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Near infrared spectroscopy as a new tool to determine cocoa fermentation levels through ammonia nitrogen quantification

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ABSTRACT

Fermentation is a key step in obtaining fine cocoa through the formation of potent aroma precursors. The fermentation level of cocoa beans is traditionally assessed by measuring the amount of ammonia nitrogen (NH_3) using the time-consuming Conway technique. Near infrared spectroscopy (NIRS), a rapid and efficient tool, was used to analyze NH_3 levels in several hundred cocoa samples at different fermentation levels from six geographical origins. Fermentation levels were expressed as the number of fermentation days and sum of temperatures. The correlation between Conway results and NIRS spectra enabled the development of a reliable and accurate NIRS calibration to determine NH_3 content. We confirm that NH_3 is produced during fermentation and its amount depends on the fermentation time, sum of temperatures and geographical origin. NIRS could be used by chocolate manufacturers as a routine method to sort cocoa samples according to their level of fermentation.

1. Introduction

Cocoa beans (*Theobroma cacao* L.) are the main raw material in chocolate and a major flavouring ingredient in the preparation of beverages, confectionery, ice creams, baked products and other general products. The trading volume of cocoa futures on the Intercontinental Exchange (ICE) is close to 4.95 million metric tons for 2011 with Côte d'Ivoire, Ghana and Indonesia as the major producing countries (World Cocoa Foundation, 2013). Nowadays, world cocoa production is shared between bulk cocoa (standard quality) and fine or flavour cocoa (highly aromatic cocoa), which accounts for about 5% of production (The International Cocoa Organization, 2013). In the highly competitive market for fine or flavour cocoa, chocolate manufacturers need to be ensured they are being supplied with high quality beans.

Cocoa bean quality is linked to the cocoa variety, soil, climate, crop management and mainly to post-harvest processing (De Brito et al., 2001; Sukha, 2008). Post-harvest processing, which includes fermentation and drying, remains empirical most of the time, even though some requirements have been proposed by scientists and manufacturers (Senanayake, Jansz, & Buckle, 1997; Vincent, 1970). Fermentation is a key step in obtaining the characteristic flavour and taste of chocolate (Aculey et al., 2010; Voigt, Heinrichs, Voigt, & Biehl, 1994).

Following harvest, farmers open the pods (fruits of the cocoa tree), then extract the cocoa seeds (Lima, Almeida, Nout, & Zwietering, 2011; Lopez & Dimick, 1995) and fill up wooden boxes or bags, or pile up the cocoa seeds to start fermentation. The fresh beans to be fermented are surrounded by a mucilaginous pulp containing more than 80% water, 11–13% carbohydrates (mostly glucose and fructose) and 0.3% citric acid. Typical alcoholic, lactic and acetic fermentations naturally occur on the external pulp and are considered to initiate biochemical and enzymatic changes within the bean (Lopez & Dimick, 1995). The initial pH of the pulp, together with low oxygen levels, are conducive to colonization by yeasts, which utilize carbohydrates to produce ethanol. As aeration of the fermenting mass increases, acetic bacteria become dominant. Ethanol is oxidized into acetic acid by bacteria in a highly exothermic reaction, increasing the temperature of the mass (Barel, 1998; Schwan & Wheals, 2004). The production of acetic acid, together with the increase in temperature generates drastic changes within the cocoa beans, such as the death of the embryo and the breakdown of the cellular compartment (Quesnel, 1965). As a result, contact between biochemical constituents is facilitated, the bean colour changes from purple to brown (Aculey et al., 2010), aroma precursors are formed, proteins are degraded (Buyukpamukcu et al., 2001; De Brito et al., 2001; Lerceteau, Rogers, Petiard, & Crouzillat, 1999), and polyphenols are oxidized

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and polymerized (Forsyth, 1952; Hansen, del Olmo, & Burri, 1998). A strong relationship has been shown between the formation of cocoa aroma precursors and protein degradation during fermentation (Kirchhoff, Biehl, & Crone, 1989). Consequently, knowledge of the fermentation level is essential in order for fine chocolate manufacturers to evaluate dry cocoa quality.

Currently, the official technique for evaluating the quality of commercial grade cocoa is called the cut-test. It consists in longitudinally cutting and counting the proportion of purple and brown beans on a representative dried sample of 300 beans (Wood & Lass, 1985). In addition, some chocolate manufacturers quantify the ammonia nitrogen (NH_3) content by the Conway technique (Conway & Byrne, 1933), in order to enhance the quality control of the cocoa beans. From manufacturers' experience, there is a positive correlation between NH_3 content and the fermentation level, but no evidence of the origin of NH_3 has been demonstrated. Moreover, the NH_3 content varies from one geographical origin to another, forcing manufacturers to refer to their own background knowledge per origin.

In fact, a significant increase has been observed depending on the fermentation time, and NH_3 has been reported as a good fermentation marker (Guehi et al., 2010a; Vincent, 1970). Some scientists hypothesized that NH_3 might probably be derived from amino acids or even from polyphenols (Guehi, Zahouli, Ban-Koffi, Fae, & Nemlin, 2010b; Zahouli, Guehi, Monké Fae, Ban-Koffi, & Gnopo Nemlin, 2010). In addition, no effect of drying on the ammonia nitrogen content has been found (Zahouli et al., 2010), and no significant differences in ammonia nitrogen content have been observed in the different parts of fermented beans (Vincent, 1970).

