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Analytical Methods

Fast and neat – Determination of biochemical quality parameters in cocoa using near infrared spectroscopy

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ABSTRACT

The qualitative heterogeneity and increasing consumption of cocoa products require fast and efficient methods for quality assessment of fermented cocoa with regard to fermentation quality and flavor potential.

To date, quality control is achieved by visual inspection (e.g., "cut test") and sensory testing. Chromatographic methods for quantification of flavor relevant substances are limited in their applicability in standard quality control due to laborious isolation and purification steps.

Therefore, the aim of this study was the development of a near infrared spectroscopic (NIRS) method for routine analytical prediction of biochemical quality parameters. Different compound classes like phenolic substances (R^2 = 0.93) or organic acids (R^2 = 0.88) as well as individual substances like epicatechin (R^2 = 0.93) or lactic acid (R^2 = 0.87) could be precisely determined just as fermentation time (R^2 = 0.92) and pH value (R^2 = 0.94) presenting NIRS as fast and reliable alternative in routine quality assessment.

1. Introduction

With a total global production volume of more than 4 million tons per season (e.g., 4.05 M t 2011/2012) cocoa (*Theobroma cacao* L.) represents a major agricultural export commodity for several producing countries in West Africa (International Cocoa Organization (ICCO), 2013). It is the key raw material in chocolate manufacturing. Since cocoa deliveries are frequently characterized by a great heterogeneity with regard to their quality attributes, a reliable quality assessment is of great importance for both, producers and purchasers (Rohsius, Elwers, & Lieberei 2010).

A key quality attribute of cocoa is the flavor profile. The latter strongly depends on the post-harvest processing i.e., fermentation and drying that the fresh cocoa is subjected to in the countries of origin. Unfermented cocoa does not develop any chocolate flavor during chocolate manufacturing because it does not contain the precursors necessary. Moreover, it is characterized by an unpleasant bitterness and astringency. Fermentation results in chocolate flavor precursor formation as well as in the reduction of bitterness and astringency. These changes in the flavor profile go along with a change in color from pale purple (unfermented) to brown (fully fermented) (Kadow, Bohlmann, Phillips, & Lieberei, 2013).

Abbreviations: ffdm, fat free dry matter; NIR, near infrared; NIRS, near infrared spectroscopy; IR, infrared; HPLC, high performance liquid chromatography; GC, gas chromatography; PLS, partial least square algorithm; PCA, principle component analysis; WMSC, weighted multiple scatter correction; RMSECV, root mean square error of cross validation; RPD, ratio of performance to deviation; SEC, standard error of calibration; SEP, standard error of prediction; STD, standard deviation; SDCV, standard deviation of cross validation; SNV, single normal variate; MSC, multiple scatter correction; MIRS, mid infrared spectroscopy; BR, biological replicate.

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Degree of fermentation and flavor profile are routinely determined in trade and industry by cut test (color check) and sensory evaluation, respectively. Both assessments require specially trained personnel. Moreover, sensory evaluation is highly subjective depending on the sensory panel (Emmanuel, Jennifer, Agnes, Jemmy, & Firibu, 2012; Guehi et al., 2010; Hamid & Lopez, 2014).

Multiple substances are known to contribute to cocoa flavor. The chocolate flavor precursors for instance have been shown to be composed of small peptides, free amino acids and reducing sugars (Kadow et al., 2013). Bitter tasting components are the methylxanthines theobromine and caffeine. However, it has been shown that also diketopiperazines contribute to the typical bitterness of cocoa (Stark, Bareuther, & Hofmann, 2006). The acidic note, also a typical flavor attribute, is mainly due to lactic- and acetic acid formed during the fermentation process. The astringency, a haptic attribute, is caused by phenolic substances that in cocoa contribute to approximately 15% of the dry weight (Kadow et al., 2013). According to the Cocoa Atlas 2010, the mentioned substances may be used as biochemical quality parameters for the detailed description of cocoa quality (Rohsius et al. 2010).

Various chromatography methods like HPLC (high performance liquid chromatography) or GC (gas chromatography) have been described for the quantification of these flavor relevant substances (Aculey et al., 2010; Elwers, Zambrano, Rohsius, & Lieberei, 2009; Humston, Knowles, McShea, & Synovec, 2010; Machonis, Jones, & Kwik-Uribe, 2014; Pereira-Caro et al., 2013; Rohsius, Matissek, & Lieberei, 2006; Synovec, Humston, Skogerboe, & Hoggard, 2012). Moreover, several traditional methods involving wet chemistry have been investigated (Afoakwa, Kongor, Takrama, & Budu, 2013a; Suazo, Davidov-Pardo, & Arozarena, 2014). Although these methods provide reliable and accurate description of individual cocoa quality, they are destructive, more or less expensive, require special time-consuming sample preparation and hence, are not applicable for routine analysis in quality control of raw materials. In addition, each analysis only provides information about single compounds or compound classes due to the different isolation and detection methodologies. Since quality of cocoa is a function of numerous different substances, for a detailed quality evaluation several analyses would have to be carried out. Therefore, fast and robust analytical alternatives allowing the consideration of the entire metabolic composition of cocoa in a single analysis are of great interest.

