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Effect of alkalization on the Maillard reaction products formed in cocoa during roasting

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ABSTRACT

Cocoa beans are used in vast variety of food products whether as raw or after alkaline treatment. In this study, cocoa beans were treated with sodium carbonate or water and then roasted together with non-treated cocoa beans. To understand the effect of alkalization on Maillard reaction during cocoa roasting, changes in the concentrations of certain Maillard reaction compounds were determined. Additionally, changes in the concentrations of sugars and modification of lysine were also monitored. Alkaline treatment favored the degradation of sugars in cocoa together with roasting. The concentration of α-dicarbonyl compounds was higher in alkaline treated cocoa compared to water-immersed and non-treated cocoa. Roasting process substantially decreased the concentrations of 3-deoxyglucosone (3-DG), glucosone, glyoxal and diacetyl in alkaline treated cocoa. The concentrations of methylglyoxal and 5-hydroxymethylfurfural (HMF) increased in cocoa samples after roasting although this increase was less in alkaline treated cocoa compared to the other treatments. Most of the lysine was modified within 30 min and fructoselysine, measured as furosine, gradually degraded independent of the treatments, but depending on temperature. *N*-ε-Carboxymethyllysine (CML) was doubled in alkaline treated cocoa although it did not change depending on the roasting.

1. Introduction

Cocoa beans, the seeds of the *Theobroma cacao* tree, are widely consumed all over the world. They are processed to obtain chocolate liquor, cocoa powder and cocoa butter which are the main ingredients of chocolate and a vast range of products like cocoa beverages, ice cream and bakery products (Payne, Hurst, Miller, Rank, & Stuart, 2010). Roasting is the key step in the production of chocolate liquor or cocoa powder as it helps in the removal of undesirable volatile compounds, provides desirable aroma and flavor and makes cocoa beans more brittle (Oliviero, Capuano, Cammerer, & Fogliano, 2009; Wollgast & Anklam, 2000).

Alkalization which is also known as Dutch processing could be applied to the cocoa beans, chocolate liquor or cocoa powder in some cases (Miller et al., 2008). The aim of alkali processing is to obtain a desirable dark brown color, to reduce the bitterness and astringency and to prevent sinking of the cocoa powder in cocoa based drinks (Andres-Lacueva et al., 2008; Miller et al., 2008).

Maillard reaction and caramelization are the most important reactions proceeding during roasting as they contribute to the aroma, flavor and color of cocoa products (Bonvehi & Coll, 2002; Hollnagel & Kroh, 1998). Although these reactions provide desirable properties to the final products, they may cause the formation of certain products which are proposed to be mutagenic, cytotoxic, or carcinogenic (Nguyen, 2006; Wong & Shibamoto, 1996). In addition to that, Maillard reaction also causes loss of nutritional value of proteins as a result of modification in essential amino acids especially lysine (Erbersdobler & Somoza, 2007). Alpha-dicarbonyl compounds, which have ability to interact with many cellular constituents giving rise to several metabolic disorders, are a group of molecules formed through both Maillard reaction and caramelization (Jiang, Hengel, Pan, Seiber, & Shibamoto, 2013). Degradation of Amadori product and dehydration of hexose sugars result in the formation of 3-deoxyglucosone (3-DG) and 1-deoxyglucosone (1-DG) (Gobert & Glomb, 2009). 3-DG could further dehydrate to form 3,4-dideoxyglucosone which consequently form 5-hydroxymethylfurfural (HMF) with loss of one molecule of H_2O (Locas & Yaylayan, 2008; Marceau & Yaylayan, 2009). HMF is an intermediate whose higher concentrations indicate high thermal load or prolonged storage (Capuano & Fogliano, 2011). Presence of HMF is also undesirable as HMF itself is cytotoxic in high concentrations or its biotransformation product 5-sulphoxymethylfurfural is mutagenic to mammalian cells (Capuano & Fogliano, 2011; Glatt & Sommer, 2006). Cleavage of hexuloses could form glyoxal, methylglyoxal and diacetyl or they may directly arise from hexoses after isomerization and retro-aldolization (Gobert & Glomb, 2009; Weenen, 1998). Glucosone, which is another α-dicarbonyl compound, is formed through oxidation of hexoses (Gobert & Glomb, 2009; Thornalley, Langborg, & Minhas, 1999).

