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Master Thesis

Precursors of chocolate aroma – flavour profile comparisons of traditionally fermented cocoa and cocoa beans from fermentation-like incubation by means of HS-SPME-GC-MS-O

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Abstract

Aroma precursors developed during fermentation are of indispensable importance for chocolate aroma formation. Generation of a standardised high-quality aroma profile of cocoa would be beneficial for producer and processing industry. Fermentation-like incubation could be an option for realization to obtain a standardised quality.

In the present study a HS-SPME-GC-MS method with olfactometry was applied to compare the flavour profile of cocoa beans from traditional fermentation and of fermentation-like incubation. Additionally, the flavour profile development over time of fermentation-like incubation was investigated.

In total 34 odour-active volatile compounds were identified in this study for traditionally fermented cocoa and for cocoa from fermentation-like incubation. Both cocoas of traditional fermentation and fermentation-like incubation are characterised by the same important odour notes green, fruity, cocoa/caramel and acid. Also, a difference in spectrum of components of cocoa beans from traditional fermentation and fermentation-like incubation was observed. Cocoa beans from traditional fermentation developed more components perceived as vanilla odours and one component perceived as cheesy. Cocoa beans from fermentation-like incubation developed more components which have cocoa/nutty, soapy, alcoholic odour notes and one component smelling musty, putrid. The investigation of odour-active substances over the time of fermentation-like incubation showed that this form of fermentation also yields fruity and flowery notes typical for fine flavour cocoa as well as cocoa and acid notes.

The aroma profile of cocoa from fermentation-like incubation seems due to lack of several vanilla notes less complex. However, aroma-profile of cocoa beans from fermentation-like incubation shows a higher stability as no indication of overfermentation was observed. Both characteristics minor complexity and better stability, could be explained by the absence of microorganisms during fermentation-like incubation.

1. Introduction

The worldwide highly appreciated aroma of cocoa and chocolate arises from their volatile fraction. Up to 600 volatiles could be identified in cocoa (Aprotosoaie et al. 2016; Counet et al. 2002; Ducki et al. 2008). Only some of these volatiles in cocoa are odour-active and contribute to the aroma. Genotype, climate and harvest conditions as well as post-harvest processes like fermentation, drying and roasting have important effects on the complex composition of volatile and non-volatile compounds (Brito et al. 2000; Caligiani et al. 2007; Rodriguez-Campos et al. 2011). Fermentation, a prerequisite for flavour development, is of utmost importance. During this key processing stage, the precursors of chocolate aroma are formed. Unfermented dried cocoa beans do not generate the typical chocolate flavour during roasting. They do not contain flavour precursors (Afoakwa et al. 2008; Tran et al. 2015; Ziegleder, Biehl 1988; Rohan 1964). Present aromatic compounds classify the aromatic quality of cocoa beans (Frauendorfer, Schieberle 2008). A high and consistent aromatic quality of cocoa beans is of great importance for the chocolate industry even though necessarily there is no general standard spectrum of flavour components (Panlibuton, Lusby 2006; Tran et al. 2015). In practice, post-harvest treatments are often not uniform enough to generate a standardised aroma profile. Especially scale and construction of fermentation, drying, preconditions of cocoa pulp and seeds as well as inoculum of microorganisms influence this post-harvest process (Ziegleder, Biehl 1988). To avoid off-flavour formation and to assure a constantly good aroma quality, fermentation-like incubation could be an option. It has the potential for standardisation and automation of cocoa fermentation (Kadow et al. 2015). As Biehl and Passern (1982) and Kadow et al. (2015) demonstrated, regarding aroma precursors, fermentation-like incubation can produce a raw cocoa with a high potential of chocolate aroma.

Several studies investigated volatiles of raw cocoa, however, without considering odour-activity (Ascrizzi et al. 2017; Bailey et al. 1962; Jinap et al. 1998; Oberparleiter, Ziegleder 1997; Ramos et al. 2014; Rodriguez-Campos et al. 2012; Ziegleder 1991). Only Frauendorfer and Schieberle (2008) identified, on the basis of the odour activity value, the key aroma compounds of unroasted Criollo cocoa beans. From the most odour-active volatiles the odour-activity value of 32 important components were presented. Among them acetic acid, 3-methylbutanoic acid, ethyl 2-methylbutanoate and 3-methylbutanal showed the highest odour activity value (ratio of concentration/odour threshold).

This study describes which odour-active aromas are produced by beans from fermentation-like incubation and traditionally fermented cocoa beans. For the fermentation-like incubation no microorganisms are utilized. The aim of this work is the comparison of the volatile flavour profile of cocoa from these two ways of fermentation. Thereby, odour-activity of volatiles is

analysed and used for comparison. Additionally, changes of odour-active volatiles over a seven-day fermentation-like incubation are investigated.

2. Theoretical background

2.1 Theobroma cacao L.

The botanical name of the cocoa tree is *Theobroma cacao L.*. Originally *Theobroma cacao L.* is indigenous to South America (Afoakwa 2010). Located in a climate belt approximately within 20 degrees of the equator *Theobroma cacao* is cultivated in small farms (McShea et al. 2008). There is a vast genetic diversity of *Theobroma cacao.* It is suggested that there are more than 14000 distinct varieties of the plant, recently classified into ten major clusters (McShea et al. 2008; Motamayor et al. 2008). Depending on the variety, cocoa seeds are separated into bulk cocoa and fine or flavour cocoa by traders. The presence of special aroma notes such as floral and fruity characterise the fine cocoas (Ziegleder 1990; Kadow et al. 2013). Fermented and dried seeds of the cocoa tree, in current language called beans, are designated as raw cocoa.

Preliminary stages of characteristic aroma precursors of chocolate are identified in fresh (unfermented) seeds, so in their cotyledon (Afoakwa 2010; Rohan 1964). However, fresh cocoa seeds do not produce chocolate flavour during the roasting process. The cotyledons' storage components consist of 50 % fat, 15 % phenol, 12 % protein, 5 % starch and 2 % sucrose on a dry weight base (Kadow et al. 2013; Kim, Keeney 1984; Reineccius et al. 1972; Nazaruddin et al. 2001; Reineccius et al. 1972). The aqueous pulp contains 12 % mono- and disaccharides, 2 % citric acid as well as further organic acids, esters, aldehydes, methyl ketones, secondary alcohols and terpenes (Kadow et al. 2013; Quijano, Pino 2009).

2.2 Fermentation and aroma development

Fermentation is essential to develop appropriate aroma precursors which generate chocolate flavour (Afoakwa 2010). Fermentation process starts after harvest of mature fruits when the pods are broken and seeds (beans) are removed to heaps, boxes or baskets (Afoakwa et al. 2008). Cocoa beans are made up of an embryo which is enveloped by a seed coat (testa) and a sweet, white, mucilaginous pulp (Afoakwa 2010; Schwan, Wheals 2004). An embryo is made up of two storage cotyledons and an embryonic axis (Duncan, Todd 1972). In the first phase of fermentation beans are exposed to numerous sources of microorganisms. On the sugar-rich acidic pulp and under anaerobic conditions these organisms, like yeasts and bacteria (*Bacillus* species), produce ethanol, lactic acid and acetic acid as well as heat (Afoakwa 2010; Aprotosoaie et al. 2016; Schwan, Wheals 2004). Under aerobe conditions and increasing temperature (45 -50 °C), the second phase, lactic acid formers are replaced with acetic acid-forming bacteria as dominant microflora. Both acids penetrate

into the cotyledons and acidification as well as heat lead to the death of the seed (Afoakwa 2010; Aprotosoaie et al. 2016; Schwan, Wheals 2004). This leads to enzymatic reactions and structural changes which yield flavour precursors (Afoakwa et al. 2008; Afoakwa 2010; Mohr et al. 1976; Rohan, Stewart 1967). Traditional fermentation is spontaneously initiated by microorganisms transferred to the seeds from surfaces and tools, used for cutting the fruit, workers hands and containers from fermentation (Rodriguez-Campos et al. 2012). Furthermore, temperature variation between inside and outside of heap fermentation can influence flavour development (Quesnel 1965; Wollgast, Anklam 2000). In total, scale and construction as well as the wild inoculum during traditional fermentation can result in a heterogenic cocoa bean quality (Ziegleder, Biehl 1988). Developed spore-forming bacteria during fermentation can sometimes be responsible for the off-flavour formation (Schwan, Wheals 2004).

As mentioned, during fermentation of cocoa seeds various biochemical and chemical processes inside the seed generate cocoa-specific aroma precursors (Afoakwa et al. 2008).

During fermentation the content of fructose and glucose is increased. These reducing sugars are increased in content due to inversion of sucrose. Reducing sugars decrease when seeds are dried and roasted (Brito et al. 2000). Furthermore, peptides and free amino acids are formed during the degradation of proteins. A correlation between the amount of produced free amino acids and the extent of developed aroma and flavour was found (Brito et al. 2000; Hashim et al. 1998; Rohan, Stewart 1966; Rohan 1964). However, formation of specific peptides is more important for flavour development rather than a high quantity of total peptides (Biehl et al. 1985). Efficiency and products of proteolysis, as specific peptides, are depending on different pH optima of endoprotease and carboxy- (exo)peptidase activities. Optimum for endopeptidase is pH 4,5 and pH 3,5 and 5,5 for carboxy- (exo)peptidase (Afoakwa et al. 2008; Biehl et al. 1985). A moderate acidification of the seeds during fermentation seems important for a good aromatic flavour development. As acidification is crucial for proteolysis, having microorganisms appears not essential for the formation of typical aroma precursors (Afoakwa et al. 2008; Brito et al. 2000; Voigt et al. 1994). In summary the interactions of amino acids, peptides and reducing sugars lead to the formation of amadori products and are therefore necessary for aroma formation (Mohr et al. 1976; Mohr et al. 1971). Amadori compounds and certain individual peptides are potent aroma precursors and therefore indispensable during roasting (Frauendorfer, Schieberle 2008; Mohr et al. 1976).

2.3 Gas chromatography-olfactometry

A majority of volatile compounds present in an odour can be identified by applying GC analysis (Delahunty et al. 2006). However, the aroma profile of a food is not automatically represented by the peak profile received from a GC (Blank 1997). There are indications that only a very small part of the complex mixture of volatile compounds occurring in food contribute to the odour and aroma (Blank 1997; Guth, Grosch (1999); van Ruth 2001). It is important to differentiate between the large number of volatile compounds and those which are odour-active volatiles. Not all volatiles are perceived the same. The amount of compounds released from the food matrix as well as the odour properties of the compounds influence the perception. A physical GC detector is not as representative as a human nose due to wide threshold variations of odour-active compounds (Delahunty et al. 2006; van Ruth 2001). It was already proved that many key aroma compounds appear at low concentrations (Blank 1997). Sniffing effluents from a gas chromatograph (GC-O) provides a selectivity for odour-activity. By specifying the extent, which food compounds or representative extract of food are above absolute threshold, a selectivity is given. Intensity and odour quality can also be described during GC-O (Delahunty et al. 2006; van Ruth 2001).

There are several preparation methods for a sample that is about to be analysed in a gas chromatograph. A flavour profile is closely related to the isolation procedure. The received sample should represent the product which is investigated (d'Acampora Zellner et al. 2008). Mincing, homogenisation, centrifugation, distillation and extraction are potential preparation steps which can be applied according to the product. Steam distillation (SD), solvent extraction (SE), simultaneous distillation–extraction (SDE), supercritical fluid extraction (SFE), pressurised-fluid extraction, Soxhlet extraction, solvent assisted flavour evaporation (SAFE), microwave-assisted hydrodistillation (MAHD), direct thermal desorption (DTD), headspace (HS) techniques, solid-phase microextraction (SPME), matrix solid-phase dispersion (MSPD) are some well-known sample preparation methods. Each method has its advantages and disadvantages. With widely applied SDE, for example, a representable sample can be obtained. The loss of highly volatile compounds, artifact formation and odour component degradation is an aspect that needs to be considered. SAFE works with low temperatures and high vacuum; without the risk of decomposition of labile compounds. However, both mentioned methods are complex in execution and time consuming. Furthermore, larger amounts of samples are needed for extraction. HS is often combined with SPME. Concentration step of static HS can be described by sampling of the atmosphere around the headspace of a food matrix. During SPME a coated fused silicea fibre collects the volatiles. Sampling time and temperature, type, thickness and length of fibre affect the chemical profile of collected volatiles. However, HS and SPME are solvent-free methods,

need small sample amounts and are, due to their easy utilization, very time effective. Another benefit of HS methods is the fact that the loss of most volatile compounds is very little. The loss of volatiles often has the biggest influence on the odour of a sample (d'Acampora Zellner et al. 2008; Plutowska, Wardencki 2008).

Next to various sample preparation methods, there are different GC-O related techniques that can be applied. There are three categories to collect and process olfactometric data. Dilution analysis, respectively dilution to threshold are methods that determine odour potency of a compound. It is based on the ratio of a compound's concentration to its odour threshold. Combined hedonic response measurement (Charm-Analysis) and aroma extraction dilution analysis (AEDA) are examples for dilution analysis methods. Detection frequency methods count the number of assessors detecting an odorant at a particular retention time. More frequently detected odours are concluded to have a greater relative importance. The detection frequency is used as an estimate for odour intensity. Direct intensity methods are methods where assessors use a scale to measure the perceived intensity of the eluting compound. One type of direct intensity is posterior intensity method. The odour intensity is recorded on a scale after the peak has eluted from the column. Second type is the time-intensity method, for example OSME and finger span. Trained assessors rate the intensity, record the duration and describe the perceived odour at the same time when the compounds are eluting (d'Acampora Zellner et al. 2008; Delahunty et al. 2006; van Ruth 2001).

3 Materials and methods

3.1 Cocoa samples

Raw cocoa - produced with fermentation-like incubation

A genotype mix of Trinitario cocoa (*Theobroma cacao L.*) from the Dominican Republic was used in the experiments. Fresh cocoa fruits for fermentation-like incubations were provided by Rizek Cacao. The experiment with fresh fruit material was started one week after harvest. The cocoa fruits were transported by express shipping. Unripe, damaged or moulded/brown fruits were rejected. To ensure viability, 2-3 seeds of each fruit were required to check germination capacity. Germination capacity was checked with a cut-test in order to see an intact embryo. In total 23 cocoa fruits were used.

Raw cocoa - traditionally fermented

Traditional raw cocoa beans (*Theobroma cacao L.*) were obtained from the Dominican Republic. It was also a typical mix of fine flavour cocoa of the genotype Trinitario used. Two repetitions of seven days fermented beans are investigated. Fermentation was executed in

wooden boxes. Each box contained 900 kg fresh cocoa seeds. A combined drying of sun-drying and infrared drying was applied over six days.

3.2 Fermentation-like incubation

Cocoa fruits were sterilised for 20-30 minutes in a water bath with 100 ml DanKlorix® sodium hypochlorite solution (CP GABA GmbH, Hamburg, Germany). After drying on sterile paper, fruits were sprayed three times with Sterillium® (Bode Chemie GmbH, Hamburg, Germany). Sterilised fruits were opened with sterilised instruments under a clean bench. Seeds from each fruit were distributed equally to sterile 500 ml glass bottles. As a result, a total of 110 seeds was placed in each glass bottle. An average of 200 g ± 10 g of 10 mmol*l^{-1} ethanol solution containing pectinase (Novozymes Pectinex® Ultra Tropical; Novozymes A/S, Bagsvaerd, Denmark) was added. The fermentation-like incubation was performed in total in seven days. Filled incubation glasses were aerated with nitrogen to assure anaerobic conditions for the first 24 hours. A water bath ensured a temperature of 30 °C for day one. On the second day the first incubation medium was replaced with a 75 mmol*l^{-1} acetic acid solution (average 190 g ± 20 g), temperature was adjusted to 45 °C and nitrogen was replaced by an airstream, having a constant air circulation. Microbial contamination was avoided by using sterile filter (Sartorius, Göttingen, Germany).

Sampling was always done at the same time every 24 hours under sterile conditions. Seeds were put on petri dishes to dry at 50 °C for five days in a drying cabinet to ensure dry beans without becoming moldy.

3.3 Preparation of samples for GC-MS

Around 25 cocoa seeds were peeled per sample. Whole cocoa seeds were peeled with a scalpel and stored in a closed container until the next step preventing a major loss of volatile compounds. After all seeds for one sample had been peeled, seeds were pestled to 2-7 mm small particles. Around 2 g of cocoa nibs were weighed into a 20 ml glass vial and sealed with a silicone septum. Samples were stored frozen at -20 °C until use.

3.4 HS-SPME-GC-MS-O

3.4.1 Method optimization – Application of a Design of Experiments

The volatile aroma profiles were recorded using HS-SPME-GC–MS-O. With a Design of Experiments (DoE, Design-Expert® Software Version 10) an appropriate equilibration and extraction time for the applied HS-SPME-GC-MS-O was determined. For DoE a response surface study type with central composite design was used. Limits for the experiment were geared on different studies for fermented and dried beans (Humston et al. 2009; Tran et al. 2015) and also for cocoa liquor (Counet et al. 2002; Ducki et al. 2008; Misnawi 2011; Pini

et al. 2004). Limits of equilibration time were 10 and 40 minutes, for extraction time 10 and 30 minutes. Equilibration time of 10 minutes was used to cover lower limits. More than 40 minutes of equilibration time was not investigated to limit the maximum preparation time. Most applied temperature for fermented and dried beans or for cocoa liquor is 60 °C (Ducki et al. 2008; Misnawi 2011; Pini et al. 2004; Tran et al. 2015). Furthermore, Humston et al. (2009) did a DoE and identified 60 °C as the best equilibration and extraction temperature for raw cocoa beans which is why this temperature is used in this investigation.

Two different SPME fibres were tested for extraction; 50/30 µm divinylbenzene/carboxene on polydimethylsiloxane (DVB/CAR/PDMS) and 65 µm polydimethylsiloxane-divinylbenzene (PDMS/DVB) fibre.

In total 26 runs were calculated for the DoE and executed in double determination with the described parameters. The experiments were carried out with each 2 g of traditional fermented cocoa nibs. Target was to achieve the highest average value of all peak areas. For evaluation, the response of the target size was analysed.

As result the optimal conditions (highest peak areas) for the HS-SPME were obtained. An equilibration time of 10 minutes, an extraction time of 28 minutes and use of the PDMS/DVB fibre was calculated as optimal conditions with the Design-Expert® Software.

3.4.2 Equipment and applied conditions

HS-SPME-GC–MS-O was performed with an Agilent gas chromatograph, type 6890N (Agilent Technologies, Waldbronn, Germany). The method of headspace solid phase micro extraction (HS-SPME) was applied to transfer volatile compounds to a DB-WAX column (250 µm, 25 µm film thickness and a length of 30 m; Agilent Technologies, Waldbronn, Germany). For HS-SPME prepared vials were incubated for 10 min at 60 °C in a thermostatic agitator; followed by an extraction of 28 min at 60 °C on a well-conditioned polydimethylsiloxane-divinylbenzene (PDMS/DVB) on a StableFlex fibre (Supelco, Bellefonte, PA, USA). These conditions are results of the previously run DoE. The following time–temperature programme was applied: Start with 40 °C (3 min), from 40 °C to 56 °C (3 °C/min) hold for 2 min, from 56 °C to 80 °C (15 °C/min), from 80 °C to 120 °C (6 °C/min), from 120 °C to 200 °C (10 °C/min) and from 200 °C to 250 °C (30 °C/min). Injector and transfer lines were maintained both at 270 °C. The detector temperature was kept at 230 °C. The gas chromatograph was equipped with one sniffing port (JAS - joint analytical systems GmbH, Moers, Germany) and a 5975C VL MSD with Triple-Axis Detector Mass Spectrometer (MS; Agilent Technologies, Waldbronn, Germany) in an electron ionisation (EI) mode at 70 eV (scan area: m/z range from 35 to 350 amu). At the end of the DB-WAX column the effluent was split 1:2 for MS and sniffing port. Hydrogen is used as a carrier gas (3,4 ml/min). To

prevent drying out of assessor's nose humidified nitrogen-air mixture is carried to the sniffing port. Detection of volatile compounds in the headspace was performed analytical with the MS and sensorial with olfactometry. For identification the reference mass spectral data base of the National Institute of Standards and Technology MS library searches (NIST/EPA/NIH Version 2.0 f, 2008) together with the MSD ChemStation software and Kovats retention index from literature (NIST library) was used. When available standard pure substances were used to confirm identification. The content of each compound was defined as the integrated peak area of the volatile compound from the GC-MS. The mean peak area was calculated from total contents of replicate analyses. Only odour-active compounds were used for calculation of total contents of chemical groups.

