lecithin. Good agreement can be seen between the two techniques.

Additionally, the ^1H NMR technique could offer an important and easy check of the phosphatidylcholine moiety of commercial lecithins.

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Formation of *N*-Nitrosoproline by Reacting Nitrite with L-Citrulline and L-Arginine

It was found that *N*-nitrosoproline (NPro) was formed from either L-citrulline or L-arginine when these amino acids were reacted with nitrite under simulated human stomach conditions. The determination and confirmation of the formed NPro were carried out after converting NPro to its methyl ester. This derivative was analyzed by gas chromatography and gas chromatography–mass spectrometry. The yield of NPro from L-arginine was 0.1% and that from L-citrulline was 27.1%.

Many *N*-nitroso compounds are known to be carcinogenic to various experimental animals, while NPro has been reported to be noncarcinogenic (Magee and Barnes, 1967; Druckrey et al., 1967). However, NPro can be converted to carcinogenic *N*-nitrosopyrrolidine by decarboxylation occurring at higher cooking temperatures (Lijinsky and Epstein, 1970).

As to the formation of *N*-nitroso compounds from amino acids reacted with nitrite under acidic conditions, Warthesen et al. (1975) reported that NPro was formed from ornithine, and Mirvish (1971) reported that *N*-nitrosocitrulline and *N*-nitrosoarginine were formed from L-citrulline and noncarcinogenic to experimental animals. The present study revealed that NPro can be formed from either L-citrulline or L-arginine by nitrosation under simulated human stomach conditions.

EXPERIMENTAL SECTION

Reagents. L-Arginine and L-citrulline were of reagent grade (Wako Chemicals Co.). Both compounds were assayed for purity by using an amino acids analyzer (Hitachi Model LLA-5), and only trace quantities of less than 0.01% proline and ornithine could be detected. NPro was synthesized by nitrosation of proline according to the Lijinsky method (Lijinsky and Epstein, 1970).

Reaction Conditions and Extraction of NPro. Nitrosation of the test amino acids was carried out in a pH 1.2 acetate–hydrochloric acid buffer solution prepared by mixing 1 M sodium acetate and 1 N hydrochloric acid. L-Arginine, L-citrulline, and sodium nitrite were separately dissolved in the pH 1.2 buffer solution, and then the solutions were readjusted to pH 1.2 with 6 N hydrochloric acid. Five milliliters of each amino acid and nitrite solution was transferred to a 20-mL glass-stoppered test tube and thoroughly mixed. Then the mixture was incubated in a 37 °C water bath for 2 h. The concentration of each amino acid in the final reaction mixture was 0.03 M, and that of sodium nitrite was 0.3 M. The reaction was stopped by addition of 0.1 mL of 10% ammonium sulfamate to the mixture. The reacted mixture was transferred to a separatory funnel, and 2–3 g of sodium chloride was added. The formed NPro was extracted 2 times with 20 mL of ethyl acetate. The combined extracts were dried over anhydrous sodium sulfate and transferred to a 100-mL round-bottom flask, followed by evaporation in a rotary evaporator to dryness.

Methylation of NPro with Diazomethane. A few milliliters of diazomethane-saturated diethyl ether was added to the dried reactants containing NPro. This is allowed to stand for 30 min at room temperature, and then it was concentrated to 1 mL under a gentle stream of nitrogen (Ishibashi et al., 1980). The derivative was analyzed in a gas chromatograph equipped with an alkali flame ionization detector (GC-AFID), and it was further identified by gas chromatography–mass spectrometry (GC–MS). The fragments observed in the authentic NPro methyl ester were m/e 128, 99, 69, and 68.

Operating Conditions for GC-AFID and GC–MS. A Shimadzu GC-4BF gas chromatograph equipped with an AFID was used for the analysis of NPro methyl ester. A glass column (2 m × 3 mm i.d.) packed with 3% DEGS–0.5% H_3PO_4 on Chromosorb W (60–80 mesh) pretreated with hexamethyldisilazane was employed. The carrier gas was nitrogen at a flow rate of 40 mL/min, and the temperatures of column oven and the detector were 170 and 200 °C, respectively. A shimadzu-LKB 9000 gas chromatograph–mass spectrometer was used for the GC–MS analysis of NPro methyl ester. A glass column (2 m × 3 mm i.d.) packed with the same material as employed for the GC-AFID analysis was used. The carrier gas was helium, at a flow rate of 30 mL/min. The temperatures of the injection port and column oven were 240 and 180 °C, and those of the separator and ion source were 250 and 260 °C, respectively. The electron energy was 20–70 eV. The accelerating voltage was 3.0 kV, and the trap current was 60 μA.

