

EFFECT OF α -TOCOPHEROL FORMULATIONS ON THE INHIBITION OF NITROSOPYRROLIDINE FORMATION IN MODEL SYSTEMS

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ABSTRACT

The effect of novel water dispersible α -tocopherol mixtures on the nitrosation of pyrrolidine was determined in an oil-aqueous-protein model system consisting of oil, water, protein, sodium chloride and sodium tripolyphosphate. α -Tocopherol dissolved in Polysorbate 20 in ratios of 1:6, 1:1, 1:0.4 and 1:0.2 inhibited nitrosopyrrolidine formation. 500 mg/L of α -tocopherol was found to be the most effective level.

INTRODUCTION

SINCE NITROSOPYRROLIDINE (NPY) was found in fried bacon (Crosby et al., 1972; Fazio et al., 1973; Sen et al., 1973), our laboratory has been investigating methods to eliminate or drastically reduce its formation. The presence of nitrosamine precursor in adipose, but not in lean, tissue (Fiddler et al., 1974) was found to be an important factor in NPY production. Water soluble sodium ascorbate and sodium erythorbate inhibited nitrosamine formation in frankfurters made with high nitrite (Fiddler et al., 1972; 1973) but were not completely effective with bacon (USDA Expert Panel). Other investigators have tested the antioxidants propyl gallate, t-butylhydroquinone and α -tocopherol in model systems and found varying degrees of inhibitory effectiveness, but have not applied these compounds to cured meat systems (Gray and Dugan, 1975; Sen et al., 1976a). Sen et al. (1976b) reported that ascorbyl palmitate applied to the surface of bacon slices prior to frying inhibited dimethylnitrosamine and NPY production; however, the method of application appears to have limited commercial feasibility. We found this compound to have limited effectiveness due to its slight solubility in fat (Pensabene et al., 1976). Therefore, in an effort to find a more fat soluble nitrosamine inhibitor, we investigated the effect of α -tocopherol in a novel water dispersible formulation on the nitrosation of pyrrolidine in two model systems and report the results herein.

EXPERIMENTAL

Reagents

α -Tocopherol was dissolved in the emulsifier Polysorbate 20, in ratios of 1:6, 1:1, 1:0.4 and 1:0.2. A second source of tocopherol was a dry powder form prepared with α -tocopherol and dextrin (a food grade emulsifier) and containing preservatives and a flow agent. Both tocopherol samples were obtained from Hoffmann-La Roche Inc. Pyrrolidine, sodium nitrite, bovine serum albumin and other chemicals were obtained from commercial suppliers and used without further purification.

Oil-aqueous-protein model system

A model system consisting of corn or coconut oil (30g), buffer (30g) (0.5N NaOH, 0.5M KH_2PO_4 , pH 6.0), bovine serum albumin (8.0g), sodium chloride (1.5g) and sodium tri-polyphosphate (0.5g) was used. This would correspond to bacon with a composition of 60% fat, 30% water, 8% protein and 2% ash. To this system was added pyrrolidine (3.0×10^{-4} mole; 214 mg/L), sodium nitrite (2.2×10^{-4} mole or 2.9×10^{-4} mole; 150 or 200 mg/L) and the α -tocopherol mixture to be

Table 1—Effect of α -tocopherol:dextrin on the nitrosation of pyrrolidine^a in an oil-aqueous-protein model system

Treatment	Nitrosopyrrolidine ($\mu\text{g/L}$)		
	Aqueous	Oil	Total
None	108	8	116
α -Tocopherol (1000 mg/L)	21	18	39
α -Tocopherol (1000 mg/L):dextrin	119	13	132

^a Pyrrolidine (3.0×10^{-4} mole) plus sodium nitrite (2.2×10^{-4} mole)

tested. The mixtures were stirred and heated at 52°C for 2 hr. The reaction mixtures were cooled to room temperature, then centrifuged to facilitate separation of the oil and aqueous layers. The individual layers were extracted as described previously (Pensabene et al., 1976).

