3 log CFU/g lower during the LS-F than during the OF-F. While species of the genera *Hansenia*, *Saccharomyces,* and *Acetobacter* dominated most of the fermentation processes, the genera dominating the drying phases were *Pichia*, *Trichosporon*, *Pediococcus,* and *Acetobacter*. Dried beans resulting from the LS-F, compared to the OF-F, were similar in contents of acetic acid, 6 times lower in lactic acid, up to 4 times higher in residual sugars, and 3–12 times higher in polyphenols. Dried beans processed at LS showed a similar flavour profile in terms of astringency, bitterness, acidity, and brown, fine, and cocoa flavours, but 2 units higher off-flavours than OF processed beans. With 81%, the share of well-fermented beans from the LS-F complied with industrial standards, whereas 7% over-fermented beans were above the threshold. Conclusively, the 5-day model fermentation and subsequent drying successfully mimicked the on-farm process, providing a high-throughput method to screen microbial strains to be used as starter cultures.

Keywords Cocoa bean fermentation · Lab-scale fermentation · Cocoa bean drying · Lactic acid bacteria · Acetic acid bacteria · Yeast

Introduction

Cocoa beans are the major raw material for chocolate production. Their fermentation is the first step in this process, and it is essential for removing the pulp that envelops the beans (Schwan and Wheals 2004; Lima et al. 2011; Pereira et al. 2016). Inside the pods of the *Theobroma cacao* tree, the beans are embedded in a sweet, white, mucilaginous pulp that is low in pH (3.0–4.0) due to the presence of citric acid. In the producing countries in the equatorial zone, the pods are opened manually and the beans with the surrounding pulp are fermented in boxes, heaps, trays, or baskets for 2–10 days (Lima et al. 2011; Schwan and Fleet 2014; De Vuyst and Weckx 2016). After the fermentation is completed, beans are dried to a water content of 6–8% in the sun or artificially in mechanical dryers (Lima et al. 2011; Hamdouche et al. 2015; Kongor et al. 2016; Visintin et al. 2016).

Despite its spontaneous nature, the fermentation generally follows a rather predictable pattern due to the composition of the pulp. Oxygen is one of the factors that determine the microbial succession. Yeasts and lactic acid bacteria (LAB) are the predominating microbial species at the initial phase of the fermentation process. In the tightly packed and mostly anaerobic pulp-bean mass, facultative anaerobic yeast produce ethanol from glucose present in the pulp. Semi-anaerobic conditions following the initial yeast growth favour microaerophilic LAB that convert glucose, fructose, and citric acid mainly into lactic acid, acetic acid, and/or mannitol. When most of the pulp has been degraded due to the pectinolytic

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activity of the yeasts, oxygen becomes available and the strictly aerobic acetic acid bacteria (AAB) develop. They produce acetic acid from ethanol in an exothermic oxidation reaction, causing the temperature of the bean mass to increase to 50 °C or higher and convert lactate into acetoin and biomass building blocks (Schwan and Wheals 2004; Leal et al. 2008; Papalexandratou et al. 2011b; Adler et al. 2014; Schwan and Fleet 2014; Ho et al. 2014; Pereira et al. 2016; Ouattara et al. 2017; De Roos and De Vuyst 2018). *Hanseniaspora opuntiae*, *Saccharomyces cerevisiae*, *Pichia manshurica,* and/or *Pichia kudriavzevii* often dominate the group of yeasts during cocoa bean fermentation and *Lactobacillus plantarum* and *Lactobacillus fermentum* the group of LAB, whereas *Acetobacter pasteurianus* has most often been reported as dominant AAB species, beside *Acetobacter ghanensis*, *Acetobacter senegalensis,* and *Gluconobacter oxydans* (Camu et al. 2007; Kostinek et al. 2008; Daniel et al. 2009; Papalexandratou et al. 2011b; Papalexandratou et al. 2013; Fernández Maura et al. 2016; Miescher Schwenninger et al. 2016; Visintin et al. 2016). To this day, only little is known about microbial communities and their dynamics during the drying of cocoa beans. Hamdouche et al. (2015) investigated the microbial species diversity on drying cocoa beans in the Ivory Coast and found species including *H. opuntiae*, *Pichia manshurica*, *Lb. fermentum*, *Acetobacter lovaniensis*, *Acetobacter cerevisiae*, *Acetobacter nitrogenifigens*, *Gluconobacter xylinum,* and *Clostridium* sp. Yeast species generally appeared less resistant to sun drying than bacterial species, since fewer yeast species were retrieved from the drying than from the fermentation process.

During fermentation, ethanol, and acetic acid diffuse into the beans, which causes pH values of the cotyledon to decrease from 6.3–7.0 to 4.0–5.5, which, in combination with the heat produced, results in the death of the seed (De Vuyst and Weckx 2016). The destruction of internal membranes of the bean cotyledon compartments gives rise to complex biochemical reactions in the cotyledons (Lima et al. 2011; Moreira et al. 2013; Schwan and Fleet 2014). While the alkaloids caffeine and theobromine impart bitterness in cocoa, polyphenolic compounds, such as the flavan-3-ols epicatechin and catechin and higher oligomeric proanthocyanidins, contribute both bitter and astringent notes depending on their degree of polymerization (Stark et al. 2005). The diffusion of polyphenols out of the beans and their oxidation and complexation with other polyphenols, proteins, and peptides significantly reduce bitterness and astringency (Camu et al. 2008a; Romero-Cortes et al. 2013). These reactions also lead to the development of precursors of chocolate flavour and the colour of the bean changes from purple to brown under favourable fermentation conditions (Camu et al. 2008a; Rusconi and Conti 2010).

Small-scale fermentation models have been described in various studies, either performed in net bags, which were buried in larger fermentations, or in small containers, e.g. wooden boxes, plastic baskets, or vessels. They are a useful tool to study the influence, e.g. of different cocoa genotypes or origins, on the cocoa bean fermentation process and the final bean quality (Hansen et al. 2000; Sukha et al. 2008; Ho et al. 2015; Hue et al. 2016) and they are often used to test starter culture strains prior to their implementation in industrial-scale fermentations (Leal et al. 2008; Lefeber et al. 2011; Crafack et al. 2013; Essia Ngang et al. 2015; Meersman et al. 2016; Sandhya et al. 2016; Pereira et al. 2017). So far, no study has compared a small-scale to a large-scale fermentation in a multiphasic approach that includes the analysis of both the drying process and the quality of fermented dried beans.

The aim of this study was to develop a model system of the cocoa bean fermentation process at lab scale, comprising both the fermentation and the drying phases, to speed up research on the influence of starter cultures on the process and final dried bean quality. Therefore, we compared our 1-kg lab-scale model fermentation to a well-performed 300-kg on-farm box fermentation, both carried out spontaneously in Honduras.

Materials and methods

Fermentation experiments and sampling

Three spontaneous, 5-day, lab-scale cocoa bean fermentations (LS-F) were performed as technical replicates in January 2016 and three spontaneous, 5-day, on-farm fermentations (OF-F) were performed as biological replicates between September and October 2013. The LS-F took place in a laboratory of the FHIA (Fundación Hondureña de Investigación Agrícola) in La Lima, Cortés, Honduras and the studied OF-F at the CEDEC (Centro Experimental Demostrativo del Cacao) located in La Masica, Atlántida, Honduras.

