

Industrial Storage of Green Robusta Coffee under Tropical Conditions and Its Impact on Raw Material Quality and Ochratoxin A Content

Peter Bucheli,^{*,†} Inge Meyer,[‡] Alain Pittet,[‡] Gilles Vuataz,[‡] and Rinantonio Viani[§]

Nestlé Research Center, 101 Avenue Gustave Eiffel, F-37390 Notre Dame d'Oé, France; Nestlé Research Center, Nestec Ltd., Vers-chez-les-Blanc, P.O. Box 44, CH-1000 Lausanne 26, Switzerland; and Nestec Ltd., Avenue Nestlé 55, CH-1800 Vevey, Switzerland

Green Robusta coffee was stored in silos for 8 months under industrial conditions in Thailand, and subjected to air-conditioning, aeration, and nonaeration, and compared to bag storage under ambient conditions. Air-conditioning clearly reduced the relative humidity (RH) of the silo atmosphere and the moisture content (MC) and the water activity (a_w) of green coffee. Overall storage behavior was better for coffee in the aerated silo (RH = 68%; MC = 13.0%; a_w = 0.69) than for coffee stored in bags (RH = 81%; MC = 13.5%; a_w = 0.72). Aeration provided an efficient means to reduce MC and a_w during the rainy period. Glucose content was linked positively with the increase of a woody/rubbery note in coffee cup quality and increased by ~50% between months 3 and 6 when green coffee MC and a_w increased the most. Glucose is a potential green coffee quality marker. Under the tested storage conditions, neither the growth and presence of ochratoxin A (OTA)-producing fungi nor consistent OTA production was found. OTA contamination appeared to have occurred before storage.

Keywords: Coffee; storage; silo; glucose; mold; ochratoxin A

INTRODUCTION

Under tropical conditions, appropriate storage of green coffee is important to avoid raw material deterioration. Green coffee storage was intensively studied on Arabica coffee in Kenya (Sterling, 1980; Kulaba, 1981). According to this work, storage should begin with well-dried [10–12% moisture content (MC)] and good-quality green coffee; deterioration is minimized when the relative humidity (RH) of the storage atmosphere ranges between 50 and 70%, and the temperature is maintained below 26 °C.

Green coffee can be stored under controlled conditions for ≥ 3 years. However, color and flavor slowly change even under the best storage conditions (Mabbett, 1990). Green Robusta coffee production and storage conditions are particularly variable under the tropical conditions existing in southeastern Asia. Frequent MC determination is a simple and useful tool for following green coffee storage under such conditions. However, its determination does not provide any information about the handling history before purchase and the gradual decline in flavor and color characteristics of green coffee. Only the use of sensitive and reliable markers could fill this gap and could help to detect early stages of coffee deterioration that are currently detected by cup tasting. By testing eight well-defined Robusta and Arabica parchment and green coffees, Bucheli et al. (1996) demonstrated recently that the determination of glucose could be a sensitive marker for green coffee quality. Glucose is present only in trace amounts in good-quality

coffee (Wolfrom et al., 1960; Viani, 1986) and is generated in higher amounts upon poor storage as a result of sucrose hydrolysis (Bucheli et al., 1996). Small increases of glucose can be precisely determined by high-performance anion exchange chromatography coupled with pulsed electrochemical detection (HPAE-PED), a methodology that was successfully used for determining soybean deterioration upon storage (Locher and Bucheli, 1998).

Mold infestation of green coffee can result from inappropriate storage conditions and lead to quality loss. Gopalakrishna Rao et al. (1971) found that it is necessary to keep the MC of monsooned coffee within a limit of 14.5% to prevent mold growth during storage. Similarly, Betancourt and Frank (1983) observed that 14% MC and 75% RH were the limits for spoilage of green coffee due to fungal growth. The microflora of stored coffee beans was studied in detail by López Garay et al. (1987). Among the fungus species identified, *Aspergillus ochraceus* and *Penicillium* spp. were found to affect bean color and beverage quality. Ochratoxin A (OTA), a nephrotoxic and nephrocarcinogenic mycotoxin in animal experiments, is potentially being produced by these microorganisms at a minimum a_w of 0.85 (Moss, 1996) and is also found in coffee beans (Pohland et al., 1992). Studer-Rohr et al. (1995) stated recently that regular coffee consumption may contribute to exposure of humans to OTA. The conclusions of that paper were seriously questioned by several studies which demonstrated that pure soluble coffee is not a major source of OTA in the diet, with estimated intakes being well within safety limits (Pittet et al., 1996; Patel et al., 1997; van der Stegen et al., 1997). The impact of green coffee storage on OTA formation is unclear, and to our knowledge no published data exist about this subject.