The split effluent part to the sniffing port is used to determine olfactometrically the aroma activity of individual volatile compounds.

3.4.3 Olfactometrically data acquisition

To collect and process olfactometrically (GC-O) data a combined method of detection frequency, intensity rating and description of the odour from Petersen (2013) is used. Detection frequency methods capture detected odours over a group of assessors and thereby overcome the limitations of a small number of assessors and the use of detection thresholds (Linssen et al. 1993; van Ruth 2001). In this experiment the noise level is set to 40 % of all panellists. This means, at least two out of six panellists must have a perception at the same time, so the detection can be regarded as meaningful. Due to possible variance between individual panellists the flavour score concept considers the mean value of intensity rating and frequency of detecting an odour (Petersen 2013).

flavour score (FS) = detection frequency (DF) * flavour intensity (FI) (Petersen 2013)

Identical gas chromatographic runs were carried out for each panellist (van Ruth 2001). A variable resistor was used to record a signal. Verbal odour descriptions and intensity ratings were recorded written by a second person. A 5-point scale from 1 = very weak to 5 = very intensive was used.

Selection criteria for defining meaningful odour-active compounds were:

- FS value ≥ 5 at least one out of two (50 %) traditional fermented samples or/and
- FS value ≥ 5 of least 75 % of fermentation-like incubation samples or/and
- FS value ≥ 5 of four consecutive samples from fermentation-like incubation or/and
- FS values ≥ 5 for unfermented beans and beans from first incubation day.

3.5 Panel

Sensory evaluation of the samples was carried out by twelve trained panellists, eight females and four males. Panellists were trained at least once or twice a week for five months. Training was carried out with standard substances on paper smelling stripes. Substances for training (in total sixteen) were selected after first screenings of samples and included at least one substances of each chemical group that can be found in cocoa nib samples. Training included simple descriptive, odour recognition, memory, pair comparison, ranking of intensities, detection and triangle tests as well as test sniffing sessions to get used to sniff at the sniffing port.

Each sample was sniffed six times by panellists. Sniffing time of each sample was split in two halves. No panellist had to sniff the same half of the sample twice. It was tried to have each panellist sniff each sample once; at least one half of the sample.

3.6 Statistic

Two-way analysis' of variance (ANOVA) and multiple range tests were used to determine effects and interactions of factors. As multiple range test, a Tukey multiple comparison at 95 % significance level, was implemented to detect differences between factor levels. The multivariate technique principle component analysis (PCA) was performed to reduce the dimensionality of the data sets from GC-MS and GC-O. Based on similarities and differences, data sets can be compressed without much loss of information. A new ordered data set of variables was converted which are the principle components or dimensions (Jolliffe 2002; Rodriguez-Campos et al. 2011). Another multiple range test, a multiple factor analysis (MFA), was applied. Both data sets are based on the same samples. Therefore, a MFA can be applied to evaluate the relationships between flavour score obtained by GCO and the content (peak area) obtained by GC-MS of selected odour-active compounds.

All treatments of fermentation-like incubation (e.g. IC2-Rep1 and IC2-Rep2) were done in duplicate. All analysis' (e.g. peak area/content) were repeated at least six times. The results of the content of chemical compounds is therefore an average value. Furthermore, the mean values of repetition one and two are reported, unless otherwise indicated.

4 Results

4.1 Volatile odour-active compounds produced during fermentation-like incubation and traditionally fermentation

In cocoa beans of fermentation-like incubation and traditionally fermentation 34 odour-active compounds were identified, respectively. They are shown sorted by chemical groups in table 1. Two identified aldehydes were 2-methylpropanal and 3-methylbutanal. From eleven

identified odour-active alcohols two were amyl alcohols 3-methyl-1-butanol and 2-pentanol and the others were ethanol, 2-methyl-3-buten-2-ol, 2-heptanol, 1,2-oxolinalool, 2-nonanol, 1,2-dihydrolinalool, phenylmethanol, 2-phenylethanol and 2-[(Z)-octadec-9-enoxy]ethanol. Some of the amyl alcohols are used to assess fermentation degree and flavour of cocoa (Oberparleiter, Ziegleder 1997). Five pyrazines and pyrroles and five esters were identified in this study (table 1). Three ketones and in each case one compound of phenols, furans, alkanes and alkenes were detected (table 1).

The following components were also identified in this investigation: 1-phenylethanone, 2-pentanol acetate, ethyl acetate, 2-phenylethyl ester acetic acid, benzaldehyde. However, the components did not match the selection criteria for meaningful odour-active (see explanation in chapter 4.4.3) substances or the allocation to other components was more appropriate. Therefore, they were not further investigated. Benzaldehyde, for example, was detected in all samples. The peak of benzaldehyde elutes shortly before the 3,5-diethyl-2-methylpyrazine peak. 3,5-Diethyl-2-methylpyrazine was not detectable in samples from fermentation-like incubation but in samples from traditionally fermented beans. Given odour descriptions from the panel show a better match with literature descriptions for 3,5-diethyl-2-methylpyrazine. On basis of the odour description, it was decided to refer to 3,5-diethyl-2-methylpyrazine. Same decision was made for 2-pentanol acetate and ethyl 3-methylbutanoate. Given signals during GC-O measurements and odour descriptions from the panel are more appropriate for ethyl 3-methylbutanoate.

Substances like 2-methylbutanal, 2,3-butanediol, butylamine, 1,3-propanediol, diacetate, epoxylinalool, 3,7-dimethyl-1,6-octadiene or pinane, limolene, beta-pinene, acetoin, dimethyl sulphide were not clearly identified, and/or it was too less often detected at GC-O. A last group of components could be identified, but were not odour-active in any sample. These were linalool, butyrolactone, hexanoic acid, 2-methoxy phenol, methyl ester benzeatic acetic acid, octanoic acid and nonanoic acid. In table 1 a listing of all detected odour-active meaningful substances is given.

Table 1 (part 1) Odour-active volatile compounds identified in cocoa bean samples from fermentation-like incubation and traditional fermentation, using HS-SPME-GC-MS-O. GC–MS was used for compound identification and the panel generated the odour description with use of GC-O. For identification the reference mass spectral data base of the National Institute of Standards and Technology MS library searches (NIST) together with the MSD ChemStation software and Kovats retention index from literature (NIST library) was used. When available standard pure substances were used to confirm identification.

Odour descriptions cited in the text are underlined. DF= Detection frequency; how often a chemical compound is detected in other studies, KI = Kovats Index from NIST library

Retention time [min]	Compound	Chemical group	Odour description (panel)	DF in studies	KI (Kovats Index)
	Aldehydes				
1,23	2-methylpropanal	Aldehyde	cocoa, chocolate, roasted, nutty, vanilla	7	543
1,81	3-methylbutanal	Aldehyde	cocoa, chocolate, roasted, caramel	8	643
	Ketones				
2,47	2-pentanone	Ketone	vanilla, sweet, butter, caramel, fruity	3	654
7,16	2-heptanone	Ketone	fruity, sweet/candy, nail polish, flowery	10	853
13,51	2-nonanone	Ketone	sweet, citrus, green, soapy, spicy, earthy, woody	4	1052
	Alcohols and Phenols				
2,11	ethanol	Alcohol	alcoholic, solvent, chemical	2	463
3,69	2-methyl-3-buten-2-ol	Alcohol	fruity, sweetish, citrus, flowery	-	600
5,7	2-pentanol	Alcohol	sweet, fruity, solvent	4	681

Table 1 (part 2) Odour-active volatile compounds identified in cocoa bean samples from fermentation-like incubation and traditional fermentation

8,5	3-methyl-1-butanol	Alcohol	banana, flowery, citrus, honey (TfC) chocolate, earthy, forest, woody, musty, nutty (IC)	4	697
9,1	2-[(Z)-octadec-9-enoxy]ethanol	Alcohol	fruity, sweet, green	-	2336
12,44	2-heptanol	Alcohol	soapy, flowery/parfumy, green, fruity, citrus	5	879
14,39	1,2-oxolinalool	Alcohol	fruity, citrus, green, sweet, chemical	-	1133
16,45	2-nonanol	Alcohol	green, savory, herbal, vegetal, green leaves, green pepper	1	1078
16,6	1,2-dihydrolinalool	Alcohol	green, herbal, grassy, peppery, woody	-	1092
21,85	phenylmethanol	Alcohol	vanilla, sweet, flowery, green	7	1036
22,35	2-phenylethanol	Alcohol	smokey, burnt, woody, vanilla, flowery (TfC) sweet, fruity, flowery, hay (IC)	11	1136
23,45	phenol	Phenol	soapy, flowery, vanilla, fruity	3	
	Esters				
2,27	propyl acetate	Esters	glue, fruity, sweet, flowery	-	686
3,95	ethyl 3-methylbutanoate	Esters	nail polish, glue, fruity	1	820

Table 1 (part 3) Odour-active volatile compounds identified in cocoa bean samples from fermentation-like incubation and traditional fermentation

5,41	3-methylbutyl acetate	Esters	banana, ice candy (TfC); fruity, flowery, sweet (IC)	1	820
10,31	2-heptanol acetate	Esters	fruity, sweet, solvent, glue, green, flowery	2	1019
14,52	ethyl octanoate	Esters	green, herbal, vegetal, savory, green peas, forest	7	1183
	Acids				
14,99	acetic acid	Acid	acid, sour, pungent, musty, sour dough bread	7	576
19,2	3-methylbutanoic acid	Acid	cheesy, musty, sour, sweet, rotten, yeasty	1	811
	Pyrazines and Pyrroles				
12,62	2,3-dimethylpyrazine	Pyrazine	sweet, vanilla, honey, musty, nutty	11	894
13,9	2,3,5-trimethylpyrazine	Pyrazine	vanilla, earthy, musty, roasted, sweet	13	1008
15,3	2,3,5,6-tetramethylpyrazine	Pyrazine	musty, earthy, chocolate	13	1121
16,23	3,5-diethyl-2-methylpyrazine	Pyrazine	earthy, herbal, green, vegetal, green leaves, woody, mushroom	10	1206
23	1-(1H-pyrrol-2-yl)ethanone	Pyrrole	overripe mango, grassy, woody, musty, smoky	8	1035

Table 1 (part 4) Odour-active volatile compounds identified in cocoa bean samples from fermentation-like incubation and traditional fermentation

	Furan				
14,91	cis-linalool oxide	Furan	musty, peas, earthy, vegetal	7	1164
	Hydrocarbons				
3,4	toluene	Hydrocarbons	glue, fruity, sweet, alcoholic	3	794
	Oxime				
20,69	methyl (Z)-N-hydroxyben-zenecarboximidate	Oxime	fruity, earthy, woody, sweet/vanilla	-	1301
	Alkanes and Alkenes				
0,99	2,2,4-trimethylpentane	Alkane	musty, putrid, sulfur	-	668
2,88	2,6-dimethyl-2-octene	Alkene	glue, sweet, fruity, vanilla, solvent	-	936

4.2 Developments in cocoa during course of fermentation-like incubation

Major compounds in terms of total content were acids, alcohols and ketones (Fig. 1). Alcohols 2-phenylethanol, 3-methyl-1-butanol and ethanol showed an increase in content on day one of fermentation-like incubation. After the first day a decrease in content of the mentioned alcohols can be observed (Fig. 1). 2-Pentanol, 2-heptanol and 2-methyl-3-buten-2-ol showed a continuous decrease in content from the fresh cocoa bean up to cocoa from day three of incubation. From third day on no change of any alcohol content, except for ethanol, was determined. 2-Nonanol, methyl (Z)-N-hydroxybenzenecarboximidate and 1,2-dihydrolinalool were alcohols with minor contents. Phenylmethanol was not developed in cocoa beans of fermentation-like incubation. Ketones showed a small but significant decrease (Fig. 1). Only 2-nonanone did not change significantly. Aldehydes and esters had no significant higher total content than pyrazines (Fig. 1). In general, all three chemical groups were the minor components (Fig. 1). All esters, besides propyl acetate, varied not significantly in content over time of fermentation-like incubation. The aldehyde 3-methylpropanal showed no significant increase in content after acidification of cocoa beans on day two. After the increase the content of the aldehyde remained constant in cocoa up to of day seven of incubation. The pyrazines 2,3,5-trimethylpyrazine, 2,3,5,6-tetramethylpyrazine and 3,5-diethyl-2-methylpyrazine were not developed in cocoa beans during fermentation-like incubation. 2,3-Dimethylpyrazine had a significant increase in content of fresh cocoa to cocoa of the second day of fermentation-like incubation, followed by no significant decrease in cocoa until the end of fermentation-like incubation. The pyrrole 1-(1H-pyrrol-2-yl)ethanone was present in all cocoa samples of fermentation-like incubation with no significant change in total content.

The furan cis-linalool oxide and 2,2,4-trimethylpentane showed a significant decrease in content of the fresh cocoa beans (FC0) to cocoa beans from the beginning until the end of fermentation-like incubation. 2,6-Dimethyl-2-octene, (Z)-n-hydroxybenzencarboximidate, toluene and phenol did not change significantly in content of cocoa during fermentation-like incubation.

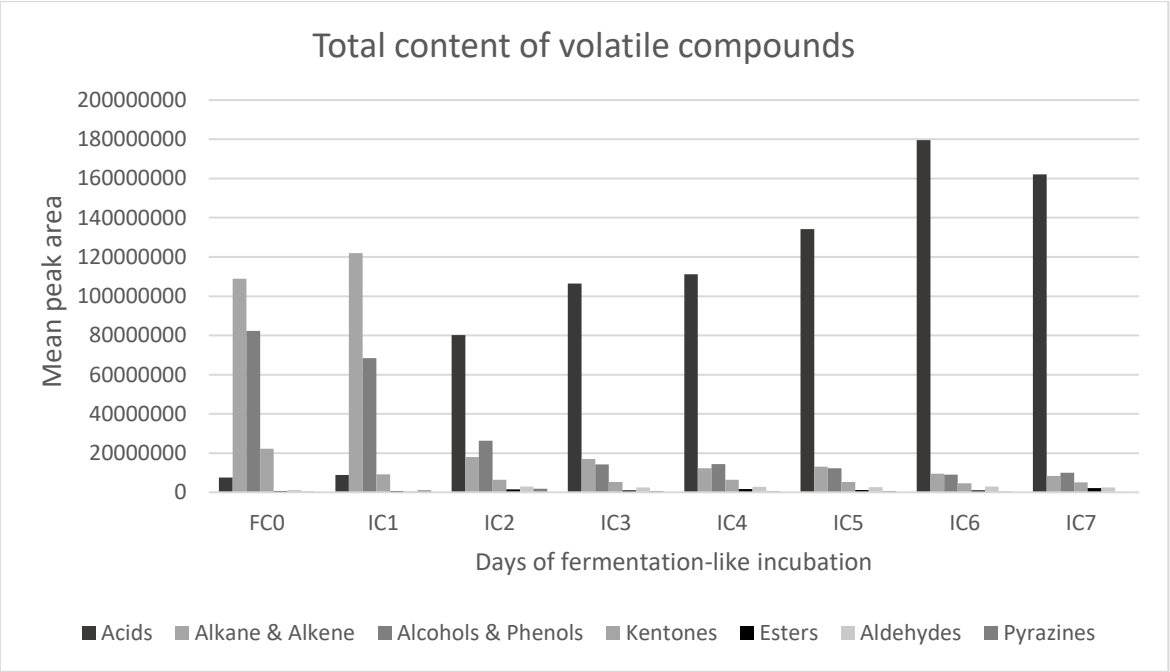

Fig. 1 Dynamics of total content of volatile compounds by chemical groups for fresh cocoa (FC0) and cocoa in the process of fermentation-like incubation (IC1, IC2, IC3, IC4, IC5, IC6, IC7)
content of compounds was defined as the integrated peak area of the volatile compound from the GC-MS; given results are the average values of data from six GC runs and the mean value of repetition one and two; associated compounds of groups can be seen in table 1; hydrocarbon, furan and oxime are excluded

Repetitions showed variations in FS. However, the magnitude of the individual FS's between repetitions was similar. Over 50 % of all FS's from the second repetition were higher than the first. A drift of the FS's was noticeable. A PCA was performed (Fig. 2). It confirms the drift of FS. A change between fresh cocoa (FC0), cocoa from first (IC1) and second (IC2) day of fermentation-like incubation in comparison to cocoa of remaining days of fermentation-like incubation was existing in both repetitions. The closer the cocoa sample FS's of repetition one and two were, the less the cocoa samples of repetition one and two were diverse in their FS's. The distance between repetition one and two of a cocoa sample and therefore the size of the ellipses can be assigned to reproducibility of repetitions (Fig. 2). The smaller the size of the ellipses the better the reproducibility is.

The order of cocoa samples from the single days of incubation from day three to seven is divers between repetition one and two (Fig. 2). A second performed PCA showed the FS mean value of repetition one and two (Fig. 3).

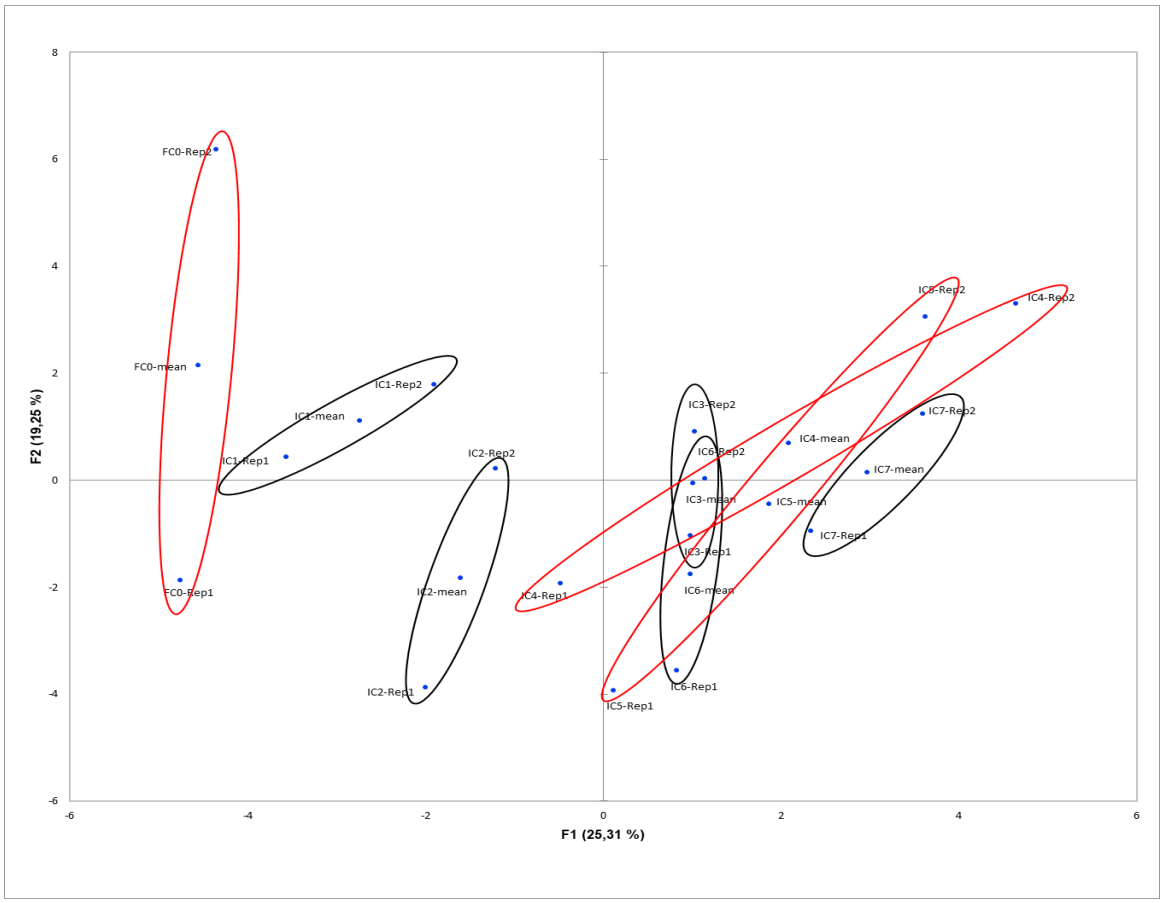

Fig. 2 Principle component analysis (PCA) of flavour scores from cocoa samples; first two principle components – score plot. Variations in flavour scores of cocoas between repetition one and two. mean = is the mean of repetition one and two of a cocoa sample. Size of ellipses can be assigned to reproducibility of repetitions.

