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Assessing cocoa aroma quality by multiple analytical approaches

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ABSTRACT

In this study the quality of 26 fermented cocoa beans from 4 different countries (Vietnam, Indonesia, Peru and Ghana) is assessed by means of both a compositional analysis (amino acids, reducing sugars, polyphenols and lactic acid) and profiling of their volatile aroma composition (Mass Spectrometry (MS)-fingerprinting and Headspace-solid Phase Micro-extraction-Gas Chromatography–Mass Spectrometry (HS-SPME-GC–MS)) following high roasting processing (30 min at 150 °C). Compared with the labour-intensive measurement of cocoa precursors, MS-fingerprinting on the cocoa headspaces appeared to be a very powerful and fast classification technique. Different cocoa groups were researched having unique organoleptic characteristics which were affected by fermentation, roasting conditions and geographical origin. A clear separation of fine flavour cocoa (*Criollo* variety), well fermented/roasted cocoa and low quality cocoa could be made. Additionally, the clustering was confirmed by more conventional HS-SPME-GC–MS aroma analyses on the cocoa samples. Several markers for the bean origin/quality were identified. Roasting degrees were also calculated based on typical pyrazine ratios.

1. Introduction

Chocolate, the most appealing confectionery item worldwide, is unique due to its specific organoleptic properties. A combination of analytical methods can be applied to determine aroma related compounds in cocoa beans (Luykx & Van Ruth, 2008). However, due to the consumer's increasing demand for high quality cocoa products, the chocolate industry is highly interested in a more rapid analytical technique for cocoa aroma quality classification. Such advanced aroma analyses would be applied for quality assessment of cocoa beans having specific genotypic, geographical and processing properties. Indeed, the quality and flavour of cocoa is not only the result of genotype and geographical origin, but also of post-harvest treatments including fermentation and roasting (Ramli, Hassan, Said, Samsudin, & Idris, 2006).

Fermentation of harvested cocoa is of utmost importance, since aroma precursors are formed during both anaerobic and aerobic fermentation phases. Indeed, roasting of non-fermented cocoa beans does not deliver sufficient aroma compounds to produce high quality chocolate. The fermentation degree of cocoa beans can be determined by several quality parameters. The fermentation index is a commonly used marker with values above 1 being indicative of well-fermented cocoa beans (Ilangantileke, Wahyudi, & Bailon, 1991). Secondly, the polyphenol content which is reduced by oxidation, condensation and complexation reactions during cocoa fermentation can also be used as cocoa quality indicator (Nazaruddin, Seng, Hassan, & Said, 2006a, 2006b). Enzymatic browning of polyphenols occurs at subsequent drying and further reduces their initial content (Kyi et al., 2005). Generally, it is accepted that with increasing fermentation time, lower amounts of polyphenols and/or epicatechin levels are present in cocoa beans (Caligiani, Cirlini, Palla, Ravaglia, & Arlorio, 2007). Polyphenol compounds reduce the cocoa flavour when binding with aroma precursors or aroma compounds formed during roasting (Misnawi, Jinap, Jamilah, & Nazamid, 2004). Fresh non-*Criollo* cocoa beans contain purple anthocyanin pigments, namely 3-β-galactosyl- and 3-α-L-arabinosyl-cyanidins which are hydrolysed by glycosidases, resulting in a bleaching of the cotyledons during fermentation (Forsyth & Quesnel, 1957). Besides phenolic substances, the presence of aroma precursors formed during

Abbreviations: MS, Mass Spectrometry; MS, Mass Spectrometry; HS-SPME-GC–MS, Headspace-solid Phase Micro-extraction-Gas Chromatography–Mass Spectrometry; TMP, tetramethylpyrazine; TrMP, trimethylpyrazine; DMP, 2,5-dimethylpyrazine; P, Peruvian clones; TD, Vietnamese clones; G, Ghanaian clones; IN, Indonesian clones.