[†] Nestlé Research Center, Tours.

[‡] Nestlé Research Center, Lausanne.

[§] Nestec Ltd., Vevey.

In this study we describe the effect of industrial silo (air-conditioned, aerated, and nonaerated) and bag storage under the existing tropical conditions of Thailand on the observed physical, microbiological, and compositional aspects of green Robusta coffee. We tested the practical use of glucose determination as a potential green coffee deterioration marker and present data on the effect of different industrial storage conditions on the development of OTA in green coffee and coffee cup quality.

MATERIALS AND METHODS

Raw Material. The green coffee used in this study was Robusta (*C. canephora*), harvested and produced during the period of January through March 1995 in the Chumphon area in the south of Thailand. Arabica (*C. arabica*) from Colombia and Robusta green coffee from Togo were used for sorption isotherm determination.

Storage Conditions and Sampling. The following four storage conditions were tested for 8 months: silo and bag storage were compared by storing 600 tons of green coffee each under air-conditioning (30 °C, 60% RH), aeration, and nonaeration in three separate silos, at Chachoengsao factory (Nestlé Thailand). Bag storage consisted of 100 tons of green coffee kept under ambient warehouse conditions. The storage trials were conducted between May 1995 and January 1996. Each month, samples of 5 kg were taken from the top and from the wall (top to bottom) of each silo. The corresponding bag sample was composed of aliquots (100 g each) taken from 50 different bags.

Defect Count. Defective coffee beans (black, partly black, broken, infested, husks, cherries, foreign matter) were determined as percentage weight on samples of 300 g of green coffee.

MC and a_w Determination. MC was determined in duplicate by drying for 6 and 4 h, respectively, at 130 °C according to the Norm International ISO 1447 (1978). Water activity was measured in duplicate according to the method of Guilbert and Morin (1986) with a DECAGON equipment based on the dew point determination. The sorption isotherm at 30 °C was established by determining the MC (in duplicate) of whole green coffee beans equilibrated for 1 month in desiccators over saturated salts at 33, 53, 62, 74, 77, and 84% RH, respectively.

Microbiological Analysis. Samples of 20 g of green coffee were soaked in 180 mL of TS diluent (tryptone 1 g/L, NaCl 8.5 g/L) for 30 min and then homogenized for 1 min in a Waring blender. Aerobic mesophilic microorganisms (AMM) and fungi were enumerated according to the method of Pitt et al. (1992). The Pastorex *Aspergillus* Kit (Sanofi Diagnostics Pasteur), a semiquantitative immunoagglutination method, was used to evaluate the viable and nonviable mold biomass. Results are expressed as the highest positive dilution. Dominant molds were identified by their aspect under a stereomicroscope according to the method of Pitt and Hocking (1985).

Soluble Sugar Analysis. Approximately 500 mg of ground green coffee was vigorously mixed with 70 mL of bidistilled water and incubated for 30 min at 70 °C. After cooling to room temperature, sample extracts were made up to 100 mL, vigorously shaken, and filtered with paper (S&S 597 1/2), and aliquots were prepared in special vials for HPLC by passing through 0.2 μ m nylon membrane filters (Supelco, Bellefonte, PA). This procedure was done in triplicate. Water-soluble extracts were separated in duplicate by HPAE-PED on an anion-exchange column (CarboPac PA-100, 4 \times 250 mm, Dionex, Sunnyvale, CA) by injecting 20 μ L at a flow rate of 1 mL/min. A linear NaOH gradient was applied, and quantification was performed as described in detail by Locher and Bucheli (1998) and Bucheli et al. (1996).

Cup Quality Evaluation. The green coffee was roasted, ground, and brewed in hot water. Cup tasting was carried out against the reference green coffee (stored frozen) used for coffee manufacturing. All samples were blindly tasted every month during the storage trial with a panel usually composed

of the same five to seven persons. The following quality attributes were evaluated and scored on a 5-point scale: coffee aroma, Robusta flavor, body, bitterness, fruity/winey, woody/rubbery, and chemical/phenolic. Sensory data are expressed as the average scores obtained by the panel for the nine tasting sessions of the trial period.