Significant changes in FS could be observed only for the three active odours soapy/flowery, cocoa/nutty and acid/sour. In order to get an overview how cocoa samples over the time of fermentation-like incubation can be characterised, a PCA of their flavour scores was performed (Fig. 3). Seven chemical compounds were removed for the PCA of samples from fermentation-like incubation. Phenol, 2,3-dimethylpyrazine, phenylmethanol, 1-(1H-pyrrol-2-yl)ethanone, 2,3,5-trimethylpyrazine, 2-phenylethanol and 2,3,5,6-tetramethylpyrazine were removed because they did not have two or more FS values ≥ 5 and therefore were not relevant for consideration (see chapter 4.4.3). First dimension (F1) of the PCA explains 34,57 % of the data and second dimension explains 22,03 %. In this graphical view (Fig. 3) the differences in odour perception of incubated cocoa samples become clear. The further the samples are away from an odour, the less this odour describes a sample. However, shorter lines and corresponding odour descriptions are not explained in this view of dimensions. They can be explained on other dimensions. The other dimensions, however, do not explain that much information than the first two dimensions do.

The flavour profile, referring to the individual substances, changes from fresh cocoa samples (FC0) over cocoa samples from the first (IC1) and second (IC2) day of incubation to

samples from the remaining days of incubation (IC3 – IC7). From the third day cocoa samples from fermentation-like incubation can be characterised more by cocoa/nutty, cocoa/caramel, acid/sour and fruity/sweet/green, fruity/solvent odours. Another distinct change in odour perception can be seen in figure 3. Cocoa samples, relating to FS, of day five and six of fermentation-like incubation are a bit further apart from cocoa samples of day three and four. Cocoa bean samples from day three and four can be described with a more vanilla/buttery/sweet and glue/alcoholic odour than samples from day five and six. They are located further in the positive direction on the second dimension. Cocoa samples from day five and six had a slightly higher cheesy and a green pepper/savory note, explained by dimension two (Fig. 3). Cocoa beans from the last day of fermentation-like incubation are in the middle between cocoa beans from day three to four and cocoa beans from day five to six. Cocoa beans from day seven had a less pronounced green pepper/savory and cheesy odour than beans from day five and six (Fig. 3). Furthermore, they had a less pronounced vanilla/buttery/caramel and glue/alcoholic odour than samples from day three and four (Fig. 3). The fresh cocoa and cocoa samples from fermentation-like incubation day one and two had in general more earthy/green leaves, musty/peas and soapy/flowery notes; especially the fresh cocoa (FC0). However, odour perception of cocoa from day one and two is different regarding dimension two. Cocoa beans from day two had a more cheesy, green pepper/savory note. Beans from day one had a more vanilla/buttery/caramel and glue/alcoholic note.

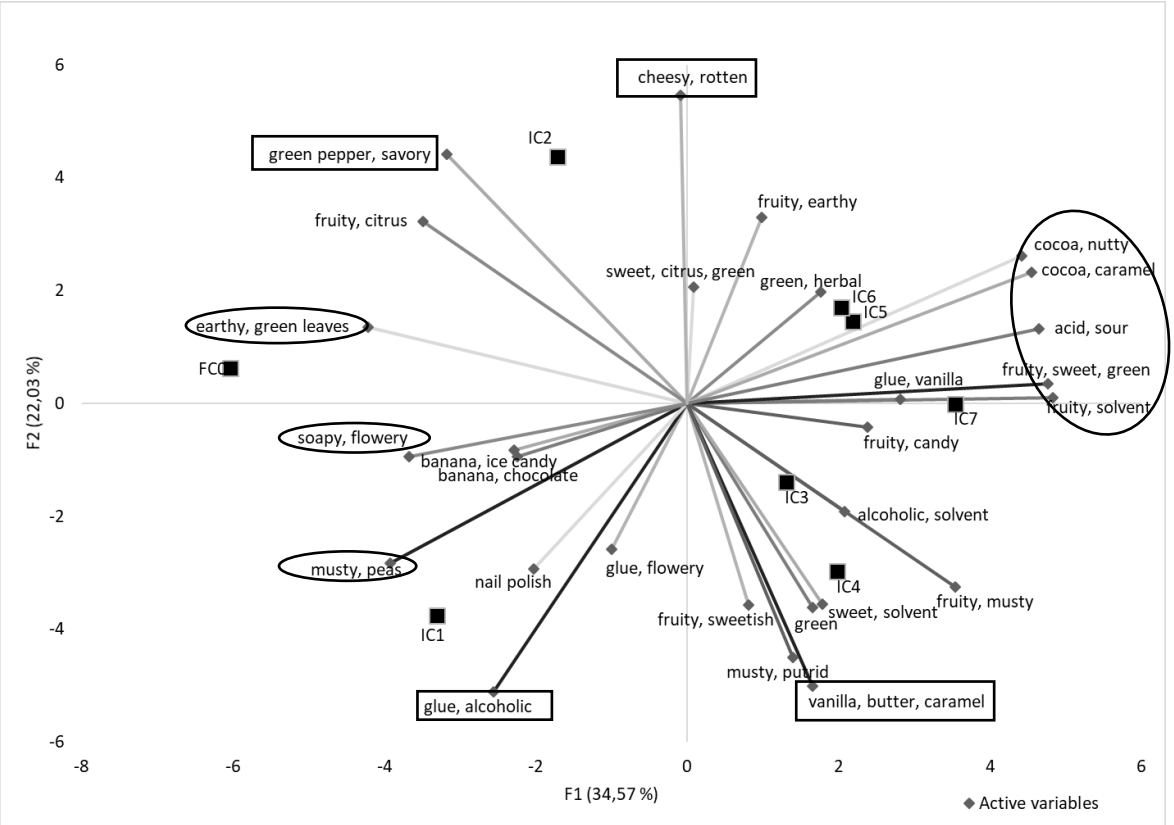

Fig. 3 Principle component analysis of flavour scores (mean of repetition one and two) with odour description; first two principle components – biplot from fresh cocoa beans at time zero (FC0), cocoa beans from fermentation-like incubation from day one to seven (IC1, IC2, IC3, IC4, IC5, IC6, IC6, IC7); odour descriptions with ellipses describe the first dimension, odour description with squared frames describe the second dimension

As it was explained, only three odours showed a significant change in their FS during course of incubation. The following graphics (Fig. 4 a) and b)), nevertheless, show the tendency of flavour scores over the course of fermentation-like incubation. In fresh cocoa beans a more musty/peas, earthy/green leaves, glue/flowery, glue/alcoholic, nail polish and a more soapy/flowery odour was perceived than in fermented bean samples. In contrast the odours alcoholic/solvent, fruity/solvent, fruity/sweet/green, cocoa/caramel, cocoa/nutty and acid/sour were perceived fewest in the fresh cocoa and higher in cocoa samples during the course of fermentation-like incubation.

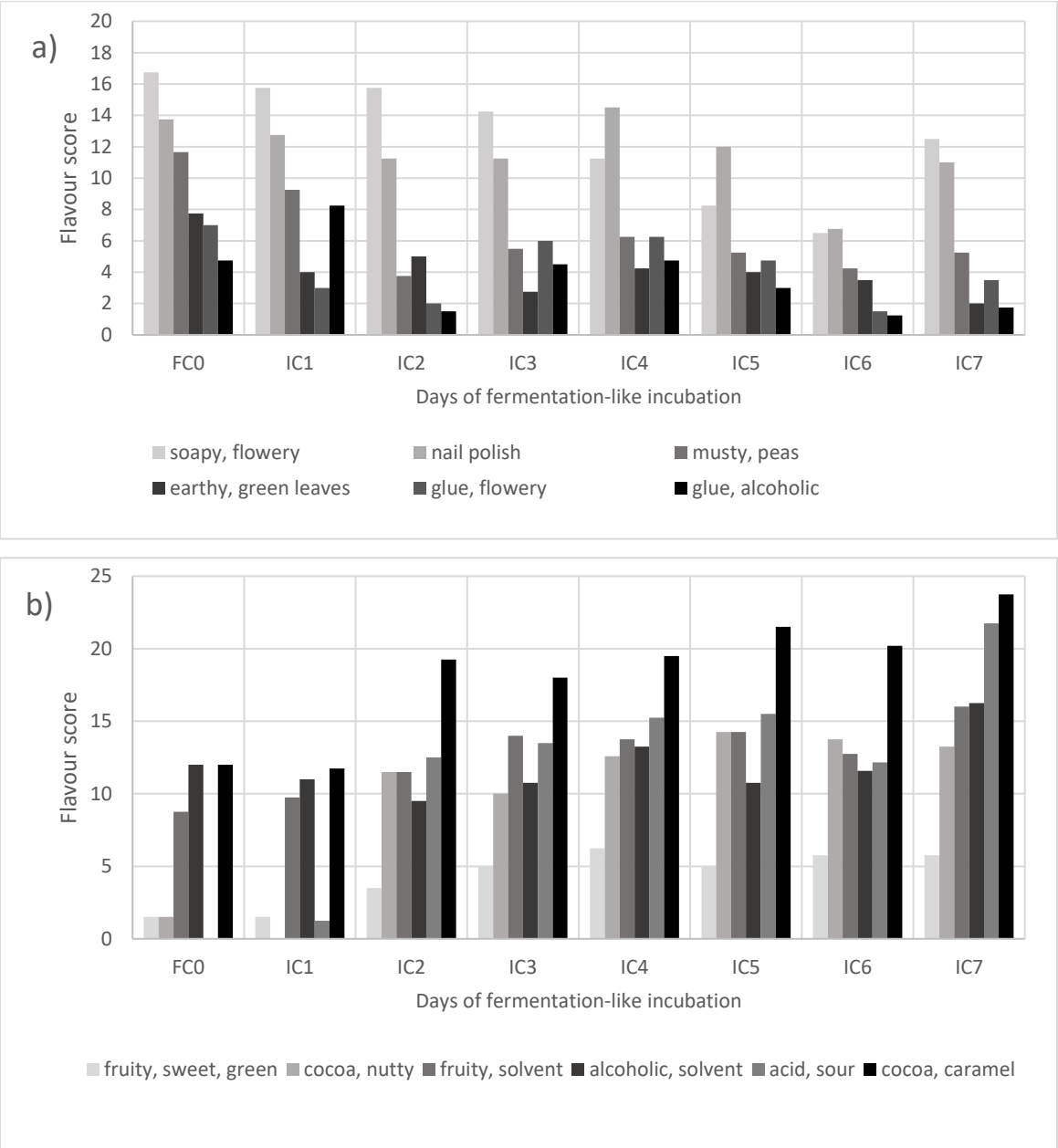

Fig. 4 a) Decreasing flavour scores from odour-active volatiles and b) Increasing flavour scores from odour-active volatiles during fermentation-like incubation of the Theobroma cacao L. from fresh cocoa at time zero (FC0) and incubated cocoa beans from day one to day seven (IC1, IC2, IC3, IC4, IC5, IC6, IC7); given results of flavour scores are mean value of repetition one and two.

4.3 Comparison traditionally fermented cocoa and cocoa from fermentation-like incubation

Traditionally fermentation was seven days. The comparison of cocoa from fermentation-like incubation was therefore with the sample from the seventh day. Significant higher contents of esters and especially pyrazines/pyrroles were detected in samples from traditionally fermented cocoa. Contents of acids, alcohols, ketones and alkanes/alkenes were not significantly higher (Fig. 5). Contents of aldehydes were significantly, and hydrocarbons were not significantly lower in traditional fermented cocoa samples.

Almost all pyrazines (2,3-dimethylpyrazine, 2,3,5-trimethylpyrazine, 3,5-diethyl-2-methylpyrazine), three esters (3-methylbutyl acetate, ethyl 3-methylbutanoate, 2-heptanol acetate), two alcohols (3-methyl-1-butanol, 2-phenylethanol) and 3-methylbutanoic acid showed a significantly higher content in traditionally fermented cocoa. 2,3,5,6-Tetramethylpyrazine was just not significant with a p-value of 0,051. Methyl (Z)-N-hydroxybenzenecarboximidate was significantly lower in incubated cocoa samples. Significantly higher contents in incubated cocoa samples were detected for all aldehydes, four alcohols (2-heptanol, 2-methyl-3-buten-2-ol, 2-[(Z)-octadec-9-enoxy]ethanol, ethanol), propyl acetate and 2,6-dimethyl-2-octene. Differences in content of all ketones were not significant. The same applies to four alcohols (1,2-dihydrolinalool, 1,2-oxolinalool, phenylmethanol, 2-pentanol, 2-nonanol), ethyl octanoate, acetic acid, toluene, phenol and 1-(1H-pyrrol-2-yl)ethenone.

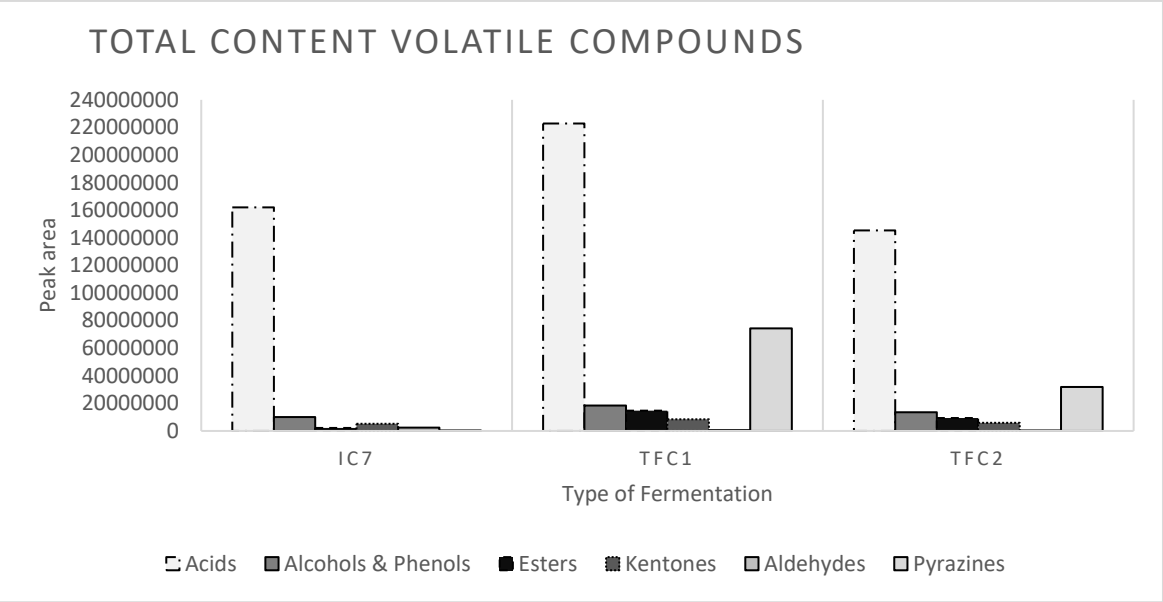

Fig. 5 Dynamics of total content of volatile compounds by chemical groups of traditionally fermented cocoa (TfC) and cocoa of the last day of fermentation-like incubation (IC7);content of compounds was defined as the integrated peak area of the volatile compound from the GC-MS; given results are the average values of data from six GC runs and the mean value of repetition one and two; associated compounds of groups can be seen in table 1; hydrocarbon, furan and oxime are excluded

The ratio of 3-methylbutyl acetate to 3-methyl-1-butanol can be used to check overfermentation. It was checked, because of the significant increase of both components. A ratio higher than 1,5 indicate overfermented beans. For incubated cocoa the ratio was between 0,5-1,0 (IC7 and IC7-Rep), whereas the ratio for traditional fermented cocoa beans was between 5,1-6,4 (TfC1 and TfC2).

When all samples are clustered with statistical tools there are nine clusters: FC0, IC1, IC2, IC3/4, IC5, IC6, IC7, TfC1 and TfC2. Seven dimensions are considered because the Eigenvalue is above 1 until the seventh dimension. It means that cocoa from every day of fer-

mentation-like incubation, except for cocoa from day three and four, were perceived different in GC-O. To get an overview how all cocoa samples from fermentation-like incubation were perceived in FS in comparison to traditionally fermented samples, a PCA was performed. Cocoa from fermentation-like incubated can be characterised differently than cocoa from traditional fermentation. Dimension F1 and F2 explain about 60 % of information (Fig. 6). Positive side of dimension F1 characterises traditionally fermented cocoa samples with following odours: vanilla/earthy/roasted, vanilla/sweet, soapy/vanilla, sweet/vanilla/honey, banana/ice candy, glue/flowery, overripe/grassy/smoky, cheesy/rotten and musty/earthy/chocolate. All samples from fermentation-like incubation can be characterised as green/herbal and alcoholic/solvent on the negative side of dimension F1 (Fig. 6).

