Effect of Aureomycin on the Behaviour of Certain Free Amino Acids in Oil Sardine (*Sardinella longiceps*) held in Ice Storage

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[The course of development of a few free amino acids under the influence of aureomycin in oil Sardine (*Sardinella longiceps*) held in ice storage was investigated. The levels of leucine(s) and valine regularly increased in the contorl and aureomycin treated fish throughout the storage period. Alanine(s) and Threonine showed similar trend in both control and fish treated with 20 ppm aureomycin. These amino acids however showed a gradual fall in fish treated at 50 ppm. level. The changes in tyrosine + tryptophane were found to be irregular. Most of the amino acids studied indicated a remarkable change in trend by about the 16th day of ice storage in the case of fish treated with 50 ppm. aureomycin]

Introduction

Free amino acids of herring flesh and their behaviour during post mortem spoilage have been studied by Hughes (1959). Shewan and Jones (1957) reported the production of free amino acids in cod muscle under sterile conditions compared with normal conditions. Production of free amino acids in herring stored under commercial conditions in plain and antibiotic ices was studied by de Silva and Hughes (1960, 1962). In this work, the behaviour of certain free amino acids like leucine(s), valine, threonine, alanine(s) arginine etc. has been investigated in a fatty fish like oil sardine stored in ice in round condition after pretreatment with aureomycin at two levels.

Experimental Procedure

Oil Sardines (*Sardinella longiceps*), from boats landed at Manassery in Cochin, were used for the study. After thoroughly washing with tap water to remove the adhering dirt and slime, the fishes were dipped in aureomycin solution ('Acronize' to give 50 ppm. or 20 ppm. antibiotic) after which they were packed in polythene bags and stored in crushed ice side by side with the control untreated fish. Sampling for bacterial counts and amino acids was done for a period of 26 days. The free amino acids were extracted from the meat with 80% ethyl alcohol and the extract concentrated. The amino acids were separated and estimated by unidimensional paper chromatography using butanol : acetic acid : water (100 : 24 : 100) as the developing solvent and 0.5% ninhydrin in water saturated butanol as the colour developing reagent. The spots were cut, eluted with 75%

alcohol containing traces of copper ions and measured at 540 mμ. Arginine was estimated by naphthol hypobromite method after Weber and tyrosine together with tryptophane was estimated using Folin-Ciocalteu Phenol reagent. Total α-amino nitrogen was determined by Pope-Stevens (1933 method and the standard plate counts followed by employing sea water agar.

Results and Discussion

Changes in total bacterial count and amino nitrogen content are shown in Table I. The bacterial counts showed considerable reduction by treatment with antibiotic. However the counts did not show appreciable difference between the samples treated at the two levels tried. In the control and treated samples, the bacterial counts regularly increased with storage period. The total α-amino nitrogen content increased by nearly three fold during the period of 26 days in ice.

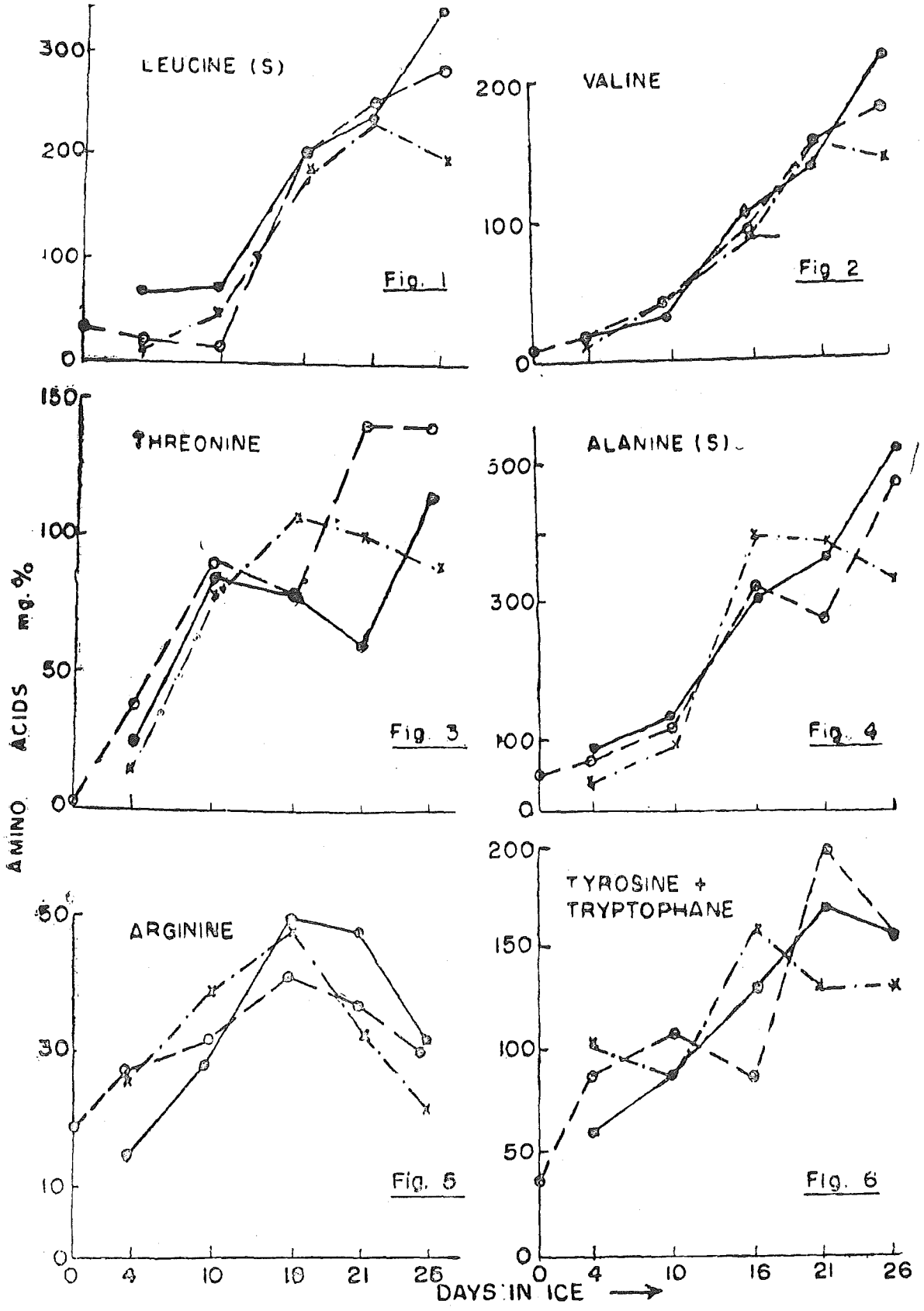
TABLE I.