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fermentation, such as free amino acids, short-chain peptides and reducing sugars can also be linked to the desired cocoa flavour development during the roasting step via the Maillard reaction (Afoakwa, Paterson, Fowler, & Ryan, 2008).

The abovementioned precursors determine the degree of Maillard reaction products, mainly aldehydes and pyrazines (Afoakwa et al., 2008), that can be formed during roasting of cocoa. Roasting results in the formation of primarily three methylpyrazines. Specifically, tetramethylpyrazines (TMP) reaches its maximum level at medium roasting levels. Trimethylpyrazine (TrMP) increases steadily while 2,5-dimethylpyrazines (DMP) only increases under strong roasting conditions. Based on sensory evaluation, a normal degree of roasting relates to a high concentration ratio of TMP/DMP and TMP/TrMP between about 1.5 and 2.5, while values below indicate over-roasted samples exhibiting a burnt, coffee-like taste (Beckett, 2011).

As mentioned earlier, another aspect that highly defines cocoa flavour is the origin of the raw cocoa beans (Frauendorfer & Schieberle, 2006). Several analytical methods have long been established to predict the flavour properties of final products. However, those involve several labour intensive procedures for determining cocoa precursors, acids and polyphenols in order to measure the degree of difference of fermented cocoa quality in relation to origins and genotypes (de Brito et al., 2001; Jinap, Jamilah, & Nazamid, 2004; Luna, Crouzillat, Cirou, & Bucheli, 2002; Noor-Soffalina, Jinap, Nazamid, & Nazimah, 2009; Rohsius, Matissek, & Lieberei, 2006). Alternatively, the use of Headspace-solid Phase Micro-extraction-Gas Chromatography–Mass Spectrometry (HS-SPME-GC–MS) has become increasingly popular in cocoa flavour studies due to a number of advantages such as high sensitivity, selectivity and reproducibility (Cambrai et al., 2010; Hernández & Rutledge, 1994). Recently, the applicability of electronic nose techniques, sometimes in combination with GC–MS and sensory evaluation has been explored for assessing the condition of food and origin. Several commercial applications were successfully introduced for differentiation of olive oils (Guadarrama, Rodrıguez-Méndez, Sanz, Rıos, & De Saja, 2001), wines (Penza & Cassano, 2004), orange juices (Steine, Beaucousin, Siv, & Peiffer, 2001), flaxseeds oils (Wei et al., 2014) and propolis (Cheng, Qin, Guo, Hu, & Wu, 2013). However, to the best of our knowledge only limited studies applied this technique for assessing the cocoa quality. Gu et al. (2013) used electronic nose technology for the evaluation of the aroma variation in fermented, dried cocoa samples from different countries including China, Indonesia and Papua New Guinea. Olunloyo, Ibidapo, and Dinrifo (2012) developed a prototype electronic nose for monitoring the quality of cocoa beans originated from Nigeria. These traditional electronic nose systems rely on a sensor array response after being exposed to a complete aroma, without separation of individual aroma components, and using pattern recognition software for data processing and correlation with origin data. Although showing numerous advantages such as simplicity, time saving and economical aspect (Franke, Gremaud, Hadorn, & Kreuzer, 2005), this system encountered major problems, i.e. drift, instability due to water vapour or carbon dioxide, need for frequent calibration, sensor poisoning and poor sensor-to-sensor and instrument-to-instrument reproducibility (Marsili, 2001). Mass fingerprinting based techniques, a hyphenated HS-SPME-MS-nose configuration, were already successfully applied on coffee powders for objective measurement of different parameters influencing roasted coffee flavour (Balaban, Buettner, Roussel, Vanthuyne, & Schieberle, 2004). The combination of mass fingerprinting and pattern recognition techniques will be referred to as 'MS-fingerprinting' throughout this manuscript.

