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# Influence of roasting conditions on the biogenic amine content in cocoa beans of different *Theobroma cacao* cultivars

Joanna Oracz *, Ewa Nebesny

*Institute of Chemical Technology of Food, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, 4/10 Stefanowskiego Street, 90-924 Lodz, Poland*

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ABSTRACT

The objective of this study was to investigate how roasting process conditions affected the content of biogenic amines in cocoa beans of different *Theobroma cacao* varieties. The raw cocoa beans were roasted at four different temperatures (ranging from 110 °C to 150 °C) and three different air humidities (0.3% to 5.0%). Roasting process may significantly modify the profile and levels of biogenic amines. Tyramine was the most abundant amine in raw cocoa beans, followed by tryptamine and 2-phenylethylamine. Serotonin and dopamine were presented only in small amounts. However, it was found that roasted cocoa beans contained mainly 2-phenylethylamine, followed by tyramine, tryptamine, serotonin and dopamine. Parameters of roasting have a significant effect on the levels of each amine in all types of roasted beans. The highest amount of biogenic amines was observed in the samples roasted at the highest temperatures and in the air with increased humidity. In addition, the results revealed that the cacao cultivars significantly affect the levels of biogenic amines.



## 1. Introduction

Cocoa beans, seeds of the tree *Theobroma cacao* L., are the primary raw material used in the manufacture of finial products intended directly for consumption such as cocoa powder, chocolate bar and other cocoa derivative products which are highly valued by consumers around the world (Belscak, Komes, Horzic, Kovacevic Ganic, & Karlovic, 2009). Cocoa beans and their derivative products found on the market are produced from three main subspecies of *T. cacao* i.e. traditional Criollo (fine or flavor) and Forastero (bulk) and their natural hybrid Trinitario (fine or flavor) (Hii, Law, Suzannah, Misnawi, & Cloke, 2009). In recent years a number of new hybrid and clonal varieties with beneficial sensory traits or/and more resistant to adverse environmental conditions have been developed (Lachenaud, Paulin, Ducamp, & Thevenin, 2007). Roasting is one of the basic technological operation in cocoa bean processing affecting the quality of cocoa derivative products. This process plays an important role in formation of the characteristic chocolate flavor, increases the intensity of brown color and causes changes in the texture of roasted beans (Oliviero, Capuano, Cammerer, & Fogliano, 2009). During roasting, the raw cocoa beans are exposed to temperatures that range from 110 to 160 °C (Farah, Zaibunnisa, & Misnawi, 2012), whereas the "fine or flavor" varieties require lower temperatures than the "bulk" ones (Ramli, Hassan, Said, Samsudin, & Idris, 2006). Many previous studies revealed that the temperature and duration of roasting substantially affected the character of chemical and physical changes occurring in cocoa beans (Farah et al., 2012). It should be noted that the transformations of bioactive compounds are mostly omnidirectional, and can lead to both the degradation and formation of new substances, as a result of transformations of their precursors. Additionally, a diversity of cacao varieties as well as geographical region of cultivation may affect the composition of cocoa seeds (Bertazzo, Comai, Brunato, Zancato, & Costa, 2011; Borchers, Keen, Hannum, & Gershwin, 2000).

The seeds of the cacao tree belong to the sources of many bioactive compounds, including biogenic amines (Bruinsma & Taren, 1999). Biogenic amines are low molecular weight organic bases that have a high biological activity. They are presented in plants in their free form (as free bases), conjugated to small molecules such as phenolic acids, or bound to high molecular weight compounds such as proteins or nucleic acids (Casal et al., 2004). Biogenic amine content depends mainly on the plant variety and region of cultivation, as well as degree of maturity and the post-harvest manufacturing processes and storage conditions (Bandeira, Evangelista, & Gloria, 2012; Casal et al., 2004; Gloria, Tavares-Neto, Labanca, & Carvalho, 2005; Oliveira, Franca, Gloria, & Borges, 2005). The main biogenic amines found in cocoa and chocolate are 2-phenylethylamine, tyramine, tryptamine, serotonin, dopamine and histamine (Guillén-Casla, Rosales-Conrado, León-González, Pérez-Arribas, & Polo-Díez, 2012; Kosman, Stankevich, Makarov, & Tikhonov, 2007; Pastore et al., 2005; Smit, 2011). 2-Phenylethylamine is an endogenous trace amine that occurs naturally in the brain of many mammalian species including humans. Tyramine, 2-phenylethylamine and tryptamine have been considered as the initiators of hypertension and dietary-induced migraine (Smit, 2011). Serotonin is an essential neurotransmitter and vasoconstrictor and plays an important role in the regulation of anger, appetite, body temperature, blood pressure, mood, sexuality and sleep (Guillén-Casla et al., 2012). Dopamine is

