TETRAZOLIUM REDUCTION AS AN INDEX OF SPOILAGE IN CURED FISH

BY

S. V. SURYANARAYANA RAO, M. RAJENDRANATHAN NAYAR

AND

A. P. VALSAN

Reprinted from "Current Science", December 1956, 25, 396-397

Reprinted from "Curr. Sci.", Dec. 1956, 25, 396-397

TETRAZOLIUM REDUCTION AS AN INDEX OF SPOILAGE IN CURED FISH*

PREVIOUS attempts to employ redox indicators like methylene blue and resazurin for assessing the bacterial contamination of fish have been unsuccessful due to the presence of trimethylamine oxide which retards the lowering of redox-potential.[1] Since it has been suggested that more electropositive dyes may be useful, we have investigated the possibility of employing triphenyl tetrazolium bromide (T.T.B.) which has been often used in recent years for studying dehydrogenase systems.[2] Laxminarayana and Iya[3] have described a tetrazolium reduction test for milk. Though the Eo of T.T.B. is reported to be -80 mV, it is reduced by succinic dehydrogenase system having a much more positive normal redox-potential due to the insoluble nature of the reduced form.[4]

3 ml. to 5 ml. of fish muscle extract at pH 7·2 (1 part of muscle for 10 parts of phosphate buffer) made up to 6 ml. were incubated after adding 0·5 ml. of 0·5% T.T.B. Time taken for the onset of a distinct pink colour was noted and formazan formed after definite periods of incubation estimated by visual comparison after extracting with toluene.[5] Standards were prepared according to the method described by Fairbridge *et al.*[6]

Muscle from fresh samples of choodai (*Sardinella* sp.) did not show any reduction even after 18 hours. Among four different samples of sun-dried choodai, one specimen which was evidently spoiled and also showed a high T.V.N. value of 264 mg.% gave 360 μg. of reduced T.T.B. in 20 hr. as against 190 μg. to 220 μg. in other cases. In another experiment eight samples of sun-dried sardines from experimental lots and obtained from local curing centres and the local market were examined. Dye reduction was observed in 5 hr. to 8 hr. and samples cured in the laboratory showed no appreciable reduction. Maximum reduction amounting to 100% was observed with the market sample.

The influence of spoilage before curing was investigated by studying the dye reduction of cured samples of *Chirocentrus dorab* dry-salted after allowing to spoil at room temperature for different intervals. A distinct gradation was observed in the onset and extent of reduction in 4, 6, 8 and 12 hr. Values recorded during 6 hr. to 8 hr. were even more sensitive than Total Volatile Nitrogen in indicating the condition of the four different samples. Amount of dye reduced in 8 hr. ranged from 22·5 μg. to 41·3 μg.

During the course of this investigation we have examined about 20 samples of cured fish from Paramakudi market comprising of all the common varieties of fish. When the amount of dye reduced in 8 hr. was compared with the T.V.N. values, there appeared to be some relationship between the extent of reduction and the quality of the samples as revealed by T.V.N. values. Results of these preliminary experiments indicate that tetrazolium reduction may prove to be useful as a presumptive test for the detection of fish spoilage since there exists a wide variation in the extent of reduction presumably related to the magnitude and nature of the bacterial population. Further work on these lines is in progress to evolve a suitable procedure by standardising the conditions.

While this work was in progress we have come across a brief report in the *World Fisheries Abstracts*[7] that similar work is in progress at Torry Research Station (U.K.).

We are deeply indebted to Dr. N. K. Panikkar, Chief Research Officer, for his keen interest and valuable suggestions.

Central Marine Fisheries Res. Station, Mandapam Camp,
September 5, 1956.

S. V. SURYANARAYANA RAO.
M. RAJENDRANATHAN NAYAR.
A. P. VALSAN.

* Published with the kind permission of the Chief Research Officer, Central Marine Fisheries Research Station, Mandapam.

1. Castell, E. M., *J. Fish. Res. Bd. Can.*, 1950, **7**, 57.
2. Kun, E. and Abood, L. G., *Science*, 1949, **109**, 144.
3. Laxminarayana, H. and Iya, K. K., *Proc. Soc. Biol. Chem.* (India), 1954, **12**, 56.
4. Jambor, B., *Nature*, 1954, **173**, 774.
5. Fahmy, A. R. and Walsh, E. O'F., *Biochem. J.*, 1952, **51**, 55.
6. Fairbridge, R. A., Willis, K. J. and Booth, R. G., *Ibid.*, 1951, **49**, 423.
7. Anon, *FAO World Fisheries Abstracts*, 1955, **6** (6), 2.