Furosine occurs as a result of acid hydrolysis of the Amadori product, fructoselysine, originating from the reaction between

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glucose and ε-amino group of lysine. It is evaluated as the most important molecule indicating the early Maillard reaction (Erbersdobler & Somoza, 2007). *N*-ε-Carboxymethyllysine (CML) is one of the advanced glycation end products (AGEs) that gives additional information about the protein damage and known as the indicator of the advanced or late stages of the Maillard reaction (Erbersdobler & Somoza, 2007; Poulsen et al., 2013). AGEs are mainly formed through the reaction of amino side chains of proteins and carbonyl compounds. The possible formation routes for CML are the oxidation of Amadori product, or the reaction of glyoxal with ε-amino group of protein bound lysine (Krause, Knoll, & Henle, 2003).

The objective of this study is to reveal the effect of alkalization process on the formation of certain Maillard reaction products in cocoa during roasting process. For that reason, 3-DG, glucosone, glyoxal, methylglyoxal and diacetyl concentrations of natural, water-immersed and alkaline treated cocoa beans during roasting were measured with LC–MS after pre-column derivatization. Moreover, HMF, furosine and CML concentrations were also determined as the indicators of the Maillard reaction.

2. Materials and methods

2.1. Chemicals and consumables

3-Deoxyglucosone (75%), quinoxaline (99%), 2-methylquinoxaline (97%), 2,3-dimethylquinoxaline (97%), o-phenylenediamine (98%), diethylenetriaminepentaacetic acid (98%), glucose (>99%), fructose (>99%), sucrose (>99%), 5-hydroxymethylfurfural (≥99%), lysine (>98%), sodium borohydride powder (≥98%) were purchased from Sigma-Aldrich (Steinheim, Germany). Furosine standard was obtained from Neosystem Laboratoire (Strasbourg, France). Methanol (≥99.9%) and acetonitrile (≥99.9%), hexane (95%) and water (ChromasolV grade) were also purchased from Sigma-Aldrich (Steinheim, Germany). Formic acid (98%) was purchased from JT Baker (Deventer, The Netherlands). Potassium hexacyanoferrate, zinc sulfate, disodium hydrogen phosphate anhydrous, sodium dihydrogen phosphate dihydrate, sodium hydroxide, sodium carbonate, boric acid, hydrochloric acid (37%), sulfuric acid (95-98%) were purchased from Merck (Darmstadt, Germany). Nylon syringe filters (pore size 0.45 μm) and OASIS HLB cartridges were supplied by Waters Corp. (Milford, MA, USA). Fermented and dried cocoa beans (*T. cacao* L.) were supplied by a food processing company in Turkey.

2.2. Alkalization and roasting process

A 160 g portion of cocoa bean was immersed in 160 mL of 7.5% Na_2CO_3 containing alkaline solution for 30 min. To make the alkalization more effective the solution was shaken every 5 min. Then, cocoa beans were dried at 70 °C for 2 h before the roasting process. The same procedure was applied to the same portion of cocoa beans by using water as control.

Roasting was performed with alkaline treated (cocoa beans that were dipped into 7.5% Na_2CO_3 containing alkaline solution) and water-immersed cocoa beans in addition to non-treated cocoa beans. Twenty grams of cocoa beans was roasted at 135 and 150 °C for 30 and 60 min. All cocoa samples were defatted by using hexane and dried before analysis.

2.3. Measurement of moisture content

Moisture content of cocoa beans was determined gravimetrically according to AOAC 925.10 (AOAC, 1990). Two grams of ground cocoa samples was dried at 105 °C until reaching to a constant weight.