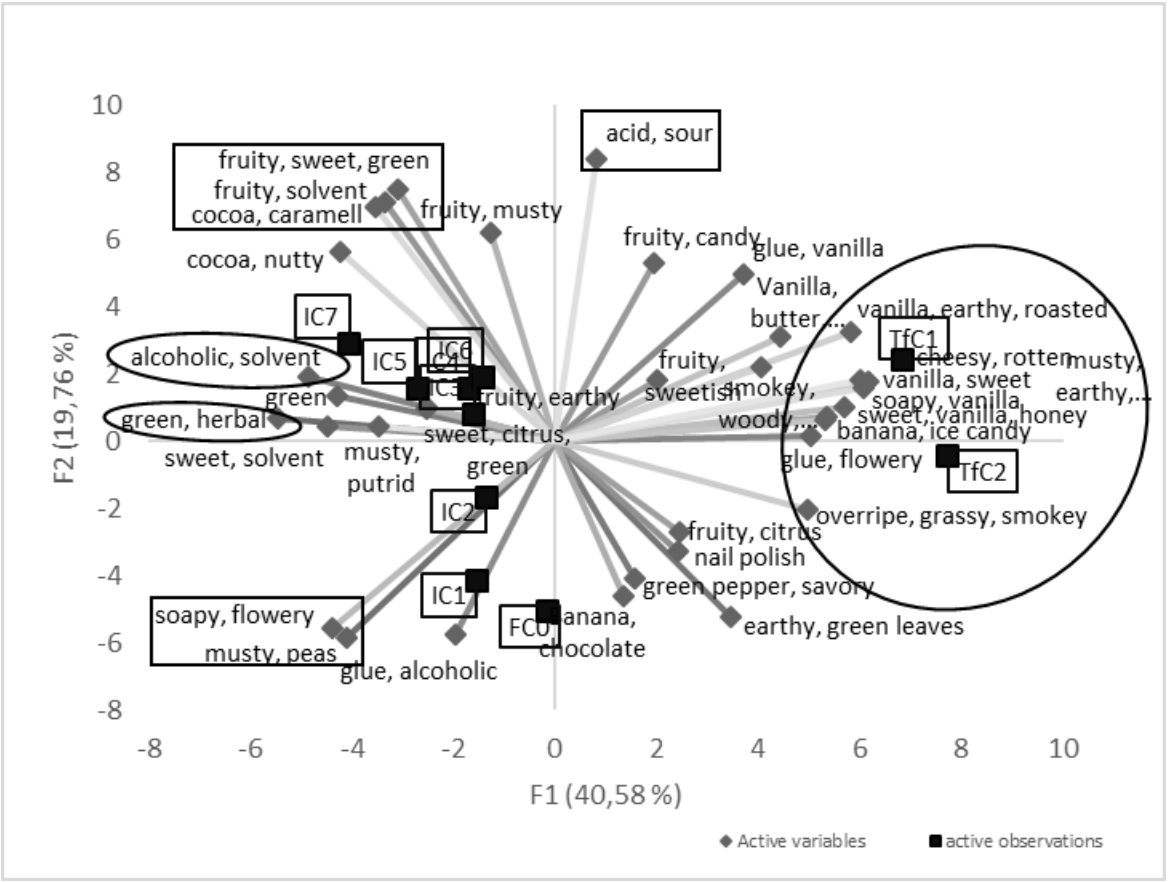

Fig. 6 Principle component analysis of flavour scores (mean of repetition one and two) with odour desription; first two principle components – biplot from fresh cocoa beans at time zero (FC0), cocoa beans from fermentation-like incubation from day one to seven (IC1, IC2, IC3, IC4, IC5, IC6, IC6, IC7) and cocoa beans produced by traditional fermentation (TfC) of Theobroma cacao L.*; odour descriptions with ellipses describe the first dimension, odour description with squared frames describe the second dimension*

To have a better comparison of odours from cocoa of the last day of fermentation-like incubation and cocoa from traditional fermentation, another PCA was performed (Fig. 7). It separated samples by FS. All odours on the negative side of dimension F1 had a higher FS for the incubated cocoa sample (IC7). Green/herbal, musty/peas, green, alcoholic/solvent,

sweet/fruity, fruity/solvent, fruity/sweet/green, sweet/citrus/green, fruity/musty, fruity/earthy, soapy/flowery, cocoa/nutty, cocoa/caramel, acid/sour, musty/putrid characterised more the incubated cocoa sample (Fig. 7).

Green pepper/savory, glue/flowery, glue/vanilla, nail polish, fruity/candy, fruity/sweetish, banana/ice candy, soapy/vanilla, vanilla/butter/caramel, sweet/vanilla/honey, vanilla/sweet, overripe/grassy/smokey, vanilla/earthy/roasted, smokey/woody/flowery, musty/earthy/chocolate, cheesy/rotten characterised more the traditional fermented samples (Fig. 7).

However, not all FS were significantly different between cocoas of fermentation-like incubation and traditional fermentation. Acid/sour, cocoa/nutty, green, fruity/solvent, green/herbal, sweet/fruity, musty/peas, musty/putrid, soapy/flowery had significantly higher FS for the incubated cocoa (IC7). Cheesy/rotten, musty/earthy/chocolate, vanilla/earthy/roasted, soapy/vanilla had significantly higher FS for traditionally fermented cocoa.

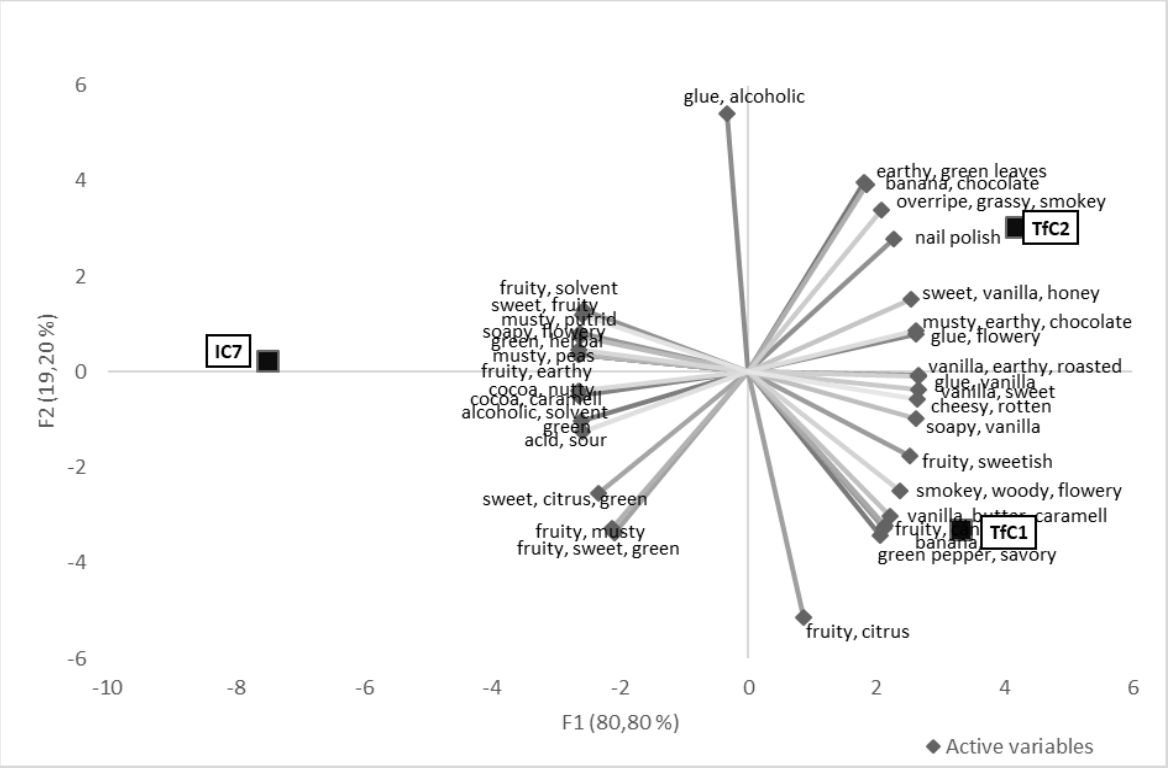

Fig. 7 Principle component analysis of flavour scores (mean of repetition one and two) with odour descriptions; biplot of cocoa bean samples from day seven of fermentation-like incubation (IC7) and traditional fermentation (TfC 1 & 2); odours on the negative side of dimension F1 have a higher FS for the incubated cocoa sample (IC7), odours on the positive side of dimension F1 have a higher FS for traditional fermented cocoa samples

On basis of these results, some odour-active compounds were defined as important odour compounds. To be defined as an important odour, the compound must be mentioned before in other studies and must have a high flavour score (FS > 10). Even if an odour was not

significantly different in FS, it was defined as important odour. In total six odours are important for cocoa samples of traditional fermentation and fermentation-like incubation: green/herbal, green pepper/savory, nail polish, acid/sour, cocoa/caramel and fruity/solvent. Vanilla/butter/caramel, vanilla/earthy/roasted, vanilla/sweet and cheesy/rotten were important for traditional fermented beans and soapy/flowery, cocoa/nutty and alcoholic/solvent for incubated samples. In figure 8 and 9 components defined as important are marked bold framed. In total ten out of 34 odour-active compounds were defined as important odours for traditional fermented cocoa beans (TfC) and nine out of 34 for the incubated cocoa sample (IC7).

The odour profiles of cocoa samples from traditional fermentation and fermentation-like incubation were illustrated in four categories (figure 8 and 9). All odour-active volatiles identified in this study were grouped into one of the four odour categories: green/alcoholic (green colour), fruity/flowery/glue (red colour), vanilla/cocoa (yellow colour) and acid/putrid (blue colour). Cocoa samples from traditional fermentation and fermentation-like incubation contained odours of all four categories (see figure 8 and 9). In general, cocoa samples of traditional fermentation and fermentation-like incubation showed similar important odours. However, the different defined important odours showed the diversity between cocoa of traditional fermentation and fermentation-like incubation.

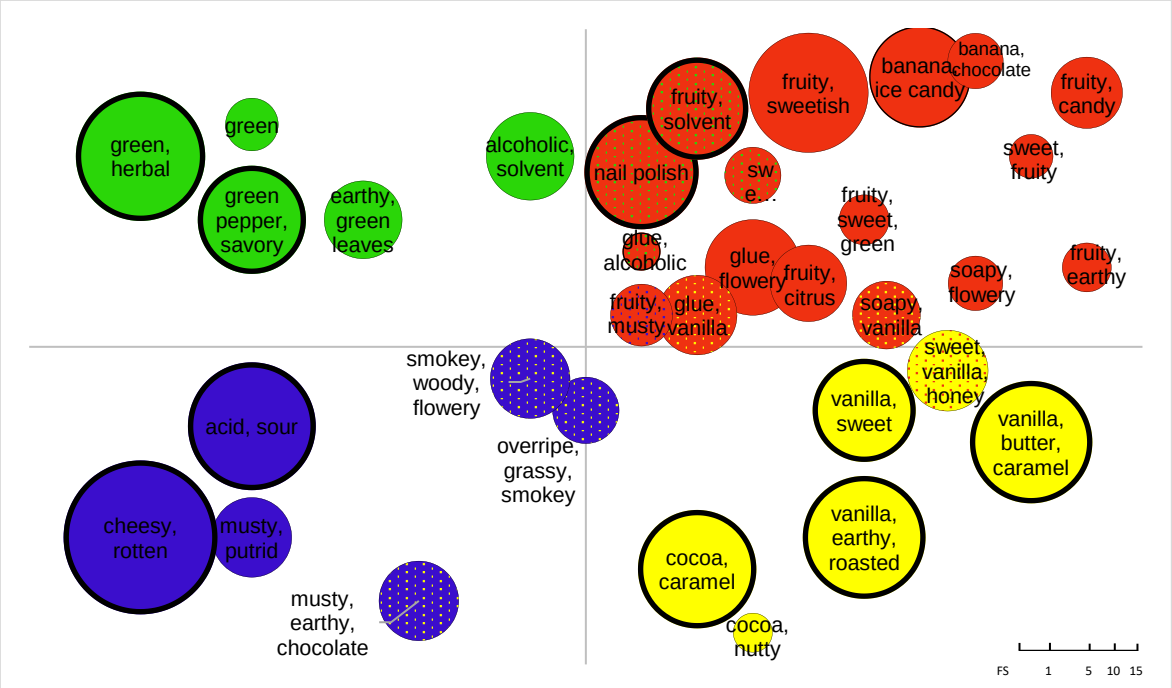

Fig. 8 Odour-active volatiles grouped in four categories by odour description – traditional fermented cocoa. Size of the circles represents the flavour score of each odour. Bold framed circles are defined as important odours.

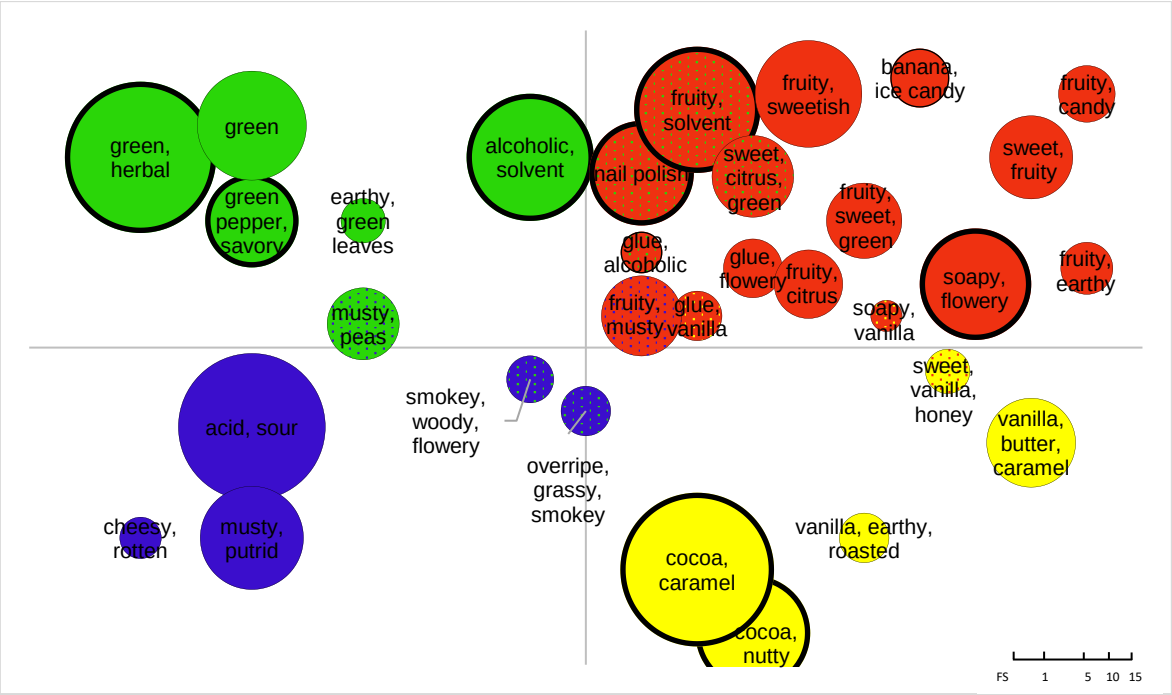

Fig. 9 Odour-active volatiles grouped in four categories by odour description – cocoa from fermentation-like incubation day seven (IC7).
Size of the circles represents the flavour score of each odour. Bold framed circles are defined as important odours.

In general, individual categories of active odours like green/alcoholic (green colour), fruity/flowery/glue (red colour), vanilla/cocoa (yellow colour) as well as acid/putrid (blue colour) cannot be assigned to one chemical class (Fig 8, Fig. 9). Green/alcoholic category was represented by alcohols, esters and furan. Fruity/flowery/glue category was represented by alcohols, esters, ketones, hydrocarbons, oximes and alkenes. Ketones, especially pyrazines and aldehydes represented vanilla/cocoa category. Acids, alkene, furan, pyrrole and alcohol represent acid/putrid category.

A multiple factor analysis (MFA) was used to analyse data from GC-MS, the chemical components (peak area), and data from GC-O (calculated FS) at the same time. MFA was executed with data sets from cocoa of traditional fermentation and from fermentation-like incubation. Both data sets were compared. Similarity of both data sets can be measured with the correlation coefficient. A correlation coefficient of 0,879 indicated a high conformity between peak area and flavour score. Additionally, the accordance of the PCA to MFA can be evaluated. PCA of GC-MS had a higher accordance with the MFA (0,971) than the PCA of GC-O with a value of 0,966. However, the accordance is very similar.

Data from chemical components and from flavour score correlate positively on the first dimension for substances like 2,3,5,6-tetramethylpyrazine, 2,3,5-trimethylpyrazine, 3-methylbutyl acetate, 3-methylbutanal and 3-methylbutanoic acid. That means when the content of

a chemical substance was increasing than the flavour score also was increasing. Correlation on the second dimension is negative. Following substances and their odour description are negatively correlated: 2,2,4-trimethylpentane, 2-heptanol acetate, 2-methylpropanal. When the content of a chemical substance was decreasing or low, then the flavour score for this substance was increasing or high.

A second MFA was executed only for samples of fermentation-like incubation. It showed a high conformity between peak area and flavour score with a correlation coefficient of 0,879. In this second MFA the accordance of the PCA to MFA was higher for GC-O data set (0,975) than for the GC-MS data set (0,963). When considering only fermentation-like incubation samples, on the first dimension a negative correlation and on the second dimension a positive correlation was observed.

5 Discussion

5.1 Methodology

HS-SPME-GC-MS/O was a suitable method to compare the odour-active volatiles of cocoa beans from traditional fermentation and fermentation-like incubation.

No quantification was performed as time was a limiting factor. Quantification would produce more precise and reliable results than referring to peak area. Additionally, confirmation of volatiles with standard pure substances could, due to cost reasons, not be conducted for all identified components. For components, where no Kovats retention indices could be found in comparable literature, it would be a desirable aim to assure more confident findings with standard pure substances.

The executed DoE, however, has led to an improved identification of components. Good identification of components with the mass spectral data base mainly depends on the peak area. The peak area of defined important components was increased by adapting parameters for HS-SPME.

The time-temperature profile of the GC method was adapted to have a relatively short GC run time but simultaneously good separated peaks. A short GC run induces a short sniff duration what is advantageous for the panellists to avoid sensory fatigue (Delahunty et al. 2006). To support a good performance the sniffing time for each panellist was bisected in comparison to the GC run time. On the one hand a short GC run can be disadvantageous for the correct identification of volatiles and on the other hand for the assignment of given panel description and the eluting compound.

On the one hand, when more than one compound is eluting from the equipment very close together or at same time, a conclusively identification is difficult to make without further analysis (Chambers, Koppel 2013). As described above a confirmation with pure standard

substances is one option. Another option to assure better identification and a higher resolution could be a two-dimensional gas chromatography. Analytes in a sample are selectively separated on two different separation columns, thereby contributing to a better separation and therefore better identification (Meinert, Meierhenrich 2012).

On the other hand, a given signal of a panellist can hardly be assigned to close eluting compounds. The perception of an eluting compound and the time to react (giving a signal) is variable for each component and for each panellist. The closer eluted compounds are the more difficult it is to identify the right component, especially when compounds smelling similar. It is also possible that panellists detect some components that cannot be detected from the GC-MS.

The facts of sensory fatigue and identification issues have to be considered but can hardly be improved when applying GC-O as a method. Both issues mainly rely on the length of the GC run. It should be investigated if a two-dimensional GC in combination with olfactometry is a possible solution.

Panellists were trained to accurately describe and rate odours. However, a panellist only has a few seconds to assess a perceived odour. This can cause a wrong attribution to a compound (Chambers, Koppel 2013). A variable description of an odour, other than found in literature, can also be explained by concentration of a component. Different concentrations can cause different odour notes (Legrum 2011). Additionally, it is known that olfactory response of an individual varies over time and with the speed of breathing (Brattoli et al. 2013).

Two repetitions were executed consecutively. Within repetition one and two, sniffing was performed randomised and blind. The mean variation of the two repetitions probably arise from the consecutively realisation and the associated familiarization with sniffing cocoa samples on the sniffing port. Training of the panellists stopped after they have reached the required amount of training sessions and results. To ensure a consistent description and especially rating of odours a continued training should occur. A short training session with standard substances after a sniffing session or a standard sample, which has to be rated and described each time the same, would be conceivable. However, limiting time of panellists is a critical factor to accomplish a continued training. Panel performance was not evaluated. Van Ruth (2001) refers to two studies explaining a great variation between assessors. Therefore, the use of a panel and taking the mean of the intensity rating is of importance (Petersen 2013; van Ruth 2001).

Despite all assessors are trained over a long period, for most concentrations of compounds, there will be a part of panellists that is not able to detect these (van Ruth, O'Connor 2001). To reduce the influence of panellists, it was aimed to have a balanced allocation of samples

and panellists. No panellist had the same sample twice and it was tried to have each panellist sniff each sample once. The aim of having each panellist sniffed all samples could not be entirely fulfilled due to limited time of panellists or illness. Therefore, more than six required panellists were trained.

Variation in results of trial repetition and a high standard deviation in these results (peak area) can result from multiple factors. Variation in sample material was tried to avoid by preparation of similar starting material for incubation and preparation of homogeneous samples for GC measurement. A higher amount of sample material would be advantageous. The equipment in a small laboratory can be limiting for a higher volume of incubation material. Nevertheless, variation of the low concentrated volatiles can still be possible. When applying SPME it also needs to be considered that the volatile profile obtained, is depending on the selectivity and capacity of the used fibre. Generally, a SPME fibre has a low quantity to absorb volatiles. Competitive binding on fibre can explain variations in results of peak area (Kadow et al. 2013; Reineccius 2010). However, HS-SPME is a fast and simple method without a solvent extraction step or a complicated purge-and-trap apparatus (Hui 2010). It needs to be considered that only one method, here HS-SPME, cannot represent a complete profile of odour-active volatiles in cocoa. The flavour profile obtained, is related to isolation method (d'Acampora Zellner et al. 2008). Several methods have different advantages and give different views. Solvent extraction and distillation methods yield an almost complete flavour profile which was not relevant for determination of an odour profile of only volatile components from cocoa. Some other methods as SDE must deal with loss of highly volatile compounds, decomposition and heat-induced artifact formation. SAFE does not have these disadvantages but requires big samples amounts, application of solvents and the availability of a SAFE apparatus (d'Acampora Zellner et al. 2008). HS-SPME requires only small sample amounts and the apparatus was available to use for this study.