OTA Determination. OTA analyses were performed according to the method of Pittet et al. (1996), which can be summarized as follows. The finely ground test portion was blended for 3 min with methanol/3% sodium hydrogen carbonate (50:50) and filtered. An aliquot of 4 mL of filtrate was diluted to 100 mL with phosphate-buffered saline (PBS) and applied to an Ochratest immunoaffinity column containing a monoclonal antibody specific for OTA (Vicom Inc., Watertown, MA). After washing with 10 mL of distilled water, the OTA was eluted with 4 mL of methanol and quantitated by reversed-phase HPLC with fluorescence detection. A Spherisorb ODS II column (5 μ m particle size, 250 mm \times 4.6 mm i.d.) and an ODS Hypersil guard column (5 μ m particle size, 25 mm \times 4.6 mm i.d.), both from Metrohm Bischoff AG (Leonberg, Germany), were used along with a mobile phase of 45% acetonitrile/55% 4 mM sodium acetate/acetic acid (19:1). The separation was performed at ambient temperature at flow rate of 1 mL/min. The fluorescence detector was operated at an excitation wavelength of 330 nm and emission wavelength of 470 nm.

RESULTS AND DISCUSSION

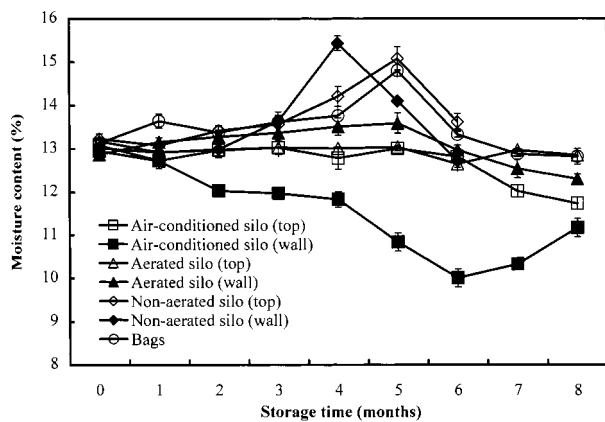
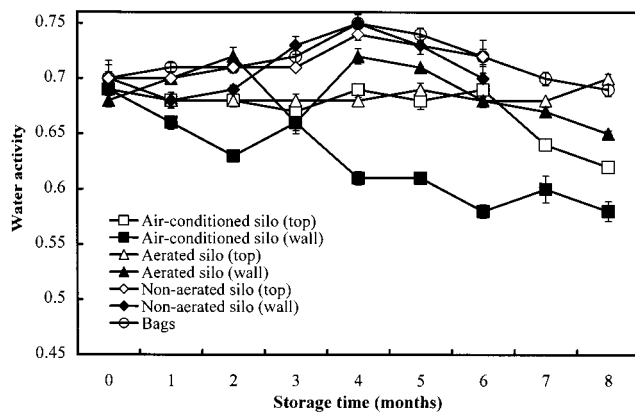
Temperature and RH Recording. The average temperature measured over 8 months ranged between 28 °C (air-conditioned, top), 28.9 °C (aerated, wall), and 30.3 °C (nonaerated, top) for the three silo conditions tested. The average temperatures recorded in the warehouse (bag storage area) and between stored bags were 29.4 and 28.4 °C, respectively (Table 1). It is unlikely that the observed average temperature differences (\sim 2 °C) between the different storage treatments were affecting selectively green coffee quality.

The RH of the storage atmosphere was strongly influenced by the applied storage treatments. It remained high in the warehouse (average = 81%) throughout the storage period, especially during the rainy period (months 2–5). Air-conditioning of the silo lowered considerably the RH, but a significant reduction was obtained only after 3 months of silo operation, when a level of \sim 60% RH was found. RH under aerated silo conditions was found to be comparable to the one measured between the stored bags and in the nonaerated silo (Table 1).

Determination of Green Coffee MC and a_w . Comparison of green coffee MC evolution under the four storage conditions tested (Figure 1) revealed that MC of green coffee in the aerated silo was relatively constant, with an average of 13.0% (top of silo) and 13.1% (silo wall) over the 8 month storage period. Silo aeration during rainy periods should avoid dangerous moisture pickup that can lead to fermentation or spoilage and to biochemical modifications inside the green coffee beans (Bucheli et al., 1996). Bag storage led to increased green coffee MC (up to 14.8%) between months 4 and 5 before declining afterward. These changes were tightly linked to atmospheric humidity (Table 1). The average MC of bag-stored green coffee was 13.5% over 8 months. Air-conditioning clearly reduced the MC of green coffee along the silo walls down to levels of \sim 10%, although drying was much less effective at the top of the silo (Figure 1). Evolution of the MC of green coffee in the nonaerated silo was similar to that observed for bags stored in the warehouse, with an important moisture

