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Investigating time dependent cocoa bean fermentation by ESI-FT-ICR mass spectrometry

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ABSTRACT

A cocoa bean fermentation series, comprising bean samples taken every 24 h of a seven-day cocoa fermentation was analyzed by ultra-high-resolution mass spectrometry using the ESI-FT-ICR technique. Data were acquired in both positive and negative ion mode. At early fermentation times around 2000 signals could be resolved raising to a maximum of 8000 signals in each ion mode towards the end of the fermentation. Molecular formulae were assigned to a large number of observed peaks leading to ion statistics classifying cocoa constituents according elemental composition. Time dependent van Krevelen diagrams were constructed allowing the tracking of chemical changes in bean composition. In particular, the number of CHON compounds assigned to short peptides and their derivatives were shown to undergo significant chemical transformations. Theoretical MS data bases were constructed containing all possible peptides up to a length of ten amino acids and putative derivatives expected to be formed during microbial fermentation. Experimental *m/z* values were compared to these databases and conclusions drawn with respect to the composition of fermenting beans with tentative fermentation products suggested.

1. Introduction

Cocoa powder and resulting chocolate products constitute the most highly processed food consumed by humans (Rusconi & Conti, 2010). Cocoa production starts with the harvesting of cocoa pods their opening followed by fermentation. In fermentation, wet cocoa beans are exposed to microorganisms in so-called heap or box fermentation (De Vuyst & Weckx, 2016). Microorganisms used in cocoa fermentation usually originate from the environment present in origin countries with a high diversity of species employed. On rare occasions defined starter cultures are used as well (Lefeber, Papalexandratou, Gobert, Camu, & De Vuyst, 2012). In fermentation yeasts are initially colonizing the fermentation heap, converting the sugar rich pulp, surrounding the wet beans, into ethanol. In a second anaerobic phase lactic acid bacteria convert pulp sugars to lactic acid. In a last anaerobic phase, acetic acid bacteria convert ethanol to acetic acid. During fermentation the temperature of the fermentation heap rises due to exothermic microbial metabolism to temperatures above 50 °C, the color of the beans changes from white to purple or brown, while the pH drops to between 4 and 4.5 (John et al., 2016). The primary microbial fermentation products ethanol, lactic and acetic acid permeate into the bean, inducing an avalanche of biochemical reactions and thus significantly altering the wet beans' chemical composition. A general defense reaction within the bean is triggered during microbial colonization, releasing stress response factors such as jasmonic acid derivatives along further antimicrobial defense compounds, most importantly polyphenols (D'Souza et al., 2017a; Fayeulle et al., 2019). Concomitantly cocoa proteases initiate degradation of storage proteins such as vicilin and albumin into shorter oligopeptides. More than 800such oligopeptides produced during fermentation have been characterized recently (D'Souza et al., 2018). Whether microorganisms, selected microbial enzymes or metabolites penetrate into the bean and play a crucial role in facilitating the fermentation process, is under intense scientific debate. Under reduced pH and increased temperatures compounds induced or produced during fermentation start reacting with each other for example to form Maillard products such as Amadori products from free sugars and free amino acids, detectable in fermenting beans.

Chemical changes during fermentation are significant and yield so-called aroma precursor molecules. Non-fermented cocoa beans do neither show the typical chocolate taste nor aroma (Voigt et al., 1994).

Following fermentation cocoa beans are dried, transported over longer period of time under harsh storage conditions from tropical countries to cocoa producing countries. Here the beans are roasted at temperatures between 100° and 180 °C, mostly temperatures of around

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120 °C are employed for periods of 50–90 min. For about half of the global cocoa powder production alkali solutions mainly K_2CO_3 is added in the "dutching process" (Payne, Hurst, Miller, Rank, & Stuart, 2010). Again, the chemical composition of the beans changes dramatically in the roasting process. Following roasting, cocoa powder and cocoa butter are separated by mechanical pressing, again remixed with further ingredients such as sugar, emulsifiers such as lecithin and milk powder, conched for prolonged period at elevated temperatures and finally tempered to furnish the final chocolate product. During all these steps cocoa constituents react to form new chemical reaction products, many of them defining the characteristics of the final product.

We have, on several occasions, demonstrated the power and suitability of ultra-high resolution mass spectrometry using electrospray ionization in combination with the Fourier Transform Ion Cyclotrone Resonance Mass Spectrometry (FT-ICR-MS) setup in the analysis and characterization of processed foods such as black tea (Kuhnert, Drynan, Obuchowicz, Clifford, & Witt, 2010) and coffee (Jaiswal, Matei, Golon, Witt, & Kuhnert, 2012). Processed foods such as black tea, roasted coffee, caramel and others are characterized by an extremely high number of chemical constituents formed during processing, ranging from several thousands to several tens of thousands. Such processed food samples have therefore been classified as complex mixtures (Kuhnert, Dairpoosh, Yassin, Golon, & Jaiswal, 2013). The high resolving power of FT-ICR-MS, being far superior to any other analytical technique, allows detection of these large number of analytes in a single experiment. As a direct result a comprehensive snapshot and chemical fingerprint of a sample on the molecular level can be obtained (Gougeon et al., 2009). The direct infusion experiments provide reliable information on the elemental composition of all analytes detected in a given sample and for selected examples such as black tea and roasted coffee. We have shown that this information is sufficient to allow formulation of a chemical model of fermentation obtaining mechanistic insight into the chemical processes occurring during food processing. Analyte numbers of around 12,000 for dried cocoa beans and around 30,000 for alkalized cocoa have been reported (Milev, Patras, Dittmar, Vrancken, & Kuhnert, 2014). At the time of publication the data were too complex for further interpretation, so that we embarked over the last years on the characterization of cocoa bean fermentation using a bottom-up approach, fractionating fermenting cocoa beans into individual compound classes such as polyphenols, proteins, peptides, carbohydrates and lipids, thus studying chemical changes in less complex samples.