In general, the applied method was able to give an overview of volatile components in cocoa with discussed limitations.

Sample setting of fermentation-like incubation with samples taken every 24 hours was appropriate to generate a first overview of changes occurring during fermentation-like incubation. Primarily between FC0, IC1-IC2 and IC3-IC7 a change in volatiles could be observed. The sample setting of 24 hours should be retained, as it gives a good first overview. Additionally, a third trial repetition should be executed to get even more reliable results. In future research it would be of advantage to continue with a roasting of cocoa beans after fermentation-like incubation to further investigate the development of volatiles in cocoa.

5.2 Literature

The applied literature consists of about 85 % primary literature from scientific journals. Books and articles in books represent about 13 %. One source is a dissertation from Petersen (2013). The author developed the model of flavour score which is used in this thesis. The work from the dissertation was executed at the same institution (HAW Hamburg) and with the same equipment as in this study. The method required for flavour score calculations, is applied as a standard method in this institution. The flavour score model was utilized as it combines the often quoted and used method detection frequency and direct intensity measurement. The author explains a better differentiation of samples with significance tests for flavour scores than only for intensity ratings. The method was developed in dependence on the odour activity value (OAV) (Petersen 2013). However, the more common used OAV method could not be utilized as no required quantification was executed. Applied flavour score method could be used without quantification. Detection frequency and therefore flavour score method can produce results for defining odour-active compounds. In combination with the intensity rating it appears to be a good alternative to OAV method. When repetitions are executed, as it was done in this study, then statistical analyses are possible. Therefore, repetitions should be applied when using flavour score model.

5.3 Results

All mentioned volatiles in this study are odour-active and therefore, contribute to the aroma profile. The results show how intense odours are perceived and how they were described as a single odour. To get a better overview of the dynamics of odours during fermentation-like incubation the mean value of both repetitions was calculated for content and FS. In general, it is assumed that the mean value is a better estimate of the investigated effect than a single measured value. Nevertheless, it needs to be considered that the reproducibility of the data is poor for repetition data of FC0, IC4 and IC5. Figure 2 in 5.2 gives a good overview. For FC0, IC4 and IC5 the ellipses are quite large and therefore, it shows that the data between both repetitions is poor (Fig.2). For cocoa samples of the other days of incubation, the ellipses are smaller and show a good reproducibility. Reproducibility for content of chemical compounds is also poor. All compounds of all cocoa samples from fermentation-like incubation and of samples from traditional fermentation show a high standard deviation of their contents. Possible factors are explained before in chapter 6.1.

It is assumed that odours, which are defined as more important, contribute more to the overall odour profile. However, with these results it is not possible to answer the question of how odours will be perceived in a mixture with all odour-active compounds.

Flavour profile comparison of traditional fermented cocoa bean samples and cocoa bean samples from fermentation-like incubation show similarities and differences.

Quality of cocoa liquor cannot be evaluated clearly as the interactions of all compounds are not evident. Nevertheless, it is assumed that cocoa from traditional fermentation and fermentation-like incubation will probably have a similar basic odour caused by the same important odour-active compounds. Fruity and sweet notes will exist but originate from different important and minor compounds. Traditional fermented cocoa beans will presumably have a vanilla and earthy note in cocoa liquor. Cocoa liquor from fermentation-like incubation cocoa beans in contrast will probably have more intense soapy, green and solvent notes. Cocoa samples from traditional fermentation and fermentation-like incubation generated an odour which is perceived as an off-flavour during GC-O. It does not mean that this odour will have a negative impact on the overall cocoa quality. A single odorant is perceived differently in a mixture of odour compounds (Hofmann et al. 2014). The cheesy-smelling 3-methylbutanoic acid seems to contribute to the cocoa aroma as it was repeatedly detected in other studies (Bonvehí 2005; Counet et al. 2004; Ducki et al. 2008; Frauendorfer, Schieberle 2006; Misnawi 2011; Rodriguez-Campos et al. 2012; Tran et al. 2015, 2015). The other, in GC-O perceived off-odour, 2,2,4-trimethylpentane was not found in other studies contributing to the cocoa aroma.

Most odour-active compounds in traditional fermented cocoa are acetic acid and 3-methylbutanoic acid. They also had the highest odour-activity values in the study of Frauendorfer and Schieberle (2008). In total six here detected odour-active compounds are also mentioned as odour-active in the study of Frauendorfer and Schieberle (2008). Three of the six detected compounds are under the first four compounds with the highest odour-activity value. Both mentioned acids and 3-methylbutanal seem highly important contributors to aroma in raw cocoa beans, independent of type of cocoa. However, most identified odour-active compounds were different in comparison to results of Frauendorfer and Schieberle (2008). As explained in the discussion of methodology 6.1, it is possible that the applied method in this study does not capture all odour-active volatiles of raw cocoa. It might just show a restricted view.

Compared to other literature investigating volatiles of raw cocoa, several equal volatiles were identified in this study. Alcohols like 2-heptanol, phenylethyl alcohol, 2-phenylethanol and 3-methyl-1-butanol were often identified in other studies. Same as here detected ketones and esters 2-heptanone, 2-nonanone, 2-heptanol acetate and ethyl octanoate. Tri- and tetramethylpyrazine, 2-methylpropanal and 3-methylbutanal were also quite often identified in volatiles of raw cocoa (Ascrizzi et al. 2017; Frauendorfer, Schieberle 2008; Jinap et al. 1998; Oberparleiter, Ziegleder 1997; Ramos et al. 2014; Rodriguez-Campos et al. 2012). This study was investigating only odour-active volatiles. Therefore, not all possibly detectable volatiles are mentioned here. Many more volatiles were identified in literature. Some components like linalool, benzaldehyde, 2,3-butanediol, acetophenone, pinene and acetoin

are additionally often detected in literature. In this study they are mentioned once (see chapter 5.1). They were either not odour-active enough or not identified clearly. In general, differences between identified compounds and the total number of compounds in literature exist. The identified volatiles depend on the genotype of cocoa and the applied extraction method and analytical conditions.

One reason for the absence of pyrazines could be explained with a maximum temperature of 45 °C during incubation. As the centre of a traditionally fermented cocoa mass can reach a maximum of 50 °C, it seems possible that pyrazines (e.g. tetramethylpyrazine) are generated by heat (Reineccius et al. 1972; Rohan 1963). Second reason can be the lack of microorganisms. *Bacillus subtilis* is often mentioned as reason for the production of pyrazines. Hashim et al. (1998) and Jinap et al. (1994) also found a correlation between the increase of *Bacillus subtilis* and the increased content of pyrazines. The concentration of methyl pyrazines in unroasted cocoa beans can be used as an index of fermentation degree and potential quality (Zak et al. 1972; Jinap et al. 1994). In the investigation of Frauendorfer and Schieberle (2008) five pyrazines are counted to important aroma compounds. All pyrazines are among the second half of the 32 aroma compounds with minor odour activity values (OAV). Four out of five pyrazines show an increase in content and in the OAV. The increase of pyrazines belongs to the minor increases in comparison to other aroma compounds. Roasting of cocoa beans seems not to have a big influence on the development of pyrazines. No other pyrazines were classified as important in roasted cocoa than in raw cocoa. However, pyrazines found in raw cocoa by Frauendorfer and Schieberle (2008) are also important aroma compounds in roasted cocoa. Even if pyrazines have a little OAV, they are classified as important aroma compounds by Frauendorfer and Schieberle (2008). Therefore, the potential quality of cocoa in samples from fermentation-like incubation seems not completely developed.

Ketones, secondary alcohols and esters, derive from fatty acid and/or amino acid metabolism (Fridman et al. 2005; Schwab et al. 2008). For example 3-methylbutanal, 2-methylpropanal, ethyl-3-methylbutanoate, 3-methylbutanoic acid, phenylmethanol and 2-phenylethanol are formed out of amino acids by a similar metabolic pathway, initiated by an aminotransaminase (Smit et al. 2005). However, microorganisms seem to be the main source of enzymes (Smit et al. 2005) as not all components were developed in cocoa of fermentation-like incubation. The detected aldehydes in cocoa bean samples from fermentation-like incubation could arise from non-enzymatic chemical conversation and/or Strecker degradation (Smit et al. 2005). It shows that there is a potential of flavour development without microorganisms.

Besides the production of metabolic end products like ethanol, acetic, citric and lactic acid yeasts are major producers of esters, aldehydes, ketones and higher alcohols. They are likely to contribute to the complex mixture of volatile aroma compounds and thus to cocoa bean and chocolate quality (Ardhana 2003; Crafack et al. 2013; Ho et al. 2014; Schwan, Wheals 2004). *K. apiculata* and *S. cerevisiae var. chevalieri* yeasts have been reported to be responsible for the formation of alcohols such as 3-methyl-1-butanol, 2-phenylethanol and 2,3-butanediol (Schwan, Wheals 2004). These mentioned alcohols are desirable for high quality cocoa products. However, they were developed less in incubated cocoa samples. Same applies for more than half of the esters and half of the alcohols. Ho et al. (2014) also describes yeasts and not acetic acid as responsible for the formation of alcohols and esters. Results of missing increase of alcohols confirms the finding. Thus, some alcohols show an overall high odour-activity. These observations confirm the assumption that fermentation-like incubation without application of microorganisms as yeasts and bacteria do not lead to an entire formation of desirable alcohols, esters, ketones and especially pyrazines in cocoa.

The ketone 2-pentanone is rated as an important odour for traditional fermented cocoa in this study. It is not rated as important odour for incubated cocoa samples. In comparison to the other two ketones 2-pentanone exhibits a higher odour-activity in incubated cocoa. It has an influence on cocoa aroma as Kadow et al. (2013) supposed, however, on the vanilla notes and not on the fruity notes in this study. The quite intense alcoholic odour in incubated samples probably arisen due to the unnaturally added ethanol solution. This concentration and/or amount of ethanol solution probably does not depict the naturally concentration of produced alcohols. A lower concentration of ethanol solution could be applied in future research.

3-Methylbutyl acetate is an amyl acetate identified in significant higher concentrations in traditional fermented samples. The formation of amyl acetates should be avoided as they are seen as indicators of flavour defects (Rodriguez-Campos et al. 2012). Furthermore esters are usually present in low concentrations apart from higher contents in overfermented cocoa beans (Oberparleiter, Ziegleder 1997). Given higher concentrations of the ester 3-methylbutyl acetate led to check the ratio of 3-methylbutyl acetate to 3-methyl-1-butanol. As the ratio of samples from traditional fermentation is higher than 1,5 and a hammy smell of the beans is confirmed, it indicates overfermented cocoa beans. Therefore, it might not be the aim to have exactly the same results for fermentation-like incubation. As cocoa beans from fermentation-like incubation show no overfermentation based on these criteria, it can be argued that cocoa from fermentation-like incubation has a better stability relating to aroma volatiles. The better stability might be the reason of an incubation without microorganisms. On the other hand, the absence of microorganisms could be the reason

for the minor complexity of odour-active volatiles of incubated cocoa in comparison to traditional fermented cocoa beans.

The assumption from Kadow et al. (2013) that fruity and floral flavour notes are fully developed in fresh cocoa seeds can only partially be supported. Almost all, as important classified fruity and flowery odours (nail polish/fruity, solvent/fruity, sweetish/soapy, flowery), have comparable high flavour scores from the beginning on. The minor fruity and flowery notes, however, are not fully developed in the fresh cocoa bean. Most odour-active volatiles with minor developed fruity and flowery notes show an at least temporary increase in flavour score during course of fermentation-like incubation. Chetschik et al. (2017) detected a parallel increase of in pulp detected odour-active fruity and flowery substances in pulp and cocoa seeds during fermentation. Therefore, fermentation and most likely the pulp have an influence on the development of fruity and flowery notes in raw cocoa. It was also the aim in this investigation to depict the flavour profile development of cocoa over time of fermentation-like incubation. As it was explained before in the PCA (see chapter 5.2), there are changes from fresh cocoa over the first and second day of incubation to the remaining days of incubation. This observation confirms literature from Afoakwa et al. (2008) and Kadow et al. (2013). It says that fine flavour cocoas need shorter fermentation times as not to lose these fine fruity and flowery flavours. To finish fermentation-like incubation without fortifying fine aromas in cocoa is presumably best at day 4 as a compromise for all fine aromas. It needs to be checked in future research. However, this final assessment can only be made with caution since the results of the repetition samples were different, especially at day 4 and 5.

When the detected correlation of chemical and sensory data is considered, a contradiction becomes apparent. One MFA was comprising data of cocoa from traditional fermentation and fermentation-like incubation, the other, only data of cocoa from fermentation-like incubation. Correlation is quite high for both considered MFAs with difference in positive or negative direction of correlation. It seems that the MFA is affected by compounds that were detected only in samples from traditional fermentation. Therefore, conclusions about a correlation of chemical and sensory data can hardly be drawn. It confirms Chambers and Koppel's (2013) prediction of the lack of a direct linear relationship between chemical compounds and sensory perceived aromas.

6 Conclusion and future prospects

This study gives a good comparable overview of odour-active volatiles in traditional fermented cocoa beans and of cocoa beans from fermentation-like incubation without microorganisms. The latter yields cocoa and acid notes as well as fruity and flowery notes typical for fermented fine flavour cocoa. Traditional fermentation developed, besides equal odours,

more cocoa typical components. Certain compounds had vanilla and cheesy odours found in traditional fermented cocoa. These compounds were not detected in cocoa beans from fermentation-like incubation. Overall cocoa from traditional fermentation has a higher complexity of odour-active volatiles probably due to the impact of microorganisms. In contrast, the absence of microorganism during fermentation-like incubation might be the reason for the higher stability in cocoa from fermentation-like incubation. The latter has a better stability of odour-active substances as no signs of overfermentation are observed.

To improve the potential development of odour-active volatiles in cocoa some adjustments could be made in future research. The temperature rise during fermentation-like incubation might be slightly too rapid (Biehl et al. 1985) from the first to the second day. Beside a slower temperature rise, a different acidification protocol may be applied in future research to improve the aroma potential in fermentation-like incubations without microorganisms. Even if the content of acetic acid was in the same magnitude than in traditional fermented beans, more conditions than the total acid content seem to be important for flavour precursor formation. To reach an optimum of aroma formation the diffusion rate, timing of the first entry and the duration of optimum pH should be examined in more detail. The usage of additional acids like oxalic acid should be considered in future fermentation-like incubations as it is suggested to have a beneficial influence on the cocoa flavour (Holm et al. 1993).

Furthermore, a comparable study of improved fermentation-like incubation without microorganisms with a defined starter culture of microorganisms should be made. As conditions will be more comparable, it will probably give new results if microorganisms are responsible for some compounds. Future research with described improvements in fermentation-like incubation should also include extended trials in roasting to evaluate the continuing development of precursors to chocolate aroma.

For a complete understanding of cocoa and chocolate sensory quality, future studies should consider aroma and taste-active substances. Given that most of these substances are produced during roasting, this processing step needs to be taken into account as well. Like for the aroma, also the key tastants in roasted cocoa nibs have been identified (Hofmann et al. 2006). The precursors for aroma and taste-active substances respectively are known. These data are the basis for this suggested future research.

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Appendix

List of appendices

Appendix 1 (part 1) extended version of table 1 with detailed overview of studies and odour description of compounds in literature; DF= Detection frequency; how often a chemical compound is detected in other studies, KI = Kovats Index from NIST library

Retention time [min]	Compound	Chemical group	Odour description (panel)	Odour description in literature (thegoodscentscompany, o.J.)	Studies	KI (Kovats Index)
	Aldehydes					
1,23	2-methylpropanal	Aldehyde	cocoa, chocolate, roasted, nutty, vanilla	fresh, aldehydic, floral, pungent	Afoakwa et al. (2008) Ducki et al. (2008), Counet (2002) J. Rodriguez-Campos et al. (2011), Tran et al. (2015) (chocolate, cocoa) Schnermann, P. Schieberle, P. (1997) (malty) van Praag	543
1,81	3-methylbutanal	Aldehyde	cocoa, chocolate, roasted, caramel	ethereal, aldehydic, chocolate, peach, fatty fruity, dry, green, peach	Afoakwa et al. (2008) (chocolate) Ascrizzi et al. (2017) (malty, chocolate, bitter) Counet&Collin (2004) Counet (2002) chocolate Ducki et al. (2008) Frauendorfer & Schieberle (2006) (malty) J. Rodriguez-Campos et al. (2012) (chocolate) Tran et al. (2015) (cocoa, chocolate, malt) Schnermann, P. Schieberle, P. (1997) (malty)	643
	Ketones					
2,47	2-pentanone	Ketone	vanilla, sweet, butter, caramel, fruity	sweet, fruity, ethereal, wine, banana, woody ethereal, sweet, banana, fermented, woody Mosciano, Gerard P&F 20, No. 1, 31, (1995)	Kadow et al. (2013) J. Rodriguez-Campos et al. (2011, 2012) (fruity) Quijano, C.,Pino, J. (2009)	654

Appendix 1 (part 2) extended version of table 1 with detailed overview of studies and odour description of compounds in literature; DF= Detection frequency; how often a chemical compound is detected in other studies, KI = Kovats Index from NIST library

7,16	2-heptanone	Ketone	fruity, sweet/candy, nail polish, flowery	cheesy, fruity, ketonic, green, banana, creamy (Mosciano, Gerard P&F 16, No. 4, 45, (1991)) fruity, spicy, sweet, herbal, coconut, woody (Luebke, William tgsc, (1989))	Afoakwa et al. (2008) Ascrizzi et al. (2017) (fruity, cheese-like) Counet&Collins (2004) und Counet (2002) Kadow et al. (2013) J. Rodriguez-Campos et al. (2012) (fruity, floral) Tran et al. (2015) (banana-like, fruity)	853
13,51	2-nonanone	Ketone	sweet, citrus, green, soapy, spicy, earthy, woody	fruity, sweet, waxy, soapy, cheese, green herbal, coconut Mosciano, Gerard P&F 16, No. 5, 71, (1991) fresh sweet green weedy earthy herbal	Counet&Collin (2004) Kadow et al. (2013) Misnawi, Ariza (2011) Ramli et al. (2006)	1052
	Alcohols					
2,11	ethanol	Alcohol	alcoholic, solvent, chemical	strong, alcoholic, ethereal, medical	Quijano, C.,Pino, J. (2009) Tran et al. (2015)	463
3,69	2-methyl-3-buten-2-ol	Alcohol	fruity, sweetish, citrus, flowery	herbal, earthy, oily	-	600
5,7	2-pentanol	Alcohol	sweet, fruity, solvent	alcoholic, fusel, fermented, musty, sweet, winey, banana, ripe, banana, apple (Mosciano, Gerard, (2009)) mild, green, oily, fusel, fermented	Counet&Collins (2004) Kadow et al. (2013) J. Rodriguez-Campos et al. (2011, 2012) (green, mild) Tran et al. (2015)	681

Appendix 1 (part 3) extended version of table 1 with detailed overview of studies and odour description of compounds in literature; DF= Detection frequency; how often a chemical compound is detected in other studies, KI = Kovats Index from NIST library

