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Strecker Degradation of Leucine and Valine in a Lipidic Model System

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Leucine and valine were reacted with fructose in a mixture of cocoa butter and water at different temperatures. The rate of the Strecker reaction is higher in cocoa butter–water than in water. In the presence of cocoa butter the two amino acids give some aldehyde also without sugar. The participation of cocoa butter in the production of flavor during the roasting of cocoa beans is therefore proposed.

Chocolate flavor is the product of several operations among which fermentation and roasting of beans are of great importance for its quality.

Fermentation produces some of the flavor components and some indispensable precursors like glucose, fructose, and free amino acids, while during roasting at well-defined temperatures (generally in the range 115–140 °C) reducing sugars and amino acids react together and the principal compounds of the flavor are obtained.

The qualitative and quantitative determination of sugars in cocoa beans before and after fermentation was studied by Rohan and Stewart (1967b) and Reineccius et al. (1972a), and the production of free amino acids during fermentation, by Rohan and Stewart (1967a). The destruction of both amino acids and sugar during roasting is well documented (Rohan and Stewart, 1966, 1967c; Reineccius et al. 1972b).

Many groups analyzed volatile constituents of roasted beans or cocoa powder and identified, among others, carbonyl compounds (Van Praag et al., 1968; Van der Wal et al., 1971).

By gas chromatographic headspace analysis, Ziegleder (1972) studied some carbonyl compounds with the aim of using them as indicators during cocoa processing, and in particular he defined the optimal concentration of 3-methylbutanal.

Darsley and Quesnel (1972) by radioactive tracer techniques showed that carbonyl compounds derive from amino acids like leucine, phenylalanine, and threonine.

The main route for the formation of aldehydes during the process of roasting is the Strecker degradation (Hodge, 1953). In this reaction amino acids react with α-dicarbonyl and α-hydroxycarbonyl compounds (Nyhammar et al., 1983) coming from the Maillard reaction, forming Schiff bases that later enolize and decarboxylate. The new Schiff base, with one C atom less, is hydrolyzed to the amine and an aldehyde containing one carbon atom less than the starting amino acid.

As the study of the Maillard and Strecker reactions in foods is very complex, in order to obtain data on the effect of different components or conditions, many authors have studied them by model systems. They are mixtures of an amino acid, a sugar, and sometimes a few other compounds. In most cases water is used as a solvent, but in some cases mixtures of water with methanol (Eichener and Karel, 1972; Lee et al., 1984), ethanol (Ledl, 1982), diethylene glycol (Koehler and Odell, 1970), and octanol (Westphal and Cieslik, 1983) are used. To our knowledge this last one is the only lipophilic solvent used.

In choosing a model system of the roasting of cocoa beans, one should take into account that they contain about 50–60% of cocoa butter and are therefore a highly lipophilic medium. Moreover, the effect of cocoa butter on the products of the Maillard and Strecker reactions is not clear nor is its role in the production of the flavor.

As 3-methylbutanal and 2-methylpropanal (the Strecker aldehydes from leucine and valine) are considered important indicators during cocoa processing, we have studied their formation at different temperatures in a model system composed of fructose, leucine, or valine in a mixture of 4% water and 96% deodorized cocoa butter. The results were compared with those obtained in the same amount of water alone.

EXPERIMENTAL SECTION

Model Systems in Cocoa Butter. Cocoa butter (96 g) was melted in a 250-mL flask equipped with a reflux condenser, a good magnetic stirrer, a pressure-equalizing valve, and a rubber septum. L-Leucine (200 mg, 1.52 mmol) and undecane (internal standard, 44.40 mg) were added. The mixture was heated at the desired temperature, and fructose (1.37 g, 7.6 mmol) and distilled water (4 mL) were added. The temperature was kept constant for 2–3 h. Every 10–13 min samples of 1 mL of the vapor phase were drawn with a gas-tight syringe (Hamilton 1001) and injected in the gas chromatograph.