Having completed this exercise, we now like to return to a top-down approach looking at the global picture of all analytes present and all analytes changing during cocoa bean fermentation using the FT-ICR-MS approach. We report on the study of a "so called" fermentation series of cocoa beans. In a fermentation series beans are fermented under controlled conditions; all contextual data are recorded and samples are taken every 24 h for a period of one week until the dried bean is obtained. Thus, a time resolved investigation of the chemical changes within a fermenting bean is obtained under maximum analytical resolution.

2. Materials and methods

2.1. Chemicals and reagents

Sodium acetate, dichloromethane, HPLC-grade acetonitrile and HPLC-grade methanol were obtained from Carl Roth (Germany). MilliQ water (18.2 MΩ cm at 25 °C) was used throughout all experiments. Acetic acid, sodiumcarbonate, arginine and internal standards were obtained from Sigma-Aldrich. Hydrochloric acid was obtained from Merck (Germany).

2.2. Sample collection

Cocoa bean samples were collected from Ivory Coast in 2015 by Barry Callebaut AG, in unfermented and fermented form taking samples every 24 h. Information on fermentation and drying times have been recorded including heap temperatures, heap pH and turning intervals as well as GPS coordinates of the producer. Samples were stored at −80 °C shipped for analysis on dry ice. Samples were stored at 4 °C prior to defatting and both stored at 4 °C after defatting.

2.3. Sample preparation

At least 30 g of cocoa beans from a sample were first de-shelled and then ground to a fine powder using a Retsch Grindomix GM200 knife mill (Haan, Germany) at 10,000 rpm. 5 g of powder was defatted using dichloromethane in an automated Büchi B-811 Soxhlet extraction apparatus (Essen, Germany) for 18 h. The defatted powder was dried under vacuum and stored at 4 °C until further use. Polyphenol extraction was carried out using an acidified methanolic solution ($MeOH:H_2O:CH_3COOH$ 70:28:2). 50 mg of defatted power was mixed with 5 mL of the extraction solution and this mixture was sonicated in an ultrasonication bath for 10 mins, stirred for a further 30 mins, and finally syringe-filtered through a PTFE membrane filter (0.45 μm). The methanolic extract was spiked with an internal standard and further diluted for FT-ICR-MS analysis.

2.4. Electrospray FT-ICR-Mass spectrometry

Ultra-high resolution mass spectra were acquired using a Bruker ScimaX mass spectrometer equipped with a 7 T Maxwell refrigerated superconducting cryo-magnet and ParaCell™ analyzer. The instrument was equipped with an Apollo II ESI source. Drying gas temperature was set to 200 °C. Spectra of the cocoa extract samples were acquired in positive and negative ion mode using direct infusion with a syringe pump at a flow rate of 120 μL/h. Initial extracts were diluted by a factor of 1:40 in 50:50 MeOH:water for negative ion mode. For positive ion mode extracts were diluted by a factor of 1:40 in 50:50 MeOH:water with 0.1% formic acid. Internal standards were added to the spray solutions for semi-quantitation. Hexakis (1H,1H,2H-perfluoroethoxy) phosphazene was added for measurements in negative ion mode. Lincomycin was added for measurements in positive ion mode. The instrument was externally calibrated with NaTFA cluster using a solution of 0.1 mg/mL NaTFA in 50% acetonitrile. After data acquisition the mass spectra were internally recalibrated with exact masses of known compounds. A sine apodization of the time domain signal was performed prior to fast Fourier transformation (FFT). The ion accumulation time was set to 30 ms. A total number of 200 single scans were accumulated for the final mass spectrum. Spectra were acquired in a mass range between m/z 151 and 3000. Spectra were acquired with mass resolution of 470.000 mt m/z 400. All samples were measured in three replicates. The RSD values were below 1% for the 50 most abundant signal intensities.

2.5. Data analysis

Mass spectra were preprocessed using Data Analysis 4.2 (Bruker Daltonik GmbH, Bremen, Germany). Spectra were mass deconvoluted and mass lists were generated using a cut off value of 0.0001% total intensity and a S/N of 10. Molecular formula assignment was performed in DataAnalysis with elemental restriction for $C_cH_hN_nO_oS_s$ of s = 2. Values of c, h, n and o were not restricted. A filters for H/C was set to max. 2.5.

The generated molecular formulae lists were exported to Excel and further filtered according to non-sensical elemental ratios and to Fiehn's Seven Golden Rules (Kind & Fiehn, 2007). The final list of annotated mass peaks contained between 5 and 8% ambiguous formula entries,

which were retained for visualization.

Known reported compounds including small peptides, polyphenolics and carbohydrates were compiled in a specific analyte list and searched using MetaboScape 4.0 (Bruker Daltonics, Bremen). Van Krevelen plots and other analyses were performed with the software language Anaconda Python using a self-developed script. Similarly, hypothetical formulae databases were coded in Python and searched versus databases using a Python algorithm available on request.

3. Results and discussion

In this contribution, a fermentation series of Ivory Coast cocoa beans was investigated in detail, using direct infusion ultra-high resolution mass spectrometry. Cocoa beans were fermented in a heap fermentation and samples taken every 24 h for seven days, frozen on dry ice and shipped to Bremen for further analysis. Samples were extracted following our reported procedure, yielding extracts containing both polyphenols and peptides as well as all further constituents of medium polarity (D'Souza et al., 2017b). Contextual data are given in the supplementary information.

Cocoa extracts were analyzed in triplicates in positive and negative ion mode using a 7 T FT-ICR mass spectrometer equipped with an electrospray ion source. An internal standard was added for single point mass calibration and relative quantification. Representative mass spectra of unfermented cocoa and fermentation at four different time points of fermentation are shown in Fig. 1a–d. In zoomed spectra the complexity and resolution of the mass spectra can be appreciated.