For both fermentation experiments, high-quality cocoa pods were harvested at the CEDEC by traditional methods and opened within 1 to 3 days. For the LS-F, fruits of the selected hybrids, i.e. IMC-67, UF-29 and UF-668 were transported to the FHIA laboratory in La Lima. After opening the fruits, beans with their adherent pulp were collected in a plastic bucket to obtain a homogenous mixture of 12 kg. One kg of the cocoa pulp-bean mass was filled into each of 12 - nine used for daily sampling and three as reserves - commercially available plastic pots and the fermentation mass was covered with pieces of banana leaves. The pots measured 16 cm in height, 13 cm in diameter at the bottom, and 19 cm in diameter at the top and contained 10 to 12 holes in the bottom to allow drainage of the sweatings. The fermentation pots were kept in a laboratory incubator (Thermo Fisher Scientific Inc., Waltham, USA) for 117 h and temperature was adjusted in 6–24 h intervals, simulating

the OF-F: 28 °C at 0 h, 30 °C at 6 h, 32 °C at 21 h, 35 °C at 30 h, 38 °C at 45 h, 42 °C at 54 h, 45 °C at 69 h, and 47 °C at 93 h. The pulp-bean mass was mixed every 24 h by manual kneading to allow efficient removal of the pulp. For the OF-F, cocoa pulp-bean mass of a mix of various hybrids was collected in plastic bags, weighed, and transferred to the fermentation site. For the three fermentations, 310, 299 and 248 kg of pulp-bean mass, respectively, were filled in wooden boxes of 89 cm × 84 cm × 60 cm and were covered with banana leaves. Every 24 h, the whole pulp-bean mass was transferred to another box allowing aeration and homogenization of the fermentation mass.

After fermentations were completed, beans were dried to final water content of 6–7%, which was checked with a portable coffee moisture tester (Agratronix Corporate, Ohio, USA). For drying at LS, fully fermented beans from the plastic pots 1 to 4, 5 to 8, and 9 to 12, respectively, were combined and dried in a laboratory drying oven (Thermo Fisher Scientific Inc., Waltham, USA) for 7 days. Temperature was increased gradually up to 35 °C on day 1, to 45 °C on day 2, to 52 °C on day 3, to 55 °C on days 4 and 5, and to 50 °C on days 6 and 7. During the nights, the beans were allowed to cool down at room temperature on days 1 to 4 and at 33–35 °C on days 5 to 7. Beans from the OF-F were placed on wooden trays of 2 m × 4 m for sun drying during 7 to 11 days, while frequently moving the mass, or, depending on weather conditions, drying was partially performed in a drying oven (Maquinaria Industrial Joca S.A., San José, Costa Rica).

During all fermentations, samples were collected every 24 h, with a first sample of fresh pulp-bean mass at 0 h and a final sample of fully dried beans. During the LS-F, the sampling took place after mixing the pulp-bean mass. At 0 and 24 h of fermentation, the samples were collected from plastic pots 1, 5, and 9, at 48 and 72 h from pots 2, 6, and 10, and at 96 and 120 h from pots 3, 7, and 11. During the LS drying process, samples were taken from the combined drying bean masses. During the OF-F, while transferring the fermentation mass to the next box, equal amounts of pulp-bean mass were collected from the centre, the side, and the corner of the fermentation box at each of three levels, i.e. bottom, middle, and top, resulting in composite samples from nine representative positions within the box.

To prevent enzymatic degradation or oxidation of polyphenols during storage and transport, fermentation samples for polyphenol analysis were stabilized. Twenty grammes of cotyledon, separated from pulp and testa, were crushed with a hand blender (Bamix AG, Mettlen, Switzerland) and the particles were mixed with a 5-g/l $CaCl_2$ solution in a sample-to-solvent ratio of 1:7 (Yang et al. 2010). The stabilized polyphenol samples and the samples for the metabolite target analysis were transported on ice to Switzerland and stored at − 20 °C until further analyses. The analyses of microbial counts, pulp pH, and pulp content were performed with fresh samples in Honduras and the dried bean samples were stored and transported to Switzerland at room temperature.

Enumeration, isolation, and maintenance of microorganisms

The dominant microbiota were enumerated in fresh samples of fermenting and drying beans in Honduras and representative isolates were selected from different agar media. Therefore, 5–10 cocoa beans with adhering pulp were mixed with nine times the weight of dilution solution (0.1% bacteriological peptone, 0.85% sodium chloride, [w/v]) in a sterile stomacher bag and either treated at 180 rpm for 1 min in a Stomacher 400 (Seward, Worthington, UK) during the LS-F or manually kneaded for 5 min during the OF-F to obtain a uniform homogenate. For serial dilutions of samples derived from the LS-F, four spots of 0.02 ml each were applied on one section of an agar plate, allowing the drops to run down slantwise. During the OF-F, aliquots of 0.1 ml from serial dilutions were plated. The enumeration and propagation of microorganisms was done on different agar media. Total aerobic counts were analysed on plate count (PC) agar, incubated at 30 °C for 3 days, yeasts on yeast extract glucose chloramphenicol (YGC) agar and incubated at room temperature, corresponding to 25–30 °C, for 3 days, and LAB on de Man-Rogosa-Sharpe (MRS) agar and incubated anaerobically at 37 °C for 2 days. For the cultivation of AAB, yeast peptone mannitol (YPM) agar and ethanol glucose mannitol (EGM) agar were prepared as described by Miescher Schwenninger et al. (2016) and incubated at room temperature for 5 days. MRS, YPM, and EGM were supplemented with cycloheximide at 40 mg/l to inhibit yeast growth and YPM and EGM were additionally supplemented with 50 mg/l penicillin G to inhibit growth of Gram-positive bacteria. All media and chemicals used for agar media were purchased from Sigma-Aldrich (Saint Louis, USA). Confirmation tests and microscopic examination were performed with representative colonies and only confirmed colonies were included in the calculation of cell counts: catalase-negative and oxidase-negative colonies from MRS as LAB, catalase-positive and oxidase-positive colonies from YPM and EGM as AAB, and colonies from YGC visually confirmed under the microscope as yeast. The results were expressed in colony forming units per gramme of sample (CFU/g).

The species diversity of LAB on MRS agar, yeast on YGC agar, and AAB on YPM and EGM agar was investigated using a qualitative approach. One to ten representative colonies that had previously been confirmed as LAB, yeast, or AAB, respectively, as described above, were selected due to their morphology from an appropriate dilution of each medium. The isolates were purified on the respective agar media, produced without supplements of penicillin and

cycloheximide, and were transferred to 1-ml slant agar of the same medium and transported on ice to Switzerland.

Identification of isolates using MALDI-TOF MS

All isolates were identified with MALDI-TOF MS preparing and measuring the samples as described by Miescher Schwenninger et al. (2016).

Measurement of pulp pH, pulp content, and temperature

The pH of the pulp was measured daily during all fermentations and throughout the drying process following the LS-F by single determinations, except on days 1, 3, and 5 during the LS-F when pulp pH was measured in triplicate. An indirect method was applied, mixing 10 g of fresh sample during the LS-F and 20 g of fresh sample during the OF-F with the same amount of dH_2O in a stomacher bag and manually kneading the mix for 2 min. The pH was measured in the pulp-water mixture with a pH meter (VWR, Pennsylvania, USA).

The content of pulp in the fermentation mass was determined by weighing ten wet cocoa beans before and after the pulp was removed by rubbing the beans with sawdust and tissue paper. Measurements were performed daily as single determinations during the LS-F and in triplicate during the OF-F.

To measure temperature during the LS-F, a digital temperature sensor (DS18B20, Maxim Integrated, CA, USA) connected to a data logger developed for this study was placed in one plastic pot of every replicate fermentation and temperature was recorded every 15 min. During the OF-F, the temperature of the fermenting mass was measured with a thermocouple (Thomas Scientific, Swedesboro, USA) every 1-6 h at the nine positions in the box that correspond to the sampling points.