With the same procedure L-valine (200 mg, 1.7 mmol) and fructose (1.53 g, 8.5 mmol) were reacted. The chro-

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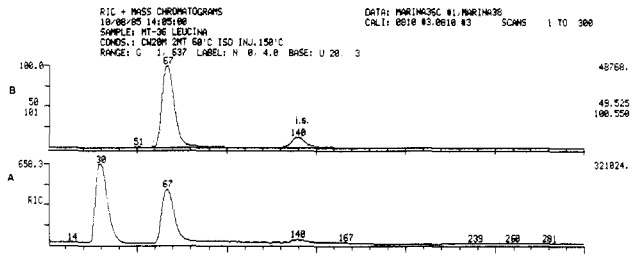

Figure 1. HS GC–MS analysis of a model system in cocoa butter with leucine: (A) reconstructed ion chromatogram (RIC); (B) mass chromatogram from m/z 50 to 150. i.s. = internal standard.

matographic analyses were performed on a DANI 3600 gas chromatograph equipped with a fid detector. A 2 m × 2 mm stainless-steel column of 10% Carbowax 20 M on Chromosorb W-AW, 80–100 mesh was used. Operating conditions: column temperature, 80 °C; injector temperature, 150 °C; detector temperature, 180 °C; carrier gas, nitrogen; flow rate, 9 mL min^{-1}. The integration was performed on a Shimadzu Chromatopac C-R 3A with the internal standard method. Correction factors for every temperature were calculated from the chromatograms of mixtures of the suitable components and the standard heated at that temperature (mean of four values).

Model Systems in Water. L-Leucine (200 mg, 1.52 mmol), water (4 mL), and 1-butanol (24.30 mg, internal standard) were heated at the proper temperature in a 50-mL flask, and fructose (1.37 g, 7.6 mmol) was added. Headspace analysis was done as already described.

With the same procedure L-valine (200 mg, 1.7 mmol) and fructose (1.53 g, 8.5 mmol) were reacted.

Gas Chromatography–Mass Spectrometry. The cold reaction mixture (7.5 g) was added with NaCl (12.50 g) and carefully mixed in a mortar. A sample (7.5 g) was transferred in a 50-mL vial with a septum cap and was heated in an oven at 80 °C for 90 min. The instrumentation was a gas chromatograph–mass spectrometer Finnigan 4021 equipped with an INCOS data system operating in EI mode with 10% Carbowax 20 M on Chromosorb W-AW, 80–100 mesh, 2 m × 2 mm glass column. Analytical conditions: injector temperature, 150 °C; column temperature, 60 °C; carrier gas, He; flow rate, 10 mL min^{-1}; ion source temperature, 200 °C; scanning from 33 to 400 AU in 1.15 + 0.05 s; electron energy, 70 eV; emission current, 0.25 mA; electron multiplier, 1.500 V.

Determination of Residual Fructose by HPLC. Fructose was determined in the cold mixture after the gas chromatographic analysis. The quantitative determinations was carried out with sucrose as internal standard. The mixture (1 g) was added with sucrose [0.30 mL of a 20.0 mg mL^{-1} solution in acetonitrile–water (3:2)] and acetonitrile–water (3:2) (9.70 mL). The mixture was stirred for 1 h and filtered. The analyses were performed on a Waters 6000 A HPLC instrument equipped with a U6K injector and a refractive index detector. A Lichrosorb NH_2 250 mm × 4 mm column was used with a 1.5 mL min^{-1} flow of acetonitrile–water (80:20). In any case the final concentration of fructose was in the range 0.2–0.45 mmol, with no significant dependence on the conditions or reagents.

RESULTS AND DISCUSSION

Gas chromatographic headspace analysis was chosen as the most direct technique for the analysis of volatile components in a complex mixture. Before the kinetic study, a model system heated at 100 °C for 2 h was submitted to GC–MS for identification of the compounds, with retention times similar to the Strecker aldehyde and checking of the purity of the peaks. Headspace analyses (Ioffe and Vitenberg, 1982) were performed by the static method: the sample was added with NaCl or LiCl and heated at a suitable temperature in a sealed vial equipped with a rubber septum.

With use of the jet molecular separator of Becker (1961) and Ryhage (1964) for the connection of a packed column to the mass spectrometer, with a carrier gas flow of 7–10 mL min^{-1} and molecular weight lower than 100–150, the transmission is about 40%, while the optimum value is 60–70%. As we have verified experimentally, this means a dramatic decrease in sensitivity. Instead of a jet separator, we used a stainless-steel microcapillary needle valve with a zero dead volume (Finnigan-Mat) having an inner diameter of 0.0500 in. This interface prevents from missing any sample, allowing all the column effluent to reach the ion source. In this way, the high vacuum measured into the analyzer was 5 × 10^{-6} Torr.

The reconstructed ion chromatograms (RIC) show a very intense peak due to air, having a retention time of 29–30 s. Mass chromatograms in the range 50–150 u clearly exhibit other peaks (Figure 1).

Cocoa paste analyzed in these conditions showed two peaks recognized as 2-methylpropanal and 3-methylbutanal. The model system with leucine showed the peak of 3-methylbutanal and a little peak of acetone, while the one with valine showed the peak of 2-methylpropanal.