3.1. Ion statistics and quantitation of change

As a first step of data interpretation we looked at the ion statistics. Firstly, predefining arbitrary thresholds for signals to be considered for analysis, allows counting of all ions detected. For a threshold value of 0.001% of the base peak and signal to noise ratio above 10 the number of detected ions in the fermentation series in both ion modes is shown in Fig. 2. Alternative threshold values, both higher and lower, are provided in the supplementary information. As can be seen in wet unfermented beans the number of detectable ions is fairly low around 2000 eg in negative ion mode, sharply increasing on day 2 and 3 of fermentation. Towards the end of fermentation, the total detectable ion number decreases slightly. The ion statistics reveals a maximum of chemical entities present towards the middle of fermentation. The trend is identical in both positive and negative ion mode data. Positive ion mode data reveal more signals if compared to negative ion mode data.

A second useful ion statistics exercise includes counting of number of ions of different elemental composition classes. From high resolution MS data elemental compositions with a mass error below 1 ppm were determined and classified into CHO, CHON, CHONS compounds. Bar charts indicating the overall elemental composition are shown in Fig. 2. It is worth noting that compounds with CHNO and CHNOS composition increase over time to a maximum and then decline again.

A further popular analysis tool to describe ion statistics is the Bray-Curtis dissimilarity score that quantifies compositional dissimilarities between two sample sets. This parameter has as well been employed for FT-ICR data sets (Koch, Dittmar, Witt, & Kattner, 2007). We calculated both dissimilarities for fermentation series if compared to the 0 h wet bean sample, as well as compared to the previous day of fermentation. A Bray Curtis score of 0 indicates identity of samples whereas a score of 1 indicates total dissimilarity or non-identity of samples. Values are given in Table 1. Values in negative ion mode are lower if compared to positive on mode indicating that changes in polyphenolics and carbohydrates are less dramatic if compared to changes in peptide profiles

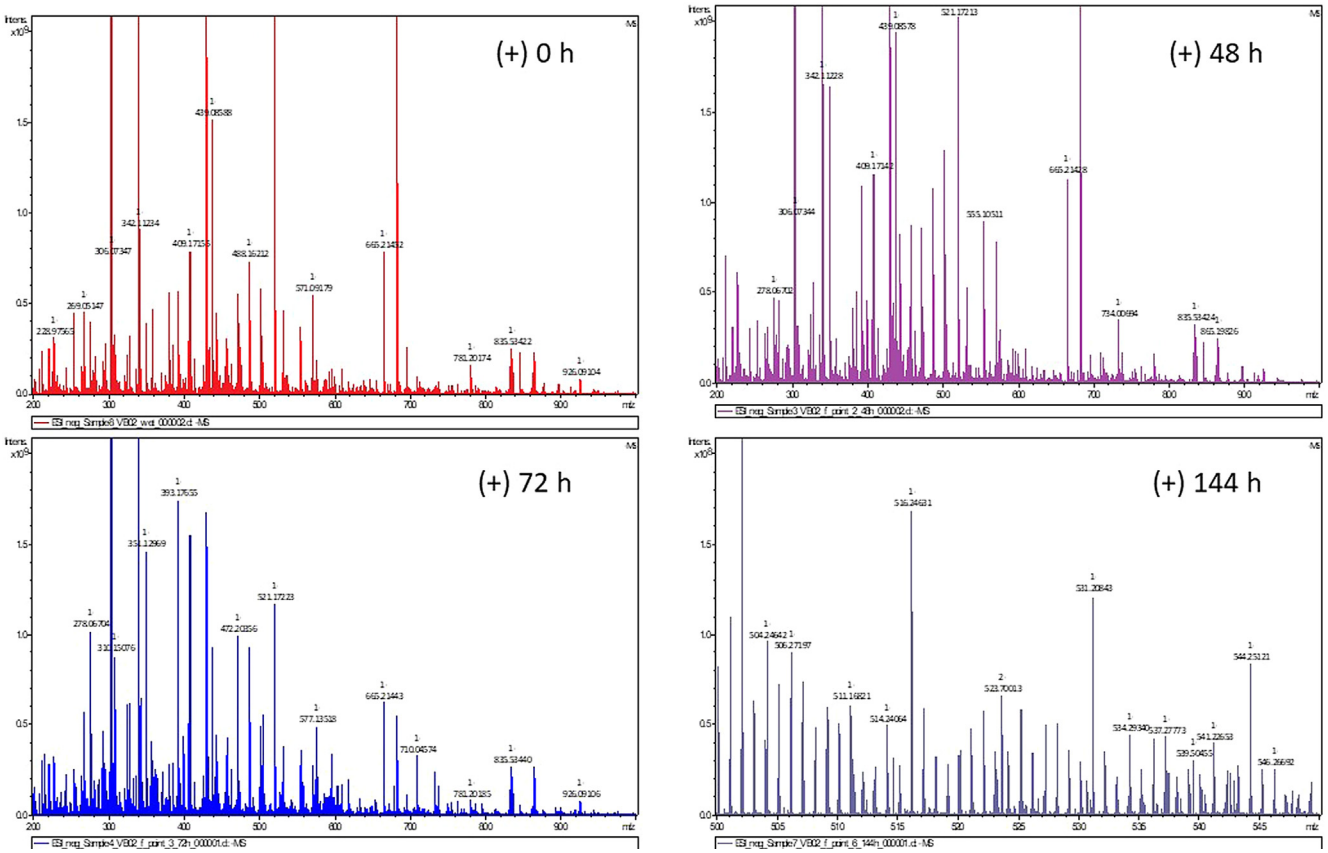

Fig. 1. Expanded ESI-FTICR mass spectra of cocoa bean extracts by direct infusion, in positive ion mode from four fermentation time points. For full series and selected expansions, please see supplementary information.