* Corresponding author. Tel.: +48 42 631 34 58; fax: +48 42 636 74 88.
*E-mail addresses:* joanna.oracz@p.lodz.pl (J. Oracz), ewa.nebesny@p.lodz.pl (E. Nebesny).

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an endogenous catecholamine that determines many physiological functions, including behavior, nerve conduction, hormone synthesis and secretion, blood pressure, and kidney function regulation (Jose et al., 1992).

It is well-known that bioactive amines are mainly formed in foods during the microbial decarboxylation of free amino acids, or by amination and transamination of ketones and aldehydes. Very recent publications also indicated that they can also be formed during thermal processes as a result of the oxidative decarboxylation of corresponding amino acid precursors (Martins & Gloria, 2010; Oliveira et al., 2005; Zamora, Delgado, & Hidalgo, 2012). Some authors have reported that the Strecker degradation is responsible for the formation of biogenic amines in the way of thermal decarboxylation of amino acids in the presence of α-dicarbonyl compounds formed during the Maillard reaction (Granvogl, Bugan, & Schieberle, 2006; Schieberle, Koehler, & Granvogl, 2005) or lipid peroxidation products (Zamora et al., 2012). According to Schieberle et al. (2005) Strecker degradation of asparagine results in the 3-aminopropionoamide formation. Other authors suggest that serotonin may also form as a result of the transformation of its precursors (tryptophan and 5-hydroxytryptophan) at very high temperatures (Martins & Gloria, 2010). However, data on the effects of thermal processes on the formation of biogenic amines in cocoa beans are still limited. Only a few studies investigating the influence of coffee roasting on the levels of biogenic amines are known (Casal et al., 2004; Cirilo et al., 2003; Oliveira et al., 2005). Furthermore, there is a lack of information on the effect of roasting conditions such as temperature and relative humidity on the changes of biogenic amine levels in different varieties of cocoa beans.

The consumption of food containing high concentrations of some biogenic amines can cause undesirable physiological and toxicological effects (Bandeira et al., 2012). Moreover, knowledge about the types and concentrations of biogenic amines in cocoa beans of different varieties as well as factors affecting the changes of these compounds levels during roasting will provide a more accurate estimation of dietary intake of biogenic amines from cocoa derived products, such as cocoa and chocolate.

Therefore, the aim of this study was to determine the profile and levels of biogenic amines in cocoa beans of various cultivars originating from selected geographic areas and to investigate the effects of different roasting conditions, including temperature and air humidity of roasting on the changes of those compounds content.

## 2. Materials and methods

### 2.1. Chemicals

Tyramine hydrochloride, tryptamine hydrochloride, 2-phenylethylamine, serotonin hydrochloride, dopamine hydrochloride, 1,7-heptanediamine, dansyl chloride, acetonitrile (HPLC), acetone (HPLC), ammonia solution (25%) and perchloric acid (70%) were purchased from Sigma-Aldrich (Poznan, Poland). HPLC grade methanol was obtained from J.T. Baker (Deventer, Netherlands). Petroleum ether (bp 40–60 °C), sodium hydroxide and sodium hydrogen carbonate (all of analytical grade) were purchased from Chempur (Piekary Slaskie, Poland). Ultrapure water was obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA). Phenomenex Strata-X 33U Polymeric Reversed Phase (3 ml, 200 mg) columns and Chromacol PTFE syringe filters (0.2 μm pore size) were purchased from Shim-Pol (Izabelin, Poland).