A simplified headspace analysis technique was applied to the kinetic studies, in order to perform many successive

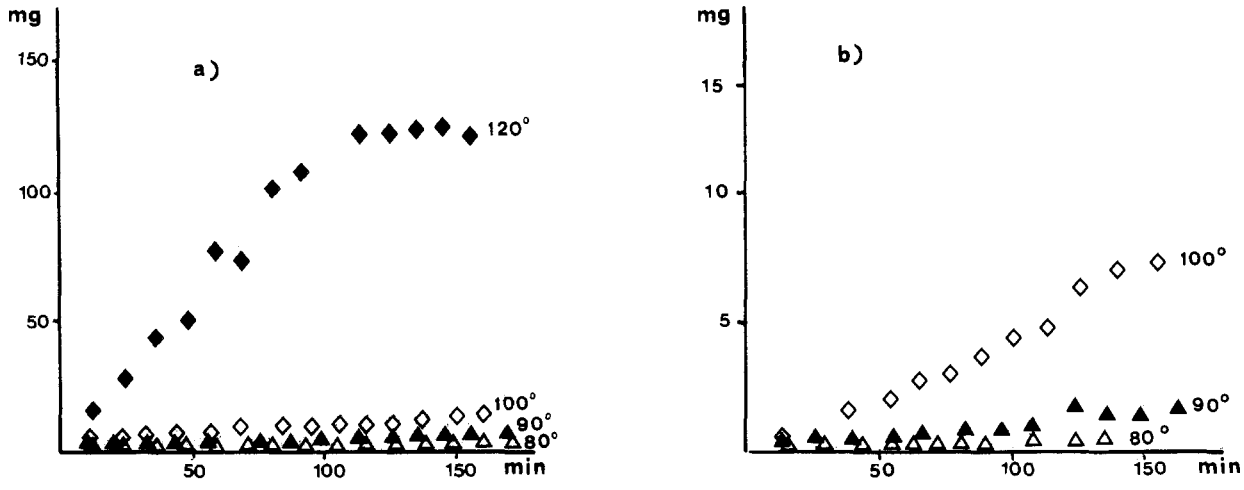

Figure 2. 3-Methylbutanal formed from leucine (200 mg): (a) in cocoa butter (96 g) + water (4 g) in the presence of fructose (1.37 g) at 80, 90, 100, and 120 °C; (b) in water (4 g) in the presence of fructose (1.37 g) at 80, 90, and 100 °C.

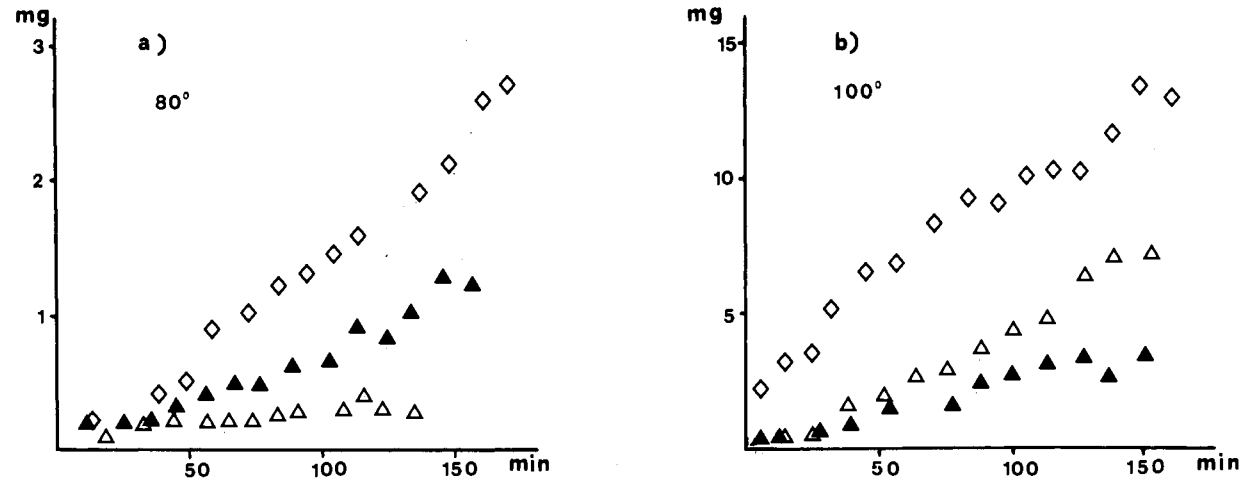

Figure 3. 3-Methylbutanal formed from leucine (200 mg) at (a) 80 °C or (b) 100 °C: ◇, in cocoa butter (96 g) + water (4 g) in the presence of fructose (1.37 g); ▲, in cocoa butter (96 g) + water (4 g) without fructose; △, in water (4 g) in the presence of fructose (1.37 g).