C = Control ; T_1 = Fish treated with 20 ppm aureomycin ;
T_2 = Fish treated with 50 ppm aureomycin.

Days in ice	Bacterial count per gram			α-amino nitrogen mg%		
	C	T_1	T_2	C	T_1	T_2
0	4.0×10^5			91.94		
4	9.9×10^5	3.2×10^3	1.9×10^3	105.5	94.40	90.58
10	2.3×10^7	2.8×10^4	3.9×10^4	123.0	123.0	114.9
16	8.2×10^6	8.7×10^5	1.2×10^5	171.7	151.5	177.1
21	2.0×10^7	1.7×10^6	3.4×10^6	221.7	181.2	190.6
26	8.9×10^7	2.9×10^7	9.9×10^6	215.0	259.8	220.0

Changes in amino acids are represented graphically (Figs. 1 - 6). Among the individual amino acids, leucine(s) and valine showed regular increase throughout the storage period in control and aureomycin treated samples. However in the case of fish treated at level of 50 ppm, these amino acids showed a tendency to fall after the 21st day. Although the level of threonine is always less in control compared to that in treated samples, changes in this amino acid follow the same pattern with storage time in both the groups, except that the level in the case of samples treated at 50 ppm showed a gradual fall after the 16th day. Alanine(s) showed regular increase in both control and fish treated at 20 ppm. level, similar in behaviour to that of threonine. These amino acids showed a gradual fall in fish treated at 50 ppm level. Arginine regularly increased upto 16th day and thereafter its level decreased gradually till the end of storage period. In the case of tyrosine and tryptophane the changes were found to be quite irregular.

de Silva and Hughes (1962) observed that the amino acids which increased during ice storage showed a greater increase in the presence of antibiotic. This fact was not ob-



○———○ C = CONTROL ●———● T_1 = FISH TREATED WITH 20 ppm. AUREOMYCIN,
×.—.—.—× T_2 = FISH TREATED WITH 50 ppm AUREOMYCIN.

served in our repeated studies with oil Sardine. An observation which was found to be interesting was that in the sardines treated with aureomycin at 50 ppm. level, most of the amino acids studied showed a tendency to decrease during the later days of ice storage. According to de Silva and Hughes (1960) the fall in the amino acids after a regular increase for a few days of storage, is probably due to the assimilation of the free amino acids by bacterial flora. During ice storage, after the initial period when the bacterial flora have been developed by using up the various nitrogenous components produced in the muscle, the strains compete amongst themselves until the dominant types become established. In the present investigation, 16th day appears to be the critical day in fish treated with aureomycin at 50 ppm level, at which almost all the amino acids studied showed a remarkable change in the trend as could be seen from the curves. It may be safely assumed that by about 16th day, the dominant bacterial strains might have got established and active bacterial proteolysis would have started. Organoleptically, the untreated fish developed considerable slime and unpleasant odour within 10 days of storage while the antibiotic treated fish continued to retain the appearance and odour satisfactorily during the period. The more or less identical pattern observed in the development of the majority of amino acids in the three groups of samples during earlier days of ice storage approximately till 10th day lends support to the suggestion (Ranke, 1960) that native enzymes play more important role in controlling their relative amounts in the tissue rather than bacterial enzymes. The fact that a critical day of storage in ice is shown clearly only in the case of fish treated with aureomycin at 50 ppm level and not in the control and the fish treated at 20 ppm level may be due to the difference in the sensitivity of bacterial species at different level of aureomycin concentration (Castell and Jacqueline Dale, 1963).

Summary

Changes in the production of certain free amino acids in oil sardines stored in ice under the influence of aureomycin at two different concentrations have been studied. Leucine (s) and valine indicated regular increase throughout the storage periods in treated and untreated fish. In fish treated with aureomycin at 50 ppm. level, most of the amino acids showed a remarkable change in trend by about the 16th day of ice storage.

Acknowledgement

The authors are greatly indebted to Dr. A. N. Bose, Director of the Institute for his keen interest and helpful suggestions.

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