### 2.2. Cocoa samples

Seven samples of fermented and dried cocoa beans of different *T. cacao* varieties obtained from Brazil (Forastero), Ecuador (Nacional), Papua New Guinea (Trinitario), Venezuela (Trinitario), Ghana (Upper Amazon Forastero hybrid, UAF), Cameroon (Trinitario × Upper Amazon Forastero hybrid, T × UAF) and Indonesia (Trinitario × Upper Amazon Forastero hybrid, T × UAF) were evaluated. All seeds were harvested in 2011 and purchased from commercial sources. Raw material was selected to discard broken or chipped seeds.

The selected cocoa seeds of the same size (200 g) were conventionally roasted in a tunnel with forced air flow without circulation, with the possibility of carrying out the process in air with elevated humidity. The operating temperature of 50–220 °C can be obtained with a 6-kW heater. Relative humidity of roasting air was increased using saturated steam at a pressure of 0.2 MPa produced in the steam generator. Temperature and relative humidity were determined using a high temperature/humidity probe Rotronic HygroPalm HP22 (Rotronic AG Bassersdorf, Switzerland) with an accuracy of ±1 °C and ±0.5%, respectively. In the experiment air velocity along the material layer was 1 m/s, measured with an accuracy of ±0.05 m/s.

The samples were roasted in triplicate at four different air temperatures: 110, 120, 135 and 150 °C and three different relative humidities of air were used: 0.3, 2.0 and 5.0%. Applied in these studies heat treatment parameters were chosen to obtain a range of roasted beans with acceptable physico-chemical and sensory properties (Krysiak, 2011, Krysiak, Majda, & Nebesny, 2007; Nebesny & Rutkowski, 1998). Thermal treatment was carried out to obtain about 2% water content in the roasted material, because then the cocoa beans gain appropriate textural properties which are important for the subsequent processing steps, such as crushing and extraction of cocoa butter (Nebesny & Rutkowski, 1998). The roasting time was determined for each batch of beans based on the initial water content and the size of the raw material. After roasting, the cocoa beans were immediately cooled, placed into hermetically sealed plastic containers (500 g) and stored at −20 °C until analysis.

### 2.3. Methods of analysis

#### 2.3.1. Biogenic amine determination

Biogenic amines were extracted from the cocoa samples according to the procedure developed by Pastore et al. (2005) with some modifications. Cocoa beans were deshelled and ground in a laboratory mill before the amine extraction. 5 g of ground samples was first defatted three times with 20 ml of petroleum ether for 20 min in an orbital shaker at 300 rpm and then the mixture was centrifuged for 10 min at 6000 rpm. The petroleum ether layer was discarded and remained ether was evaporated under a stream of nitrogen. 2 g of each dried and defatted sample was extracted twice with 10 ml of 0.2 M perchloric acid containing a known amount of internal standard solution (1,7-heptanediamine) for 20 min in an ultrasonic bath, and then centrifuged for 45 min at 6000 rpm. The clear supernatants were combined and the final volume was adjusted to 20 ml with 0.2 M perchloric acid. A 1-ml aliquot of the extract was quantitatively transferred to the 5 ml Reacti-vial containing a Reacti-vial magnetic stirrer and mixed with the 200 μl of 2.0 M sodium hydroxide solution and 300 μl of sodium hydrogen carbonate solution. For preparing DNS-Cl derivatives, subsequently 2 ml of dansyl chloride solution (10 mg/ml in acetone) was added. The vial was capped and placed in the Reacti-Block aluminum block within a Reacti-Therm III Heating/Stirring Module (Thermo Fisher Scientific, Rockford, IL) and left for 20 h at room temperature with constant stirring (600 rpm). After an incubation time, in order to remove residual dansyl chloride the 100 μl of 25% ammonia solution was added and the sample was centrifuged for 15 min at 6000 rpm (Kim, Byun, & Mah, 2012). Isolation of the amine derivatives from aqueous solution was performed by using the Phenomenex Strata-X 33U Polymeric Reversed Phase columns (200 mg, 3 ml), previously conditioned with 6 ml of methanol and 4 ml of Milli-Q water. The supernatant was loaded onto the column and washed with 6 ml of Milli-Q water:acetone (80:20, v/v). Then the extraction column was dried under vacuum. Finally, the amines were eluted from the column with 6 ml of acetonitrile. The eluted fraction was concentrated by evaporation to dryness under a