8,5	3-methyl-1-butanol	Alcohol	banana, flowery, citrus, honey (TfC) chocolate, earthy, forest, woody, musty, nutty (IC)	fusel, oil, alcoholic, whiskey, fruity, banana fusel, oil, alcoholic, pungent, fruity, banana, molasses, ethereal, cognac (Mosciano, Gerard P&F 22, No. 4, 75, (1997))	Ascrizzi et al. (2017) J. Rodriguez-Campos et al. (2012) (malty, bitter, chocolate) van Praag et al. (1968) Ziegleder, G. (1991)	697
9,1	2-[(Z)-octadec-9-enoxy]ethanol	Alcohol	fruity, sweet, green			2336
12,44	2-heptanol	Alcohol	soapy, flowery/parfumy, green, fruity, citrus	fresh, lemon, grass, herbal, sweet, floral, fruity, green	Ascrizzi et al. (2017) (citrus, fresh, lemongrass-like) Counet&Collin (2004) Counet (2002) Frauendorfer & Schieberle (2006) Ramli et al. (2006)	879
14,39	1,2-oxolinalool	Alcohol	fruity, citrus, green, sweet, chemical	-	-	1133
16,45	2-nonanol	Alcohol	green, savory, herbal, vegetal, green leaves, green pepper	waxy, green, creamy, citrus, orange, cheesy, fruity (Mosciano, Gerard P&F 15, No. 2, 69, (1990))	Misnawi, Ariza (2011)	1078
16,6	1,2-dihydrolinalool	Alcohol	green, herbal, grassy, peppery, woody	bois de rose, woody, citrus, blueberry, weedy (Luebke, William tgsc, (1994))	-	1092
21,85-22,16	phenylmethanol	Alcohol	vanilla, sweet, flowery, green	floral, rose, phenolic, balsamic Luebke, William tgsc, (1981) sweet, floral, fruity, chemical Mosciano, Gerard P&F 15, No. 4, 59, (1990)	Afoakwa et al. (2008) Counet&Collin (2004) & Counet 2002 Ducki et al. (2008) Quijano, C.,Pino, J. (2009) J. Rodriguez-Campos et al. (2012) sweet, floral Ziegleder, G. (1991)	1036

Appendix 1 (part 4) extended version of table 1 with detailed overview of studies and odour description of compounds in literature; DF= Detection frequency; how often a chemical compound is detected in other studies, KI = Kovats Index from NIST library

22,35	2-phenylethanol	Alcohol	smokey, burnt, woody, vanilla, flowery (TfC) sweet, fruity, flowery, hay (IC)	floral, rose, dried rose, flower, rose water Luebke, William tgsc, (1981) sweet, floral, fresh, bready, rosey honey Mosciano, Gerard P&F 18, No. 4, 51, (1993)	Afoakwa et al. (2008) (caramel-like, sweet, honey) Ascrizzi et al. (2017) (flowery, spicy, honey-like, rose) Counet 2002 Ducki et al. (2008) Frauendorfer & Schieberle (2006) flowery Misnawi, Ariza (2011) Jinap et al. (1998) J. Rodriguez-Campos et al. (2012) honey, spice, rose, lilac, flowery, caramel Schnermann, P. Schieberle, P. (1997) sweet, yeast-like van Praag et al. (1968) Ziegleder, G. (1991)	1136
23,45-23,60	phenol	Phenol	soapy, flowery, vanilla, fruity	phenolic, plastic, rubbery	Afoakwa et al. (2008) Counet 2002 van Praag et al. (1968)	
	Esters					
2,27	propyl acetate	Esters	glue, fruity, sweet, flowery	solvent, celery, fruity, fusel, raspberry, pear solvent, pungent, fusel, sweet, fruity Mosciano, Gerard P&F 21, No. 5, 49, (1996)		686

Appendix 1 (part 5) extended version of table 1 with detailed overview of studies and odour description of compounds in literature; DF= Detection frequency; how often a chemical compound is detected in other studies, KI = Kovats Index from NIST library

3,95	ethyl 3-methylbutanoate	Esters	nail polish, glue, fruity	fruity, sweet, apple, pineapple, tutti frutti Luebke, William tgsc, (1987) sweet, diffusive, estry, fruity, sharp, pineapple, apple, green, orange Mosciano, Gerard P&F 23, No. 2, 43, (1998)	Quijano, C.,Pino, J. (2009)	820
5,41	3-methylbutyl acetate	Esters	banana, ice candy (TfC) fruity, flowery, sweet (IC)	sweet, fruity, banana, solvent sweet, banana, fruity, ripe, estery (Mosciano, Gerard P&F 16, No. 6, 43, (1991))	Ascrizzi et al. (2017) Counet&Collin (2004) Jinap et al. (1998) Misnawi, Ariza (2011) Quijano, C.,Pino, J. (2009) Ramos et al. (2014) fruity, banana J. Rodriguez-Campos et al. (2012) van Praag et al. (1968)	820
10,31	2-heptanol acetate	Esters	fruity, sweet, solvent, glue, green, flowery	green, waxy, fatty, citrus, aldehydic, winey, woody (Mosciano, Gerard P&F 21, No. 5, 49, (1996)) fresh, green, rum, ripe, fruit, pear, apricot, woody Luebke, William tgsc, (1984)	Ascrizzi et al. (2017) Kadow et al. (2013)	1019
14,52	ethyl octanoate	Esters	green, herbal, vegetal, savory, green peas, forest	fruity, wine, waxy, sweet, apricot, banana, brandy, pear Luebke, William tgsc, (1986) waxy, sweet, musty, pineapple, fruity, creamy, dairy Mosciano, Gerard P&F 22, No. 2, 69, (1997)	Ascrizzi et al. (2017) (fruity, floral, pineapple) Bonvechi(2005) fruity, floral, pineapple Jinap et al. (1998) Counet 2002 Misnawi, Ariza (2011) Quijano, C.,Pino, J. (2009) J. Rodriguez-Campos et al. (2012) fruity, floral	1183

Appendix 1 (part 6) extended version of table 1 with detailed overview of studies and odour description of compounds in literature; DF= Detection frequency; how often a chemical compound is detected in other studies, KI = Kovats Index from NIST library

	Acids					
14,99	acetic acid	Esters	acid, sour, pungent, musty, sour dough bread	sharp, pungent, sour, vinegar	Afoakwa et al. (2008) (astringent, vinegar) Ducki et al. (2008) Misnawi, Ariza (2011) J. Rodriguez-Campos et al. (2012) Schnermann, P., Schieberle, P. (1997) (sour) Tran et al. (2015) (sour, vinegar-like) Ziegleder, G. (1991)	576
19,2	3-methylbutanoic acid	Acids	cheesy, musty, sour, sweet, rotten, yeasty	sour, stinky feet, sweaty, cheese, tropical Luebke, William tgsc, (1989) cheese, dairy, acidic, sour, pungent, fruity, stinky, ripe, fatty, fruity Mosciano, Gerard P&F 18, No. 5, 39, (1993)	Ascrizzi et al. (2017) (rancid, cheesy) Bonvechi(2005) (rancid, cheesy, faecal, hammy) Counet&Collin (2004) Ducki et al. (2008) Frauendorfer & Schieberle (2006) (sweaty) Misnawi, Ariza (2011) J. Rodriguez-Campos et al. (2012) (sweat, acid, rancid) Tran et al. (2015) (unpleasant blue cheese, sweaty) van Praag et al. (1968) Ziegleder, G. (1991)	811

Appendix 1 (part 7) extended version of table 1 with detailed overview of studies and odour description of compounds in literature; DF= Detection frequency; how often a chemical compound is detected in other studies, KI = Kovats Index from NIST library

	Pyrazines and Pyrroles					
12,62	2,3-dimethylpyrazine	Pyrazine	sweet, vanilla, honey, musty, nutty	nutty, coffee, peanut, butter, walnut, caramel, leather musty, nut skin, cocoa, powdery, roasted, potato, coffee (Mosciano, Gerard P&F 21, No. 6, 49, (1996))	Afoakwa et al. (2008) (cooked, nutty) Ascrizzi et al. (2017) (caramel, cocoa) Bonvechi(2005) (caramel, cocoa, sweet chocolate) Counet&Collin (2004) Counet (2002) (hazelnut, roasted) Ducki et al. (2008) Jinap et al. (1998) Ramli et al. (2006) Tran et al. (2015) (hazelnut, roasted, baked) van Praag et al. (1968) Ziegleder, G. (1991)	894
13,9	2,3,5-trimethylpyrazine	Pyrazine	vanilla, earthy, musty, roasted, sweet	nutty, musty, powdery, cocoa, potato, musty Mosciano, Gerard P&F 15, No. 6, 35, (1990) nutty, musty, earthy, powdery, cocoa, roasted, peanut	Afoakwa et al. (2008) (cocoa, roasted, cooked) Ascrizzi et al. (2017) (earthy, peanuts, cocoa, roasted nuts) Bonvechi(2005) (cocoa, rusted nuts, peanut) Counet&Collin (2004) Counet (2002)Ducki et al. (2008) Jinap et al. (1998) Frauendorfer & Schieberle (2006) (earthy) Ramli et al. (2006) Schnermann, P. Schieberle, P. (1997) (earthy, potato-like) Tran et al. (2015) van Praag et al. (1968) Ziegleder, G. (1991)	1008

Appendix 1 (part 8) extended version of table 1 with detailed overview of studies and odour description of compounds in literature; DF= Detection frequency; how often a chemical compound is detected in other studies, KI = Kovats Index from NIST library

15,3	2,3,5,6-tetramethylpyrazine	Pyrazine	musty, earthy, chocolate	musty, nutty, chocolate, coffee, cocoa, soybean, lard, burnt nutty, musty, vanilla, dry, brown, cocoa (Mosciano, Gerard P&F 22, No. 2, 69, (1997))	Afoakwa et al. (2008) (milk-coffee, roasted) Ascrizzi et al. (2017) (roasted, green, coffee, cocoa) Bonvechi(2005) (chocolate, cocoa, coffee) Counet&Collin (2004) Counet 2002 (milk coffee, mocha, roasted, green) Ducki et al. (2008) Jinap et al. (1998) Misnawi, Ariza (2011) Ramli et al. (2006) J. Rodriguez-Campos et al. (2012) Tran et al. (2015) (milk coffee, mocha, roasted, green) van Praag et al. (1968) Ziegleder, G. (1991)	1121
16,23	3,5-diethyl-2-methylpyrazine	Pyrazine	earthy, herbal, green, vegetal, green leaves, woody, mushroom	nutty, meaty, vegetable	Counet (2002) (cocoa, chocolate, rum, sweet, roasted) Jinap et al. (1998)	1206
23	1-(1H-pyrrol-2-yl)ethanone	Pyrrole	overripe mango, grassy, woody, musty, smoky	musty, nutty, coumarin, licorice, walnut, bread musty, nutty, coumarinic (Mosciano, Gerard P&F 16, No. 2, 49, (1991))	Bonvechi (2005) (bread, walnut, licorice) Counet&Collin (2004) Counet 2002 (cocoa, chocolate, hazelnut, roasted) Ducki et al. (2008) Misnawi, Ariza (2011) Tran et al. (2015) (popcorn-like, musty, nutty-like, coumarin-like) J. Rodriguez-Campos et al. (2012) (chocolate, hazelnut) Ziegleder, G. (1991)	1035

Appendix 1 (part 9) extended version of table 1 with detailed overview of studies and odour description of compounds in literature; DF= Detection frequency; how often a chemical compound is detected in other studies, KI = Kovats Index from NIST library

	Furan					
14,91	cis-linalool oxide	Furan	musty, peas, earthy, vegetal	earthy, floral, sweet, woody	Afoakwa et al. (2008) (fruity, flowery/floral) Ascrizzi et al. (2017) (sweet, nutty) Bonvechi(2005) (nutty) Counet 2002 Quijano, C.,Pino, J. (2009) Ziegleder, G (1990) Ziegleder, G. (1991)	1164
	Hydrocarbons					
3,4	toluene	Hydro-carbons	glue, fruity, sweet, alcoholic	sweet	Afoakwa et al. (2008) Counet&Collin (2004) Counet 2002	794
	Oxime					
20,69	methyl (Z)-N-hydroxybenzenecarboximidate	Oxime	fruity, earthy, woody, sweet/vanilla	-	-	1301
	Alkanes and Alkenes					
0,99	2,2,4-trimethylpentane	Alkane	musty, putrid, sulfur	-	-	668
2,88	2,6-Dimethyl-2-octene	Alkene	glue, sweet, fruity, vanilla, solvent	-	-	936

Appendix 2 integrated peak area of each chemical compound of all investigated cocoa sample; repetition one and two is listed separately; integration was performed with the integration tool in the ChemStation software

Chemical group	Compound	FC0-Rep 1	FC0-Rep 2	IC1-Rep 1	IC1-Rep 2	IC2-Rep 1	IC2-Rep 2	IC3-Rep 1	IC3-Rep 2	IC4-Rep 1	IC4-Rep 2	IC5-Rep 1	IC5-Rep 2	IC6-Rep 1	IC6-Rep 2	IC7-Rep 1	IC7-Rep 2	TfC1-Rep 1	TfC1-Rep 2	TfC2-Rep 1	TfC2-Rep 2
Acids	acetic acid	8107535	6642745	7012007	10287237	88340580	70918951	107509792	104793791	124878159	96992558	171599586	96151545	229317684	129439324	195014923	128557958	163936785	220003962	112062582	125589361
Acids	3-methylbutanoic acid	233095	163563	375659	170665	364093	821261	234548	326889	320283	347501	213926	301708	207605	235071	257701	257709	31251446	30691783	24042999	29245683
Alcohol	ethanol	20824171	11156722	20850462	19656487	8668454	6416655	5125627	1968958	2531261	2316523	1219467	3323341	443211	1269953	407718	817607	296193	228738	140766	124022
Alcohol	2-methyl-3-buten-2-ol	4528776	2789388	1441140	2131907	258863	423105	242727	167056	159641	150876	131950	179540	154125	136695	308218	319215	205040	172416	92052	63557
Alcohol	2-pentanol	40930315	27736913	11186100	13143863	4628683	4645364	1526459	1137300	1190816	1172349	816609	1182442	265970	424874	365292	393663	1218998	1243223	687037	757294
Alcohol	3-methyl-1-butanol	5887536	3377925	8231287	4620857	193976	891850	60815	675327	86979	123740	60097	115853	65778	80743	62538	98195	1562311	1549808	1015907	1305794
Alcohol	2-[(Z)-octadec-9-enoxy]ethanol	636081	688669	599787	962318	760624	922461	467726	644289	400386	822971	780280	310022	661171	615515	620907	716793	359742	501054	222875	368391
Alcohol	2-heptanol	16484486	13208507	11637333	14124216	11282222	5652877	6342527	4714978	4985132	7996837	4668249	5923257	3779680	4536527	4510168	6723474	2190857	3077609	2441886	2648051
Alcohol	1,2-Oxolinalool	780075	527384	317187	422188	971365	553803	755538	523595	477344	595069	483158	622898	472308	503686	396427	383176	440057	548691	392717	403184
Alcohol	2-nonanol	1316625	589485	963859	1268146	1771456	335442	852348	370178	1529780	623378	1000207	414287	936359	670312	461194	647331	1172871	2355984	1031255	665485
Alcohol	1,2-Dihydrolinalool	1243819	415527	888275	1289548	1436894	797007	1373319	913208	583109	893513	1450702	1047308	1525710	878240	801434	1410416	624827	1848917	568489	947827
Alcohol	phenylmethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1167942	316806	369639	282395
Alcohol	2-phenylethanol	7823763	3236800	14686499	8037806	729500	422121	264463	252330	361889	210509	258809	229555	215696	181944	263661	173763	7578957	8094080	6000454	6311155
Aldehydes	2-methylpropanal	219210	68205	122963	32155	196265	403472	270415	248147	457895	342795	280768	322990	373181	314569	255435	372219	90612	35649	38371	27368
Aldehydes	3-methylbutanal	1614324	346666	642847	194381	2934664	2272154	2391765	1930584	2627974	2090466	2789842	1949380	3289830	1811879	2013369	2390647	522399	468529	381082	370303
Alkane	2,2,4-trimethylpentane	147752734	66478196	145368925	95885907	25505838	5753898	23732085	5947594	14748754	7337931	16576583	4696828	8146458	6762589	8480511	3741817	84725313	37096214	79988709	22670683
Alkene	2,6-dimethyl-2-octene	2864944	788881	666824	1878540	3339223	1344996	2045510	2444796	1141119	1191053	2783466	2059814	2751785	1445450	1547919	2784292	920347	849774	624018	718112
Esters	propyl acetat	87300	41003	63949	84319	165938	308230	186834	216207	97687	192983	163525	374237	276639	221806	1238472	1357456	195181	63486	38482	26604
Esters	ethyl 3-methylbutanoate	0	33690	36304	48647	56644	35296	25964	40972	45846	29876	18806	39006	33884	26202	22781	55416	185158	194780	153523	161080
Esters	3-methylbutyl acetate	268475	115906	174470	112358	77342	54573	69472	61103	60053	56061	74279	39977	140394	38799	63946	49390	9620752	10433111	5011644	6829095
Esters	2-heptanol acetate	653583	448833	397004	414418	774220	556998	610367	511140	734765	811551	663501	455652	528590	459388	611097	716016	2343696	4301230	3016379	2708505
Esters	ethyl octanoate	273509	71190	233134	237358	874994	227128	387215	231415	1045970	321170	327740	299201	340076	252434	225811	269062	1162662	868571	289024	336764
Furan	cis-Linalool Oxide	812984	566803	318100	631462	777658	417300	443511	158491	189778	343234	852	190127	1955	94781	6068	4705	5933	8481	26541	8791
Hydrocarbons	toluene	686413	369462	629345	459514	768058	505826	912778	437028	746961	470394	744990	519484	1294806	521500	923950	610427	209901	498292	583390	359654
Ketones	2-pentanone	13153049	8087845	2225666	2313080	453400	626706	303091	416881	202979	392766	292622	421849	384117	278493	119569	485534	1570893	730391	692142	396917
Ketones	2-heptanone	10326900	9365692	5238307	5095145	5664305	3483173	3784032	2908811	3313515	5465727	2593128	3836893	2257488	2517935	2510893	4323187	2515333	2659242	2358097	2821335
Ketones	2-nonanone	2070353	1494430	1389365	1977918	1685742	1029019	2295115	678355	906146	2445939	2629970	824637	2818008	1015169	888180	1839111	1890969	7337513	2752682	2721925
Oxime	methyl (Z)-N-hydroxybenzenecarboximidate	1553613	212071	819968	517725	832417	610091	877287	576735	834971	399192	528465	924030	752641	508849	859925	883482	1713283	1208295	1757488	1569401
Phenol	phenol	384652	154310	226229	200869	650948	80779	52665	73276	1393142	155239	160948	64522	124900	56002	90503	82023	176956	27322	146253	26202
Pyrazine&Pyrroles	2,3-dimethylpyrazine	318185	166583	997590	829229	1823725	1186322	815797	253780	257027	312918	235131	615213	115357	155571	116766	125333	638611	571982	426972	533429
Pyrazine&Pyrroles	2,3,5-trimethylpyrazine	239517	141660	83117	110623	233088	113676	198332	144311	126654	128157	173215	168018	143485	137099	139502	125136	2626712	2655176	1259099	1812263
Pyrazine&Pyrroles	2,3,5,6-tetramethylpyrazine	1279586	1452712	487152	819048	346909	537571	550235	521723	216925	482688	379552	545441	362608	401014	252640	391372	70759728	70258575	24543416	34390958
Pyrazine&Pyrroles	3,5-diethyl-2-methylpyrazine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	304290	305065	111233	147346
Pyrazine&Pyrroles	1-(1H-pyrrol-2-yl)ethanone	192863	114363	261670	69194	308317	201866	200154	127487	202169	127734	141483	421800	159853	139131	142672	142053	398518	283055	178266	248634

Appendix 3 (part 1) Calculation of flavour scores; M =mean value of intensity rating, DF= detection frequency; FS = flavour score

