Table I. Effect of Enzyme Treatment on Mercury Content of Soluble and Insoluble Fractions of FPC Made from Pacific Hake and Swordfish

Enzyme	Digestion time, hr	% FPC in		Mercury content, ppm		% recovery	
		Soluble fraction	Insoluble fraction	Soluble fraction	Insoluble fraction	FPC	Hg
		Pacific hake, 0.49 ± 0.03 ppm of Hg					
Pancreatin	1	63.3	34.4	0.08 ± 0.02	1.33 ± 0.06	97.7	104
	6	84.3	16.8	0.12 ± 0.01	2.49 ± 0.10	101.1	106
Pronase	1	57.6	41.3	0.14 ± 0.01	1.13 ± 0.07	98.9	112
	6	89.6	11.8	0.15 ± 0.02	3.71 ± 0.15	101.7	116
Monzyme	1	49.3	47.6	0.19 ± 0.04	0.84 ± 0.04	96.9	100
	6	77.7	23.4	0.05 ± 0.01	2.05 ± 0.07	101.1	106
		Swordfish, 6.39 ± 0.51 ppm of Hg					
Monzyme	1	50.3	43.8	0.64 ± 0.01	13.63 ± 0.92	94.1	99
	6	77.9	15.5	1.04 ± 0.01	31.94 ± 0.63	93.4	90

gut unabsorbed. Either the mercury is bound to an extremely enzyme-resistant fraction or the presence of mercury in the protein lends the property of enzyme resistance.

FPC has poor functional properties for incorporation into food systems. Enzymatic solubilization has been suggested as a method for improving its functionality (Archer *et al.*, 1973; Cheftel *et al.*, 1971). We have shown that an additional benefit gained by enzyme digestion is a significant reduction in mercury levels in the soluble product.

ACKNOWLEDGMENT

We thank Steven Kahn and John Ferguson for excellent technical assistance.

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Received for review March 12, 1973. Accepted June 22, 1973. This study was supported by the National Marine Fisheries Service, U. S. Department of Commerce.

Long-Term Preservation of Carotene in Alfalfa Meal

Alfalfa stems and leaves were steam-blanched, dried, and ground to a meal containing 374 ppm of carotene. Samples stored *in vacuo* in darkness at 90° for 27, 35, and 55 weeks retained 98% of the carotene. A final sample, kept 27 years at room temperature, retained 95.5% carotene. Isomerization of carotene was negligible in all samples. Possible importance for space trips is suggested.

It is well established that molecular O_2 is responsible for most loss of carotene in the preparation of alfalfa meals (Griffith and Thompson, 1949) and other dried plant products (Booth, 1957; Seshan *et al.*, 1942). Enzymic oxidation is very important, hence the need for blanching soon after harvest (Goodwin, 1954; Zscheile *et al.*, 1943). Zscheile and Whitmore (1947) found no appreciable loss in dried alfalfa meal during 4 months of storage in bottles at −18°.

EXPERIMENTAL SECTION

Soon after the development of a reliable and simple method for determination of carotene in alfalfa (Zscheile and Whitmore, 1947) samples of first-cutting alfalfa were prepared for accelerated storage tests at the Department of Botany, University of Chicago. Stems with leaves were harvested in mid-afternoon of a bright sunny day (May 8, 1945) and blanched with steam for 3 min at 15 lb within 10 min of cutting. After drying in a circulating hot air

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 characteristic 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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Received for review March 21, 1973. Accepted July 6, 1973.

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 *Helminthosporium 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