HEAT INACTIVATION OF THIAMINASE IN WHOLE FISH

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ABSTRACT

The time required at various temperatures to inactivate all of the thiaminase in several species of whole fish was studied. Some effects of pH and enzyme concentration on the time-temperature inactivation were also determined. Whole raw fish were ground, sealed in specially-constructed metal cans, heated at various temperatures for various lengths of time, and analyzed for residual thiaminase activity. Results indicate that a minimum time-temperature of 5 minutes at 180° F. is required to inactivate all the thiaminase of whole fish. Enzyme concentrations, pH, and possibly oil content of fish influence the time required to destroy thiaminase.

INTRODUCTION

The heating conditions employed by commercial mink-food producers and mink ranchers to destroy thiaminase in whole fish are empirical. The conditions are not based on predetermined time-temperature relations for the thermal inactivation of this antimetabolite. A common practice, for example, is to cook the fish at 180°-200° F. for 15 minutes (Borgstrom 1962).

Most of the specific data available on the time-temperature relation is found in various research publications dealing with the occurrence of thiaminase in fish, or with studies on the chemistry of the enzyme. Deutsch and Hasler (1943) used 15 minutes at 100° C. (212° F.) to inactivate thiaminase in whole fish and viscera homogenates. Sealock, Livermore, and Evans (1943) destroyed most of the thiaminase in carp by heating at 100° C. for 30 minutes. They found that with dilute or purified preparations, thiaminase can be destroyed completely in a bath of boiling water in 5 minutes. Melnick, Hochberg, and Oser (1945) used 20 minutes at 100° C. to destroy thiaminase in ground fish suspended in water at pH 4.5. Somogyi (1949) used only 10 minutes at 100° C. to destroy the thiaminase in a carp intestinal extract. Similarly, Kuusi (1963) used 10-30 minutes in a bath of boiling water to inactivate thiaminase in Baltic herring and bream.

The time-temperature conditions used by those researchers can be used, however, only as general guidelines by commercial processors. There appears to be no published data regarding minimum times and temperatures that are sufficient to inactivate all of the thiaminase in whole fish. Such data would be important in the development of improved fish-reduction methods that are designed to yield safe, high-quality products.

The purpose of this study was to determine minimum time-temperature relations required to inactivate all of the thiaminase in several species of fresh-water fish. Some effects of pH and enzyme concentration on time-temperature relations were also studied.

EXPERIMENTAL

PREPARATION OF WHOLE-FISH SAMPLES: Fresh whole fish were passed twice through a meat grinder, first using a $\frac{1}{4}$-inch plate and finally using a $\frac{1}{8}$-inch plate. The homogenous ground material was then immediately packed and sealed in metal 208 x 006 Thermal Death Time (T.D.T.) cans, was frozen, and stored at 0° F. until used. Sufficient ground material was used so that no air space remained in the cans. These cans (which have a capacity of about 20 grams) are specially constructed so that the entire contents can be heated (or cooled) very rapidly and uniformly, thereby eliminating the need to measure the internal temperature of the cans; thus, the internal temperature is assumed to be equal to the temperature of the heating medium.

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HEAT TREATMENT OF SAMPLES: The filled cans were removed from frozen storage and equilibrated in an ice-water bath prior to their being heated; thus, the starting temperature was about 32° F. for all samples. The cans were then submerged in a thermostatically-controlled water bath for the desired length of time and at the desired temperature. At the end of the heating period, the cans were immediately placed again in the ice-water bath until cooled. The samples were subsequently frozen and held at 0° F. until analyzed for residual thiaminase activity.

ANALYSES OF SAMPLES: Thiaminase analyses were carried out according to the procedure of Gnaedinger (1964). Proximate composition analyses were carried out according to the Association of Official Agricultural Chemists Official Methods of Analysis (1960).

RESULTS AND DISCUSSION

The times required to inactivate all of the thiaminase in various species of whole fish at various temperatures are shown in the table. In most cases, the results are reported as two numbers (for example, 3-4, which indicates that some activity was observed at 3 minutes but none at 4 minutes). Thiaminase activities of the unheated starting materials, the pH and proximate composition of the raw fish, including date and location of their capture, are also included in the table.

Time-Temperature Relationships of Inactivating Thiaminase in Whole Fish

Species1/	Date of Capture	Location	pH	Thiaminase Activity	Time to Inactivate Thiaminase at:						Proximate Composition			
					200° F.	190° F.	180° F.	170° F.	160° F.	150° F.	Water	Oil	Ash	Protein
				2/	(Minutes)						(Percentage)			
Bowfin	1/-/64	Arkansas	6.60	206	1-2	1-2	1-2	4	-	-	76.16	1.54	3.58	15.53
Carp	12/10/64	Lake Erie	6.75	2,003	2-3	3-4	4-5	9-10	105	-	71.59	9.56	3.15	13.97
Shad	10/28/64	Lake Erie	6.65	112	2-3	3-4	4-5	32	-	-	70.58	14.16	2.24	12.01
Smelt	10/28/64	Lake Erie	6.85	47	1	1	1	1	1	1-2	75.54	7.60	2.01	12.71
Shiner	12/3/64	Lake Michigan	6.70	1,418	1-2	1-2	1-2	2-3	4-5	-	69.90	11.92	2.22	12.99
Alewife A	11/-/64	Lake Michigan	6.90	173	1-2	2-3	4-5	26-27	-	-	71.99	13.49	1.89	11.20
Alewife B	11/-/64	Lake Michigan	5.9	124	1-2	2-3	4-5	74-75	-	-	70.15	13.30	2.39	11.87
Alewife C	11/-/64	Lake Michigan	8.2	162	1-2	2-3	3-4	3-4	-	-	70.63	13.27	2.82	10.86
Alewife fillets3/	11/-/64	Lake Michigan	6.4	152	1-2	1-2	1-2	6	-	-	71.63	13.78	1.26	11.72
Alewife viscera4/	12/3/64	Lake Michigan	6.9	572	2-3	3-4	6-7	45-46	-	-	70.57	16.15	1.96	9.34
Alewife diluted3/	11/-/64	Lake Michigan	6.9	152	1-2	2-3	3-4	7	-	-	85.38	6.76	1.06	5.83