Determination of sugars, organic acids, and ethanol using HPLC-RI

Carbohydrates, organic acids, and ethanol were extracted as described by Camu et al. (2007) with minor modifications and determined by analytical high-performance liquid chromatography with refractive index detector (HPLC-RI). Aqueous extracts were prepared from frozen samples of fermenting and dried cocoa beans. In the case of the fermentation samples, the pulp was separated from the cotyledons by manual peeling of the beans with a blade, resulting in two fractions. Twenty grammes of each fraction was mixed with 80 ml of Milli-Q water. For the homogenization of the cotyledons, a hand blender (Bamix AG, Mettlen, Switzerland) was applied for 1 min and the pulp was homogenized with a polytron (Kinematica AG, Lucerne, Switzerland) for 3 min. Dried beans were mixed with Milli-Q water including the shells. The homogenate was centrifuged at 14,000×*g* at 4 °C for 15 min and the supernatant was put aside. The sediment was washed with 20 ml of Milli-Q water and centrifuged as described above. The resulting supernatant was combined with the supernatant from the first washing step, diluted 1:1 with Milli-Q water, filtered with a 0.45-μm filter (Sartorius AG, Goettingen, Germany), and stored at − 20 °C until further analysis. Aqueous extracts were analysed by HPLC (Hitachi, Merck KGaA, Darmstadt, Germany) using an Aminex HPX-87H column (7.8 mm i.d. × 300 mm, 5 μm; BioRad, Rheinach, Switzerland) with a Cation-H guard cartridge (4.6 mm i.d. × 30 mm, 5 μm; BioRad). The mobile phase was 10 mM sulphuric acid (Fluka, Buchs, Switzerland) in Milli-Q water at a flow rate of 0.4 ml/min at 40 °C. Glucose, fructose, sucrose, citric acid, lactic acid, acetic acid, ethanol, mannitol, and gluconic acid were determined with a refractive index detector.

Determination of alkaloids and polyphenols using LC-DAD-ESI/MS

The contents of the alkaloids theobromine and caffeine and of the polyphenols epicatechin, proanthocyanidin B2, proanthocyanidin C1, cinnamtannin A2, cyanidin-3-*O*-arabinoside, and quercetin-3-*O*-arabinoside (Extrasynthèse S.A., Lyon, France) were analysed using liquid chromatography coupled to diode array detection and electrospray ionization spectrometry (LC-DAD-ESI/MS) according to Pedan et al. (2016). Samples collected after 0, 48, and 120 h of the LS-F and dried bean samples resulting from both the LS-F and the OF-F were analysed.

Cut test and sensory analysis of dried cocoa beans

A cut test was performed by trained staff of the FHIA, cutting 50 dried beans from each LS-F and 300 beans from each OF-F lengthwise through the middle to expose the maximum cut surface of the cotyledons. Both halves were examined in full daylight and placed in one of the following categories: brown (well-fermented), slightly violet (partly fermented), violet (under-fermented), over-fermented, internal mould, slaty, and other defects, i.e. insect damaged, germinated, flat, twins, or others.

After storing dried beans for 3–4 months at room temperature, their sensory profile was evaluated in Switzerland in Mai 2016 for the LS-F and in January 2014 for the OF-F. Therefore, a composite sample from three technical replicate LS-F, consisting of equal amounts of fermented dried beans, and dried bean samples from each OF-F were processed to cocoa liquor in the pilot plant of the company Chocolats Halba (Wallisellen, Switzerland). The beans were roasted for 50 min in a pilot roaster using air temperatures between 100 and

146 °C. After de-shelling by breaking and winnowing, 200 grammes of nibs were ground into cocoa liquor in a thermomixer (Vorwerk & Co. KG, Wuppertal, Germany). The cocoa mass was mixed with 20% (v/v) powdered sucrose and processed again in the thermomixer. After a final refining step with a three-roll mill (Bühler AG, Uzwil, Switzerland) to reduce particle size, the resulting cocoa liquor was stored at 45 °C until sensorial evaluation. The tasting of the cocoa liquor was performed by a trained panel of Chocolats Halba, with four of seven members of the panel being the same in both tastings. Characteristic flavour notes of the samples were recorded and discussed during the sessions. The flavour descriptors were cocoa flavour, roast intensity, acidity, fine flavour, i.e. fruity, berry-like, dried fruit, and floral, brown flavours, i.e. aromatic, woody, earthy, and roasty, bitterness, astringency, characteristic off-flavours, i.e. musty, fusty, mouldy, earthy, and fungous, and uncharacteristic off-flavours, i.e. chemical, medicinal, smoky, animal, ham-like, and bacon-like, and were expressed as values between 0 and 10 with half points possible. A maximum of two cocoa liquor samples were evaluated during each session to reduce perception fatigue. Water and pieces of white bread were used to neutralize the mouth between different samples.

Statistics

Unless otherwise stated, mean values were calculated from the three technical replicates of the LS-F and from the three biological replicates of the OF-F. When applicable, the standard deviation is depicted as an error bar. Values under the detection limit were not included to calculate mean values, and—in these cases, when less than three data points were used for the calculation of the mean value—no standard deviation is shown.

Results

Pulp content, pulp pH, and temperature of the fermentation mass

The content of pulp amounted for 37.8 ± 1.9 and 38.8 ± 2.7% of the pulp-bean mass at the beginning of the LS-F and the OF-F, respectively (Fig. 1). The pulp was degraded more rapidly during the LS-F, resulting in a lower final pulp content on day 5 with 10.9 ± 3.3% than in the OF-F with 19.9 ± 1.7%.

The initial pH of fresh pulp was comparable in both types of fermentations, with 3.8 ± 0.04 measured in the three technical replicates of the LS-F and 3.9 ± 0.03 in the three biological replicates of the OF-F (Fig. 2). The pulp pH increased steadily to a final level of 4.9 ± 0.06 on day 5 of the LS-F. A comparable trend was observed in the OF-F, but resulting in a lower final pH of 4.4 ± 0.02 on day 5. During the drying stage following the LS-F, the pulp pH increased to a maximum of

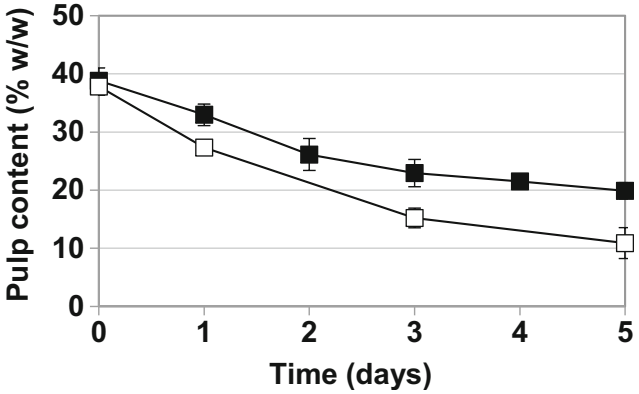

Fig. 1 Pulp content (%, *w*/*w*) during the LS-F (empty square) and the OF-F (filled square) carried out in Honduras

7.2 ± 0.55 on day 7, before stabilising between 6.1 and 6.3 between day 8 and the end of the drying process. The pH of the cotyledons was similar in dried beans resulting from both processes with 5.4 ± 0.12 after the LS-F and 5.5 ± 0.13 after the OF-F (data not shown).

The temperature of the fermentation mass during the LS-F and the OF-F is shown in Fig. 2, for the latter of which the temperatures of one fermentation are shown, representative for the three fermentations analysed. An increase in temperature was observed right from the beginning of the LS-F to 34 °C after 23 h, whereas at the onset of the OF-F, temperature increased slightly to 30 °C after 23 h. After a steady increase in both fermentations, maximal temperatures were reached at 90 h of the LS-F with 46 °C and at 71 h of the OF-F with 45 °C. An influence from daily turning of the pulp-bean mass was observed in both fermentations, in most of the cases causing the pulp-bean-mass temperature to drop temporarily.