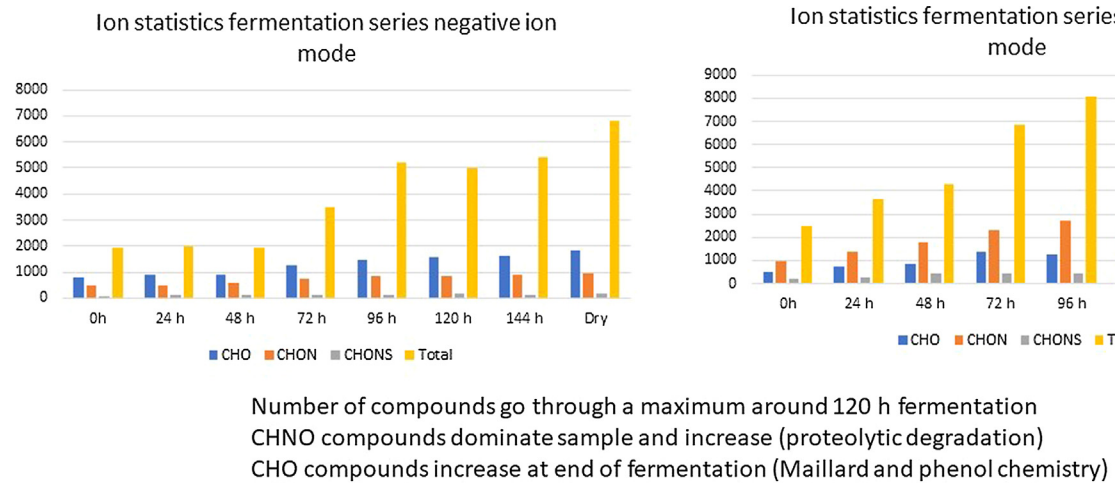

Fig. 2. Bar charts of ion statistics indicating number of peaks with associated elemental compositions.

Table 1
Bray Curtis similarity of ESI-FTICR mass spectra.

	Compared to	24 h	48 h	72 h	96 h	120 h	144 h	Dried
(+)-Ion mode	Wet (0 h)	0.22	0.35	0.59	0.66	0.71	0.73	0.74
	previous day	0.22	0.31	0.41	0.21	0.18	0.15	0–15
(−)-Ion mode	Wet (0 h)	0.29	0.39	0.52	0.61	0.66	0.68	0.67
	previous day	0.29	0.33	0.31	0.22	0.20	0.19	0.15

based on their ionization ability. In both positive and negative ion mode the dissimilarity scores are highest between day 2 and 3 of fermentation, indicating a remarkable change at this time point coinciding with the death of the cocoa embryo.

We attempted a second line of data analysis for the quantification of changes of spectral features in course of the fermentation, by generating a so-called fold change diagram. Here relative intensities are compared and plotted on a fold change diagram indicating only compounds that increase or decrease by a factor of at least ten in relative intensities. A total of 3988 signals increase by a factor of more than ten, whereas a total of 5106 signals decrease by a factor of more than ten. Among these rather complex data we could not identify a clear trend, other than that the set of well-known 800 peptides formed during fermentation follow the identical concentration time behavior as described previously (Kumari et al., 2018). An illustrative plot is provided in the supplementary information.

3.2. Van Krevelen plots

For all data points of the cocoa time fermentation series van Krevelen plots using H/C and O/C ratios were created in both positive and negative ion mode (Wu, Rodgers, & Marshall, 2004). Data points were color coded according to elemental composition class and relative intensities represented in bubble plots, with bubble size representing relative intensities. Selected examples are shown in Figs. 3 and 4, the full data are given in the supplementary information.

The van Krevelen formalism allows a rough classification according to compound classes, based on characteristic elemental ratio boundaries, observed in a sample. Most classes of natural products are defined by characteristic elemental compositions and hence are found in a defined area on the plot (Kuhnert, 2010). From the data shown here, it becomes apparent that during fermentation in particular compounds with a CHNO elemental composition appear and change in large numbers during the fermentation and change their overall pattern (points coded in green on plots). We assign these to peptides produced by proteolysis from storage proteins and their reaction products of unknown structure. Furthermore, compounds of polyphenolic character appear in larger relative quantities at 24 and 48 h time points, indicating the antibacterial response of the cocoa bean.

The van Krevelen formalism was as well extended towards nitrogen containing compounds with plotting H/C versus N/C ratios as shown in Fig. 5. From these plots, the peptidic nature of the majority of cocoa constituents can be concluded, with N/C ratios typical for peptidic elemental compositions.

3.3. Data matching against cocoa constituents database

Literature information on cocoa constituents is gradually increasing. Over the last decades an estimated 1 500 fully or partially characterized compounds from cocoa or chocolate have been reported. While investigating cocoa chemistry in depth, we have collected an in-house database of the majority of known cocoa constituents over the last seven years and compared their entries with experimental data from the FT-ICR MS data recorded in this contribution. Among the *m/z* values observed 804 could be matched to known peptides, 134 to reported polyphenolics, 3 to alkaloids, 35 to carbohydrates, 21 to diketopiperazines and 12 to terpenes using compound lists exported to MetaboScape 4.0 software (D'Souza et al., 2017b; Megías-Pérez, Grimbs, D'Souza, Bernaert, & Kuhnert, 2018; Andruszkiewicz, D'Souza, Altun, Corno, & Kuhnert, 2019).

3.4. Data matching against hypothetical databases

We assume that the analytes observed fall into two categories of compounds formed: Firstly secondary metabolites from the cocoa bean itself and secondly fermentation products originating from reactions between secondary metabolites. Following the production of microbial fermentation products such as ethanol and acetic acid, small peptides are generated from cocoa storage proteins. Concomitantly, polyphenolics are biosynthesized as stress response compounds. Both polyphenolics and peptides react with other products present to yield "fermentation processing compounds". Since van Krevelen and Kendrick analysis does not provide a clear trend with respect to the chemical nature of these processing products, we decided to follow an approach of compound matching to hypothetical databases. A hypothetical database in the case of oligopeptides contains a mass list of all possible di- to decapeptides. All di- to decapeptides resulting from amide bond formation from the twenty proteinigenic amino acids are contained in this database (Berna et al., 2012). This database can hence be viewed as a virtual list of compounds that are theoretically present following the rules of biochemistry. The experimental mass lists from all fermentation time points, containing between 2000 and 8000 entries, were compared to the hypothetical database, containing around