In the last two decades, vibrational spectroscopy methods have proven to be fast and routinely applicable alternatives for simultaneous qualitative and quantitative determination of various primary and secondary constituents in between a complex plant matrix (Baranska & Schulz, 2009; Krähmer et al., 2013; Schulz, 2004; Schulz & Baranska, 2007; Schulz & M., 2009). Early studies on commercially available cocoa powder and chocolate mass showed the applicability of near infrared spectroscopy (NIRS) for the quantification of the main constituents – fat, protein, moisture and carbohydrates (Kaffka, Norris, Kulcsar, & Draskovits, 1982; Moros, Inon, Garrigues, & de la Guardia, 2007; Permanyer & Perez, 1989; Tarkosova & Copikova, 2000; Vesela et al., 2007). NIRS also showed to be suitable for the determination of minor valuable components in the mg kg^{-1} range like procyanidins (Alvarez et al., 2012; Whitacre et al., 2003). Other studies investigated directly cocoa for the prediction of fermentation quality by correlation of NIRS data with information about the micro flora of the cocoa seeds or the content of ammonia (Hue et al., 2014; Nielsen, Snitkjaer, & van den Berg, 2008). Additionally, quality attributes of a geographical origin can be determined by NIRS in combination with chemometric methods (Aculey et al., 2010; Teye, Huang, Dai, & Chen, 2013). A very recent work has further developed chemometric algorithms for the prediction of the fermentation status on the basis of NIRS data (Teye, Huang, Lei, & Dai, 2014).

Nevertheless, all presented spectroscopic methods to date are limited to subjective quality assessment or characterize only few chemical parameters contributing to the complex aroma profile.

Hence, the aim of this study is to develop a (near) infrared spectroscopic approach for the determination of overall cocoa quality based on global chemical composition (i.e., biochemical quality parameters), sensory parameters and also insect or fungi infestation.

In the present work, the first results for the determination of biochemical quality parameters like free amino acids), carbohydrates, protein, methylxanthines, organic acids and phenolic substances as well as fermentation time, pH-value and moisture content obtainable from single NIRS experiments are presented.

2. Experimental

2.1. Sample material

Cocoa samples were provided by the Cocoa Research Centre (The University of the West Indies, St. Augustine campus, Trinidad & Tobago). The sample set consisted of nine biological replicates (BR) of cocoa with subsamples obtained at different times (0, 2, 4, 6, 8, 10 days) during the fermentation process, resulting in 48 individual samples. Twenty (20) intact seeds of each cocoa sample were carefully peeled and ground to a fine powder using a commercial coffee grinder. To avoid pasting, seeds were homogenized by repeated short intervals of grinding and the resulting fine powder was sieved with a test sieve (DIN 1171, 1.0 mm) to a final particle size <1 mm. The freshly milled raw cocoa powder was directly analyzed by NIRS and subsequently stored at −20 °C until reference analyses.

2.2. Reference analysis

Unless otherwise specified, each analysis was performed in two independent replicates. The results of the related reference analyses for the 48 individual samples were summarized in Table 1.

The determination of moisture, pH value, free amino acids, organic acids (acetic and lactic acid) and methylxanthines (theobromine, caffeine) were performed according to the literature (Rohsius, 2007).

Individual phenolic substances like epicatechin, the sum of the four most representative phenolic substances (cyanidin-3-galactoside, cyanidin-3-arabinoside, catechin and epicatechin) as well as the content of total phenolic substances were quantified by HPLC and photometry, respectively, according to the protocol introduced by Elwers et al. (2009).

All chemicals and solvents were of analytical grade and obtained from Merck KGaA (Germany), unless otherwise specified.

2.2.1. Fat

The fat content was indirectly determined by reweighing the fat free samples prepared for the quantification of free amino acids, phenolic substances and methylxanthines. The difference value of the initial weight and the resulting fat free fraction were set as fat content. The defatting itself was performed as described in literature using exclusively petrol ether as solvent (Elwers et al., 2009).

2.2.2. Carbohydrates

Carbohydrates were isolated from the defatted material by aqueous extraction. Therefore, 1 mL of distilled water was added to 0.1 g of the fat free cocoa powder and heated for 1 h at 80 °C. After subsequent centrifugation (10 min, 13,000 rpm, Biofuge 6, Heraeus Instruments, Germany) 250 µL of the supernatant were