Microbial counts and composition of the microbiota

Total aerobic germs during the LS-F followed the same trend as in the OF-F, but with 1.0–1.6 log CFU/g lower cell counts (Fig. 3a, b). Maximal values were reached on day 1 with 7.4 ± 0.38 log CFU/g in the LS-F and 8.4 ± 0.10 log CFU/g in the OF-F. Total aerobic counts decreased to 6.0 ± 1.30 and 7.2 ±

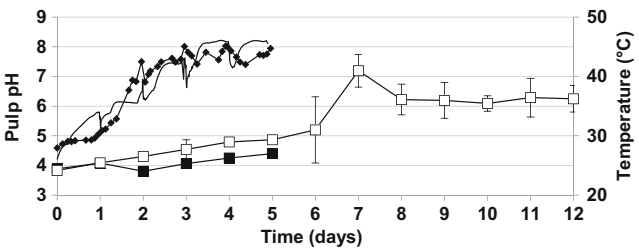

Fig. 2 Pulp pH (left axis) during the LS-F (empty square) and the OF-F (filled square) carried out in Honduras. Temperature (right axis) in the pulp-bean mass of the LS-F (continuous line) and of one OF-F (filled diamond), representative for one of the three OF-F analysed

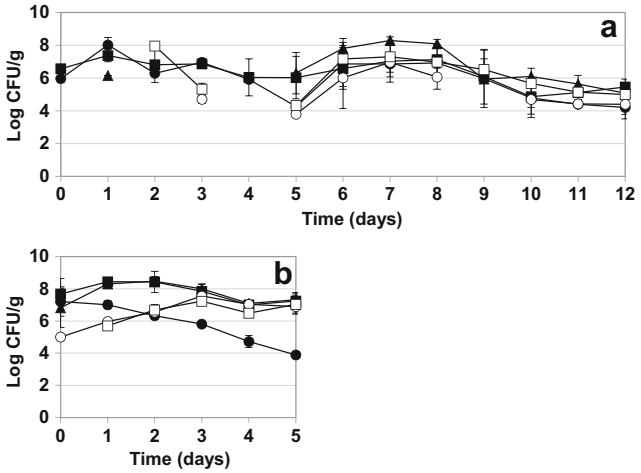

Fig. 3 Plate counts (log CFU/g) of total aerobic bacteria (PCA, filled square), yeast (YGC, filled circle), LAB (MRS, filled triangle), and AAB (EGM, empty square; YPM, empty circle) during the LS-F and the subsequent drying process (**a**) and the OF-F (**b**) carried out in Honduras

0.44 log CFU/g on day 5 of the LS-F and the OF-F, respectively. Maximal yeast cell concentrations were found on day 1 of the LS-F with 8.0 ± 0.46 log CFU/g and on day 0 of the OF-F with 7.2 ± 0.91 log CFU/g. The yeast counts decreased towards the end of both processes, reaching comparable values of 4.3 log CFU/g in the LS-F and 3.9 ± 0.03 log CFU/g in the OF-F on day 5. Due to problems with anaerobic incubation and yeast overgrowing MRS agar plates, LAB during the LS-F were only detected on day 1 with 6.1 log CFU/g and on day 5 with 6.3 ± 1.27 log CFU/g (Fig. 3a). In the OF-F, LAB were counted with 6.8 ± 1.22 log CFU/g on day 0, increased to a maximum of 8.5 ± 0.30 log CFU/g on day 2 and reached a final value of 7.3 ± 0.46 log CFU/g on day 5 (Fig. 3b). Cell counts determined for AAB were comparable on both media applied in parallel, YPM and EGM. AAB were only detected on days 2, 3 and 5 of the LS-F at concentrations between 3.8 and 8.0 ± 0.18 log CFU/g. In the OF-F, AAB on YPM were present on the fresh beans on day 0 at a concentration of 5.0 log CFU/g and increased to a maximum of 7.6 ± 0.14 log CFU/g on day 3. The final values of 7.0 ± 0.53 log CFU/g on EGM and 6.9 ± 0.47 log CFU/g on YPM on day 5 were 3 log higher than in the LS-F.

During the LS drying process, microbial counts were in a narrow range (Fig. 3a). On the first drying day (day 6), counts increased on all culture media to 6.6–7.8 log CFU/g and remained more or less stable until day 8. Towards the end of the drying process, bacterial and fungal counts decreased to final levels between 4.6 and 5.5 log CFU/g on day 12. Similar trends of LAB and yeast counts were observed for OF drying processes, when analysing yeast counts in one of the three replicate OF drying processes and LAB counts in a Honduran drying process that was performed in the same way a year later: Yeast and LAB counts increased to maximal values of 7.2 and 9.0 log CFU/g after 3 to 5 days and decreased towards the end of drying to final levels of 6.7 and 8.0 log CFU/g, respectively (data not shown).

To analyse the microbial flora of both types of fermentation, representative microorganisms were isolated from one of three technical replicate LS-F and from all three biological replicates of the OF-F (Table 1). The cultivation of microorganisms resulted in a total of 708 isolates, of which 428 were identified with a MALDI score of 1.700 or higher: 177 out of 238 isolates from YGC agar, 135 out of 241 isolates from MRS agar, 98 out of 119 isolates from EGM agar, and 79 out of 110 isolates from YPM agar.

In terms of yeast species, the genus *Hanseniaspora* dominated the beginning of both types of fermentation. Until day 2 of the LS-F, *Hanseniaspora opuntiae* and *Hanseniaspora guilliermondii* were the dominant species, while *H. opuntiae* prevailed until day 1 of the OF-F. On day 3 of the LS-F, a shift of the dominant yeast species towards *Pichia kudriavzevii* was observed. With a total of 68 isolates, *P. kudriavzevii* was also the only yeast species isolated during the LS drying process. In contrast, *Saccharomyces cerevisiae* was the dominant yeast species from day 2 to 4 during the OF-F and the OF drying process was dominated by *Trichosporon asahii* and *P. kudriavzevii*.

Table 1 shows that only few LAB isolates were identified during the LS-F and *Lactobacillus cacaonum* and *Lactobacillus plantarum* were the dominant species until day 1. In contrast, the OF-F were dominated until day 3 by isolates identified as *Lactobacillus* sp. *Pediococcus acidilactici* was the dominant species at day 5 of the LS-F, while *Lactobacillus amylovorus* was isolated at day 5 of the OF-F. Represented by a total of 51 isolates, *P. acidilactici* was the only species identified during the drying phase at LS. Similarly, the dominant LAB species of the OF drying process was *P. acidilactici* (24 isolates), but additionally, *Lb.* sp. (4), *Lb. amylovorus* (4), *Lactobacillus fermentum* (4), *Lactobacillus pentosus* (2), and *Lb. plantarum* (6) occurred.

A comparable species diversity of acetic acid bacteria (AAB) was retrieved from both growth media, EGM and YPM (Table 1). Only few isolates of AAB could be isolated from the LS-F: seven isolates of *Acetobacter senegalensis*, two of *Acetobacter pomorum* and one representative of each *Acetobacter calcoaceticus*, *Acetobacter ghanensis,* and *Acetobacter pasteurianus*. During the OF-F, most of the AAB isolates originated from day 1 to 5. *A. pasteurianus* was isolated at each day, *Acetobacter fabarum* prevailed until day 2, *A. ghanensis* until day 3, and *A. senegalensis* appeared from day 2 onwards and was the dominant AAB species on days 4 and 5. In the LS drying process, *A. senegalensis* was the dominant species isolated from both EGM and YPM, whereas EGM further revealed *A. pomorum* and *A. pasteurianus* as prevailing AAB species.

Table 1 Microbial species isolated during one of three technical replicate LS-F and the subsequent drying process and during each of the three biological replicate OF-F. Yeast and LAB species listed for the OF drying process were isolated from one of the three OF drying processes and from another Honduran OF drying process, respectively, performed and analysed in the same way a year later