			FC0- Rep1			FC0- Rep2			IC1- Rep1			IC1- Rep2			IC2- Rep1			IC2- Rep2			IC3- Rep1			IC3- Rep2			IC4- Rep1		
Retention time	Odour description	Chemical comound	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS
0,99	musty, putrid, sulfur	2,2,4-trimethylpentane	1,9	5,0	9,5	2,0	3,0	6,0	2,2	6,0	13,2	2,1	6,0	12,5	1,2	3,0	3,5	1,4	4,0	5,5	2,0	3,0	6,0	1,8	6,0	11,0	1,4	5,0	7,0
1,33	cocoa, chocolate, roasted, nutty, vanilla	2-methylpropanal	0,0	0,0	0,0	3,0	1,0	3,0	0,0	0,0	0,0	0,0	0,0	0,0	2,6	5,0	13,0	2,5	4,0	10,0	2,6	4,0	10,5	2,4	4,0	9,5	2,2	5,0	11,0
1,81	cocoa, chocolate, roasted, caramel	3-methylbutanal	2,0	4,0	8,0	2,7	6,0	16,0	2,1	5,0	10,5	2,6	5,0	13,0	2,8	6,0	16,5	3,7	6,0	22,0	3,5	6,0	21,0	3,0	5,0	15,0	2,9	6,0	17,5
2,11	alcoholic, solvent, chemical	ethanol	1,8	4,0	7,0	2,8	6,0	17,0	1,8	5,0	9,0	2,6	5,0	13,0	1,5	6,0	9,0	2,5	4,0	10,0	2,6	4,0	10,5	2,2	5,0	11,0	1,7	6,0	10,0
2,27	glue, fruity, sweet, flowery	propyl acetat	1,5	2,0	3,0	2,2	5,0	11,0	1,5	2,0	3,0	1,5	2,0	3,0	0,0	0,0	0,0	1,3	3,0	4,0	2,0	3,0	6,0	2,0	3,0	6,0	1,8	2,0	3,5
2,47	Vanilla, sweet, butter, caramell, fruity	2-pentanone	1,0	3,0	3,0	2,0	2,0	4,0	2,1	5,0	10,5	3,0	4,0	12,0	1,3	2,0	2,5	3,2	3,0	9,5	2,5	3,0	7,5	2,6	4,0	10,5	1,9	6,0	11,5
2,88	glue, sweet, fruity, vanilla	2,6-dimethyl-2-octene	1,0	3,0	3,0	1,0	1,0	1,0	1,0	1,0	1,0	2,0	1,0	2,0	1,0	2,0	2,0	0,0	0,0	0,0	1,7	6,0	10,0	2,0	1,0	2,0	1,5	3,0	4,5
3,4	glue, fruity, sweet, alcoholic	toluene	1,0	2,0	2,0	2,5	3,0	7,5	1,6	5,0	8,0	2,1	4,0	8,5	1,0	2,0	2,0	1,0	1,0	1,0	1,8	5,0	9,0	0,0	0,0	0,0	1,5	4,0	6,0
3,69	fruity, sweetish, citrus, flowery	2-methyl-3-buten 2-ol	2,1	4,0	8,5	2,8	6,0	16,5	2,2	6,0	13,0	2,6	6,0	15,5	1,8	5,0	9,0	2,8	6,0	17,0	2,9	5,0	14,5	2,8	6,0	17,0	2,3	6,0	14,0
3,9	nail polish, glue, fruity	ethyl 3-methylbutanoate	1,8	6,0	11,0	2,8	6,0	16,5	2,3	6,0	14,0	2,3	5,0	11,5	1,6	6,0	9,5	2,6	5,0	13,0	2,4	6,0	14,5	2,0	4,0	8,0	2,1	4,0	8,5
5,41	banana, ice candy (I & J) fruity, flowery, sweet	3-methylbutyl acetate	2,0	1,0	2,0	2,3	5,0	11,5	1,0	1,0	1,0	2,0	1,0	2,0	0,0	0,0	0,0	1,0	1,0	1,0	1,3	2,0	2,5	1,5	2,0	3,0	0,0	0,0	0,0
5,7	sweet, fruity, solvent	2-pentanol	2,3	3,0	7,0	0,0	0,0	0,0	1,8	4,0	7,0	1,9	5,0	9,5	1,2	5,0	6,0	2,3	3,0	7,0	2,2	5,0	11,0	1,9	5,0	9,5	1,2	6,0	7,0
7,16	fruity, sweet/candy, nail polish, flowery	2-heptanone	1,0	1,0	1,0	1,8	4,0	7,0	1,0	2,0	2,0	1,3	3,0	4,0	1,0	1,0	1,0	1,7	3,0	5,0	1,5	2,0	3,0	1,5	4,0	6,0	1,0	2,0	2,0
8,58	Banana, flowery, citrus, honey (I & J) chocolate, earthy, forest, woody, musty, nutty	3-methyl-1-butanol	1,0	2,0	2,0	2,5	2,0	5,0	2,0	4,0	8,0	1,5	2,0	3,0	1,0	2,0	2,0	1,5	2,0	3,0	1,7	3,0	5,0	1,3	2,0	2,5	1,0	2,0	2,0
9,11	fruity, sweet, green	2-[(Z)-octadec-9-enoxy]ethanol	1,0	3,0	3,0	0,0	0,0	0,0	1,0	1,0	1,0	1,0	2,0	2,0	1,0	3,0	3,0	2,0	2,0	4,0	1,7	3,0	5,0	1,7	3,0	5,0	1,0	4,0	4,0
10,31	fruity, sweet, solvent, glue, green, flowery	2-heptanol acetate	1,4	5,0	7,0	2,6	4,0	10,5	2,0	6,0	12,0	1,9	4,0	7,5	2,1	5,0	10,5	2,5	5,0	12,5	2,7	6,0	16,0	2,4	5,0	12,0	1,7	6,0	10,0
12,44	soapy, flowery/parfumy, green, fruity, citrus	2-heptanol	2,5	6,0	15,0	3,1	6,0	18,5	2,6	6,0	15,5	2,7	6,0	16,0	2,5	6,0	15,0	2,8	6,0	16,5	2,9	5,0	14,5	2,3	6,0	14,0	2,1	5,0	10,5

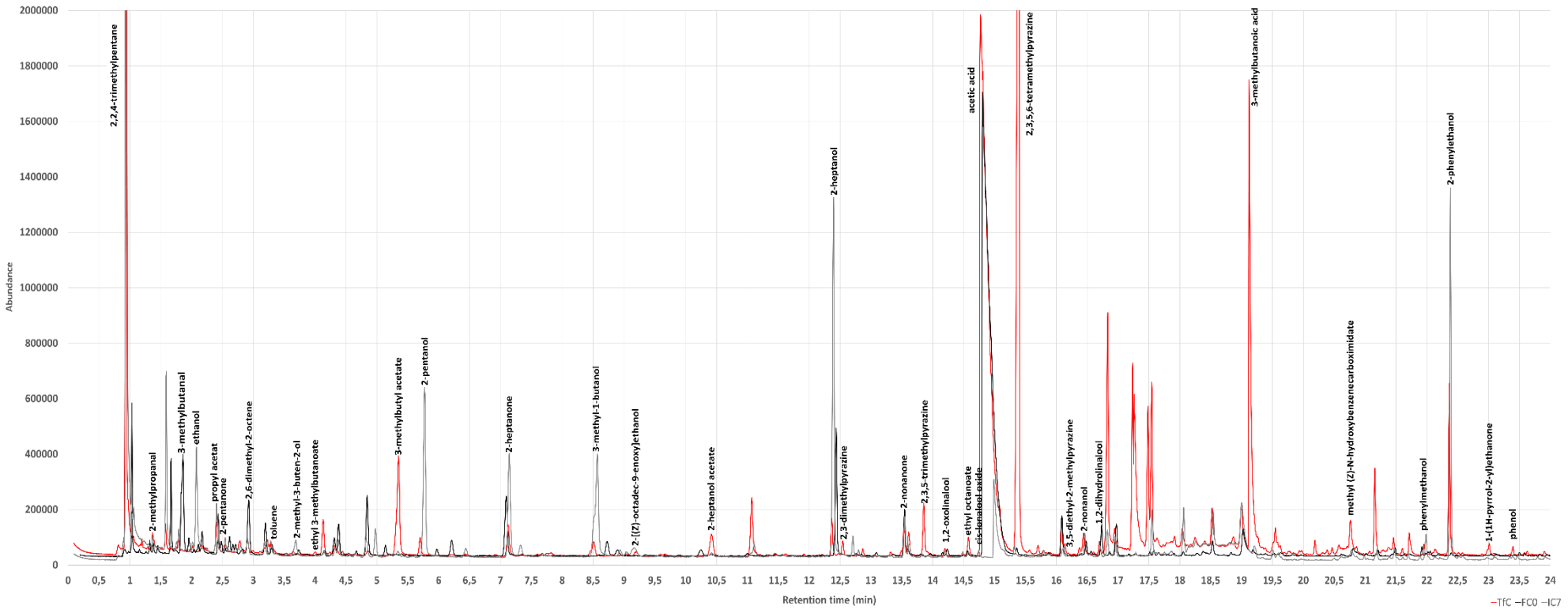
			IC4- Rep2			IC5- Rep1			IC5- Rep2			IC6- Rep1			IC6- Rep2			IC7- Rep1			IC7- Rep2			TfC1- Rep1			TfC1- Rep2			TfC2- Rep1			TfC2- Rep2		
Retention time	Odour description	Chemical comound	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS
0,99	musty, putrid, sulfur	2,2,4-trimethylpentane	2,7	5,0	13,5	1,8	5,0	9,0	2,3	6,0	13,5	2,3	4,0	9,0	2,0	5,0	10,0	1,8	5,0	9,0	3,1	4,0	12,5	1,6	4,0	6,5	2,0	3,0	6,0	1,2	5,0	6,0	2,0	4,0	8,0
1,33	cocoa, chocolate, roasted, nutty, vanilla	2-methylpropanal	2,8	5,0	14,2	2,3	4,0	9,0	3,3	6,0	19,5	2,4	6,0	14,5	2,6	5,0	13,0	2,5	3,0	7,5	3,8	5,0	19,0	0,0	0,0	0,0	3,0	1,0	3,0	0,0	0,0	0,0	1,8	2,0	3,5
1,81	cocoa, chocolate, roasted, caramel	3-methylbutanal	4,3	5,0	21,5	3,0	6,0	18,0	4,2	6,0	25,0	3,4	6,0	20,4	3,3	6,0	20,0	3,6	6,0	21,5	4,3	6,0	26,0	2,3	6,0	13,5	3,4	5,0	17,0	1,8	5,0	9,0	3,1	6,0	18,5
2,11	alcoholic, solvent, chemical	ethanol	3,3	5,0	16,5	1,3	3,0	4,0	2,9	6,0	17,5	2,7	4,0	10,7	2,1	6,0	12,5	3,1	6,0	18,5	2,8	5,0	14,0	1,8	2,0	3,5	2,8	5,0	14,0	1,5	4,0	6,0	2,8	3,0	8,5
2,27	glue, fruity, sweet, flowery	propyl acetat	2,3	4,0	9,0	1,0	1,0	1,0	2,1	4,0	8,5	1,0	1,0	1,0	1,0	2,0	2,0	1,7	3,0	5,0	2,0	1,0	2,0	2,0	2,0	4,0	2,7	5,0	13,5	2,4	4,0	9,5	2,2	5,0	11,0
2,47	Vanilla, sweet, butter, caramell, fruity	2-pentanone	2,3	5,0	11,5	1,5	4,0	6,0	2,1	3,0	6,4	1,5	2,0	3,0	2,0	5,0	10,0	1,8	3,0	5,5	2,6	4,0	10,5	2,3	6,0	14,0	4,4	5,0	22,0	2,8	4,0	11,0	3,8	4,0	15,0
2,88	glue, sweet, fruity, vanilla	2,6-dimethyl-2-octene	1,0	1,0	1,0	1,1	4,0	4,5	2,5	2,0	5,0	1,5	2,0	3,0	2,3	4,0	9,0	1,0	2,0	2,0	1,5	2,0	3,0	1,0	3,0	3,0	2,0	5,0	10,0	1,3	2,0	2,5	2,2	5,0	11,0
3,4	glue, fruity, sweet, alcoholic	toluene	1,8	2,0	3,5	1,2	3,0	3,5	1,3	2,0	2,5	1,3	2,0	2,5	0,0	0,0	0,0	1,8	2,0	3,5	0,0	0,0	0,0	1,5	1,0	1,5			0,0	1,3	2,0	2,5	1,0	2,0	2,0
3,69	fruity, sweetish, citrus, flowery	2-methyl-3-buten 2-ol	3,1	6,0	18,5	1,7	6,0	10,0	2,9	6,0	17,5	2,3	5,0	11,3	2,6	6,0	15,5	2,1	4,0	8,5	2,9	5,0	14,5	2,2	6,0	13,0	2,9	6,0	17,5	2,3	5,0	11,5	2,8	6,0	17,0
3,9	nail polish, glue, fruity	ethyl 3-methylbutanoate	3,4	6,0	20,5	1,8	4,0	7,0	3,4	5,0	17,0	2,5	2,0	5,0	2,1	4,0	8,5	2,1	4,0	8,5	2,7	5,0	13,5	2,3	5,0	11,5	2,7	5,0	13,5	2,1	6,0	12,5	3,3	5,0	16,5
5,41	banana, ice candy (I & J) fruity, flowery, sweet	3-methylbutyl acetate	2,0	2,0	4,0	0,0	0,0	0,0	1,0	1,0	1,0	1,0	3,0	3,0	1,0	1,0	1,0	0,0	0,0	0,0	2,3	3,0	7,0	2,1	5,0	10,5	3,2	5,0	16,0	1,9	4,0	7,5	2,8	3,0	8,5
5,7	sweet, fruity, solvent	2-pentanol	2,7	5,0	13,5	1,2	5,0	6,0	2,0	5,0	10,0	1,3	3,0	4,0	2,0	1,0	2,0	2,0	3,0	6,0	2,7	3,0	8,0	1,0	3,0	3,0	0,0	0,0	0,0	1,5	2,0	3,0	1,0	2,0	2,0
7,16	fruity, sweet/candy, nail polish, flowery	2-heptanone	1,6	6,0	9,6	1,0	2,0	2,0	2,3	4,0	9,0	2,0	2,0	4,0	1,5	5,0	7,5	1,3	3,0	4,0	1,3	2,0	2,5	1,4	4,0	5,5	1,6	4,0	6,5	1,3	5,0	6,5	1,3	2,0	2,5
8,58	Banana, flowery, citrus, honey (I & J) chocolate, earthy, forest, woody, musty, nutty	3-methyl-1-butanol	1,0	1,0	1,0	1,0	1,0	1,0	1,5	1,0	1,5	2,3	3,0	7,0	1,5	3,0	4,5	0,0	0,0	0,0	0,0	0,0	0,0	1,0	1,0	1,0	1,0	1,0	1,0	1,8	5,0	8,8	2,0	1,0	2,0
9,11	fruity, sweet, green	2-[(Z)-octadec-9-enoxy]ethanol	1,7	5,0	8,5	2,3	2,0	4,5	1,8	3,0	5,5	1,8	4,0	7,0	2,3	2,0	4,5	1,6	4,0	6,5	1,7	3,0	5,0	1,8	3,0	5,5	1,5	2,0	3,0		0,0	0,0	2,0	1,0	2,0
10,31	fruity, sweet, solvent, glue, green, flowery	2-heptanol acetate	2,9	6,0	17,5	2,4	5,0	12,0	2,8	6,0	16,5	2,3	6,0	14,0	2,3	5,0	11,5	2,6	6,0	15,5	2,8	6,0	16,5	2,5	4,0	10,0	3,7	3,0	11,0	2,1	5,0	10,5	2,3	5,0	11,5
12,44	soapy, flowery/parfumy, green, fruity, citrus	2-heptanol	2,4	5,0	12,0	2,3	3,0	7,0	3,2	3,0	9,5	2,5	2,0	5,0	2,7	3,0	8,0	2,0	5,0	10,0	3,0	5,0	15,0	1,0	2,0	2,0	1,8	2,0	3,5	2,3	2,0	4,5	1,3	2,0	2,5

Appendix 3 (part 2) Calculation of flavour scores; M =mean value of intensity rating, DF= detection frequency; FS = flavour score

