EVALUATION OF CHEMICAL TESTS FOR THE QUALITY OF PRAWNS

FATIMA SHAIKHMAHAMUD and N. G. MAGAR,
Department of Biochemistry, Institute of Science, Bombay

[The object of this study was to determine the value of physical, bacteriological and chemical tests used to find out and compare the indices of quality of prawns stored at 0° C and at 18°C. pH value, nature of drip, the total bacterial count, presence or absence of tryptophane, trimethylamine content, glycogen, lactic acid, vitamins such as thiamin, riboflavin and niacin were estimated periodically to evaluate the quality of prawns stored at 0°C., whereas in addition to organoleptic changes, pH, bacterial count, nature of growth in peptone water, soluble protein in salt solution and loss of moisture, glycogen, lactic acid, and changes in vitamin B contents were noted periodically for prawns stored at —18°C. Riboflavin and niacin were not affected appreciably but the retention of thiamin in prawns was very low.]

Introduction

A thorough comparison of the different tests available for evaluating the quality of stored prawns is not available in the literature. It would be an immense task to use simultaneously all the methods now available for evaluating spoilage. Considerable work has been done by various workers to develop freshness tests for shrimps (Campbell et al. 1952; Fieger et. al. 1950 and 1954; Alford et. al. 1954; Faulhner et. al. 1954; Bailey et. al. 1956). Some of the most useful criteria for judging the quality of prawns are the bacterial count, pH, trimethylamine (TMA) and soluble protein contents. However, these tests are not always reliable under different environmental conditions e. g. a low value for the bacterial count or TMA content does not necessarily indicate that the prawns are in good condition. The use of the different tests for determining the quality of prawns on storage forms the subject of this paper.

Experimental and Procedure

The prawns used in these experiments were caught during daily trips by mechanised vessels off the Bombay coast. Immediately after landing, the prawns were beheaded, washed, divided into 2 batches, wrapped in moisture proof plastic bags and stored at 0°C and — 18°C. The various tests were carried out on fresh prawns and again after certain intervals of storage depending on the temperature.

For analyses, the prawns after shelling were well ground and samples were taken from this uniform mixture. The pH was determined on a Beckman pH meter. Glycogen was extracted according to the method of Kemp et. al. (1954) and estimated by Loewns's method (Loewns's, 1952) while lactic acid was determined as suggested by Bonting (1955). Trimethylamine was estimated by Dyer's (1945) method and tryptophan by the histo-chemical test according to the Romien reaction as described by Glich (1951). Thiamine, riboflavin and niacin were determined by the methods of Jansen (1936), Scott et. al. (1946) and Sweeney (1951) respectively and the soluble protein content was estimated by Salvin's (1954) method.

Total bacterial counts were made by shaking the prawns with water to wash off almost

all the bacteria adhering to them. Suitable dilutions were made of this solution and the total number of bacteria were enumerated by plate counts on sea water agar.

Results and Discussion

The results have been presented in Tables I to III.

Storage at 0°C.

Textural changes: In fresh prawns, the exoskeleton was firm and easy to remove, there was no off-flavour, the gut (vein) came out automatically with the head on removal of the carapace and the muscle was firm, elastic and white. On storage the exoskeleton became soft and difficult to remove while the muscle lost its elasticity and became soft, moist and yellowish in colour.

Oxidative changes: On storage, blackening started first in the head and then progressed downwards. A slight blanching effect was noticed in the original red colour after 7 to 8 days.

Changes in pH: Fresh prawns had a pH varying between 6.5 and 6.9. On storage the pH increased, gradually at first and more rapidly later on. When the values reached 7.3 to 7.8, the sample was considered to be of doubtful quality.

Colour of the drip: In the initial stages the drip was clear fluid, changing to yellowish, brownish and ultimately to a blackish coloured dirty fluid with an offensive odour.

Bacteriological tests: Though bacterial counts showed considerable variations, values of 10×10^6 and above hinted towards spoilage. The bacterial counts increased gradually for the first 6 days and later at a more rapid rate.

Estimation of tryptophan: This was a reliable test as tryptophan was destroyed much more rapidly than the other amino acids. In fresh prawns the colour was bright red, changing gradually to violet light pink and ultimately to a colourless solution.

Estimation of trimethylamine: In fresh samples, TMA was present in negligible quantities, but when it reached a value of 0.9 to 1.2 mg/100 g. there was a characteristic smell, and at values of 1.3 to 1.8 mg/100 g. the sample was of either doubtful value or spoiled.

Glycogen and lactic acid: As can be seen from Table III, the initial glycogen and lactic acid contents were low, but registered a considerable increase during the next 2 to 3 days followed by a subsequent decrease on further storage.

Thiamine, riboflavin and niacin: These three vitamins decreased continuously on storage, though appreciable amounts were present even at the end of the storage period. Thiamine was the most affected while niacin was the most stable.

Storage at — 18°C

Textural changes: On storage at this temperature the exoskeleton became harder and more brittle, while the muscle too turned hard.

Oxidative changes: Melanosis was retarded, blackening appeared very slowly and its progress too was slow. But decolouration was well marked as storage time increased, and ultimately the prawns became whitish.