	Time (days)																									
	LS-F						LS drying							OF-F						OF drying						
Species	0	1	2	3	4	5	6	7	8	9	10	11	12	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Yeasts																											
Candida colliculosa	–	–	–	–	–	–	–	–	–	–	–	–	–	1 (2)	–	–	–	–	–	–	–	–	–	–	n.d.	n.d.	–
Saccharomyces cerevisiae	–	–	–	–	–	–	–	–	–	–	–	–	–	–	(1)	(3)	(3)	2	–	–	–	–	–	–	n.d.	n.d.	–
Hanseniaspora guilliermondii	2 (2)	3 (1)	(3)	–	–	–	–	–	–	–	–	–	–	1 (1)	–	–	–	–	–	–	–	–	–	–	n.d.	n.d.	–
Hanseniaspora opuntiae	*1* 4 (3)	4 (2)	2 (4)	(1)	–	–	–	–	–	–	–	–	–	7 (2)	9 (3)	–	–	–	(1)	–	–	–	–	–	n.d.	n.d.	–
Pichia kudriavzevii	–	–	*1*	*3* 4	*7* 1	*3* 2	*8* 2	*8* 2	*9* 1	*9* 1	*9*	*10*	*6* 3	–	1	–	–	–	1	–	1	1	*1*	*1*	n.d.	n.d.	1
Trichosporon asahii	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	1	*2*	*2*	3	n.d.	n.d.	*12*
Lactic acid bacteria																											
Fructobacillus pseudoficulneus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–
Lactobacillus sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	1 (3)	3 (6)	2 (7)	2 (1)	–	–	–	1	2	–	1	–	–	–
Lactobacillus amylovorus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*2*	3	–	–	–	–	–	–	*1*
Lactobacillus cacaonum	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lactobacillus fermentum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	(1)	–	–	–	–	1 (1)	(1)	–	1	–	–	–
Lactobacillus pentosus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*1*	–	–	–	–	–
Lactobacillus plantarum	1	1 (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	(1)	–	–	–	–	1	–	2	*1*	1	*1*	–
Leuconostoc pseudomesenteroides	–	(1)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pediococcus acidilactici	–	–	–	–	–	1 (4)	(7)	1 (7)	(4)	(7)	(9)	(9)	2 (5)	–	–	–	–	–	–	(1)	(2)	–	(1)	2 (2)	1 (4)	(3)	3 (5)
Weissella ghanensis	–	–	–	–	–	–	–	–	–	–	–	–	–	*1*1	–	–	–	–	–	–	–	–	–	–	–	–	–
Acetic acid bacteria (EGM)																											
Acetobacter calcoaceticus	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetobacter fabarum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*3*	*2*	–	–	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetobacter ghanensis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	*5*	*3*	–	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetobacter orleanensis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	(1)	–	–	–	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetobacter pasteurianus	–	–	–	–	–	(1)	(1)	(1)	(2)	–	(2)	(1)	(1)	–	–	*1*1	5 (3)	*1* 5 (2)	3 (1)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetobacter pomorum	–	–	(1)	1	–	–	–	(1)		2	–	–	2 (1)	–	–	–	1	(1)	2 (3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetobacter senegalensis	–	1	–	1 (1)	–	1	1	1 (1)	1 (2)	1 (2)	(1)	3 (1)	(1)			2	*1* 1 (1)	3 (3)	1 (5)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetic acid bacteria (YPM)																											
Acetobacter cerevisiae	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetobacter fabarum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*2*	*3*	–	–	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetobacter ghanensis	–	–	–	*1*	–	–	–	–	–	–	–	–	–	–	–	*6*	*1*	–	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetobacter pasteurianus	–	–	–	–	–	–	–	–	–	–	–	–	–	*1*	(1)	2 (1)	*2* 3 (2)	4 (1)	(1)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetobacter senegalensis	–	–	–	(2)	–	(1)	(1)	2 (2)	1	–	2	1 (1)	1 (2)	–	–	1	2 (4)	4 (3)	7 (7)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gluconobacter oxydans	–	–	–	–	–	–	–	–	–	–	–	–	–	–	(2)	–	–	–	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

All isolates were identified by MALDI-TOF MS and are listed according to their identification as italicized numbers (MALDI score ≥ 2.300; highly probable species identification), regular numbers (MALDI score 2.000–2.299; secure genus identification, probable species identification) and numbers in brackets (MALDI score 1.700–1.999; probable genus identification)

– no isolates, *n.d.* not determined

Substrates, metabolites, and polyphenols during fermentation and in dried beans

The main substrates and metabolites of yeast, lactic acid bacteria, and acetic acid bacteria fermentation were analysed during the LS-F and the OF-F in the pulp and the cotyledon of the fermenting pulp-bean mass (Fig. 4). Glucose and fructose were present at high levels between 44.0 and 58.5 mg/g in the fresh pulp in both types of fermentation and were mostly consumed in the first 2 days (Fig. 4 (a1, b1)). The levels of sucrose and citric acid, initially at 0.6 ± 0.1 and 5.2 mg/g in the LS-F and at 1.3 g/kg and 3.9 ± 0.7 mg/g in the OF-F, respectively, remained low until day 5. With concentrations of 16.3 ± 1.5 and 15.9 ± 4.9 mg/g on day 0 in the LS-F and the OF-F, respectively, sucrose was the major carbohydrate in the cotyledons of fresh beans besides glucose and fructose, which were present in lower concentrations (Fig. 4 (a2, b2)). Sucrose was hydrolysed by 82% during the LS-F and by 61% during the OF-F, whereas only small changes were noticed in the concentrations of glucose and fructose. Citric acid concentrations in the cotyledons decreased from initial levels of 5.1–6.6 mg/g to 1.9 mg/g on day 5 in both types of fermentation. Ethanol was produced in the pulp and reached maximal concentrations of 18.0 ± 1.0 mg/g on day 2 of the LS-F and of 21.9 ± 4.3 mg/g on day 1 of the OF-F (Fig. 4 (a3, b3)). In the LS-F, ethanol was reduced by 50% from day 2 to 5, whereas in the OF-F, it was completely degraded and/or had evaporated by day 5. The higher production and degradation of ethanol in