2.4. Measurement of pH

One gram of ground defatted cocoa sample was diluted with 20 mL of deionized water. After vortexing for 10 min, the cocoa samples were centrifuged at 5000 ×*g* for 3 min. The pH of the clear supernatants of the cocoa samples was measured by using a pH-meter (PHM 210 Meterlab, France).

2.5. Analysis of sugars

Ground defatted cocoa sample (0.25 g) was weighted into tubes and triple extraction was performed by using 10 mL water (5, 2.5, 2.5 mL) at 70 °C. The tube was vortexed for 3 min and centrifuged at 5000 ×*g* for 5 min at each step of extraction. Then, supernatants were transferred to another tube and centrifuged at 5000 ×*g* for 3 min. After then, 1 mL of the clear supernatant was transferred to a centrifuge tube and 50 μL Carrez I and 50 μL Carrez II, which were prepared by dissolving 15 g potassium hexacyanoferrate in 100 mL water and 30 g zinc sulfate in 100 mL water, were added onto it. The centrifuge tube was vortexed for 3 min and centrifuged at 5000 ×*g* for 3 min. Then, 1 mL of the clear extract was passed through a pre-conditioned (by passing 1 mL methanol and 1 mL water) OASIS HLB cartridge. The first 7–8 drops of the eluent were discarded and the rest was collected into a vial for the analysis.

Analysis was performed on an Agilent 1100 HPLC system (Agilent Tech., Waldbronn, Germany) containing a refractive index detector, quaternary pump, temperature controlled column oven and an autosampler. The extracts were injected on Shodex Sugar SH1011 column (300 × 8 mm, 6 μm) at 40 °C. The mobile phase was 10 mM sulfuric acid in water and the flow rate was 1 mL/min. Standard solutions of sucrose, glucose and fructose with concentrations ranging between 0.1 and 1 g/100 mL were prepared.

2.6. Analysis of HMF

The extracts used for sugar analysis were also used for HMF analysis. The extract was passed through a 0.45 μm nylon filter and collected in a vial. Analysis was performed by using a Shimadzu Ultra Fast Liquid Chromatography system (Shimadzu Corp., Kyoto, Japan) consisting of a quaternary pump, a temperature controlled column oven, an autosampler and a diode array detector. Waters Atlantis dC18 column (250 × 4.6 mm, 5 μm) was used for chromatographic separation. The mobile phase was isocratic mixture of 10 mM formic acid in the mixture of water:acetonitrile (90:10, v/v) and the flow rate was 1 mL/min. The column temperature was 25 °C and the injection volume was 10 μL. The diode array detector was collected signals at 285 nm. Quantification was performed with standard solutions of HMF prepared in concentrations ranging from 1 to 10 mg/L.

2.7. Analysis of α-dicarbonyl compounds

Analysis of α-dicarbonyl compounds was carried out as described by Kocadağlı and Gökmen (2014). The extracts used for sugar analysis were also used for the analysis of α-dicarbonyl compounds. The co-extracted colloids were precipitated by mixing with acetonitrile. Two hundred μL of the extract was diluted with 800 μL of the mixture of acetonitrile:water (5:3, v/v), and centrifuged at 5000 ×*g* for 5 min. The clean supernatant was used for derivatization. Derivatization of α-dicarbonyl compounds was carried out with o-phenylenediamine (o-PDA). Quinoxaline derivatives were formed by the reaction of α-dicarbonyl compounds with 0.2% o-PDA solution containing 11 mM diethylenetriaminepentaacetic acid. Five hundred μL of supernatant was mixed with 150 μL of o-PDA and 150 μL of 0.5 M sodium phosphate buffer (pH 7). The mixture was immediately filtered through 0.45 μm nylon syringe filter and kept at room temperature in the dark for 2 h prior to HPLC-ESI-MS measurement.