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Sensory and GC-O analyses of cocoa and chocolate along the cocoa production chain

S. Nottelmann[a], B. Bisping[b], D. Kadow[c] , Ch. Krabbe[a], A. Krähmer[d], S. Rohn[b], B. Rudolph[f], F. Tietz[b], K. Zug[f], F. Sobotta[a], C. v. Wallbrunn[e], K. Riehn[a]

[a] Department Nutrition & Home Economics, Faculty of Life Sciences, Hamburg University of Applied Sciences, Hamburg, Germany
[b] Institute for Food Chemistry, Hamburg School of Food Science, University of Hamburg, Hamburg, Germany
[c] August Storck KG, R&D Chocolates, Berlin, Germany
[d] Julius-Kühn-Institut, Federal Research Centre for Cultivated Plants, Berlin, Germany
[e] Department of Microbiology and Biochemistry, Hochschule Geisenheim University, Geisenheim, Germany

[f] Department of Biology, Biocenter Klein Flottbek, Biodiversity, Evolution and Ecology, University of Hamburg, Germany

Introduction: The identification of flavor and aroma relevant components in cocoa pulp and seeds, raw cacao, and cocoa based products is a key factor in cocoa research. There is strong evidence that (i) the genotype, (ii) the fermentation conditions, and (iii) the post-harvest processing have a significant impact on the flavor development in cocoa.

To further enlighten these correlations the CORNET project "Quality improved Cocoa and Cocoa-based Products with Flavor Profiles on Demand – From Farm to Chocolate Bar" (*Federal Funding Advisory Service on Research and Innovation* (IGF) Project No. 169EN/2 funded by the *Federal Ministry for Economic Affairs and Energy* (BMWI) through the *German Federation of Industrial Research Associations* (AiF) represented by the *Research Association of the German Food Industry* (FEI)) focuses on investigating the sensory profiles of both well-known and new cocoa genotypes from four selected Peruvian cocoa farms that are exposed to different fermentation conditions and post harvest treatments.

Methods: To ensure a high level of internal and external validity and reliability of the results, an international sensory panel with experts in the field of cocoa production and research was recruited and trained on the basis of a newly developed standardized protocol for molded liquors. Established protocols usually focus on the evaluation of liquid liquors. The training involves both, traditional sensory tests as well as gas-chromatography olfactometry methods for detecting and evaluating aroma and flavor-relevant components.

Expected Results and Discussion: Combined with the characterization of the chemical composition, the project aims to generate a new sensory evaluation procedure and a related evaluation scheme for both, cocoa and cocoa products. This protocol can be used to guarantee a quality based cocoa and chocolate production with flavor profiles on demand from bean to chocolate. It enables experts to perform sensory analyses all over the world with a higher comparability between the results. A key factor for that is, besides a standardized protocol, the tasting of molded cocoa liquors.

Introduction

Cocoa (*Theobroma cacao L.*) can be classified into three groups (Criollo, Trinitario and Forastero) based on (a) genetic origin, (b) morphologic pod appearance, (c) pod color and (d) bean flavor. Another way to characterize cocoa is the distinction between common grade cocoas („bulk“) and fine and flavor cocoas. While bulk cocoas usually originate from Forastero genotypes, the more characteristic fine and flavor cocoas originate from Criollo and Trinitario types. However, flavor development is also strongly influenced by post-harvest treatments (1) (2) (3) (4). In other terms, this means that a classification that is only based on genetic backgrounds is not always an adequate solution to distinguish aromatic „fine and flavor“ cocoas from common grade cocoas (5) (6).

Materials and Methods

The Cocoa Chain project was started to retrace aroma development in samples from different regional cocoa origins from Peru along the complete production chain. All intermediate goods and finished products (fresh fruit material, cocoa pulp, unfermented and fermented dried nibs, roasted fermented nibs, liquor and chocolate prototypes) are evaluated with regard to different biological, physical, microbiological, and chemical criteria (Fig. 1). In addition sensory trials and analyses as well as different processing parameters are used to complete the picture of aroma development in different cocoa products from specified origins along the process chain.