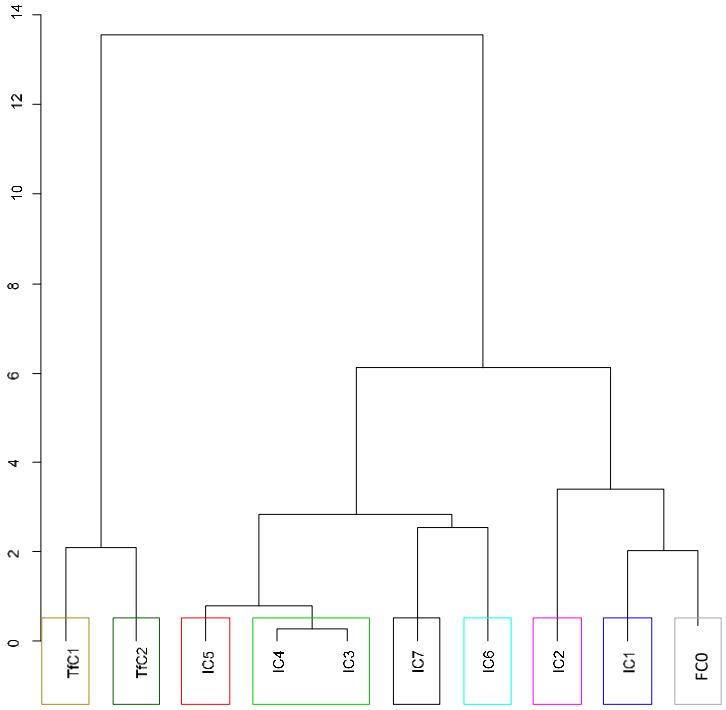
			FC0- Rep1			FC0- Rep2			IC1- Rep1			IC1- Rep2			IC2- Rep1			IC2- Rep2			IC3- Rep1			IC3- Rep2			IC4- Rep1			IC4- Rep2		
Retention time	Odour description	Chemical comound	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS
12,62	sweet, vanilla, honey, musty, nutty	2,3-dimethylpyrazine	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,0	4,0	8,0	1,5	2,0	3,0	0,0	0,0	0,0	2,0	2,0	4,0	0,0	0,0	0,0	0,0	0,0	0,0	2,0	2,0	4,0
13,51	sweet, citrus, green, soapy, spicy, earthy, woody	2-nonanone	1,5	2,0	3,0	2,3	4,0	9,0	1,3	2,0	2,5	2,0	2,0	4,0	2,5	2,0	5,0	2,0	1,0	2,0	1,8	2,0	3,5	1,0	2,0	2,0	1,0	1,0	1,0	2,5	2,0	5,0
14	vanilla, earthy, musty, roasted, sweet	2,3,5-trimethylpyrazine	2,0	1,0	2,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,5	1,0	1,5	0,0	0,0	0,0	2,0	1,0	2,0	1,7	3,0	5,0	1,3	3,0	4,0
14,39	fruity, citrus, green, sweet, chemical	1,2-Oxolinalool	1,7	5,0	8,5	2,3	3,0	7,0	1,5	2,0	3,0	2,5	2,0	5,0	1,3	4,0	5,0	2,1	4,0	8,5	1,7	3,0	5,0	2,0	2,0	4,0	1,7	3,0	5,0	1,0	1,0	1,0
14,52	green, herbal, vegetal, savory, green peas, forest	ethyl octanoate	2,9	6,0	17,5	4,0	6,0	24,0	3,0	6,0	18,0	3,5	6,0	21,0	3,0	6,0	18,0	3,8	6,0	22,5	3,3	6,0	19,5	3,8	6,0	22,5	3,3	6,0	20,0	3,6	5,0	18,0
14,91	musty, peas, earthy, vegetal	cis-Linalool Oxide	2,3	4,0	9,3	2,8	5,0	14,0	1,8	5,0	9,0	2,4	4,0	9,5	0,0	0,0	0,0	2,5	3,0	7,5	2,0	0,0	0,0	3,7	3,0	11,0	2,3	2,0	4,5	2,7	3,0	8,0
14,99	acid, sour, pungent, musty, sour dough bread	acetic acid	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,5	1,0	2,5	2,3	6,0	14,0	2,8	4,0	11,0	2,3	6,0	14,0	3,3	4,0	13,0	3,1	6,0	18,5	3,0	4,0	12,0
15,3	musty, earthy, chocolate	2,3,5,6-tetramethylpyrazine	0,0	0,0	0,0	0,0		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,8		0,0	3,0	1,0	3,0	0,0	0,0	0,0	0,0	0,0	0,0	1,7		0,0
16,23	earthy, herbal, green, vegetal, green leaves, woody, mushroom	3,5-diethyl-2-methylpyrazine	1,3	3,0	4,0	1,9	6,0	11,5	1,0	4,0	4,0	1,3	3,0	4,0	1,4	4,0	5,5	1,5	3,0	4,5	1,0	1,0	1,0	2,3	2,0	4,5	2,3	3,0	7,0	1,5	1,0	1,5
16,45	green, savory, herbal, vegetal, green leaves, green pepper	2-nonanol	2,7	5,0	13,5	3,6	4,0	14,5	2,7	5,0	13,5	2,3	4,0	9,0	3,8	4,0	15,0	3,1	5,0	15,5	2,5	3,0	7,5	3,3	3,0	10,0	2,3	3,0	7,0	2,7	3,0	8,0
16,6	green, herbal, grassy, peppery, woody	1,2-Dihydrolinalool	0,0	0,0	0,0	3,3	4,0	13,0	2,0	1,0	2,0	3,1	6,0	18,5	0,0	0,0	0,0	3,0	1,0	3,0	2,5	3,0	7,5	1,8	3,0	5,5	2,5	2,0	5,0	3,2	3,0	9,5
18,50-18,70	fruity, overripe, flowery (I & J) flowery, green, earthy, musty	1-phenylethanone	1,0	2,0	2,0	1,5	2,0	3,0	1,0	3,0	3,0	2,5	2,0	5,0	2,0	1,0	2,0	1,5	1,0	1,5	1,5	2,0	3,0	2,3	3,0	7,0	1,8	2,0	3,5	3,0	2,0	6,0
19,2	foul-smelling, cheesy (cheesy feet), rotten	3-methylbutanoic acid	0,0	0,0	0,0	1,7	3,0	5,0	1,0	1,0	1,0	0,0	0,0	0,0	1,5	1,0	1,5	2,4	4,0	9,5	0,0	0,0	0,0	1,0	1,0	1,0	0,0	0,0	0,0	0,0	0,0	0,0
20,69	fruity, earthy, woody, sweet/vanilla	methyl (Z)-N-hydroxybenzene carboximidate	1,3	3,0	4,0	2,0	1,0	2,0	1,0	1,0	1,0	1,5	2,0	3,0	2,2	5,0	11,0	1,8	3,0	5,5	1,5	2,0	3,0	2,1	5,0	10,5	1,3	3,0	4,0	2,0	3,0	6,0
21,85-22,16	vanilla, sweet, flowery, green	phenylmethanol	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
22,35	smokey, burnt, woody, vanilla, flowery (I & J) sweet, fruity, flowery, hay	2-phenylethanol	0,0	0,0	0,0	1,8	3,0	5,5	1,0	1,0	1,0	2,5	1,0	2,5	2,5	2,0	5,0	2,0	0,0	0,0	1,0	1,0	1,0	0,0	0,0	0,0	0,0	0,0	0,0	2,0	1,0	2,0
23	overripe Mango, grassy, woody, musty, smokey	1-(1H-pyrrol-2-yl)ethanone	0,0	0,0	0,0	1,9	4,0	7,5	1,5	1,0	1,5	1,3	3,0	4,0	1,5	2,0	3,0	0,0	0,0	0,0	1,5	1,0	1,5	2,3	2,0	4,5	1,0	3,0	3,0	1,0	1,0	1,0
23,45-23,60	soapy, flowery, vanilla, fruity	phenol	1,0	2,0	2,0	1,5	2,0	3,0	1,0	1,0	1,0	0,0	0,0	0,0	1,0	0,0	0,0	1,5	2,0	3,0	0,0	0,0	0,0	1,2	3,0	3,5	1,8	2,0	3,5	1,0	2,0	2,0

		IC5- Rep1			IC5- Rep2			IC6- Rep1			IC6- Rep2			IC7- Rep1			IC7- Rep2			TfC1- Rep1			TfC1- Rep2			TfC2- Rep1			TfC2- Rep2		
Retention time	Chemical comound	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS	M	DF	FS
12,62	2,3-dimethylpyrazine	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,0	1,0	2,0	0,0	0,0	0,0	4,0	1,0	4,0	3,0	2,0	6,0	1,4	4,0	5,5	2,0	3,0	6,0	2,4	4,0	9,5
13,51	2-nonanone	2,0	2,0	4,0	2,8	2,0	5,5	1,4	4,0	5,5	2,0	2,0	4,0	1,5	3,0	4,5	2,3	4,0	9,0	1,3	4,0	5,0	2,0	2,0	4,0	1,0	2,0	2,0	2,0	1,0	2,0
14	2,3,5-trimethylpyrazine	1,5	2,0	3,0	1,7	3,0	5,0	0,0	0,0	0,0	2,2	3,0	6,5	1,5	2,0	3,0	2,0	1,0	2,0	2,8	5,0	14,0	3,3	5,0	16,5	2,8	5,0	14,0	3,0	6,0	18,0
14,39	1,2-Oxolinalool	1,0	3,0	3,0	1,5	2,0	3,0	1,5	3,0	4,5	2,3	2,0	4,5	2,0	3,0	6,0	1,8	2,0	3,5	1,8	4,0	7,0	2,3	4,0	9,0	1,8	2,0	3,5	2,5	2,0	5,0
14,52	ethyl octanoate	3,1	6,0	18,5	4,3	6,0	26,0	3,1	5,0	15,6	4,0	6,0	24,0	3,8	6,0	22,5	3,9	6,0	23,5	3,1	6,0	18,5	3,2	5,0	16,0	3,0	6,0	18,0	3,3	5,0	16,5
14,91	cis-Linalool Oxide	0,0	0,0	0,0	3,5	3,0	10,5	0,0	0,0	0,0	2,8	3,0	8,5	4,0	1,0	4,0	3,3	2,0	6,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
14,99	acetic acid	3,0	6,0	18,0	3,3	4,0	13,0	3,3	4,0	13,3	2,8	4,0	11,0	3,7	6,0	22,0	3,6	6,0	21,5	3,1	6,0	18,5	3,4	5,0	17,0	3,1	5,0	15,5	2,8	6,0	16,5
15,3	2,3,5,6-tetramethylpyrazine	0,0	0,0	0,0		0,0	0,0	0,0	0,0	0,0		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,5	3,0	7,5	2,0	2,0	4,0	2,0	5,0	10,0	2,5	2,0	5,0
16,23	3,5-diethyl-2-methylpyrazine	1,3	3,0	4,0	2,0	2,0	4,0	3,0	1,0	3,0	2,0	2,0	4,0	1,5	2,0	3,0	1,0	1,0	1,0	1,5	3,0	4,5	2,0	1,0	2,0	1,7	5,0	8,5	2,0	5,0	10,0
16,45	2-nonanol	2,5	4,0	10,0	3,1	5,0	15,5	2,3	3,0	7,0	3,0	5,0	15,0	1,0	4,0	4,0	3,4	4,0	13,5	2,8	6,0	17,0	3,3	3,0	10,0	3,1	4,0	12,5	4,5	2,0	9,0
16,6	1,2-Dihydrolinalool	2,8	4,0	11,0	2,0	2,0	4,0	3,2	5,0	16,0	3,0	1,0	3,0	3,5	4,0	14,0	3,3	3,0	10,0	0,0	0,0	0,0	4,3	2,0	8,5	0,0	0,0	0,0	1,5	2,0	3,0
18,50-18,70	1-phenylethanone	1,5	2,0	3,0	2,0	2,0	4,0	1,0	2,0	2,0	2,3	3,0	7,0	2,0	3,0	6,0	2,3	3,0	7,0	2,3	2,0	4,5	3,0	2,0	6,0	0,0	0,0	0,0	1,8	3,0	5,5
19,2	3-methylbutanoic acid	1,4	3,0	4,3	2,5	3,0	7,5	1,5	2,0	3,0	1,3	2,0	2,5	1,8	2,0	3,5	0,0	0,0	0,0	4,0	6,0	24,0	4,3	6,0	26,0	4,3	6,0	25,5	3,8	6,0	22,5
20,69	methyl (Z)-N-hydroxybenzene carboximidate	1,5	2,0	3,0	2,0	4,0	8,0	2,0	3,0	6,0	2,0	1,0	2,0	1,0	2,0	2,0	1,8	2,0	3,5	1,5	2,0	3,0	2,0	1,0	2,0	2,5	2,0	5,0	0,0	0,0	0,0
21,85-22,16	phenylmethanol	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,1	6,0	18,5	1,2	3,0	3,5	2,8	6,0	17,0	2,5	2,0	5,0
22,35	2-phenylethanol	2,0	1,0	2,0	0,0	0,0	0,0	2,0	3,0	6,0	2,5	4,0	10,0	1,5	1,0	1,5	1,5	2,0	3,0	2,0	3,0	6,0	2,3	4,0	9,3	3,0	1,0	3,0	2,7	3,0	8,0
23	1-(1H-pyrrol-2-yl)ethanone	1,0	2,0	2,0	0,0	0,0	0,0	2,5	2,0	5,0	0,0	0,0	0,0	2,0	2,0	4,0	1,0	1,0	1,0	2,0	2,0	4,0	1,5	2,0	3,0	2,2	3,0	6,5	2,5	2,0	5,0
23,45-23,60	phenol	1,0	2,0	2,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	1,0	1,0	1,0	2,0	2,0	0,0	0,0	0,0	2,3	2,0	4,5	2,8	2,0	5,5	1,3	3,0	4,0	2,5	2,0	5,0

Appendix 4 Chromatograms of a cocoa sample from traditionally fermented sample (TfC, red), fresh cocoa (FC0, grey) and cocoa sample of seven days incubation (IC7, black) peaks of odour-active compounds are labelled



Appendix 5 Hierarchical clustering of traditionally fermented cocoa samples (TfC), fresh cocoa sample (FC0) and samples from fermentation-like incubation (IC1, IC2, IC3, IC4, IC5, IC6, IC7)



Appendix 6 ANOVA results of flavour scores for samples of traditional fermentation and day seven of incubated sample

Odours	p-value	signifi-cant
acid, sour	0,007	Yes
alcoholic, solvent	0,086	No
banana, chocolate	0,319	No
banana, Ice candy	0,116	No
cheesy, rotten	0,000	Yes
cocoa, caramel	0,055	No
cocoa, nutty	0,038	Yes
earthy, green leaves	0,206	No
fruity, candy	0,251	No
fruity, citrus	0,520	No
fruity, earthy	0,885	No
fruity, musty	0,295	No
fruity, solvent	0,001	Yes
fruity, sweet, green	0,153	No
fruity, sweetish	0,322	No
glue, alcoholic	0,861	No
glue, flowery	0,130	No
glue, vanilla	0,290	No
green	0,048	Yes
green pepper, savory	0,441	No
green, herbal	0,004	Yes
musty, earthy, choco-late	0,030	Yes
musty, peas	0,002	Yes
musty, putrid	0,033	Yes
nail polish	0,325	No
overripe, grassy, smo-key	0,216	No
smokey, woody, flo-wery	0,110	No
soapy, flowery	0,006	Yes
soapy, vanilla	0,009	Yes
sweet, citrus, green	0,120	No
sweet, fruity	0,015	Yes
sweet, vanilla, honey	0,062	No
vanilla, butter, caramel	0,121	No
vanilla, earthy, roasted	0,001	Yes
vanilla, sweet	0,135	No

Appendix 7 ANOVA results of flavour scores for samples of all incubated sample (IC1, IC2, IC3, IC4, IC5, IC6, IC7) and fresh cocoa (FC0)

Odours	p-value	significant
acid, sour	0,000	Yes
alcoholic, solvent	0,892	No
banana, chocolate	0,091	No
banana, ice candy	0,606	No
cheesy, rotten	0,318	No
cocoa, caramel	0,098	No
cocoa, nutty	0,043	Yes
earthy, green leaves	0,546	No
fruity, candy	0,956	No
fruity, citrus	0,150	No
fruity, earthy	0,477	No
fruity, musty	0,355	No
fruity, solvent	0,322	No
fruity, sweet, green	0,084	No
fruity, sweetish	0,935	No
glue, alcoholic	0,366	No
glue, flowery	0,630	No
glue, vanilla	0,473	No
green	0,839	No
green pepper, savory	0,433	No
green, herbal	0,943	No
musty, earthy, chocolate	0,493	No
musty, peas	0,755	No
musty, putrid	0,221	No
nail polish	0,829	No
overripe, grassy, smokey	0,978	No
smokey, woody, flowery	0,156	No
soapy, flowery	0,005	Yes
soapy, vanilla	0,691	No
sweet, citrus, green	0,631	No
sweet, solvent	0,192	No
sweet, vanilla, honey	0,859	No
vanilla, butter, caramel	0,216	No
vanilla, earthy, roasted	0,283	No

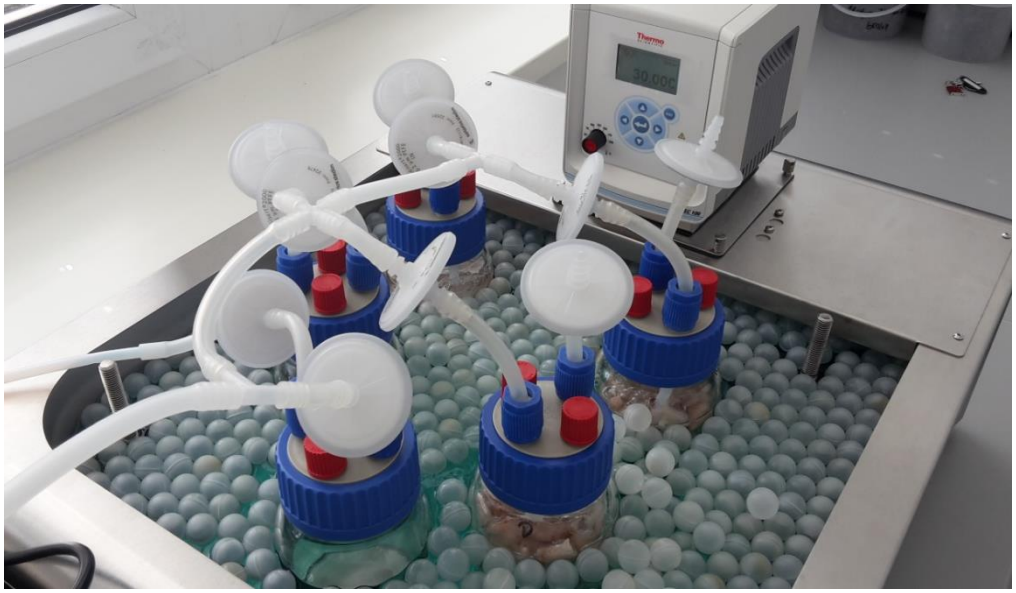
Appendix 8 ANOVA results of content (peak area) for samples of traditional fermentation and day seven of incubated sample

Chemical component	p-value	significant
1-(1H-pyrrol-2-yl)ethanone	0,122	No
1,2-dihydrolinalool	0,833	No
1,2-oxolinalool	0,353	No
1-phenylethanone	0,165	No
2,2,4-trimethylpentane	0,098	No
2,3,5,6-tetramethylpyrazine	0,051	No
2,3,5-trimethylpyrazine	0,018	Yes
2,3-dimethylpyrazine	0,003	Yes
2,6-dimethyl-2-octene	0,024	Yes
2-[(Z)-octadec-9-en-oxy]ethanol	0,027	Yes
2-heptanol	0,015	Yes
2-heptanol acetate	0,019	Yes
2-heptanone	0,223	No
2-methyl-3-buten-2-ol	0,022	Yes
2-methylpropanal	0,003	Yes
2-nonanol	0,244	No
2-nonanone	0,286	No
2-pentanol	0,054	No
2-pentanone	0,240	No
2-phenylethanol	0,001	Yes
3,5-diethyl-2-methylpyra-zine	0,047	Yes
3-methyl-1-butanol	0,003	Yes
3-methylbutanal	0,000	Yes
3-methylbutanoic acid	0,000	Yes
3-methylbutyl acetate	0,013	Yes
acetic acid	0,885	No
cis-linalool Oxide	0,311	No
ethanol	0,041	Yes
ethyl 3-methylbutanoate	0,000	Yes
ethyl octanoate	0,260	No
methyl (Z)-N-hydroxy-benzenecarboximidate	0,021	Yes
phenol	0,900	No
phenylmethanol	0,575	No
propyl acetat	< 0,0001	Yes
toluene	0,085	No

Appendix 9 ANOVA results of content (peak area) for samples of all incubated sample (IC1, IC2, IC3, IC4, IC5, IC6, IC7) and fresh cocoa (FC0)

Chemical component	p-value	significant
1-(1H-pyrrol-2-yl)ethanone	0,736	No
1,2-dihydrolinalool	0,844	No
1,2-oxolinalool	0,279	No
1-phenylethanone	0,034	Yes
2,2,4-trimethylpentane	0,010	Yes
2,3,5,6-tetramethylpyrazine	0,002	Yes
2,3,5-trimethylpyrazine	0,415	No
2,3-dimethylpyrazine	0,004	Yes
2,6-dimethyl-2-octene	0,729	No
2-[(Z)-octadec-9-en-oxy]ethanol	0,779	No
2-heptanol	0,007	Yes
2-heptanol acetate	0,102	No
2-heptanone	0,002	Yes
2-methyl-3-buten-2-ol	0,000	Yes
2-methylpropanal	0,057	No
2-nonanol	0,934	No
2-nonanone	0,995	No
2-pentanol	< 0,0001	Yes
2-pentanone	0,000	Yes
2-phenylethanol	0,007	Yes
3-methyl-1-butanol	0,002	Yes
3-methylbutanal	0,066	No
3-methylbutanoic acid	0,213	No
acetic acid	0,010	Yes
cis-linalool Oxide	0,014	Yes
ethanol	0,000	Yes
ethyl octanoate	0,507	No
methyl (Z)-N-hydroxy-benzenecarboximidate	0,992	No
phenol	0,523	No
phenylmethanol	0,253	No
propyl acetat	< 0,0001	Yes
toluene	0,874	No

Appendix 10 pictures of fermentation-like incubation - from top to bottom- sterilized cocoa fruits and sterilized instruments under a clean bench; equally distribution of cocoa seeds to sterile glass bottles; set-up of filled incubation glasses with sterile filter in a water bath with connected nitrogen flow



Appendix 11 – visual changes of cocoa seeds in incubation glasses from time zero, day one (IC1), day two (IC2), day three (IC3) and day four(IC4) (from left to right, from top to bottom)

Appendix 12 – visual changes of cocoa seeds in incubation glasses from day five (IC5), day six (IC6) and day seven (IC7) (from left to right)

Eidesstattliche Erklärung

Hiermit erkläre ich, dass die von mir eingereichtete Masterarbeit mit dem Title „**Precursors of chocolate aroma – flavour profile comparisons of traditionally fermented cocoa and cocoa beans from fermentation-like incubation by means of HS-SPME-GC-MS-O“** selbständig verfasst und ausschließlich die angegebenen Hilfsmittel und Quellen verwendet habe. Wörtlich oder dem Sinn nach aus anderen Werken entnommene Stellen sind unter Angabe der Quellen kenntlich gemacht.

Ort, Datum	Franziska Sobotta