Changes in pH: At this temperature, pH seemed to be of little value, although it increased very slowly.

Bacteriological tests: In the initial stages the count was considerably reduced to a figure much lower than the initial values, but after 5 months storage it increased and kept on increasing, very slowly but steadily. There was no off-flavour, nor was bacterial spoilage pronounced, yet the material was considered spoiled after 8 or 9 months because of other undesirable changes.

Soluble proteins: One of the factors limiting the sale of frozen prawns was their tendency to become tough during frozen storage. It has been shown (Anon, 1953) that toughening is related to structural changes in the proteins. The second important factor was the hardening of the material. To study these changes, the soluble protein content and the percentage loss of moisture were determined each month. The soluble protein fraction decreased on storage from 9.5% to 3.2% while moisture loss increased from 0.84% to 2.9% (Table II).

Glycogen and lactic acid: These increased for the first 3 months of storage by about 50% of the original value. On further storage, these values decreased continuously.

Thiamine, riboflavin and niacin: On storage for 6 months, riboflavin and niacin were retained almost quantitatively while thiamine exhibited a retention of about 50%.

Glycogen, lactic acid and the B-group vitamins decreased more rapidly at 0°C than at —18°C.

From the results obtained it appeared that on storage at temperatures above the freezing point and below room temperature the most suitable tests were the determinations of pH, total bacterial count, the general condition of the prawns, TMA, glycogen, lactic acid and vitamin B contents. However, on storage at sub-zero temperatures, some of these tests were not reliable because of the relatively rapid rise in protein denaturation. Thus, at —18°C, the most reliable tests were the solubility of proteins, loss of moisture and textural changes. These tests may be supplemented by the total bacterial count, glycogen, lactic acid and vitamin B contents.

References

1. Campbell, L. L. Jr. & Williams, O. B., *Food Tech.*, 6, 125, (1952).
2. Fieger, E.A., Green, M., Lewis H., D'Holmes & C. D. U. Bois, *Refug. Eng.*, *58*, 244, (1950).
3. Alford, A. J. & Fieger, E. A., *Food Tech.*, 8, 35, (1954).
4. Fieger, E. A., & Friloux, J. J., *Food Tech.*, 8, 35, (1954).
5. Faulhner, M. B., Watts, B. M. & Humm, H. J., *Food Res., 19* (3), 302, (1954).
6. Bailey, M. E., Fieger, E. A. & Novak, A.F., *Food Res., 21*(6), 611, (1956).
7. Kemp, A. & Kitsvan, H., *Biochem. J. 56*, 646, (1954).
8. Loewns, A., *Anal. Chem, 24*, 219, (1952).
9. Bonting, S., *Arch. Biochem. Biophys., 56*(2), 307, (1955).
10. Dyer, W. J., *J. Fish. Res. Bd. Can., 4*, 351, (1945).
11. Glick, D., *Techniques of Histo-and Cytochemistry*, 58, (1951).
12. Jansen, B. C. P., *Rec. trav. chim. Pays. Bas.*, 55, 1046, (1936).
13. Scott, M. K., Hill, F. W., Norris, L. C. & Henser, G. F., *J. Biol. Chem., 165*, 65, (1946).
14. Sweeney, J. P., *J. Assoc. Offic. Agric. Chem. 34*, 380, (1951).
15. Salvin, H., *Food Res., 19*, 235, (1954).
16. Anonymous, *Com. Fish. Rev., 17*(8), 10, (1953).

TABLE — I

EFFECT OF STORAGE ON pH, DRIP, TOTAL BACTERIAL COUNT PER G. OF MATERIAL. PRESENCE OF TRYPTOPHANE AND TRIMETHYLAMINE CONTENT IN MG. NITROGEN PER 100 g. OF PRAWNS AT 0°C.

Storage time in days	pH	Drip	Bacterial count	T. M. A. content in mg.	Tryptophan	
0	6.5	Very clear	0.54×10^8	0.15	+ ve	Red
3	6.6	Slightly yellow	37×10^8	0.65	+ ve	Violet
6	6.8	Not clear, Brownish	45×10^9	0.98	+ ve	Light pink
9	7.1	Dirty brown	25×10^6	1.3	— ve	
12	7.3	Blackish	39×10^7	1.95	— ve	

TABLE — II

EFFECT OF STORAGE ON COLOUR CHANGE, pH, DRIP, TOTAL BACTERIAL COUNT PER G. OF MATERIAL GROWTH IN PEPTONE WATER, SOLUBLE PROTEIN IN G. PER 100 G. OF MUSCLE AND % LOSS OF MOISTURE AT—18°C