Aqueous model system

Pyrrolidine (1.5×10^{-4} mole; 214 mg/L) and sodium nitrite (1.1×10^{-4} mole; 150 mg/L) were added to buffer (50g, 0.5N HCl, 0.5M $\text{KHC}_8\text{H}_4\text{O}_4$, pH 3.0) or pyrrolidine (3.0×10^{-4} mole) and sodium nitrite (2.9×10^{-4} mole) were added to buffer (30g, pH 6.0) and the α -tocopherol mixture in a 100 ml round bottom flask and heated at 52°C for 2 hr with stirring. After being cooled, the reaction mixture was extracted 3X with methylene chloride (75 ml), the extracts were combined and dried by being passed thru anhydrous sodium sulfate into a Kuderna-Danish flask equipped with a concentrator tube and three bubble Snyder column.

The extract was concentrated to 1.0 ml in a steam bath. Conditions for quantitation of NPY by gas-liquid chromatography (GLC) using an alkali flame ionization detector and confirmation by GLC-high resolution mass spectrometry were reported previously (Pensabene et al., 1976). All the data reported herein are the average of three experiments.

RESULTS & DISCUSSION

INITIALLY, the dextrin dry powder formulation containing 33 1/3% α -tocopherol was dispersed in water and added to the oil-aqueous-protein model system (Table 1). Pure α -tocopherol reduced the overall amount of NPY formed from 116 to 39 $\mu\text{g/L}$. Whereas, the dextrin dry powder formulation with an equivalent α -tocopherol concentration (1000 $\mu\text{g/L}$) enhanced the formation to 132 $\mu\text{g/L}$. In the case of α -tocopherol alone a considerably larger ratio of NPY was found in the oil phase than in the aqueous phase of the reaction mixture.

A similar experiment was conducted in an aqueous model system (Table 2). Here, two sources of tocopherol were used: the dry, dextrin-based powder and a liquid mixture of Polysorbate 20 with tocopherol. The α -tocopherol:Polysorbate 20 used in a ratio of 1:6 was found to keep the α -tocopherol completely solubilized for an indefinite period of time. Both tocopherol sources exhibited a small reduction in nitrosamine formation compared to their respective controls, but the dextrin formulation again showed a substantial increase in overall NPY production over the aqueous control reaction with no treatment. This suggested that the dextrin itself was exhibiting an unknown influence on the model system and further work with this preparation was not pursued.

During the course of these investigations we observed that significantly less NPY was formed in the aqueous model system than in the oil-aqueous-protein model system under the same conditions. This prompted an examination of the components of the oil-aqueous-protein model system. It was found that oils

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from different sources had little effect on NPy formation (Table 3); however, presence of water soluble bovine serum albumin enhanced the reaction.

The use of Polysorbate 20 in combination with α -tocopherol (Table 2) had only a small effect on the nitrosation reaction in the aqueous model system. The α -tocopherol:

Table 2—Effect of α -tocopherol formulations on the nitrosation of pyrrolidine^a in an aqueous model system

Treatment	Nitrosopyrrolidine (μ g/L)
None	80
α -tocopherol (1000 mg/L):dextrin	112
dextrin ^b	126
α -tocopherol (1000 mg/L):Polysorbate 20 (1:6)	66
Polysorbate 20 ^b	72

^a Pyrrolidine (1.5×10^{-4} mole) plus sodium nitrite (1.1×10^{-4} mole) in pH 3.0 buffer

^b Same concentration used in formulation

Table 3—Effect of oil and protein on the nitrosation of pyrrolidine

Reactants ^a	Nitrosopyrrolidine (μ g/L)		
	Aqueous	Oil	Total
None	2.0	—	2.0
Safflower oil	2.0	1.3	3.3
Coconut oil	1.6	0.8	2.4
Corn oil	1.5	0.6	2.1
Bovine serum albumin ^c	N.D. ^b	—	N.D.
Bovine serum albumin ^d	167	—	167
Bovine serum albumin + corn oil	101	6	107

