DETERMINATIONS OF FREE AND BOUND ASCORBIC ACID IN FISHERY PRODUCTS[1]

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ABSTRACT

Asorbic acid is found in two different forms in fish tissue. One form, soluble in 95% ethanol, constitutes approximately 85% of the total vitamin C content, and the other form, which is insoluble in 95% ethanol, constitutes approximately 15% of the total. Certain fish products have sufficient ascorbic acid to be of importance as dietary sources. Retention of the vitamin on processing varies with different fish products.

INTRODUCTION

Fresh and processed fish products have been assayed for ascorbic acid, but few have been found to be major dietary sources of this nutrient. In view of the fact that scurvy-free inhabitants of arctic regions exist largely on whole fish or animal diets, it has been suggested that ascorbic acid is bound in animal tissues but is not available for assay by standard acid extraction procedure. It has also been suggested that visceral organs of fish may contain a higher proportion of vitamin C than muscle tissue.

This is a preliminary report of a survey of ascorbic acid in fish and shellfish. A modification of the method used by Roe and Kuether (1943), by which amounts of "bound" and "free" ascorbic acid are determined separately, has been used. The procedure, obtained from Sumerwell and Sealock (1952), has been further modified for use with the materials studies.

METHOD

The residue from ethanol extractions, from which free ascorbic acid is determined, was digested with trichloracetic acid. The alcoholic and acidic extracts containing free and bound ascorbic acid were cleared, chilled, saturated with carbon dioxide, treated with thiocarbamide and dinitrophenylhydrazine, and incubated. They were then chilled again, sulfuric acid was added, and after aging, the color of the mixture was read in a spectrophotometer at 540 mμ, using a blank with dye addition at the end of incubation as 100% transmitancy. The ascorbic acid osazones which were measured at this wave length

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include l-ascorbic acid, dehydroascorbic acid and diketogulonic acid. Measurements of degradation products of ascorbic acid by the method of Roe, et al. (1948) showed that 0–4% of the total osazone color is due to diketogulonic acid. We believe that the modified Roe and Kuether method as used gives values of ascorbic acid that are more in line with actual *in vivo* levels than does a substraction of diketo form when it shows up in such small amounts.

RESULTS

Table I presents data on samples assayed for bound, free and total ascorbic acid as well as the percentage of moisture. Fresh silver salmon muscle is typical of fish products, being low in total ascorbic acid and showing no appreciable proportion in the bound form. Fresh Pacific herring contains about 3 mg/100g, with one fifth bound. Fresh tuna muscle appears fairly high with five mgs%, one third of this being in the bound complex.

TABLE I.—Free, Bound and Total Ascorbic Acid and Moisture in Fish and Shellfish
(Ascorbic acid expressed in mgs/100g material)

Product	*Ascorbic Acid*			*Moisture*
	Free	*Bound*	*Total*	(%)
Albacore (*Thunnus germo*)				
Fresh muscle	3.5	1.6	5.1	63
Pre-cooked muscle	3.5	1.9	5.6	55
Canned muscle	3.0	2.1	5.1	—
Liver	10.5	11.5	22.0	—
Silver salmon (*Oncorhynchus kisutch*)				
Fresh muscle	1.13	0.17	1.3	67
Liver	8.1	1.8	9.9	75
Roe	11.5	6.5	18.0	57
Milt	7.1	2.7	9.8	72
Pacific herring (*Clupea pallasii*)				
Fresh muscle	2.0	0.63	2.6	78
	3.0	0.81	3.8	73
Canned muscle	0.25	0	0.25	79
Fresh roe	11.8	2.2	14.0	75
Canned roe	1.5	0.62	2.12	75
Pacific oysters (*Ostrea gigas*)				
Fresh	20.0	2.3	22.3	80
Canned meat	10.5	3.3	13.8	76
Canned liquor	11.7	0.13	11.8	93
Olympia oysters (*Ostrea lurida*)				
Fresh	36.2	1.9	38.1	83

Whole fresh Pacific oysters show significant amounts of ascorbic acid, mostly in the free form. Whole fresh Olympia oysters give even higher amounts, assaying 38 mgs%, which is equal to the average for citrus fruit juices. Tuna liver is high in total ascorbic acid, with 52% present in the bound form. Salmon and herring eggs are high in total amounts, salmon sperm and liver being somewhat less. All these organs contained significant amounts in the bound form.

Canned Pacific oysters, processed at the time when the fresh material was assayed, showed a retention of 80% found in fresh material (see calculations for the free, bound and total ascorbic acid content of oyster meat, oyster liquor and moisture in Table I). Retorting was done at 250° F for 13 minutes in 211 x 309 cans, as recommended by the National Canners Association. Note that 99% of the total ascorbic acid in canned oyster liquor was in the free form as compared to 76% in canned oyster meat. Canned herring and their eggs showed considerable loss after retorting.

DISCUSSION

The chief purpose of the data shown in Table I is to add information on ascorbic acid sources. The value of determining "free" and "bound" ascorbic acid is not understood. Total levels obtained by adding free and bound ascorbic acid are not significantly different from those determined by the photometric method recommended by The Association of Vitamin Chemists, Inc. (1951, pp. 81–87), which uses a single acid extraction. Yet, up to 15 extractions with ethanol, with no appreciable ascorbic acid after the fifth, have been followed by acid extraction resulting in considerable amounts of "bound" ascorbic acid. It is inconceivable that a single chemical would show this property. More ascorbic acid is recovered from the ethanolic extracted residue by a short heat digestion than by a cold extraction. This is true of canned as well as fresh fish products.

Recent tests with sand sole, *Psettichthys melanostictus*, not shown in Table I, showed 6.0 mgs% ascorbic acid in the liver. After the same species, caught at the same time, had lived in a salt water aquarium for five months, with little food intake, it showed 6.4 mgs% ascorbic acid in liver samples.

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