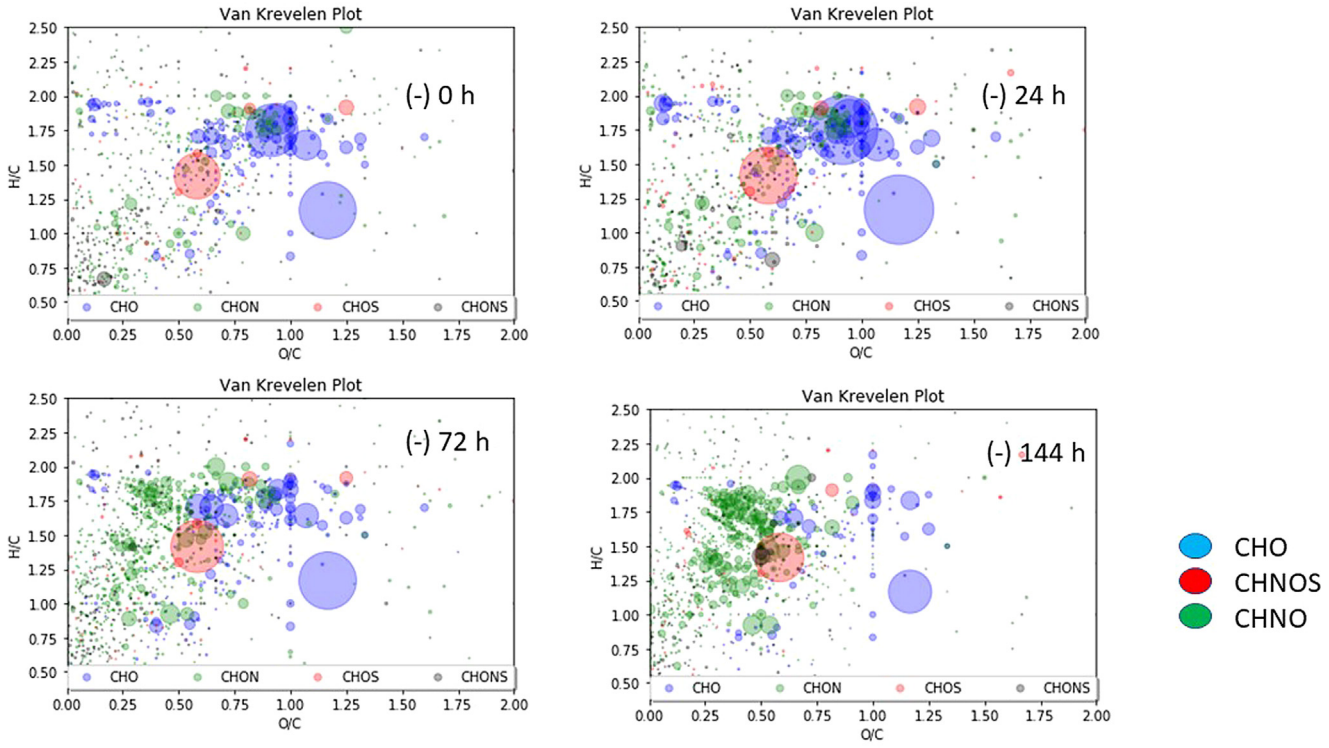

Fig. 3. Van Krevelen diagrams from ESI-FTICR mass spectra of cocoa bean extracts by direct infusion, in positive ion mode from four fermentation time points. For full series and selected expansions, please see supplementary information. Color codes for elemental composition and bubble size for signal intensities. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