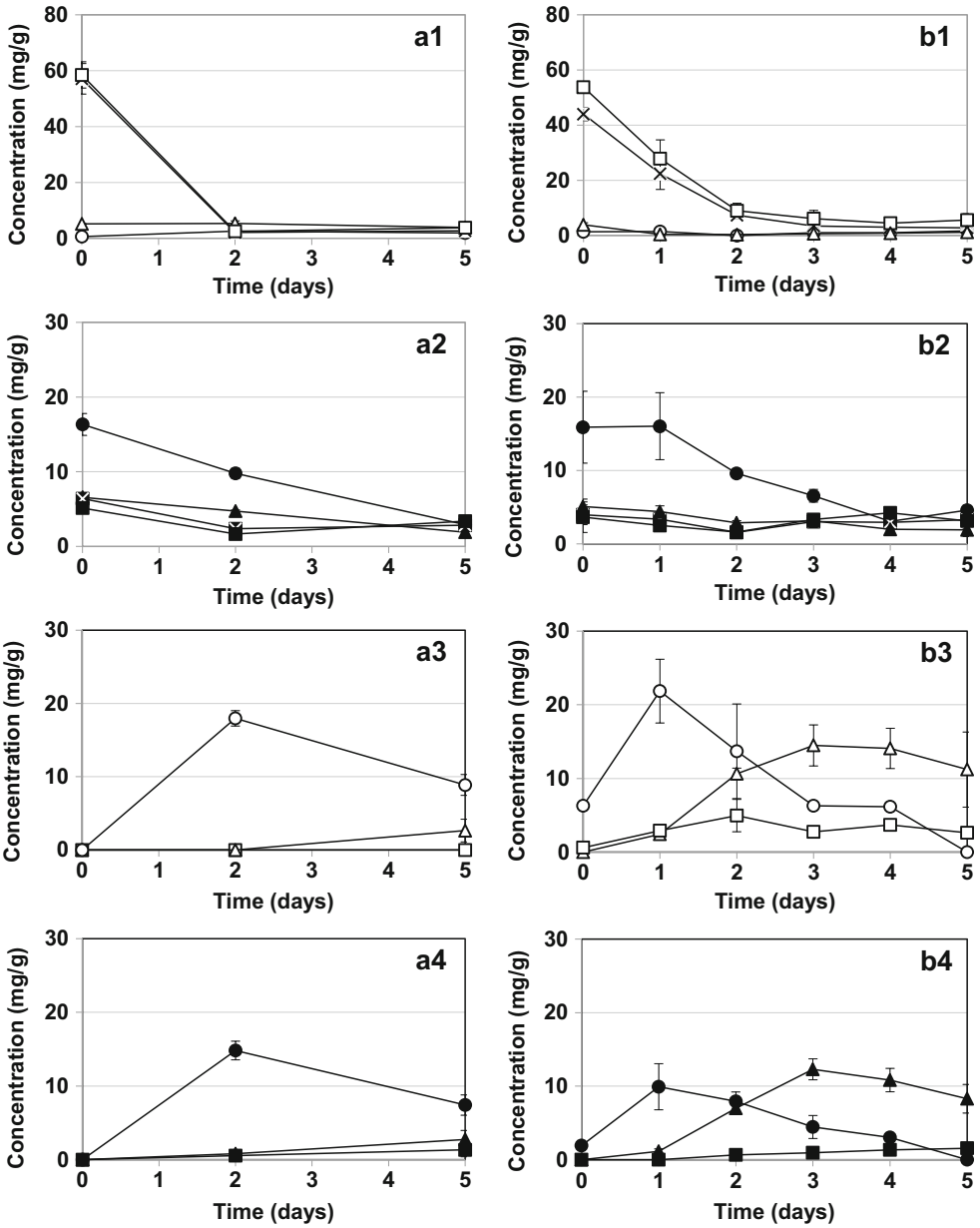

Fig. 4 Substrates and metabolites during the LS-F (**a**) and the OF-F (**b**) carried out in Honduras. Concentrations of sucrose (empty circle), glucose (multiplication sign), fructose (empty square) and citric acid (empty triangle) in the pulp during the LS-F (a1) and the OF-F (b1) and concentrations of sucrose (filled circle), glucose (multiplication sign), fructose (filled square), and citric acid (filled triangle) in the cotyledon during the LS-F (a2) and the OF-F (b2). Concentrations of ethanol (empty circle), acetic acid (empty triangle), and lactic acid (empty square) in the pulp during the LS-F (a3) and the OF-F (b3) and concentrations of ethanol (filled circle), acetic acid (filled triangle), and lactic acid (filled square) in the cotyledon during the LS-F (a4) and the OF-F (b4)

the OF-F resulted in a higher amount of acetic acid in the pulp, with a maximum of 14.5 ± 2.8 mg/g on day 3 compared to a maximum of 2.7 ± 1.5 mg/g of acetic acid measured on day 5 of the LS-F. Moreover, lactic acid was not detected in the LS-F, in contrast to a concentration of up to 5.0 ± 2.2 mg/g on day 2 in the OF-F. During both types of fermentation, ethanol, lactic acid, and acetic acid diffused into the cotyledons and the concentrations of these metabolites in the cotyledons followed the trend of the respective concentrations in the pulp (Fig. 4 (a4, b4)). During the OF-F, acetic acid levels in the cotyledons were almost as high as in pulp. Lactic acid, in contrast, reached lower levels in the cotyledon compared to the pulp, indicating a slower diffusion from the pulp into the cotyledons.

Beans fermented and dried at LS contained levels of residual sugars which were up to four times higher, with 6.2 mg/g sucrose, 3.3 ± 0.2 mg/g glucose, and 5.7 ± 1.6 mg/g fructose than in OF processed beans with 2.9 ± 0.8 mg/g of sucrose, 0.8 ± 0.3 mg/g of glucose, and 4.6 ± 0.5 mg/g of fructose (Fig. 5). The concentrations of citric acid were comparable in both processes, with 4.2 ± 1.1 mg/g in beans fermented and dried at LS and 4.7 ± 0.5 mg/g in beans fermented and dried OF. Mannitol was only detected in dried beans resulting from the OF-F with 1.9 ± 1.2 mg/g. The amount of lactic acid was almost six times lower in beans fermented and dried at LS with 0.7 mg/g than in beans fermented and dried OF with 4.1 ± 0.9 mg/g. Acetic acid was measured with concentrations of 3.8 ± 1.9 mg/g in dried beans processed at LS and 4.4 ± 1.6 mg/g in dried beans processed OF. Ethanol was only detected in beans from one of three LS processes, with 1.0 mg/g. No gluconic acid was measured in the dried beans of neither of the processes (data not shown).

Except for an increase of the higher molecular weight polyphenols proanthocyanidin B2, proanthocyanidin C1, and cinnamtannin A2 from day 0 to day 2, polyphenols decreased throughout the LS post-harvest processing from day 0 to 5 by rates ranging from 20% for quercetin-3-*O*-arabinoside to 72% for cinnamtannin A2 (Fig. 6). Cyanidin-3-*O*-arabinoside decreased from day 0 to 2 and was under the detection limit from day 5 on of the LS-F. In contrast, the alkaloids theobromine and caffeine remained more or less stable during the fermentation and drying at LS. In comparison to the LS-F, OF processed dry cocoa beans were between 3 times for cinnamtannin A2 and 12 times for epicatechin higher in polyphenols, except for cyanidin-3-*O*-arabinoside, which was under the limit of detection and quercetin-3-*O*-arabinoside, which was similar for both types of post-harvest processes. The alkaloids seemed not to be affected by the fermentation and concentrations were in a comparable range of 15.7 ± 1.24 and 15.5 ± 0.87 mg/g for theobromine and 2.7 ± 0.08 and 3.7 ± 0.23 mg/g for caffeine in dried beans from the LS-F and the OF-F, respectively. As cotyledon samples from the OF-F were not stabilized with calcium chloride, no results can be shown for day 0 to day 5.

Quality of dried beans

Cut tests with fermented and dried beans revealed comparable percentages of well-fermented beans in both processes, with 81 ± 11% after the LS-F and 76 ± 8% after the OF-F (Fig. 7). A lower share of slightly violet beans resulted from beans fermented and dried at LS with 3% than in beans fermented and dried OF with 13 ± 7%, while the share of violet beans was comparable for the LS-F and the OF-F with 6 ± 0% and 7 ± 3%, respectively. With 7 ± 5% and 1%, higher percentages of beans were rated as over fermented and slaty, respectively, after the LS-F, whereas neither over-fermented nor slaty beans were found after the OF-F.

The flavour profile showed only slight differences between the two fermentation systems, with maximum 1 unit difference, except for off-flavours, which were rated 2 units higher in LS fermented beans (Fig. 8). Beans fermented and dried at LS were similar to beans fermented and dried OF in regards to cocoa notes and roast intensity, slightly lower in acidity, bitterness and astringency, and higher in brown flavours and

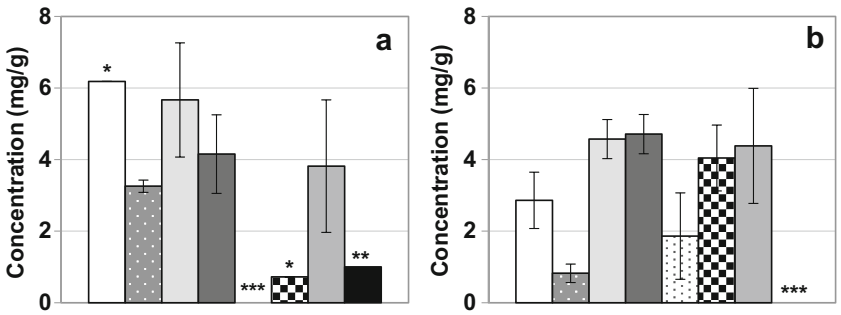

Fig. 5 Residual levels of substrates and metabolites in dried beans resulting from the LS-F (**a**) and the OF-F (**b**) carried out in Honduras. Concentrations are indicated in milligrammes per gramme for sucrose (white), glucose (white dots on grey background), fructose (light grey), citric acid (dark grey), mannitol (grey dots on white background), lactic acid (chequered), acetic acid (mid grey), and ethanol (black). Single asterisk, one out of three values under the detection limit; double asterisks, two out of three values under the detection limit; triple asterisks, three out of three values under the detection limit