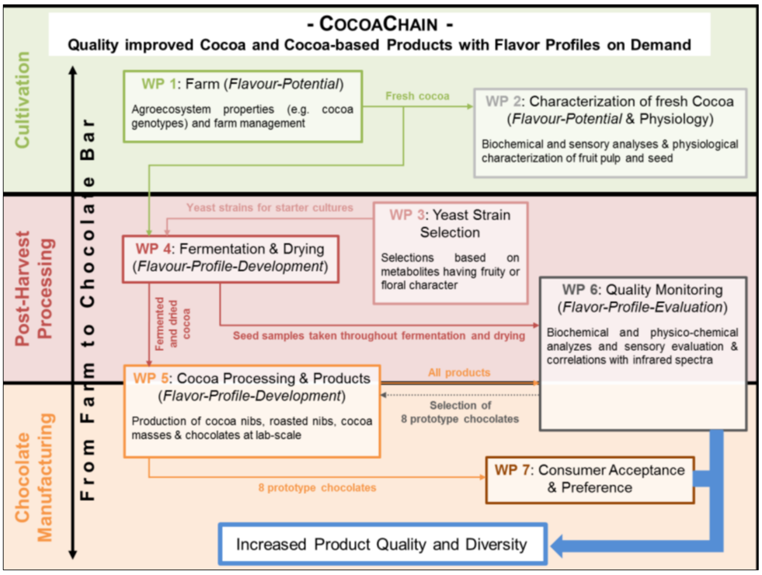

Figure 1 Flow chart of the CocoaChain Work Packages

Sample Preparation for sensory evaluation

A standardized protocol for roasting and milling of fermented and dried cocoa beans was developed at University of Applied Sciences Hamburg [7]. In addition a customized sensory protocol for cocoa molded liquors is in progress. Traditional evaluation protocols stipulate the tasting of liquid cocoa liquors. However, this method is prone to errors because, for example, the sample quantities may vary. The tasting of molded samples enables considerably better standardization and comparability of data.

Sensory analysis

Our own results as well as numerous reports in the literature [9, 10,11] demonstrate that the parallel analysis of chemical markers by HS-SPME-GC-MS-O and classic sensory profiling can significantly improve the accuracy of the assessment of flavor profiles.

Two panels are trained according to ISO norms: one panel in Lima, Peru and a second panel in Hamburg, Germany. Potential panel members are screened with taste and odor recognition tests as well as threshold detection tests. Applicants have to identify aqueous solutions of the basic tastes “sweet” (c = 5,76 g/l), “sour” (c = 0,28 g/l), “salty” (c = 1,19 g/l), “bitter” (c = 0,195 g/l), “umami” (c = 0,29 g/l) and “metallic” (c = 0,0036 g/l). Detection thresholds and training thresholds of the aforementioned basic tastes are also tested by all panel members referring to ISO 3972:2013-12 [9]. Panel applicants who successfully passed the screening tests are selected for a specific sensory training with regard to cocoa relevant attributes. This training is based on the „Flavor wheel with main categories and sub categories for liquor and chocolate“ developed by Ed Seguine and Darin Sukha [12]. A catalogue of suitable reference material was created by a group of experts at HAW Hamburg in 2017 and is applied in panel trainings in Peru and Germany.

For GC-O tests a further panel is selected and trained in a similar way. Panelists here have to meet the threshold limits as well as identify and differentiate the odors that are characteristic for cocoa products on different processing stages.

<u>Gaschromatography-Massspectrometry-Olfactometry</u>

Cocoa samples are analyzed with a Hewlett Packard HP 6890 (Agilent) device, a 5975C VL MSD detector (Agilent) and a JAS sniffport. The column is a DB-WAX (length: 30m, diameter: 250 µm, Agilent) and the solidphase micoextraction fibre a Supelco PDMS/DVB, Stableflex 23 Ga with a 50/30 µm coating and 1 cm length. Evaluation of GC-MS and GC-O data is carried out with Hewlett Packard ChemStation Software (Version A.03.00).

Depending on the analysed cocoa material the oven heating programs and headspace-solid phase microextraction methods have been adapted (Tab. 1). For cocoa fruit pulp oven temperature starts at 40 °C and is held for 3 minutes. With a heating rate of 3 °C per minute temperature rises to 56 °C, held for 4 minutes, followed by a 3 °C/min-heating rate until the oven has reached 80°C, then heated with 15 °C/min up to 120 °C, with a rate of 40 °C/min up to 200 °C and 18 °C/min up to 260 °C. Last heating rate is 30 °C/min until the oven has a final temperature of 300 °C.