To improve the sustainable management of fermented cocoa selection in relation to the NH_3 content, it is crucial to discover the kinetics of its formation during fermentation and to develop alternative analytical tools that are less expensive and faster than the Conway method.

Near-infrared spectroscopy (NIRS) is a very efficient technique for high-throughput screening of agronomic and agrifood products for their chemical characteristics. It is based on the vibrational properties of organic molecule chemical bonds and their interactions with infrared radiation. The NIR spectrum is a fingerprint related to the chemical composition of the product (Cen & He, 2007; Pasquini, 2003). This alternative, efficient, fast and non-destructive tool is already used in various fields. It has been used to monitor fermentation in wine (Di Egidio, Sinelli, Giovanelli, Moles, & Casiraghi, 2010), to identify specific components (fat profiles in shea nuts) (Davrieux et al., 2010), fat, caffeine, theobromine and (−)-epicatechin in cocoa (Alvarez et al., 2012)), to estimate fruit ripening stages (Le Moigne, Maury, Bertrand, & Jourjon, 2008), or to characterize coffee sensorial quality (Ribeiro, Ferreira, & Salva, 2011).

The aim of our paper was to study the potential of NIRS as a new approach for efficient estimation of the NH_3 content and, thereby, the fermentation level. To that end, a large number of samples uniformly covering the range of variability of the trait involved were used (Davrieux et al., 2010; Shenk & Westerhaus, 1991).

2. Materials and methods

2.1. Fermentation trials and sampling

Thirty micro-fermentation trials were carried out in three different countries (Ecuador, Madagascar and Dominican Republic), according to the producers' usual practices. A micro-fermented sample was represented by about 700 g of fresh cocoa beans placed in nets and positioned in fermentation boxes at three different defined levels (close to the top, in the middle, close to the bottom) (Sukha, Butler, Umaharan, & Boult, 2008). The temperature of each micro-fermented sample was recorded every 15 min by sensors (Thermo-boutons IP65 −40/+85 ±0.5, Laboratories Humeau, France) placed in the nets. The nets were surrounded by the mass of fresh cocoa beans in order to ensure a sufficient volume of cocoa for good fermentation. Fermentation was carried out for 6 days with stirring every 2 days (48 and 96 h). One sample per position and per fermentation was taken each day and then sun-dried to a final moisture content close to 7%. In addition, the cocoa mass was sampled every 2 days during stirring for each fermentation trial and then sun-dried like the micro-fermented samples.

The experimental design led to 635 samples, including 524 micro-fermented samples for which the fermentation temperature was recorded. Additional micro-fermented samples from Cameroon, Ghana, Indonesia and Trinidad and Tobago, from other experiments, were also used. Those samples were prepared and handled in the same way as the others. The difference was that the temperature was only recorded before each stirring, thus no sum of temperatures was calculated. This led to a total number of 718 samples (Table 1).

Each sample was identified by its origin, fermentation batch number, phenotype, fermentation time (in days) and type of sample (micro-fermented or mass). The sum of temperatures was calculated for each sample by the integral of the temperature curve over 20 °C from the beginning of fermentation to the time of sampling.

About 100 g of unshelled dried cocoa was ground in a "Valentin" blender (SEB, France) under liquid nitrogen, sifted to 0.5 mm and stored at −20 °C prior to analysis.

2.2. Chemical analysis

To investigate the NH_3 content, and for NIR calibration, wet chemistry was carried out on 190 selected samples out of the 718. Within those 190 samples, 135 were randomly selected from the 635 micro-fermented samples under the constraint of having the same sample percentage per class (fermentation time, origin, position in the box). The remaining 55 samples corresponded to randomly selected samples within the micro-fermented samples coming from other experiments.

The NH_3 content was determined by the Conway technique (Conway & Byrne, 1933). Cocoa powder was precisely weighed (about 500.0 ± 0.1 mg) and placed in the outer chamber of a Conway cell. Reagents were pure for analysis and purchased from Panreac (Barcelona, Spain). Each determination was done in triplicate.

2.3. Near infrared spectroscopy

NIRS acquisitions were obtained on a Foss 6500 monochromator (Foss, Silver Spring, MD) using a spin cell sample module. About 3 g of cocoa powder was analyzed by diffuse reflectance from

Table 1
Description of the experimental design.

Fermentation time (h)	CAM[a]	ECU	GHA	IND	MAD	RD	TRI	*N*
0	2	21	1	1	12	10	7	54
24	3	36	1	1	17	30	7	95
48	3	48	1	1	26	40	7	126
72	3	36	1	1	17	29	7	94
96	3	48	1	1	26	40	7	126
120	3	36	1	1	18	30	7	96
144	3	48	1	1	27	40	7	127
N	20	273	7	7	143	220	49	718

[a] CAM: Cameroon, ECU: Ecuador, GHA: Ghana, IND: Indonesia, MAD: Madagascar, RD: Dominican Republic, TRI: Trinidad and Tobago, *N*: total number of samples.