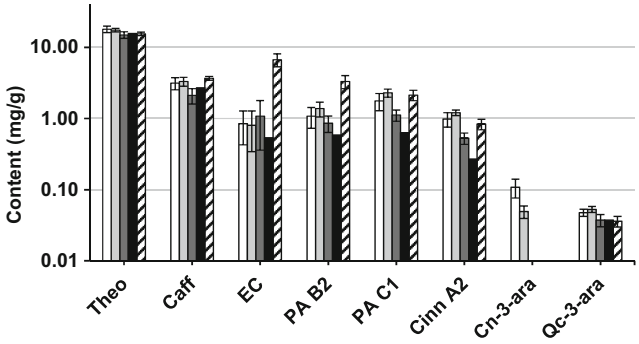

Fig. 6 Alkaloid and polyphenol contents during the LS-F and in dried beans resulting from the LS-F and the OF-F carried out in Honduras. Values are expressed in milligrammes per gramme of the non-fat dry matter of cotyledons as detected at 280 nm using LC-DAD-ESI/MS during the LS-F on day 0 (white), day 2 (light grey), and day 5 (dark grey) and in dried beans from the LS-F (black) and the OF-F (black hatched). Values are displayed on a logarithmic scale as concentrations of Cn-3-ara and Qc-3-ara are close to the detection limit. Theo theobromine, Caff caffeine, EC epicatechin, PA B2 proanthocyanidin B2, PA C1 proanthocyanidin C1, Cinn A2 cinnamtannin A2, Cn-3-ara cyanidin-3-*O*-arabinoside, Qc-3-ara quercetin-3-*O*-arabinoside

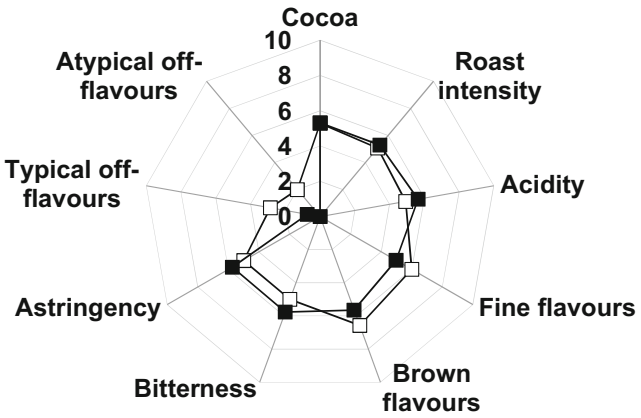

Fig. 8 Flavour profile of cocoa liquors produced with beans from the LS-F (empty square) and the OF-F (filled square) carried out in Honduras. Cocoa liquors were prepared with a composite sample of dried beans from the three technical replicate LS-F and with dried bean samples from each of the three biological replicate OF-F

typical and atypical off-flavours. The off-flavours in LS processed beans were described as earthy, musty, mouldy, medicinal, chemical, animal, ham-like, and smoky (attributes mentioned at least by two out of seven panellists; data not shown).

Discussion

The cocoa bean fermentation is a spontaneous process and the quality of the fermented dried cocoa beans and, hence, the chocolate produced thereof, largely depends on agricultural and farm practices, cultivars, and environmental conditions (Lefeber et al. 2011; Kadow et al. 2013; De Vuyst and Weckx 2016). Model systems are a helpful tool to research the influence of these factors on process parameters and the final product quality. In the present study, a lab-scale model system was developed based on a farm-scale fermentation process and the results of our multiphasic approach show the

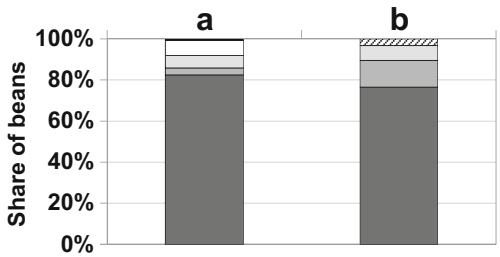

Fig. 7 Cut test performed with 50 dried beans from the LS-F (**a**) and with 300 dried beans from the OF-F (**b**) carried out in Honduras. The beans were classified as fully fermented (dark grey), slightly violet (mid grey), violet (light grey), over fermented (white), slaty (black), or with other defects (hatched)

successful mimicking of the spontaneous cocoa bean fermentation process in Honduras by means of the 1-kg fermentation in plastic pots followed by drying.

A succession of yeasts, LAB, and AAB took place in both the LS-F and the OF-F and was accompanied by an increase in pulp pH and temperature resulting from carbohydrate fermentation, citric acid assimilation and ethanol oxidation as previously described by Papalexandratou et al. (2011b). Yeasts were the dominant microorganisms at the onset of the fermentation processes and decreased towards the end of the LS-F and OF-F, which has been described before as a result of higher temperatures, rise in pH, and depletion of substrates (De Vuyst and Weckx 2016). In the OF-F, LAB growth was observed during the early semi-anaerobic phase as reported by Pereira et al. (2016). The lower LAB counts of the LS-F might have been caused by the higher relative surface to mass ratio of the fermentation mass and the faster decreasing pulp content, leading both to a reduced semi-anaerobic phase and, in the case of the pulp content, to a faster depletion of substrates. During the OF-F, the population of AAB was low at the beginning and increased from day 2 onwards when oxygen became more available, as earlier described by De Vuyst and Weckx (2016). The reason for a weaker growth of AAB in the LS-F is not completely clear, as oxygen and ethanol, the main substrate for energy production for AAB, were available. A possible explanation could be the low lactate concentration in the pulp, which serves AAB to form acetoin and biomass building blocks (Adler et al. 2014).

MALDI-TOF MS was used as a high-throughput method to identify representative isolates from both fermentation types at low cost as described before by Miescher Schwenninger et al. (2016). In our study in Honduras, comparable species were found independent of the size of the fermentation, i.e. the LS-F and the OF-F. This was also

described by Pereira et al. (2012), who compared a 500-g lab-scale fermentation to a 10-kg pilot-scale steel tank fermentation in Brazil. The dominant yeast species of the LS-F and the OF-F form part of the core microbial group described by Ozturk and Young (2017). *H. opuntiae* was the predominant yeast species at the beginning of both fermentations, possibly due to its good acid tolerance and its non-competitiveness with citrate- and fructose-converting LAB species and disappearing because of its low ethanol and heat tolerance in the course of the fermentations (Daniel et al. 2009; De Vuyst and Weckx 2016). In the second half of the LS-F, *H. opuntiae* was replaced by *P. kudriavzevii*, which has been described as tolerant towards ethanol, acid and heat (De Vuyst and Weckx 2016) and which persisted until the end of the drying process. In contrast, in the second half of the OF-F, it was replaced by *S. cerevisiae*, known for its rapid growth at a slightly higher pH, pectinolytic activity and ethanol and heat tolerance (De Vuyst and Weckx 2016). At the beginning of the LS-F and the OF-F, the prevailing LAB species were *Lb. plantarum*, *Leuconostoc pseudomesenteroides*, *Lb. cacaonum,* and *Fructobacillus pseudoficulneus* due to their microaerophilic, acid-tolerant, ethanol-tolerant, and fructophilic characteristics. Of the strictly heterofermentative *Lb. fermentum,* that often follow in a later stage of the fermentation and form part of the core group of microbes (De Vuyst and Weckx 2016; Ozturk and Young 2017), only one isolate at day 2 of the OF-F was identified. Possibly, this species was not identified with MALDI-TOF MS to species level and forms part of the large group of *Lactobacillus* sp. found from day 0 to day 3 of the OF-F. With *A. pasteurianus*, *A. ghanensis*, *A. senegalensis,* and *A. pomorum* as dominant AAB species of the LS-F and the OF-F, our findings agreed with data from cocoa bean fermentations in other countries (Pereira et al. 2012; De Vuyst and Weckx 2016; Miescher Schwenninger et al. 2016; Ozturk and Young 2017). To the authors' knowledge, *A. calcoaceticus*, which was isolated from the LS-F, has not previously been mentioned as a cocoa bean derived isolate.

