Antioxidative Properties of Arginine-Xylose Maillard Reaction Products

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Maillard reaction products are known to inhibit oxidative degradation of natural organic compounds. In reactions of arginine•HCI and xylose in boiling aqueous solution at autogenic pH, the liberation of carbon dioxide was maximal at equimolar ratio of the reactants. The rate of such evolution was maximum at about 3-5 hr reaction time. Dialysis of the products gave a retentate that was more antioxidative than either the dialysate or the crude material, but continuation of the process gradually reduced the antioxidative effect of the retentate; the dialysate also lost approximately half its activity.

INTRODUCTION

Maillard reaction compounds have been found to possess antioxidative activity. They are produced in reactions between sugars and amino acids or peptides. Neither the structures of Maillard reaction products (MRP) nor the mechanism of their formation is understood. They are found in most cooked foods and have a characteristic brown color. MRP have been shown to inhibit lipid oxidation in model systems (1-3) and food systems (4,5). Recently Lingnert and Waller (6) and Lingnert et al. (7) reported on MRP antioxidants formed from histidine and glucose. Other such antioxidants have been produced from arginine and xylose, and the effects of reaction time, initial pH, and molar ratio of reactants were described (8,9). This is a report on the carbon dioxide evolution and dialysis of the antioxidative products formed from arginine•HC1 and xylose.

MATERIALS AND METHODS

Synthesis

MRPs were prepared by refluxing 100-mL portions of doubly distilled water containing L-arginine•HC1 (Sigma Chemical Co., St. Louis, MO) and D-xylose (also from Sigma) in various molar ratios for up to 20 hr. The total concentration was 3.0 M.

Measurement of liberated carbon dioxide

The amount of carbon dioxide liberated was measured over various reaction times. Carbon dioxide evolved during the refluxing was trapped in 0.1 N NaOH (250 mL), and the resulting carbonate was titrated with 0.1 N HC1. phenolphthalein being used as an indicator.

Dialysis of MRP

The Maillard reaction mixtures were dialyzed against degassed distilled water (100 times the volume of MRP solution) under a nitrogen atmosphere for different times, with Spectropor 6 dialysis tubing with a molecular weight cutoff of 1000 (Spectrum Medical Industries, Inc., Los Angeles, CA).

Measurement of antioxidative activity

The antioxidative activity was measured by oxygen uptake (Lingnert et al., 1979) with linoleic acid as substrate in the presence of hemin and 25 μg of MRP. The oxygen level was measured in a 3.2-mL vessel equipped with a YSI 53 oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, OH).

RESULTS AND DISCUSSION

Antioxidative activities of whole MRPs prepared from different molar ratios of reactants, and of fractions of each MRP are shown in Fig. 1. The activity is seen to be maximum in all three materials (crude mixtures, retentates, and dialysates) when arginine/xylose molar ratio is 1. This confirms and extends the work of Beckel and Waller (8) on this ratio.

The antioxidative activity of the retentate during dialysis was 5.1 (arbitrary units) after 24 hr and gradually declined to 1.4 after 168 hr. During the dialysis some instability of the retentate was observed, which

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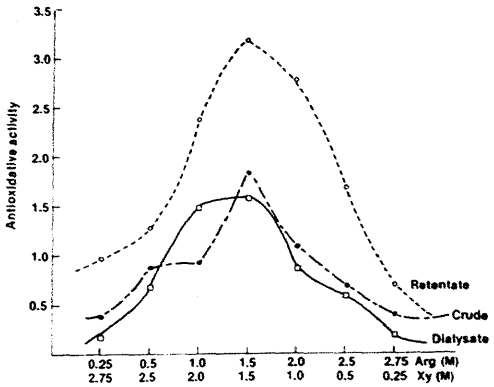

FIGURE 1. Antioxidative activity of retentate, dialysate, and crude MRP versus molar ratio of arginine/xylose. A 250–μg sample of each was used for antioxidative measurement. Time of reaction was 20 hr. Dialysis was done twice, 24 hr each time.

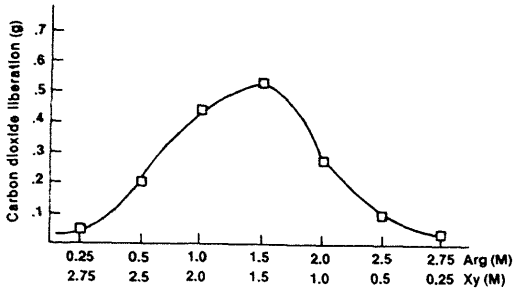

FIGURE 2. Variation of carbon dioxide liberation with molar ratio of arginine/xylose in 20–hr reaction.

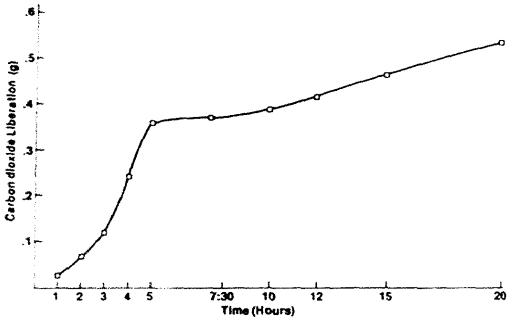

FIGURE 3. Carbon dioxide liberation with time. Ratio of reactants was 1:1.

increased the passage of compounds below 1,000 molecular weight into the dialysate. However, preliminary work on the structure(s) of these antioxidative compounds done at Oklahoma State University and two other laboratories using the fast atom bombardment technique combined with tandem mass spectrometry showed that the molecular weights of retentates never exceeded 645 even though these were prepared with dialysis tubing having nominal 1,000 molecular weight cut-off (10,11). This discrepancy is attributable to the unstable nature of the MRP(s)(6).

The evolution of carbon dioxide during the formation of carbon dioxide within 20 hr occurred at the 1:1 ratio of reagents (Fig. 2), suggesting that formation of antioxidants and release Of CO_2 are related. However, when carbon dioxide evolution is computed on the basis of the carboxyl groups initially present in arginine, maximum percentage of decarboxylation occurred at a ratio of 1:2 for arginine/xylose. The liberation of carbon dioxide increased with time, especially for the 1:1 ratio of reactants (Fig. 3), where it had not ceased after 20 hr reflux. It is notable that in all cases most of the CO_2 evolution occurred within the first 5 hr. Beckel and Waller (8) reported that 10-20 hr was required to produce maximum antioxidative effect. It is not clear how the evolution of CO_2 and the production of antioxidative effect are related, but it does seem possible that a precursor molecule is formed; however, attempts to characterize such a precursor have been unsuccessful so far in this laboratory.

No evidence of the liberation of ammonia or methylamine was observed during the synthesis of the antioxidants. This result was expected, since concurrent evolution of carbon dioxide and gaseous bases would not be predicted.

Attempts using IR, NMR, mass spectrometry and x-ray diffraction to determine the structures of MRP antioxidative compounds have so far been unsuccessful, and the mechanism of their action has not been determined.

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