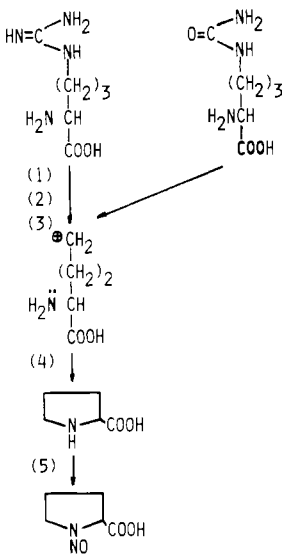

Figure 1. Proposed mechanism for the formation of *N*-nitrosoproline by reacting nitrite with L-citrulline and L-arginine. (1) N-Nitrosation; (2) diazotization; (3) deamination; (4) cyclization; (5) N-nitrosation.

°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.

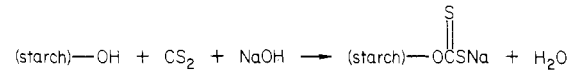
Diazinon and other pesticides have been encapsulated at this laboratory with starch xanthate to control target organisms more effectively, reduce exposure to nontarget organisms, and reduce environmental pollution (Shasha

Table I. Extent of Decomposition of Encapsulated Diazinon at 70 °C When Various Indicators Changed to Yellow

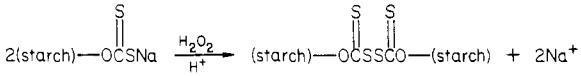
indicator	change time, days	diazinon content, % initial	diazinon content, % final	pH of aqueous dispersions initial	pH of aqueous dispersions final	diazinon decomposition, %
A[a]	13	23.9	3.0	6.0	2.0	87
B[b]	7	25.8	16.1	6.5	3.7	38
C[c]	1-2	22.9	15.3	8.2	4.5	33

[a] Bromcresol green: pH 3.8, yellow; pH 5.4, blue. [b] Bromcresol purple: pH 5.2, yellow; pH 6.8, purple. [c] Bromthymol blue: pH 6.0, yellow; pH 7.6, blue.

et al., 1976). Encapsulation was achieved by dispersing pesticides into a water solution of starch xanthate followed by oxidative cross-linking:



starch xanthate



starch xanthide

Preliminary trials have shown that encapsulated diazinon is more stable than unencapsulated technical diazinon (Feldmesser et al., 1976; Doane et al., 1977). However, various preparations were found to decompose upon storage along with a decrease in pH (Trimnell et al., 1981). When various pH indicators were added to the starch xanthate encapsulated diazinon granules, color changes were observed during accelerated aging at 70 °C that correlated with the extent of diazinon decomposition.

MATERIALS AND METHODS

Diazinon MG8 (supplied by Ciba-Geigy Corp.) was encapsulated by the starch xanthate procedure as follows: a suspension of starch (45 g) in water (250 mL) was mixed with carbon disulfide (10 mL), and then sodium hydroxide (10 g) in water (75 mL) was stirred in until the mixture gelatinized. After the mixture was allowed to stand for 1 h at room temperature, Diazinon MG8 (20 g) was added and the dispersion was thoroughly mixed. The mixture was acidified and cross-linked by portionwise addition of a solution of acetic acid (16 mL) and 30% hydrogen peroxide (10 mL) in ice water (100 mL). The coagulated product was filtered through cheesecloth by using suction and a rubber dam to press water from the resulting moist cake. The cake was resuspended in water (200 mL) and refiltered 3 more times by using suction and a rubber dam. The moist material was broken up in a Waring Blendor and sieved to pass 12 mesh. Indicator solutions were prepared by dispersing separately 0.1 g each of bromcresol green, bromcresol purple, and bromthymol blue in water, adding enough 0.01 N NaOH (14–19 mL) to neutralize each dispersion, and finally diluting each to 250 mL with water. The moist encapsulated diazinon granules (45 g) were mixed separately with each indicator solution (10 mL) and air-dried overnight. Bromcresol purple and bromthymol blue particle formulations were treated with a few drops of 0.1 N NaOH to yield purple and blue colors, respectively, prior to air-drying (see Table I). Two-gram samples of each were sealed in glass pressure flasks and kept in an oven at 70 °C. When the indicators changed to yellow, the flasks were removed and were allowed to cool. Diazinon content prior to and after the color change was determined by triturating the particles with methanol, followed by gas chromatographic analysis of the methanol solution (Trimnell et al., 1981). The pH was measured on aqueous dispersions of the granules before and after heating to a color change.

RESULTS AND DISCUSSION

Results of the accelerated aging tests are summarized in Table I. The longer the aging prior to the indicator color change, the lower the pH of the final water-suspended product. A correlation was observed between the extent of diazinon decomposition and the pH of the indicator color change. For example, more diazinon had decomposed (87%) at the color change of bromcresol green (pH 3.8–5.4) and less had decomposed (33%) at the color change of bromthymol blue (pH 6.0–6.7). The procedure has potential application for visually detecting when samples of encapsulated diazinon have decomposed during prolonged storage.

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