To this day, little is known about the microbiota of drying cocoa beans and more research should be done on microbial growth during the cocoa bean drying process (Schwan and Fleet 2014). In the present study, we analysed microbial counts and species diversity during the drying process in the laboratory oven with beans resulting from the LS-F and compared these to the natural sun drying process that followed the OF-F. The studied microbial groups were present on the bean surface throughout the whole drying processes, including on finally dried beans. Contrasting with the findings of our work, other studies reported a sharp decrease in microbial populations during the drying process and ultimately only microorganisms that form spores, bacilli, and filamentous fungi survived (Schwan and Wheals 2004). For each, yeast and LAB, a shift in the microbial species diversity was observed when drying was started both at LS and OF, probably due to changing microenvironmental conditions such as the 2 units higher pulp pH, decreasing moisture content, increasing temperatures, higher availability of oxygen and, in the case of the sun drying, natural ultraviolet radiation. *P. kudriavzevii* was the predominant species on drying beans in the laboratory oven, contrasting with the OF sun drying, where the dominant yeast species was *T. asahii*. The yeast species that we isolated from drying beans in Honduras were different from the ones reported by Hamdouche et al. (2015), who found *H. opuntiae* on drying cocoa beans in the Ivory Coast. The dominant LAB species of both the LS and the OF drying processes was *P. acidilactici*, which has been isolated from fermenting cocoa beans in different parts of the world (Kostinek et al. 2008; Garcia-Armisen et al. 2010; Papalexandratou et al. 2011a; Papalexandratou et al. 2011b; Miescher Schwenninger et al. 2016), but so far not from drying beans. During the OF sun-drying process, additionally *Lb. amylovorus*, *Lb. fermentum,* and *Lb. plantarum* prevailed, the latter two species also mentioned by Hamdouche et al. (2015) as being present on sun-drying beans in the Ivory Coast. In contrast to yeast and LAB species, the species diversity of AAB did not seem to change, when beans were dried at LS, which corresponds to the finding of Hamdouche et al. (2015), who found similar AAB species in the fermentation and the drying steps. The study of AAB species during OF sun drying was not included in our study (Table 1).

The succession of microorganisms in the pulp led to a similar consumption of substrates, i.e. sucrose, glucose, fructose, and citric acid in the LS-F and the OF-F. Lower acetic acid concentrations in pulp and cotyledons during the LS-F than during the OF-F, as well as fermentations studied in other countries by Camu et al. (2007) and Lefeber et al. (2011), indicate that ethanol mainly diffused from the pulp into the cotyledons or evaporated and that only a minor part was oxidized into acetic acid. This corresponds with the 2–3 log lower AAB counts in the second part of the LS-F compared to the OF-F, as AAB use ethanol as a substrate and are considered to be responsible for most of the acetic acid production (Ho et al. 2015). The low LAB counts during the LS-F resulted in amounts of lactic acid in dried beans that were almost six times lower than during the OF-F. This, together with the low amounts of acetic acid produced, possibly led to the difference of a 0.5-units higher final pulp pH on day 5 of the LS-F. The low acid production might as well have caused the residual sucrose levels in LS dried beans to be two times higher compared to OF dried beans, as sucrose hydrolysis into glucose and fructose partly occurs as non-enzymatic reaction at acid environment according to Camu et al. (2008a).

The concentration of epicatechin measured in the cotyledons of unfermented beans at the onset of the LS-F was rather low with 0.85 ± 0.42 mg/g, compared to values reported in other works in ranges of 21.9–43.3 mg/g (Kim and Keeney

1984), 14.4–43.9 mg/g (Niemenak et al. 2006), and ± 15 mg/g (Camu et al. 2008b). Probably due to its low initial concentration, epicatechin remained more or less stable during the fermentation, contrasting with other studies, which reported either a decrease (Nazaruddin et al. 2006; Camu et al. 2008b) or a strong increase of epicatechin in the course of the fermentation (D'Souza et al. 2017). The decrease of proanthocyanidins, i.e. proanthocyanidin B2, proanthocyanidin C1, and cinnamtannin A2, during the LS-F process was in accordance with findings of other researchers and might have been caused by diffusion out of the beans and oxidation and complexation reactions (Camu et al. 2008a; Mayorga-Gross et al. 2016). Comparing the contents of proanthocyanidins of dried cocoa beans after both processes, a higher level was revealed for the OF-F. A possible reason is a lower pH of the cotyledon, indicated by the lower pulp pH measured at the end of the OF-F, as polyphenols in cocoa powder have been described to remain stable at acidic pH but decompose at alkali pH (Miller et al. 2008). Furthermore, the influence of seasonal variation might have contributed to the different polyphenolic contents, as observed by Camu et al. (2008a) in Ghana.

The quality of the dried beans resulting from both processes was assessed by a cut test, which is a traditional method to evaluate the degree of fermentation based on the evaluation of colour change inside the cotyledon (Ganeswari et al. 2015; Sandhya et al. 2016), and by a sensorial evaluation of cocoa liquors. The requirement of industry in terms of well-fermented, slightly violet and violet beans were met for both fermentations, while the amount of over-fermented beans from the LS-F was above the threshold for quality A cocoa beans (Chocolats Halba, personal communication). The high share of over-fermented and less slightly violet beans indicate that the LS-F was more advanced after 5 days of fermentation than the OF-F, which could be due to the smaller fermentation volume. Besides this, the lower bean loading in the LS drying process compared to the sun-drying process OF could also have led to the higher degree of fermentation as observed before by Hii et al. (2006). The higher off-flavours in liquors produced from LS-F beans were possibly caused by *Bacillus* spp. and filamentous fungi, which are known to appear when microbial activities last more than 4 days by producing short chain fatty acids (Schwan and Wheals 2004; Bonvehí 2005; Guehi et al. 2010; De Vuyst and Weckx 2016). By reducing the duration of fermentation, the quality of the beans might be improved, as Schwan (1998) and Guehi et al. (2010) reported less off-flavours and Senanayake et al. (1995) less over-fermented beans when the fermentation time was shorter. With regard to cocoa and fine flavours, astringency, bitterness, acidity, roast intensity, and brown flavours, the results achieved with the lab-scale model system were favourable and in accordance with the rating of OF post-harvest processed beans. The slightly lower bitter and astringent notes in beans processed at LS might have been caused by the lower amounts of polyphenols, which were found in liquors derived from the LS-F and are known to contribute to bitterness and astringency of roasted beans (Camu et al. 2008a).

When compared to cocoa bean fermentations in other countries, the Honduran box fermentation studied in the present work shows similar courses in terms of yeast, LAB, and AAB counts and species diversity, substrate degradation and metabolite production, and pH and temperature development, and the quality of resulting dried cocoa beans complied with the industrial standards of chocolate producing industry. To the best of our knowledge, we showed in our study in Honduras for the first time the comparison of a small-scale to a large-scale fermentation in a multiphasic approach, including the drying process and a quality assessment of the fermented dried beans. To conclude, it could be demonstrated that cocoa beans fermented spontaneously at LS with 1 kg of pulp-bean mass, using a defined temperature profile, showed similar total aerobic counts and comparable yeast counts, while LAB and AAB counts differed substantially. The species diversity of the LS-F and the OF-F were similar in terms of AAB, and overlapping species were found for yeast and LAB during the fermentation and drying steps. Changes in pulp contents, pH, and temperature in the LS-F and the OF-F showed comparable trends. The degradation of substrates was similar in both types of fermentations and also a similar trend for ethanol formation was seen, while the amounts of lactic acid produced differed, and even more so the amounts of acetic acid. The concentrations of substrates and metabolites in dried beans from the LS-F and the OF-F were in a comparable range for fructose, citric acid, acetic acid, and ethanol, while differences were found for sucrose, glucose, mannitol, and lactic acid. The quality of dried beans resulting from the LS-F showed a highly similar flavour profile except for stronger off-flavours. Similar shares of well-fermented and violet beans, minor differences in the amounts of slightly violet and slaty beans and major differences in the over-fermented bean shares were evidenced by a cut test. The results also suggest that microorganisms survived at the surface of the beans until the end of the drying process and that the microbial species diversity was often limited compared to the fermentation process. The findings of our analysis of the cocoa bean drying processes revealed that yeast and LAB species, such as *P. kudriavzevii*, *T. asahii* and *P. acidilactici* are typical microorganisms for the drying process. The similarities between the two fermentation processes, above all regarding the quality of dried beans, suggest that the lab-scale fermentation provides a suitable model for larger-scale fermentations and may be used in selecting plant hybrids representing different genetic material, screening and/or evaluating starter cultures under controlled and reproducible conditions or to develop tailor-made end products.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The article does not contain any studies with human participants or animals performed by any of the authors.

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