oven 2.5-4 hr, they were ground (to 40-mesh) and the meal was mixed. Analysis showed 374 ppm of carotene, primarily a mixture of all-*trans*-β-carotene (81%) and neo-β-carotene (*ca.* 19%) (Beadle and Zscheile, 1942).

Four samples were sealed in Pyrex tubes *in vacuo* after pumping for 2.5 hr to a pressure of 1 mm or less. Each sample of *ca.* 6 g was sealed in a 25-ml glass tube.

RESULTS AND DISCUSSION

One sample, after storage in darkness at 90° for 27 weeks, contained 365 ppm, indicating 98% retention of carotene. The characteristic curve (Beadle and Zscheile, 1942; Zscheile and Whitmore, 1947) of this sample was superposable on that of the original material at wavelengths of 400-500 nm, indicating negligible additional isomerization of the all-*trans* form of β-carotene. Similar results were obtained on two other samples stored 35 and 55 weeks at 90°; characteristic curves of the carotene fraction were similar but had higher absorption at the shorter wavelengths. Other samples in potentially commercial bags, sealed but containing air, retained only 21-56% carotene after 27 weeks.

A final sample, sealed *in vacuo* in Pyrex, was kept wrapped in cloth in a desk drawer at room temperature for 27.5 yr before analysis on Nov 8, 1972. The vacuum was still good, as tested with a Tesla coil before breaking the tube open. Analysis by the same method showed a carotene content of 356 ppm, or 95.5% retention. The writer is not aware of any other controlled study of carotene stability covering such a long period of time. The charac-teristic curve of the carotene fraction matched that for the original sample very closely from 400 to 500 nm. When analyzed at 478 nm, the all-*trans*-β-carotene fraction was 79%.

It may be concluded that carotene of such food materials, blanched before drying and kept in darkness in the absence of O_2, will be retained essentially unchanged at room temperature or below for indefinite periods of time. This fact might assume importance in the future for possible application in the maintenance of vitamin A activity in foods prepared for space trips of many months or years duration.

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Suppression of Fungal Growth by Isolated Trypsin Inhibitors of Corn Grain

Isolated corn trypsin inhibitor retarded growth of six fungi when added to dextrose agar cultures at time of inoculation. Greatest activity was against *Fusarium roseum* and least was against *Helmin-thosporium maydis*. It is proposed that the inhibitor may protect the seed against fungal invasion under moist conditions.

Early interest in plant trypsin inhibitors was concerned with possible adverse nutritional effects when legumes containing them were consumed by animals (Vogel *et al.*, 1969). With more recent work showing them to be widely distributed in plants (Chen and Mitchell, 1973; Vogel *et al.*, 1969), interest in their physiological functions in the plant has increased. There has been speculation, based on site of occurrence and concentration changes during development, that they inhibit proteinases of seeds during synthesis of storage proteins prior to dormancy (Liener and Kakade, 1969), that they prevent autolysis in dormant seeds (Vogel *et al.*, 1969), or that they prevent the plant from being overrun by symbiotic bacteria by protecting the plant tissue from bacterial proteinases at the colonization site. Green and Ryan (1972) suggested a defense mechanism against insects after they found that either mechanical or insect damage to potato and tomato leaves induced rapid accumulation of an inhibitor which was active against intestinal proteinases of animals. We present data which indicate that trypsin inhibitors of corn can retard growth of certain fungi.

METHODS

Trypsin inhibitors were isolated from a normal hard endosperm corn and from an opaque-2 strain. The isolation procedure was that of Chen and Mitchell (1973), which consists of grinding and defatting the whole grain, extracting with 0.2 M NaCl, precipitating the inhibitor by adding $(NH_4)_2SO_4$ to 40% saturation, and removing inactive proteins by passing the isolated material consecutively through columns of Sephadex G75 and CM-cellulose. Inhibitory activity was measured by Method II of Erlanger *et al.* (1961), using benzoyl-DL-arginine-*p*-nitroanilide as trypsin substrate.

In the first experiment, the effects of the two inhibitors were compared by adding them to sterile potato dextrose agar medium at 0, 25, 50, and 100 μg/ml of medium. In the second experiment, only the opaque-2 inhibitor was used, at concentrations of 200 and 400 μg/ml of medium. Each fungus species was plated in the center of a petri dish on the solidified medium, with three replications for each concentration of inhibitor. The dishes were kept at

Table I. Percent Retardation of Radial Growth of Fungi by Corn Trypsin Inhibitor Isolated from an Opaque-2 Source

Fungus	Inhibitor, μg/ml	Days after inoculation				
		1	2	3	4	5
Experiment 1						
Fusarium roseum	25		9	16	10	3
	50		16	21	15	12
	100		31	27	24	19
Fusarium moniliforme	25		20	18	20	20
	50		20	25	23	25
	100		20	30	29	29
Aspergillus glaucus	25		19	19	15	9
	50		19	13	15	13
	100		25	19	17	16
Helminthosporium maydis	25		0	0	0	0
	50		0	9	7	7
	100		0	12	9	12
Experiment 2						
Fusarium moniliforme	200	67	40	36	33	32
	400	67	43	38	35	34
Alternaria tenuis	200	71	67	57	51	50
	400	100	71	60	56	58
Periconia circinata	200	100	45	39	32	31
	400	100	64	50	36	45

room temperature and daily growth of the fungi was determined by measuring diameters of the fungal colonies. Bacterial growth was not detected during the course of the experiments. Percent retardation of radial growth was calculated by the formula

$$D_u - D_i/D_u \times 100$$

where D_u is diameter of colony without inhibitor and D_i is diameter of colony with inhibitor.

RESULTS

The inhibitors depressed, but did not prevent, growth of each fungus (Table I). Effects of the inhibitors from the two corn sources were quite similar, and for brevity only the data for the opaque-2 source are shown. The inhibitors were most effective against *Fusarium roseum* (Lk. ex Fr.) emend. Synd. Hans. 'Culmorum' and *Fusarium moniliforme* Shield., and less inhibitory to *Aspergillus glaucus* Lk. ex Fr. and *Helminthosporium maydis* Nisikado & Miyake. Inhibition was apparent after 2 days for all except *H. maydis*, which was not inhibited until the third day. *H. maydis* was not inhibited at the 25 μg/ml level, whereas the others were. Inhibition generally increased as concentration of inhibitor was increased. With most of the organisms, retardation at a given level decreased with time, even when the inhibitor was added at 400 μg/ml of medium.

The results suggest that corn trypsin inhibitors may function by reducing fungal growth during seed germination or during other periods when seeds are moist enough to support fungal growth. Little is known about inhibitor distribution in the seed, except that it is located principally in the endosperm (Halim et al., 1973). Distribution of inhibitors within endosperms may vary, and there may be localized concentrations which are high enough to duplicate or exceed the inhibitions found in this study.

Resistance to ear rot has been observed in corn (Hayes, 1933; Jenkins, 1936; Koehler and Holbert, 1938) and may be associated with variations in trypsin inhibitor content. We have found high trypsin inhibitor activity in corn silk (Halim et al., 1972), which may help reduce ear infection by those fungi which cause damage through and around the silks.

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