RESULTS AND DISCUSSION

We found that NPro was formed when either L-arginine or L-citrulline was reacted with nitrite at pH 1.2 and 37

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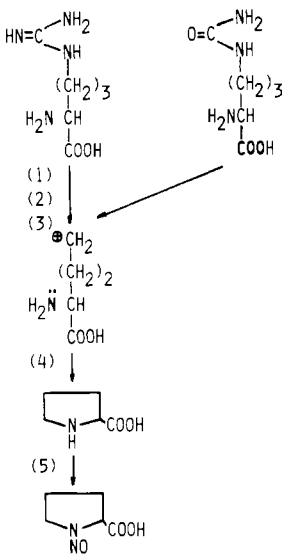

Figure 1. Proposed mechanism for the formation of *N*-nitrosoproline by reacting nitrite with L-citrulline and L-arginine. (1) N-Nitrosation; (2) diazotization; (3) deamination; (4) cyclization; (5) N-nitrosation.

°C. The yield of NPro obtained from L-arginine under those conditions was 43.6 μg, which is equivalent to 0.1% of the theoretical yield. In contrast, a much higher yield of NPro was observed in the nitrosation of L-citrulline: 11.7 mg, equivalent to 27.1%.

A proposed mechanism for the formation of NPro from either L-arginine or L-citrulline is illustrated in Figure 1. First urea is split away from the L-citrulline or L-arginine molecules to form ornithine. The presence of this amino acid was confirmed by means of thin-layer chromatography (TLC) and amino acid analysis. As pointed out by Warthesen, ornithine may undergo a series of reactions, i.e., nitrosation, diazotization, and cyclization, in the presence of nitrite under acidic conditions. Then the resultant proline can be easily nitrosated by nitrite to give NPro. The yield of NPro from L-citrulline was 270 times as much as the yield from L-arginine. This might be attributable to a difference in chemical reactivities of the ureido group in the L-citrulline molecule and the guanido group in the L-arginine molecule with nitrite under the same acidic conditions.

L-Arginine and L-citrulline are known to be widely distributed in nature. Especially L-citrulline has been reported to be present in large amounts in water melons, soy sauce, and peppers (Wada, 1930. Ogasawara et al., 1963). In addition, this amino acid is known to be a component in the Krebs–urea metabolic cycle. L-Arginine is a component of various proteins, and this amino acid has been reported to occur in soy sauce together with L-citrulline (Ogasawara et al., 1963). When we ingest a food containing L-arginine or L-citrulline and nitrite simultaneously, NPro may be formed in the human stomach. However, as already mentioned, there is a large difference between L-arginine and L-citrulline in the rate of nitrosation yielding NPro. Based on our data, it seems that L-citrulline should be considered to be a much more important precursor for potential NPro formation in the human stomach.

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Shelf Life Indicators for Encapsulated Diazinon

The addition of pH indicators to starch xanthate encapsulated diazinon provides a convenient method for judging the extent of diazinon decomposition in accelerated aging tests. As decomposition takes place, the pH of the formulation decreases and the indicators change color. Indicators used were bromcresol green, bromcresol purple, and bromthymol blue.

Indicators have been used to monitor the stability of perishables such as foods and pharmaceuticals (Bio-medical Sciences, Inc., 1975; Patel et al., 1976; Larsson, 1977). The effectiveness of the insecticide DDVP was monitored by using methyl red indicator (Kuderna and Saliman, 1977).

We have found that pH indicators can be incorporated into starch xanthate encapsulated diazinon formulations to monitor diazinon decomposition during accelerated aging. The technique provides a method for detecting, by color change, when significant amounts of diazinon have decomposed during storage.

Diazinon and other pesticides have been encapsulated at this laboratory with starch xanthate to control target organisms more effectively, reduce exposure to nontarget organisms, and reduce environmental pollution (Shasha

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