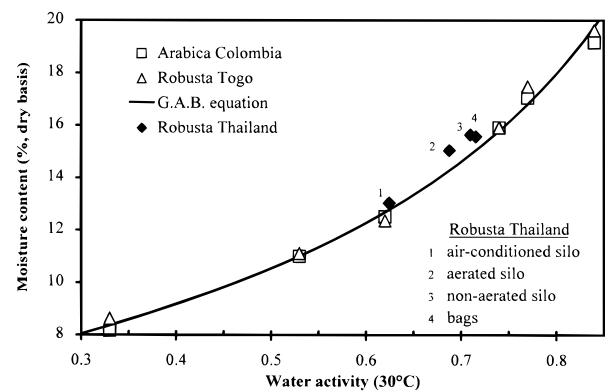
Table 1. Temperatures and Relative Humidities of Different Silo and Bag Conditions Used for Green Coffee Storage under Tropical Conditions

storage condition	month										
	0	1	2	3	4	5	6	7	8	av	
Temperature (°C)											
air-conditioned silo (top)	32.0	27.3	27.4	26.8	27.1	26.9	28.8				28.0
air-conditioned silo (wall)	31.9	27.0	28.1	27.1	29.3	29.3	28.6	25.5	27.9		28.3
aerated silo (top)	31.8	31.1	30.1	29.5	29.9	29.0	28.9				30.0
aerated silo (wall)	30.7	31.0	29.7	29.0	29.8	28.9	27.4	25.5	28.3		28.9
nonaerated silo (top)	33.0	29.4	30.6	29.5	29.9	29.8	30.0				30.3
warehouse	27.4	31.0	30.0	28.5	31.0	30.0	31.0	26.0	30.0		29.4
between bags	29.5	29.3	30.2	28.7	29.3	27.9	28.0	24.9	27.4		28.4
Relative Humidity (%)											
air-conditioned silo (top)	71.1	69.9	70.4	62.8	61.3	59.9	45.3				63.0
air-conditioned silo (wall)	70.9	67.5	67.9	58.5	48	48.8	45.8	48.5	49.1		56.1
aerated silo (top)	71.3	74.4	73.5	64.7	66.0	64.6	64.2				68.4
aerated silo (wall)	74.2	72.8	71.4	67.0	67.8	69.2	63.0	63.3	62.6		67.9
nonaerated silo (top)	72.2	76.1	72.8	64.7	64.8	65.0	65.1				68.7
warehouse	83.4	68.0	86.0	93.0	85.0	90.0	76.0	77.5	72.6		81.3
between bags	75.0	79.9	73.2	73.2	74.2	72.7	61.1	62.5	62.0		70.4

**Figure 1.** Moisture content of green coffee stored for 8 months under different silo and bag storage conditions. Error bars represent the standard deviation from the mean.**Figure 2.** Evolution of water activity of green coffee upon storage for 8 months under different silo and bag conditions. Error bars represent the standard deviation from the mean.

pickup after 3–4 months of storage, reaching a peak of 15.4% at month 4.

The most extreme variations in water activity values were found for air-conditioned silo treatment ($a_w = 0.58$) and for bag and nonaerated silo treatment ($a_w = 0.75$) over the 8 month storage period (Figure 2). Water activity increased the most during the rainy season (months 3–5) in green coffee stored in bags and in the nonaerated silo. On average, it tended to be consistently lower in green coffee stored in the aerated silo ($a_w = 0.69$) than in green coffee kept in bags ($a_w = 0.72$). As

**Figure 3.** Sorption isotherm of green coffee at 30 °C.

expected, air-conditioning considerably reduced the water activity in green coffee, especially along the silo wall, where values of 0.58 were reached (Figure 2). The water activity of green coffee stored in the nonaerated silo ($a_w = 0.70$) was on average comparable to that of bag-stored coffee during the 8 month storage period.

The predictive model of the sorption isotherm (GAB equation) shown in Figure 3 gives the water content on a dry basis of both Arabica and Robusta green coffee beans ranging from 0.30 to 0.85 a_w . The average data obtained from our industrial trials [(1) air-conditioned silo ($a_w = 0.624$; MC = 11.5%), (2) aerated silo ($a_w = 0.688$; MC = 13.0%), (3) nonaerated silo ($a_w = 0.710$; MC = 13.5%), and (4) bag ($a_w = 0.716$; MC = 13.5%)] fit the model relatively well. At 30 °C and ~70% RH, the model predicts that the MC would equilibrate at 12.8% with a variation of 0.2% MC per %RH variation; in reality, however, the close conditions existing in Thailand (bag storage, 70.4% RH, and 28.4 °C, see Table 1) led to a green coffee MC of 13.5%.