To the best of our knowledge, no research was done on the application of MS-fingerprinting to assess cocoa quality and origin. The aim of this work was to develop a rapid and reliable analytical method to differentiate roasted fermented cocoa beans according to their origins and fermentation degree. The results were linked to compositional differences and roasting degree.

2. Materials and methods

2.1. Cocoa samples

Dried and fermented cocoa beans were collected from four different origins as specified in Table 1. Samples from Vietnam were prepared under identical fermentation and drying conditions by inserting net-bags containing different clones in one homogenous batch. Specific information on fermentation and drying conditions for the other origins is not available, as this information was kept confidential by its suppliers. From the moment of arrival the dried cocoa beans were manually deshelled and stored in sealed glass recipients under cooled and dark circumstances until further sample preparation and analysis.

2.2. Chemicals

Unless otherwise specified, all chemicals used were of analytical grade and purchased at Merck (Darmstadt, Germany). Water was purified in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.3. Quality determination of unroasted fermented cocoa beans

The fermentation quality was determined using several analyses according the methods given in Atlas (2010). The fermented cocoa beans were deshelled, milled to a fine powder with a particle size of ca. 1 μm in a Retsch MM 200 (Germany) laboratory mill and defatted using n-hexane and petroleum ether prior to analysis. The total phenolic substance was determined with an adapted Folin–Ciocalteu procedure (Singleton & Rossi, 1965). Polyphenol profile was analysed using RP-HPLC (Reversed Phase High Performance Liquid Chromatography) equipped with a Photodiode Array Detector (PDA). 100 mg of the defatted cocoa powder was weighed in a centrifuge tube (16 × 100 mm), 5 mL of methanol added and the mixture stirred for 20–30 s with an Ultra-Turrax T25 (Ika Labortechnik, Staufen, Germany) agitator. The agitator was then rinsed with 2 mL of methanol and the solutions combined. The extracted sample was sonicated for 3 min, cooled for 15 min at 0 °C, and then centrifuged for 10 min at 4100 rpm. The methanolic supernatant containing phenolic compounds was decanted into a 50 mL pear-shaped flask. The extraction was repeated 3 additional times, with a cooling phase of only 2 min. The methanol was removed from the combined extracts by rotary evaporation under partial vacuum at 40 °C and 100 mbar. Subsequently, the residue was dissolved in 1.5 mL methanol (Lichrosolv®). The sample was transferred into an HPLC vial through a 0.45 μm syringe filter (PTFE Multoclear, CS-Chromatographie Service, Langerwehe, Germany). The vial was sealed hermetically and the sample stored at −20 °C until further analysis. The probe was then measured against a calibrated series by HPLC. Identification and quantification of the peaks were done using standard solutions of catechin and epicatechin as references. Chromatographic analyses were carried out on a HPLC system equipped with an AS-4000 (Merck, Darmstadt, Germany) automatic injector, two Knauer (Berlin, Germany) HPLC pumps 64, a Knauer HPLC programme 50 solvent controller, a Waters (Eschborn, Germany) 996 Photodiode Array Detector (PDA) and analysed by means of Millennium TM 3.2 software (Millipore Corporation, Milford, MA, USA). Separation of polyphenols was performed on a Waters Novapac C18 column at 26 °C. The binary mobile phase (consisted of 2% acetic acid in water (A) and a mixture of acetonitrile, water and concentrated acetic acid (400/90/10 *v*/*v*/*v*) (B). Gradient (A + B = 100% *v*/*v*): (1) 8 min 90% A, (2) 8–38 min 90% A, (3) 38–41 min 77% A, (4) 41–49 min 10% A and (5) 49–60 min 90% A.

Twenty microliter of sample was injected onto the column. The separation of polyphenols was monitored using a PDA detector at 280 nm and anthocyanins were recorded at 540 nm. Identification of each peak was confirmed by comparison of retention time and coelution with authentic standards of protocatechiuc acid (Aldrich), catechinhydrate