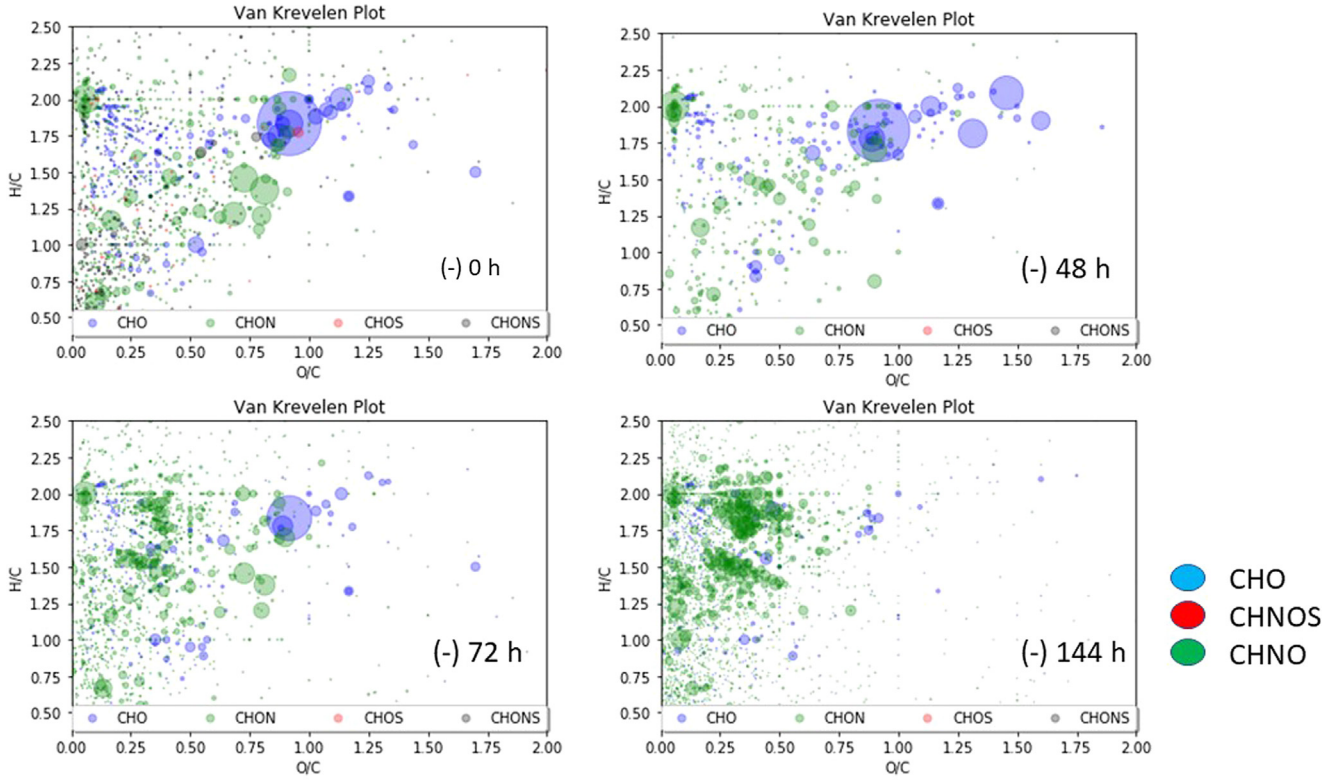

Fig. 4. Van Krevelen diagrams from ESI-FTICR mass spectra of cocoa bean extracts by direct infusion, in negative ion mode from four fermentation time points. For full series and selected expansions, please see supplementary information. Color codes for elemental composition and bubble size for signal intensities. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

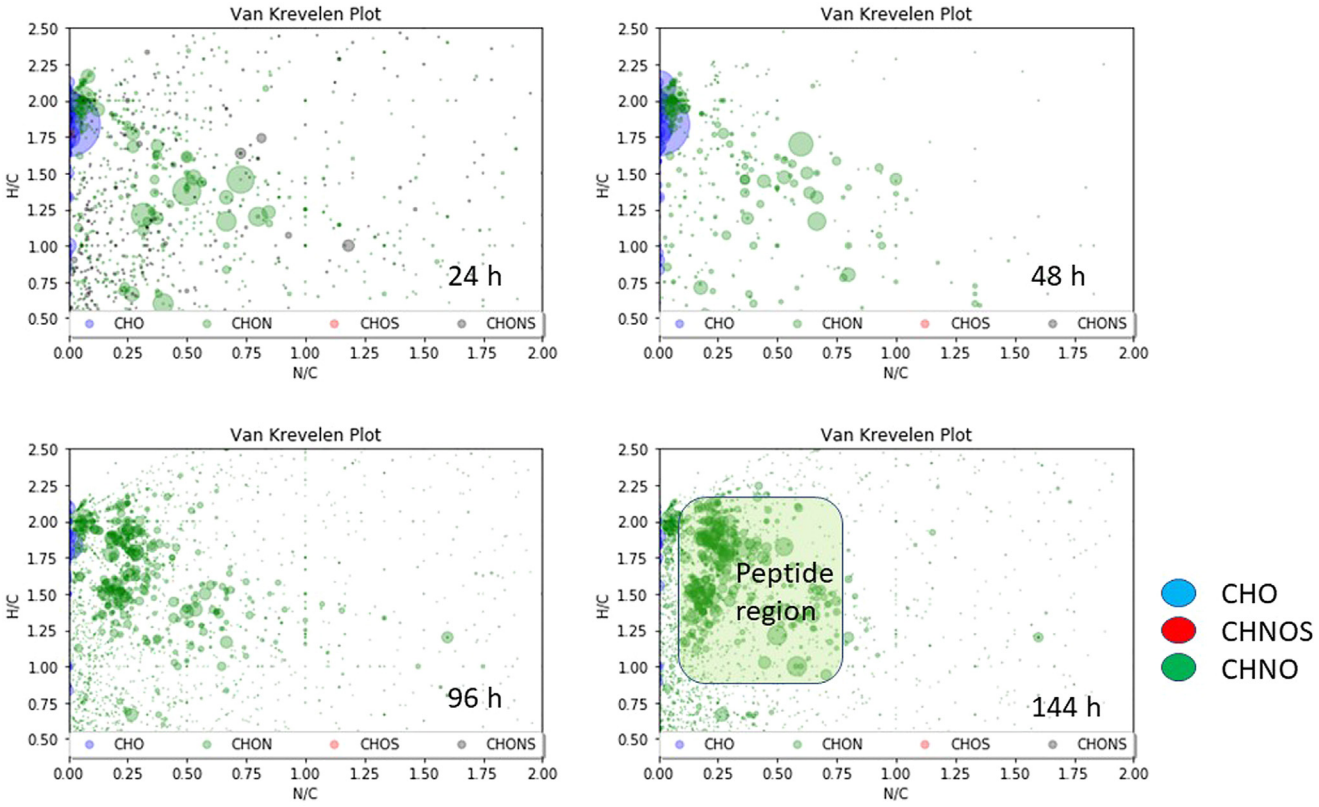

Fig. 5. Van Krevelen diagram displaying H/V versis N/C ratio from ESI-FTICR mass spectra of cocoa bean extracts by direct infusion, in positive ion mode from four fermentation time points. Color codes for elemental composition and bubble size for signal intensities. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

25 million entries, to identify all candidate compounds in the samples that could be oligopeptides. This matching exercise reveals that over all eight spectra accumulated in the fermentation series 5177 peaks detected in the positive ion mode and 1 699 detected in the negative ion mode show a high-resolution *m/z* value in line with possible peptide formulae. Similarly, we speculated that peptides react with selected fermentation products to form glucose or fructose Amadori products. We have recently shown a kinetic study on the depletion of key carbohydrates in cocoa fermentation (Roberto Megías-Pérez et al., 2020). Furthermore we suggest that cocoa oligopeptides react with acetic acid to yield acetates or N-acetamides and with lactic acid lactates of N-lactamides. For these compounds, once again, hypothetical databases were created using the predicted mass increment (peptide + $C_6H_{10}O_5$ for Amadori, peptide + C_2H_3O for acetamides and peptide + $C_3H_5O_2$ for lactamides) and matched against experimental data. Structures of tentative reaction products are shown in Fig. 6.

