

Flavor Components in the Maillard Reaction of Different Amino Acids with Fructose in Cocoa Butter-Water. Qualitative and Quantitative Analysis of Pyrazines

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Eight amino acids were reacted with fructose in deodorized cocoa butter-water as a model of the Maillard reaction in cocoa beans. Detailed GC/MS analysis of the volatile compounds obtained is reported with particular reference to similarities and differences in the patterns obtained with the amino acids. A quantitative analysis of pyrazines is described. Among the 22 pyrazines detected, 15 had been already reported in roasted cocoa beans. 2-(3-Methylbutyl)-3,6-dimethylpyrazine was formed in the reaction of leucine, while lysine gave three 2-methoxy-3-alkylpyrazines not reported in cocoa.

Pyrazines are important components of cocoa flavor (Van Praag et al., 1968; Van der Wal et al., 1971; Gill et al., 1984; Carlin et al., 1986). Two recent reviews (Maga, 1982; Fors, 1983) report the literature data on all the pyrazines isolated in foods and, when known, the sensitivity thresholds for taste or smell.

Many theories on the formation of pyrazines during the heating of foods have been proposed. A recent review (Hoskin and Dimick, 1984a) deals with this topic with particular reference to the processing of chocolate. Dawes and Edwards (1966) using model systems with amino acids and fructose identified 2,5-dimethyl- and trimethylpyrazine and concluded that pyrazines come from the condensation of amino acids and sugars. Newell et al. (1967) proposed a mechanism of pyrazine formation involving the production of two aminoacetone molecules that then condense together.

The possible role of ammonia in pyrazine formation was proposed by Van Praag et al. (1968). They reacted fructose with some amino acids or ammonia and found the same pyrazines. They concluded, therefore, that ammonia was an intermediate of the reaction. Because of these results in recent years, the reaction of ammonia with sugars has been studied (Shibamoto and Bernhard, 1977).

Koehler et al. (1969) used radioisotopic labeling techniques with the aim of establishing the origin of pyrazine atoms. They concluded that sugars give the carbon atoms and amino acids only nitrogen atoms.

Walradt et al. (1971) deduced that some dihydrocyclopentylpyrazines, characterized by them, are derived probably from the reaction of amino acids with 2-hydroxy-3-methyl-2-cyclopenten-1-one, a sugar degradation product.

Shibamoto and Bernhard (1977) depicted a general scheme for the formation of alkylpyrazines in the reaction of sugars with ammonia, proposing the intermediates that react to give rise to every pyrazine. It must be observed that with these reagents they could not justify the formation of some alkylpyrazines with large substituents (isobutyl, etc.).

More recently with ESR techniques Namiki and Hayashi (1983) succeeded in detecting in the reaction mixture some substituted pyrazine radical cations that retain the amino acid structure. These intermediates then lose CO₂ and the alkyl group to give pyrazines. In this way the authors proposed a reaction pathway in which free am-

monia is not formed in the mixture. In a following paper the same authors (Hayashi and Namiki, 1986) detected methyl glyoxal dialkylimine in the reaction of sugars with *n*-butylamine and proposed that similar intermediates give rise to substituted pyrazine radical cations.

The formation of pyrazines and other Maillard products in the reaction of amino acids and sugars has generally been studied in model systems. Water or mixtures of water with alcohols have been used as solvents: methanol (Lachener and Karel, 1972; Lee et al., 1984), ethanol (Lee, 1982), diethylene glycol (Koehler and Odell, 1970), octane (Westphal and Cieslik, 1983). The last is the only lipophilic solvent used.

As we are interested in the effect of lipids on the formation of flavor during the roasting of cocoa beans containing up to 55% cocoa butter, in the preceding paper we proposed a model system in which a large amount of cocoa butter was present and we have studied the rate of formation of the Strecker aldehydes from leucine and valine (Arnoldi et al., 1987). We observed that this rate in the presence of cocoa butter was higher than without it and that the aldehydes are also formed without sugars. In this paper we report on a detailed analysis of the flavor components obtained from eight amino acids present in cocoa beans and fructose, which is one of the most abundant sugar in cocoa beans, in the presence of cocoa butter as a quantitation of some of the pyrazines formed. Fructose and the amino acid (alanine, valine, leucine, phenylalanine, threonine, lysine, aspartic acid, glutamic acid) were heated for 3 h at 120 °C with good stirring. Water was added in order to have the cocoa butter to water ratio generally present in cocoa beans. In order to extract the compounds formed from cocoa butter, we chose steam distillation and continuous extraction with dichloromethane. In this way we obtained concentrated solutions suitable for GC/MS analysis.

EXPERIMENTAL SECTION

General Procedure for the Model System. In a 250-mL flask equipped with a reflux condenser, effective magnetic stirrer, and a hydraulic valve, deodorized cocoa butter (23 g obtained from Nestlé) was melted, and to it the amino acid (500 mg), fructose (a stoichiometric amount), and water (1 mL) were added. The mixture was stirred at 120 °C for 3 h. Then warm water (100 mL) was added, and the mixture was steam-distilled from the flask: 100 mL of distillate was collected. The aqueous solution (50 mL) was continuously extracted for 8 h with dichloromethane (200 mL) in an apparatus for the continuous extraction of a liquid with a heavier liquid. The organic layer was dried on anhydrous Na₂SO₄ and

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