^a Pyrrolidine (3.0×10^{-4} mole), NaNO_2 (2.2×10^{-4} mole), buffer (30g of pH 6.0), oil (60g) when used and bovine serum albumin (8.0g) when used

^b None detected

^c No pyrrolidine or oil

^d No oil

Table 4—Effect of α -tocopherol:polysorbate formulation (1:6) on the nitrosation of pyrrolidine^a in a model system

α -Tocopherol, mg/L	Nitrosopyrrolidine (μ g/L)			
	Oil-aqueous-protein model system			Aqueous model system ^b
	Aqueous	Oil	Total	Total
None	630	15	645	82
250 mg/L	560	9	569	79
500 mg/L	211	3	214	36
1000 mg/L	466	16	482	57

^a Pyrrolidine (3.0×10^{-4} mole) plus sodium nitrite (2.9×10^{-4} mole)

^b Aqueous buffer (30g, pH 6.0)

Table 5—Effect of α -tocopherol with varying ratios of Polysorbate 20 on the nitrosation of pyrrolidine^a in an oil-aqueous-protein model system

α -Tocopherol:Polysorbate 20 ^b	Nitrosopyrrolidine (μ g/L)		
	Aqueous	Oil	Total
None	630	15	645
1:6	211	3	214
1:1	113	3	116
1:0.4	165	2	167
1:0.2	51	1	52

^a Pyrrolidine (3.0×10^{-4} mole) plus sodium nitrite (2.9×10^{-4} mole)

^b α -Tocopherol (500 mg/L)

Polysorbate mixture (1:6) reduced NPy from 80 μ g/L in the control to 66 μ g/L. To further evaluate the effectiveness of this combination, several concentrations of α -tocopherol, ranging to 1000 mg/L were used in the oil-aqueous-protein model system (Table 4). All levels of α -tocopherol reduced the total NPy formed: the 500 mg/L α -tocopherol reduced NPy significantly from 630 μ g/L to 211 μ g/L in the aqueous phase and from 15 μ g/L to 3 μ g/L in the oil phase. This same trend was observed in the aqueous model system. However, it was found that the 1000 mg/L α -tocopherol formulation, while decreasing the overall amount of NPy formed, was not as effective as the 500 mg/L. This might be due to the higher concentration of Polysorbate 20 used in the 1000 mg/L formulation. It was also found that the difference in NPy values for the controls in Table 1 and Table 4 was due solely to the additional sodium nitrite. 200 mg/L of sodium nitrite was used instead of 150 mg/L to produce higher concentrations of NPy so that minor differences could be detected in the oil phase resulting from the various treatments. Since the inhibitory effectiveness of α -tocopherol may be dependent on the amount of Polysorbate 20 used, other α -tocopherol:Polysorbate 20 concentrations were evaluated to determine whether a more effective inhibitory mixture could be developed by varying the emulsifier ratio (Table 5). All the mixtures show an inhibitory effect on the nitrosation of pyrrolidine; with the 1:0.2 α -tocopherol:Polysorbate 20 mixture reducing NPy formation from 630 μ g/L to 51 μ g/L in the aqueous phase and from 15 μ g/L to 1 μ g/L in the oil phase. These emulsions were not as stable as the 1:6 formulation since the α -tocopherol separated from the aqueous solution over a period of 2–8 hr. However, even if the emulsion is stable for a short period of time it can still be used in the production of bacon.

Currently, combinations of α -tocopherol and Polysorbate 20 added to conventional cure solutions containing salt, sugar and sodium tripolyphosphate are being used to process pork bellies into bacon. Work is also underway to evaluate α -tocopherol distribution in the bacon and the resulting effect on nitrosamine formation.

NOTE: Precaution should be exercised in the handling of nitrosamines since they are potential carcinogens.

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