For possible Amadori products, 297 matching peaks in positive ion mode, and 97 in negative ion mode were identified. A subsequent LC-tandem MS run using a LC-Q-TOF mass spectrometer in positive ion mode allowed unambiguous identification of 31 of these compounds as Amadori products of oligopeptides based on fragment ions characteristic for this class of compounds (Weenen, 1998; Fay & Brevard, 2005; Andruszkiewicz et al., paper within this special edition), hence confirming the initial structural hypothesis for a small selection of tentative structures. For acetamides 784 peaks in positive ion mode and 383 peaks in negative ion mode matched putative acetamides. For acetamide elemental composition it should be noted that acetylation yields isobaric ions if compared to peptides, in which alanine was replaces by glutamic acid (eg. Ac-NAVAFG by NAVEFG). For lactamides 265 peaks in the positive ion mode and 81 peaks in the negative ion mode matched putative lactamides from the hypothetical database.

The approach is further illustrated by structures in Fig. 6 showing reaction of dipeptide VL and tetrapeptide NGRF reacting at its N-terminus to produce VL-Amadori, VL-acetamide and VL-lactamide. In the case of these two peptides all three predicted *m/z* values could be located in the spectra and the structures for the VL series confirmed by LC-tandem-mass spectrometry. Table 2 summarizes some selected results.

In order to visualize putative lactamides, acetamides and Amadori products, we created a van Krevelen diagram that contains the 500 most abundant peptides already identified in cocoa along with 150 amides and 50 Amadori products of the highest peak intensity from the previous analysis, colour coded by compound class (see Fig. 7). Additionally, the plots contain 150 of the most abundant unknown compounds with a CHNO elemental composition. This type of plot allows to draw a variety of conclusions. Firstly, cocoa peptides produced during fermentation proteolysis can be found in a restricted area on the van Krevelen diagram. Derivatives that are obtained by simple known transformations such as N-amides of acetic and lactic acid as well as Amadori products lie as well in the same elemental ratio area of this plot. Without exception, all further 150 unknown compounds with assigned CHNO composition (in red Fig. 7) can be found in this area of the plot suggesting additional “small chemical modifications” of short peptides at any of their functional groups. We therefore suggest that the majority of remaining unknown compounds formed in cocoa fermentations are small peptide derivatives, formed by reaction pathways yet to be elucidated. A close inspection of assigned molecular formulae suggests that decarboxylation, deamination, acetaldehyde condensation or reaction with sugars other than hexoses might be included in cocoa’s fermentation reaction repertoire. For each at least three reaction molecular formulae can be attributed to a well precedented peptide precursor.

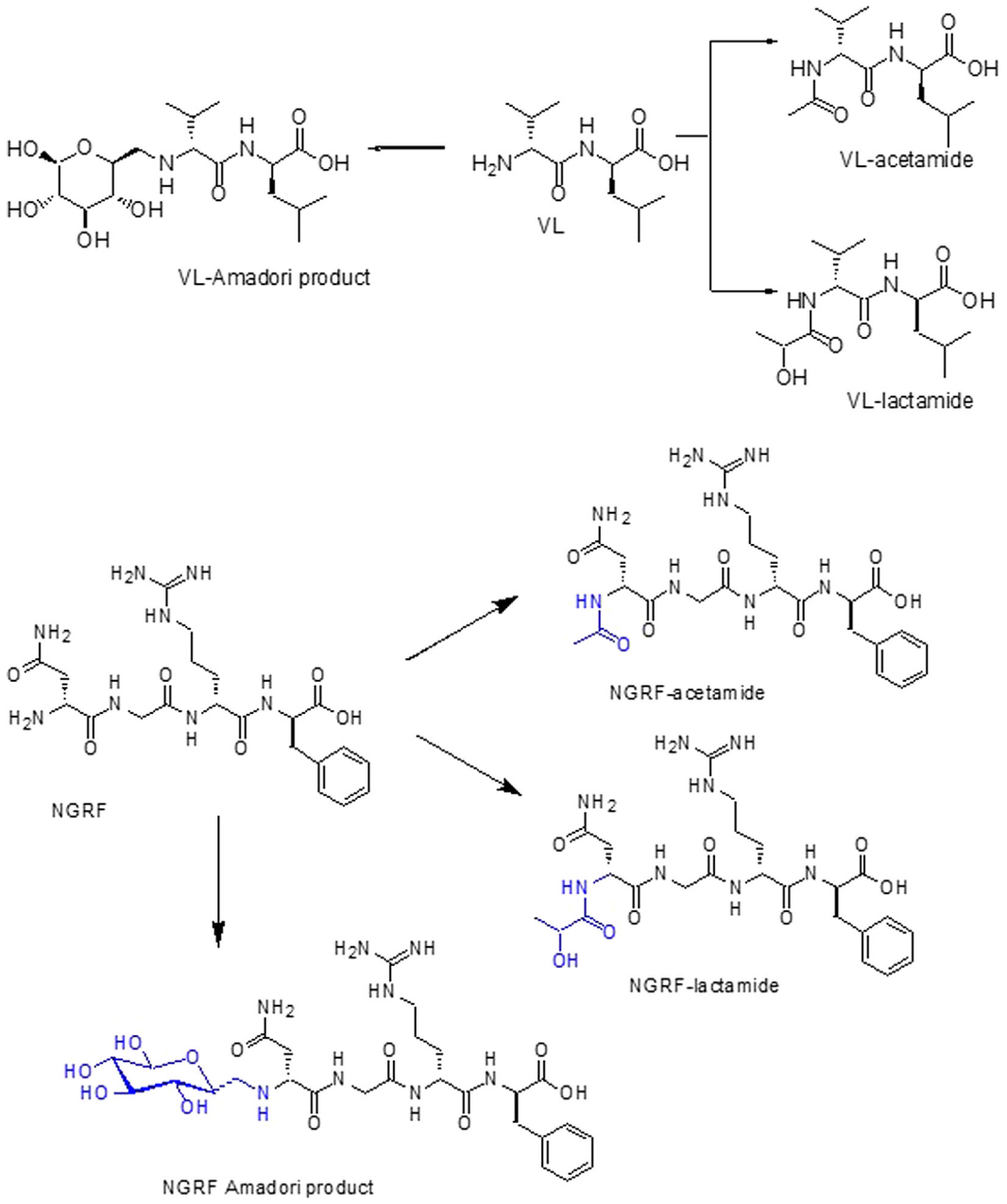

Fig. 6. Reaction scheme of selected peptides VL and NGRF forming Amadori compounds, acetamides and lactamides. Reactions representative for in text given number of further cocoa processing compounds.