Microbiological Evaluation. The results shown in Table 2 indicate that the load of AMMs was similarly high for all green coffees examined at the beginning of the storage trial [range of 10^6 – 10^7 colony-forming units (CFU)/g]. Under the various storage conditions tested, the amount of AMM decreased in most cases during storage (on average 7-fold), indicating no additional microbial growth during the storage period. A similar situation was encountered for mold growth, with an 18-fold average decrease in fungi count during all storage treatments tested. There was little change in the data from the immunoagglutination method evaluating vi-

Table 2. Microbiological Evaluation of Green Coffee Stored under Different Silo and Bag Storage Conditions in Thailand

storage condition	month 0			month 3			month 6			month 8		
	AMM log CFU/g	fungi log CFU/g	IT ^a	AMM log CFU/g	fungi log CFU/g	IT	AMM log CFU/g	fungi log CFU/g	IT	AMM log CFU/g	fungi log CFU/g	IT
air-conditioned silo (top)	7.29	5.92	1/2430	6.78	5.34	1/2430	6.48	5.29	1/2430	7.28	5.45	1/2430
air-conditioned silo (wall)	6.92	5.85	1/7290	5.9	6.26	1/7290	6.72	5.72	1/7290	6.39	5.07	1/7290
aerated silo (top)	6.94	5.67	1/7290	5.85	5.72	1/7290	6.08	4.64	1/7290	5.67	4.16	1/7290
aerated silo (wall)	6.89	5.48	1/7290	6.04	5.34	1/7290	5.87	5.04	1/7290	6.83	5.09	1/7290
nonaerated silo (top)	6.38	6.18	1/7290	5.51	5.58	1/7290	6.78	5.88	1/7290	5.49	4.94	1/7290
nonaerated silo (wall)	6.98	5.91	1/2430	5.38	5.72	1/7290	6.81	5.15	1/7290	5.86	4.19	1/7290
bag storage	6.89	5.70	1/2430	6.43	5.54	1/7290	6.04	5.94	1/7290	6.01	4.53	1/7290

^a IT, immunological titer.

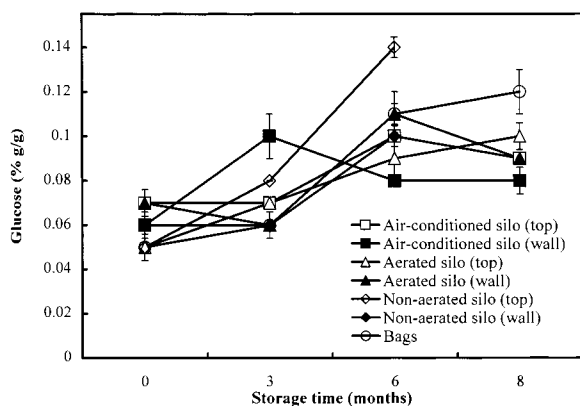


Figure 4. Glucose content of green coffee stored for 8 months under different silo and bag storage conditions. Error bars represent the standard deviation from the mean.

able and nonviable mold biomass, confirming that there was no mold biomass increase under any of the conditions examined. The principal mold species found were *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus series restrictus*, *Penicillium* sp., and *Wallemia sebi* (data not shown). No evidence was found for the presence of mold species that could potentially produce aflatoxins and OTA, respectively.

Determination of Soluble Sugars in Green Coffee. Determination of sugars by HPAE-PED chromatography revealed at first sight no dramatic changes in sugar composition during storage conditions. Nevertheless, we have shown earlier on a laboratory scale that under accelerated storage conditions (37 °C, 82% RH) a high RH storage atmosphere will generate glucose in green coffee as a result of sucrose hydrolysis (Bucheli et al., 1996). This observation led to the idea that glucose might be a reliable green coffee quality marker.