1/Alewife (Pomolobus pseudoharenqus); Bowfin (Amia calva); Carp (Cyprinus carpio); Shad (Dorosoma cepedianum); Smelt (Osmerus mordax); Shiner (Notropis hudsonius).
2/Micrograms of thiamine hydrochloride destroyed in 20 minutes per gram of protein of the unheated raw fish.
3/Low concentration of enzyme.
4/High concentration of enzyme.

EFFECT OF TIME AND TEMPERATURE ON THIAMINASE DESTRUCTION: The results of this study indicate that a minimum time-temperature relation of 5 minutes at 180° F. is required to obtain a thiaminase-free product from whole raw fish. From the standpoint of commercial application, this relation implies that the coldest part of any fish particle must be held at 180° F. for at least 5 minutes at some time during processes relying upon heat to inactivate the antimetabolite. When fish offal (rather than whole fish) is being processed, the minimum time should be increased to at least 7 minutes at 180° F., as indicated by the results obtained in the use of alewife viscera.

At temperatures above 180° F., the thiaminases of different species of fish show relatively small differences in their heat labilities. Below 180° F. however, marked differences begin to appear. At 170° F., for example, the results indicate that the thiaminases of carp and shiner are destroyed much more readily than are those of whole alewife and shad, even though the latter two species had relatively lower initial thiaminase activities. In smelt, the species with the lowest activity, thiaminase was destroyed in less than 2 minutes, even at 150° F. Thus, processing conditions for this species would probably not have to be as severe as, for example, those for alewife. There was some indication that a very high oil content exerted a protective action against heat destruction of thiaminase, although exceptions were evident, notably that of the shiner. This species possessed both high initial activity and high oil content, but the thiaminase was destroyed in 5 minutes even at 160° F.

EFFECT OF ROOM-TEMPERATURE INCUBATION ON THIAMINASE DESTRUCTION: A st was conducted (the results of which are not shown in the table) to determine the stability thiaminase at room-temperature incubation. Samples of ground whole alewife, carp, shad, iner, and smelt (at their natural pH) were sealed in T.D.T. cans and stored at 73°-83° F. t monthly intervals, the samples were analyzed for residual activity. The samples, of urse, were putrid when analyzed, and all the cans were greatly distended. Carp, shad, and iner gave a positive thiaminase test even after 3 months of incubation. Smelt, which had a w initial enzyme activity, was inactive at the end of one month. Alewife, which was incubated r only 2 months, was still active at the end of that time. These results suggest that the iaminase enzyme is not readily destroyed at room temperature by the various proteolytic nzymes of fish.

EFFECT OF ENZYME CONCENTRATION ON THIAMINASE DESTRUCTION: The intital ctivities of the unheated raw fish samples are expressed as the number of micrograms of amine hydrochloride destroyed per gram of protein in 20 minutes under the conditions of e assay procedure. The values are expressed on a protein basis so that some standard reference point can be assumed and comparisons between species can be made more meaningfully. As was indicated in a preceding paragraph, the initial activity of the raw fish is not ecessarily related to the time required to destroy the enzyme when different species are ompared. However, enzyme activity appears to be related to processing time when a single pecies is considered. Alewife viscera, for example, which had an initial activity of 572, required 46 minutes at 170° F. for total destruction of thiaminase; whereas, diluted alewife and lewife fillets (initial activity of 152 units) required only 7 minutes at 170° F.

EFFECT OF pH ON THIAMINASE DESTRUCTION: The pH values reported in the table re of the raw unheated fish, except in the cases of Alewife B and Alewife C, which were adusted to 5.9 and 8.2 with HCl and NaOH, respectively, prior to heat treatment. All values ere read by inserting the electrodes (glass and standard reference) of a pH meter into the round fish. The results obtained with Alewife A, Alewife B, and Alewife C indicate that pH as some effect on thiaminase destruction. High pH tends to increase the heat lability of the nzyme; whereas, low pH tends to decrease its heat lability. As with the effect of enzyme oncentration, the effect of pH was studied on only a single species, so no conclusions can be rawn on its effects between species.

CONCLUSIONS

A minimum time-temperature relation of 5 minutes at 180° F. is required to destroy all the thiaminase in whole raw fish. At temperatures above 180° F., thiaminase is very heat abile; only small differences are evident between species. At temperatures below 180° F., owever, the thiaminases of different species show marked differences in heat sensitivity. hese differences are as yet unexplained, although they may be related in part to protection gainst heat destruction by a high content of oil.

Enzyme concentration (as determined by the initial activity in the raw fish) is not necesarily related to processing time when different species are compared, but it is related to rocessing time when members of a single species are compared.

The effects of pH on heat sensitivity of thiaminase was studied on alewife only. High pH ended to increase the heat lability of thiaminase; low pH tended to decrease the heat lability.

The thiaminase of canned raw fish, stored at room temperature, can remain active for at least 3 months.

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