Table 2
Selected *m/z* values and molecular formulae of cocoa fermentation peptides and their resulting Amadori products, acetamides and lactamides formed during fermentation.

Peptide *m/z* exp	VL	PL	FPV	NGRF	DLLN	GINDY	FDNK
Mol. Formulae	231.1704	229.1547	362.2074	493.2516	474.2561	581.2566	523.2512
	$C_{11}H_{23}N_2O_3$	$C_{11}H_{21}N_2O_3$	$C_{19}H_{28}N_3O_4$	$C_{21}H_{33}N_8O_6$	$C_{20}H_{36}N_5O_8$	$C_{25}H_{37}N_6O_{10}$	$C_{23}H_{35}N_6O_8$
Amadori *m/z* exp	313.2486	391.2075	524.2603	655.3046	536.1776	743.3093	685.3039
Mol. Formulae	$C_{17}H_{33}N_2O_8$	$C_{17}H_{31}N_2O_8$	$C_{25}H_{38}N_3O_9$	$C_{27}H_{43}N_8O_{11}$	$C_{26}H_{46}N_5O_{13}$	$C_{31}H_{47}N_6O_{15}$	$C_{29}H_{45}N_6O_{13}$
Acetamide *m/z* exp	273.1809	271.1852	404.2170	551.2572	516.2664	NA	565.2617
Mol. Formulae	$C_{13}H_{25}N_2O_4$	$C_{13}H_{23}N_2O_4$	$C_{21}H_{30}N_3O_5$	$C_{23}H_{35}N_8O_7$	$C_{22}H_{38}N_5O_9$	NA	$C_{25}H_{37}N_6O_9$
Lactamide *m/z* exp	304.1993	302.1836	NA	566.2807	557.3042	654.2855	NA
Mol. Formulae	$C_{14}H_{28}N_2O_5$	$C_{14}H_{26}N_2O_5$	NA	$C_{24}H_{38}N_8O_8$	$C_{23}H_{41}N_5O_{10}$	$C_{28}H_{42}N_6O_{12}$	NA

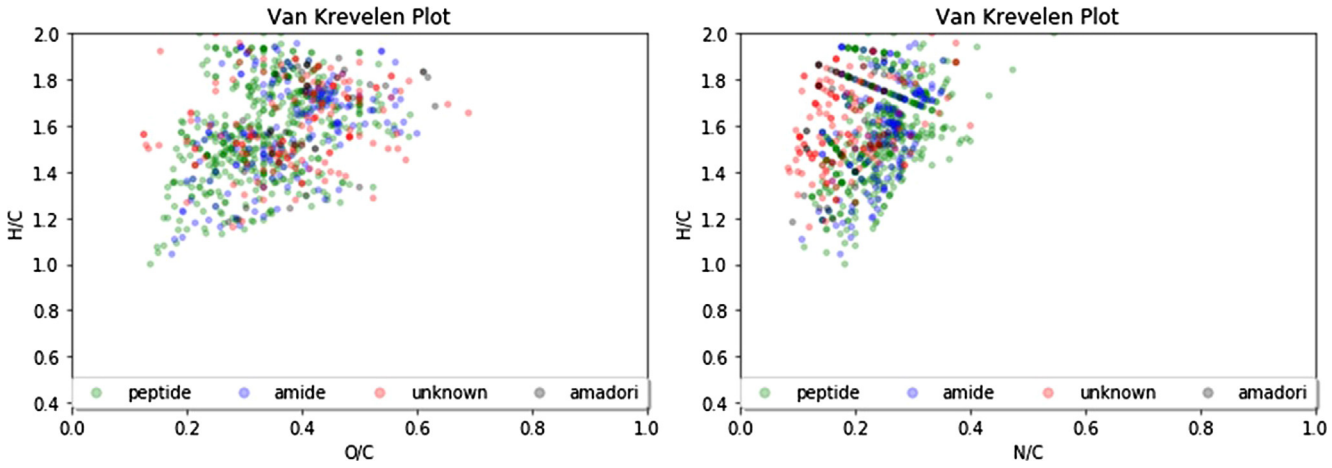

Fig. 7. van Krevelen diagram with O/C versus H/C (left) and N/C versus H/C ratio (right) colour coded according to putative class of CHNO compounds of 500 literature reported peptides (green), terminal N-amides (blue, acetamides and lactamides), Amadori compounds (black) and 150 most intense unknown constituents with CHON composition. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Conclusion

In summary we have provided for the first time a time resolved view at ultimate analytical resolution into an important food processing step, cocoa bean fermentation. The data clearly provide insight into dramatic chemical changes occurring during microbe driven fermentation. A comparison to the established cocoa literature reveals that only 15% of observed signals can currently be accounted for. Established molecular formulae assignment and elemental ratio plots reveal that the majority of cocoa constituents derive from peptides formed during proteolysis from cocoa storage proteins. Next to shorter peptides as established, we propose from the data, the formation of putative peptide derivatives formed by a series of chemical pathways including amide formation and Amadori reactions.

Author contribution

Nikolai Kuhnert wrote the manuscript devised and planned the experiments and carried out the data interpretation. Roy d'Souza designed the hypothetical libraries and carried out data interpretation with the libraries. Britta Behrends carried out the sample extraction and sample preparation. Matthias Witt carried out the FT-ICR measurements and carried out compound searches in Metaboscape to identify known cocoa constituents.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.109209.

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