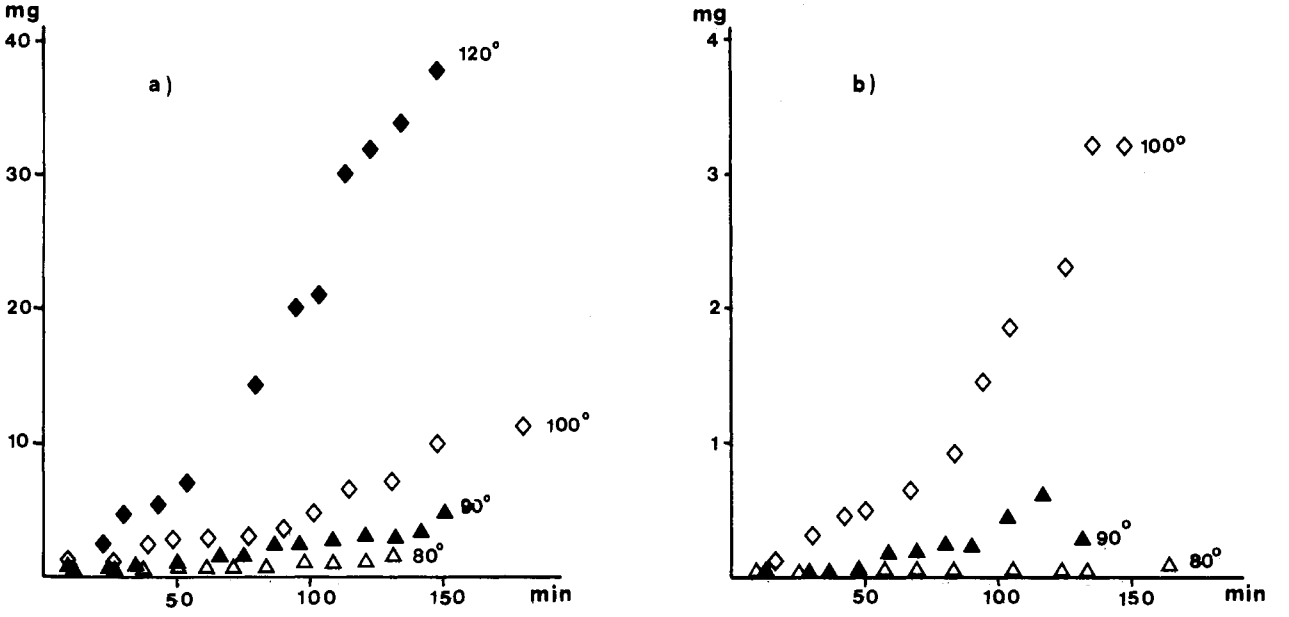

Figure 4. 2-Methylpropanal formed from valine (200 mg): (a) in cocoa butter (96 g) + water (4 g) in the presence of fructose (1.52 g) at 80, 90, 100, and 120 °C; (b) in water (4 g) in the presence of fructose (1.52 g) at 80, 90, and 100 °C.