The oven heating programme for cocoa nibs has the following heating rates and temperature holding times: Start at 40 °C, held for 3 minutes, heat with 3 °C/min to 56 °C, hold for 2 min, heat with 15 °C/min to 80 °C, heat with 6 °C/min to 120°, heat with

10 °C/min to 200 °C and with 30 °C/min to a final oven temperature of 250 °C.

The method for cocoa liquor GC-MS-O analyses has the following heating programme: Oven temperature starts at 40 °C, held for 3 minutes, continued by heating with 3 °C/min to 47 °C, then 10 °C/min to 70 °C, 3 °C/min to 107 °C, held for 2 minutes, heat with 13 °C/min to 165 °C and then with 25 °C up to 250 °C oven end temperature.

Table 1 Sample Preparation for HS-SPME-GC-MS-O Analyses of cocoa pulp, nibs and liquor

	cocoa pulp	cocoa nibs	cocoa liquor
Sample quantity per vial (g)	5	2 g	2 g
SPME fibre	PDMS/DVB	PDMS/DVB	PDMS/DVB
Equilibration temperature (°C)	35	60	60
Equilibration time (min)	40	10	30
Extraction time (min)	20	28	50
Desorption	5 min/270 °C	5 min/270 °C	5 min/270 °C

Preliminary results and expected outcome

Screening analyses with the described GC-O method for Peruvian cocoa nibs from three different origins have produced varying contents of the supposed volatile aroma-active compounds in fine and flavor cocoa (Tab. 2).

Table 2 Screening for volatile aroma-active compounds in Peruvian cocoa nibs of different origins 1

	San Martin	Piura	Amazonas	Description *
2-Pentanone	x			Solvent, sweet

2-Heptanone	x	x	x	Sweet, caramel
2-Nonanone	x		x	Flowery, soapy
2-Pentanol	x	x		Apple, citrus
2-Heptanol	x	x	x	Flowery, perfumey, aromatic, citrus, clove, green
2-Nonanol	x		x	Green, earthy
2-Pentanol acetate	x		x	Fruity, alcoholic, glue, sweet
2-Heptanol acetate	x	x	x	Fruity, herbal, sweet,
β-Linalool			x	Soapy, floral, citrus

*GC-O panelists descriptions, HAW Hamburg

Fermented and dried cocoa beans from Peruvian San Martin, Piura and Amazonas regions, are described by the GC-O panelists with attributes like fruity, banana, fruitdrop, apricot, overripe, chocolatey, nutty, earthy, green, herbal, flowery, perfumey, citrus, solvent, Vanilla, musty, cheesy (Tab. 3 and Tab. 4) and match with odour descriptions

Table 3 Screening for volatile aroma-active compounds in Peruvian cocoa nibs of different origins 2

	San Martin	**Piura**	**Amazonas**	**Description***
3-Methyl butanal	x	x	x	Cocoa, chocolaty, nutty
Ethyl acetate	x	x	x	Herbal, flowery
Pyrazine, trimethyl-	x	x	x	Grassy, green

Octanoic acid, ethyl ester	x	x	x	Beany, peas, green
Phenylethyl Alcohol	x	x	x	Vanilla, fruity, woody
1-Butanol, 3-methyl-, acetate	x	x	x	Banana, fruity, sweet, fruitdrop
Butanoic acid, 3-methyl-	x	x	x	Cheesy, stinky, musty

*GC-O panelists' descriptions, HAW Hamburg

Expected outcome

The Cocoa Chain project is expected to highlight the single effects of origin, genotype, fermentation and further post-harvest processing on the aroma development in the beans and liquors. With using not only classic sensory techniques, like profile methods and distinguishing features, but also analytical analyses like the HS-SPME-GC-MS-O method and the data from other work packages in the project (Fig. 1) the Cocoa Chain project is supposed to draw a detailed picture of the relationship of the many different aroma development influencing parameters. Especially the improved standardization of sensory analysis will help to significantly increase the comparability of data across countries.

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