In the storage trials described here, we show that initial glucose content at month 0 was on average 0.06% and that it almost doubled to 0.10% g/g within 8 months of storage (Figure 4). Most of the observed increase occurred between months 3 and 6 (~50%), exactly during the period of important moisture pickup (rainy season). Glucose levels increased under all storage conditions examined, indicating that the metabolic conditions needed for sucrose hydrolysis were attained (Figure 4). It was shown by Bucheli et al. (1996) that sufficient water activity is the trigger for enzymatic sucrose degradation. Fructose concentration did not follow the trend of increasing glucose content and was stable throughout storage (data not shown). A similar observation was made for accelerated soybean storage

Table 3. Ochratoxin A Content (Micrograms per Kilogram) of Green Coffee Stored under Different Silo and Bag Storage Conditions

storage condition	month				
	0	3	5	6	av
air-conditioned silo (top)	9.8	4.5	1.3	5.8	5.4
air-conditioned silo (wall)	3.3	3.9	2.9	4.4	3.6
aerated silo (top)	3	13.5	6.9	3.7	6.8
aerated silo (wall)	2	2.2	2.9	4.4	2.9
nonaerated silo (top)	3.5	2.2	1.4	1.8	2.2
nonaerated silo (wall)	3	5.2	2.6	2.3	3.3
bag storage	2.6	1.5	2.2	1.3	1.9
av	3.9	4.7	2.9	3.4	

conditions, where generation of fructose was detected only after substantial oligosaccharide degradation (Locher and Bucheli, 1998).

OTA Determination. OTA was detected in all green coffee samples analyzed during the storage trials (Table 3), with averages of 4.9 µg/kg (aerated silo), 4.5 µg/kg (air-conditioned silo), 2.8 µg/kg (nonaerated silo), and 1.9 µg/kg (bags). The observed differences in OTA contamination of silo- and bag-stored green coffee appeared to be directly linked to the amounts of defects. In fact, defect count was consistently lower over the 8 month period in green coffee stored in bags (12%) than in silos (18%). It is possible that source and postharvest handling of coffee affected the defect count. The substantial fluctuations in OTA content (e.g., for aerated silo) were certainly due to raw material inhomogeneity and not caused by microbial growth during storage as indicated by our results on microbiological evaluation (Table 2). The problem of inhomogeneity of OTA contamination in green coffee beans was highlighted in a recent paper published by Blanc et al. (1998); they found a relative standard deviation of 39% for OTA contamination in Thai coffee. Clearly, OTA contamination had taken place before storage as underscored with our data at month 0 (average OTA of 3.9 µg/kg) and is possibly linked to defect count. In addition, our OTA data and microbiological evaluation during the different industrial storage conditions tested strongly favor the idea that OTA contamination is not the result of storage but most likely linked to the conditions present during green coffee production (harvesting and drying of coffee fruits).

Cup Quality Evaluation. The results of sensory evaluation of cup quality are presented in Table 4. Sensory scores were obtained monthly and averaged over the 8 month storage period. The woody/rubbery

Table 4. Average Sensory Scores (5-Point Scale^a) of Monthly Cup Quality Evaluation of Green Coffee Stored for 8 Months under Different Silo and Bag Storage Conditions

attribute	ref	air-conditioned			bag
		silo	aerated silo	nonaerated silo	
coffee aroma	4	3.9	3.8	4	3.6
Robusta flavor	1	1.1	1	1	1
body	3	3	2.9	3	2.9
bitterness	3	2.9	2.9	3	3
fruity/winey	1	1.1	1	0.9	1
woody/rubbery	0	1.3	0.7	0.6	0.8
chemical/phenolic	0	0	0	0	0.3

^a Intensity scale: 0 = none, 1 = low, 2 = low/medium, 3 = medium, 4 = medium/high, 5 = high.

note was the only cup quality aspect that changed during green coffee storage. Increases in the woody/rubbery note were perceived only after month 3 (during the rainy season). The increase of the woody/rubbery note was linked to a marked increase of glucose (Figure 4) between months 3 and 6. Compared to the reference (defined note = 0), the average woody/rubbery note between months 4 and 8 was highest for coffee from the air-conditioned silo (2.1), followed by aerated silo (1.4), bag storage (1.1), and nonaerated silo (1.0). Only minor changes for coffee quality attributes (aroma, Robusta flavor, body, and bitterness) and characteristics (fruity/winey, chemical/phenolic) were perceived, and no off-flavors were detected at any time under the storage conditions examined (Table 4).

Summary. This study provides essential data, not previously available, on the impact of different industrial storage conditions in a tropical environment on the physical, microbiological, compositional, and sensory aspects of green Robusta coffee. The existing tropical storage conditions of Thailand did not significantly affect green coffee quality. We found no indications for the presence and growth of OTA-producing fungi or the production of OTA upon storage.

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