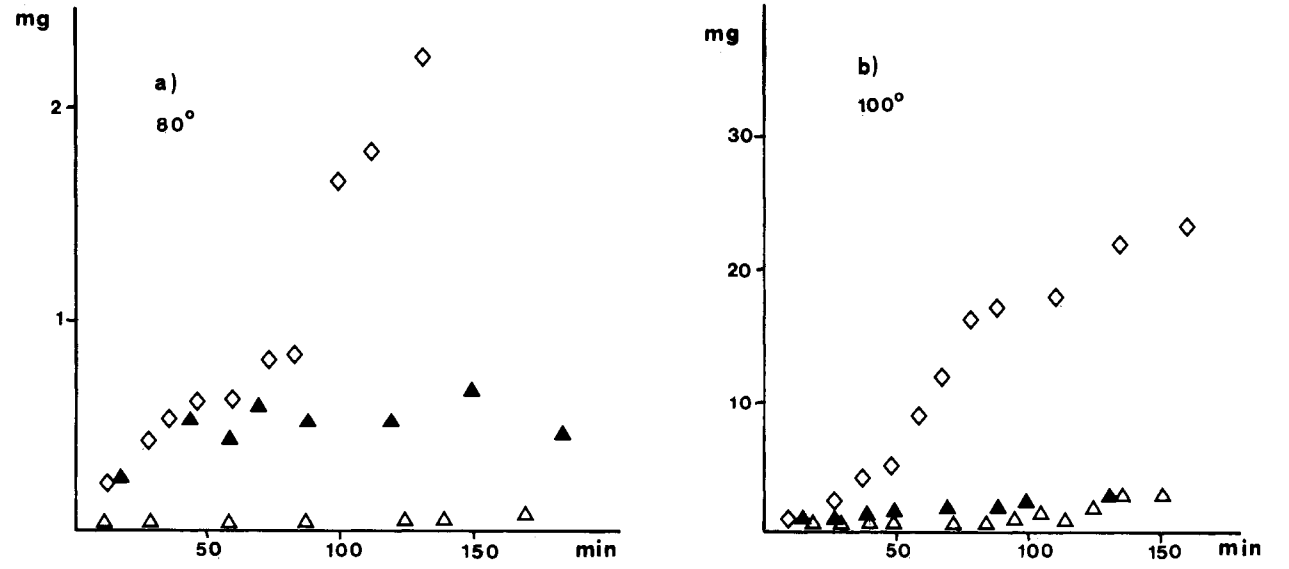

Figure 5. 2-Methylpropanal formed from valine (200 mg) at (a) 80 °C or (b) 100 °C: ◇, in cocoa butter (96 g) + water (4 g) in the presence of fructose (1.52 g); ▲, in cocoa butter (96 g) + water (4 g) without fructose; △, in water (4 g) in the presence of fructose (1.52 g).

samplings at short intervals: we analyzed directly the vapor phase over the reaction mixture. Samples of the vapor phase were drawn with a gas-tight syringe through a rubber septum and injected in the gas chromatograph. The reaction flasks were kept in equilibrium with the external pressure, but isolated by a valve. An internal standard was introduced to have a quantitative evaluation of the concentration of the aldehydes. For their inertness in the reaction condition and suitable solubility in the liquid phase, undecane was used in the model system with cocoa butter and 1-butanol with water. Desired components and the standard were heated at the working temperatures in order to calculate the gas chromatographic correction factors for every temperature.

The mixtures composed of cocoa butter–water (96:4), fructose, and leucine or valine in ratio similar to the ratio in cocoa bean in the presence of the internal standard were reacted at 80, 90, 100, and 120 °C for 2–3 h. Similar reactions were carried out in water at 80, 90, and 100 °C. Samplings were drawn every 10–13 min, and the amount of the aldehyde formed was calculated from the amount of the internal standard and the correction factors. The results of the lipidic model system with leucine at different temperature are shown in Figure 2a. 3-Methylbutanal is formed regularly during the reaction in increasing amount at higher temperature. The curve has a sigmoid feature suggesting that the aldehyde is formed from consecutive reactions. At 120 °C the curve reaches a plateau. Assuming that all the amino acid has reacted and that the plateau is the maximum possible amount of 3-methylbutanal, this value is 123 ± 3.4 mg. This result means that 94% of the amino acid is decomposed to the Strecker aldehyde. The dependence of the amount of 3-methylbutanal from reaction time at three different temperatures in water is shown in Figure 2b. The trend is similar. Figure 3 allows a comparison of the reaction rates in water and in cocoa butter at 80 °C (a) and 100 °C (b). In these plots also the amount of 3-methylbutanal formed from the amino acid without fructose in the presence of cocoa butter is reported. At 80 °C the decomposition of leucine without fructose is so high that more aldehyde is formed in these conditions than in the Maillard reaction in water. Nevertheless, the increase of the rate of the latter reaction with the temperature is higher; in fact, at 100 °C the Maillard reaction in water becomes faster. At both temperatures the Maillard reaction in cocoa butter is the fastest. The amount of the aldehyde formed in this condition is the sum of the amount deriving from the reaction with fructose and the amount deriving from the reaction with cocoa butter. Analysis of the residue fructose by HPLC showed that in every situation the sugar is almost completely destroyed after 3 h.

Similar results were obtained in the model system with valine (Figures 4 and 5). The 3-methylpropanal amount is generally lower than that of 3-methylbutanal. In particular, after 2.5 h at 120 °C in cocoa butter and in the presence of fructose only 33% of the theoretical amount of 2-methylpropanal is formed.

In conclusion, it has been demonstrated that cocoa butter has a strong effect on the rate of formation of the Strecker aldehyde from leucine and valine. Work is in progress in order to understand better the role of cocoa lipids in amino acid degradation and to verify their effect on the formation of other components of cocoa flavor.

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