Storage time in months	Colour change (surface)	pH	Bacterial count	Growth in Peptone $H_2 L_0$	Soluble protein in g./100 g. muscle	% loss of moisture
1	Red	6.8	6.3×10^4	Negligible	9.5	0.84
2	Red	7.0	0.1×10^4	,,	8.9	1.44
3	Reddish	7.0	0.5×10^4	,,	8.5	1 78
4	Orange	7.1	0.96×10^4	Very slight	7.9	1.83
5	Orange	7.2	1.9×10^4	Slight	6.8	1.95
6	Brownish	7.2	3.3×10^4	More Sediment	6.2	2.1
7	Brownish	7.2	7.5×10^4	More Sediment	4.5	2.25
8	Yellow	7.3	37×10^4	Thin pellicle	4.3	2.39
9	Yellow	7.3	45×10^4	Thin pellicle Sediment	3.9	2.58
10	Yellow	7.4	56×10^4	,,	3.7	2.73
11	Whitish yellow	7.4	68×10^4	,,	3.5	2.81
12	Whitish yellow	7.5	78×10^4	,,	3.2	2.9

TABLE — III

EFFECT OF STORAGE ON GLYCOGEN, LACTIC ACID, THIAMIN, RIBOFLAVIN AND NIACIN CONTENTS OF PRAWNS STORED AT 0°C AND —18°C

Storage temp.	Storage time	Glycogen	mg /100 g of muscle Lactic acid	Thiamin	Riboflavin	Niacin
Room	0	120	110	0.051	2.9	4.6
0°C	4 days	215	158	0.025	2.3	4.0
,,	8 days	115	90	0.011	2.0	4.0
,,	12 days	50	55	0.009	1.8	3.5
—18°C	1 month	135	165	0.011	3.5	4.8
,,	2 ,,	165	180	0.09	3.5	4.8
,,	3 ,,	185	175	0.05	3.6	4.6
,,	4 ,,	125	150	0.06	3.5	4.5
,,	5 ,,	120	145	0.06	3.4	4.5
,,	6 ,,	110	130	0.06	3.4	4.4

Discussion

During the discussion which followed, the usefulness of pH as a spoilage index was brought up. Shri Velankar pointed out that pH readings have been considered for spoilage tests ; but there are conflicting opinions on its effectiveness. Some feel that the changes in pH with spoilage are very significant. But on the contrary there is evidence to show that by the time significant changes in pH occur the fish would have been spoiled to such an extent that no spoilage test is needed.

The significance of free amino acids in relation to spoilage was discussed. There was a question as to whether the high level of free amino acids is exclusively responsible for the high rate of spoilage in prawns. Shri Velankar said that it has been experimentally shown that it is so. Dr. A. N. Bose intervened and said that for starting bacterial activity only very small quantities of amino acids are necessary. To this Shri Velankar said that the fact that prawns contain about ten times more free amino acids than teleost fish should have some significance. On an enquiry made, Shri Velankar answered that prawns contain on an average 300 mg. amino N%. There was a question as to whether there is any significant change in the pattern of amino acids in prawns and fish and whether the differences in pattern do in any way affect the spoilage pattern. To this Shri Mahadeva Iyer intervened and said that there are no differences in the qualitative distribution of the amino acids between fish and prawns and that the differences are only in quantity.

Dr. A. N. Bose stated that spoilage usually measured is bacteriological spoilage. However along with microbial spoilage lot of biochemical changes are often taking place. These latter result not only in the production of amino acids but also minor compounds like sugars, sugar phosphates etc. and these latter changes will materially affect the flavour.

It was pointed out that the problem as it relates to processing has not so far been given the adequate attention it deserves, and in fact there are no published records on the subject. Of course a good deal of work has been done on fish, particularly on codfish. It is more or less established that some of the breakdown products have a bitter taste and that sometimes spoiled prawns have got the same bitter taste.

Shri John P. George appreciated the significance of spoilage on all these changes but emphasized that from the commercial point of view there is need for a rapid test for the decision of spoilage. Dr. A. N. Bose pointed out in this connection that an equipment has been designed for the measurement of spoilage in fish, the details of which were made known at the recent FAO symposium in W. Germany and that is an electronic equipment. However that is not being put to use in the case of prawns as they are covered by a hard outer shell.

Dr. M. V. Rajgopal wanted to know what type of spoilage happens first in prawns. Shri N. K. Velankar answered that the immediate post-mortem change is invariably glycolytic and it takes comparatively long time to start proteolysis.

The Chairman then led the discussion to the consideration of the important problem of the employment of preservatives in processing fish and shell fish, particularly the antibiotic CTC on the use of which a great deal of interest has been shown in recent years in many countries.

Mr. Velankar pointed out that there is no special advantage in treating prawns with antibiotics if the period of storage is not to exceed a week or so. The difference between the control and the treated becomes significant only after prolonged storage.

On our present day condition of fishing and fish supplies, the prawns never remain in ice for more than one day and as such there is no need now for the use of antibiotics in ice. However, with the development of offshore fisheries, the need may arise to keep the prawn or fish in the fish-hold of the vessel for periods of two to three weeks. The use of antibiotics has no effect on melanosis. There is also the likelihood of misusing antibiotics. The use of antibiotics has therefore to be considered with great caution.

Dr. V. K. Pillai detailed at length the latest trend in the opinion regarding the use of CTC in fishery products.

First and foremost is the possible misuse and the tendency to use the compound on spoiled material. Another important factor is the selective destruction of gram negative organisms alone in CTC treated fish. The latter often results in the predominance of certain groups of organisms which ultimately may cause the production of undesirable odours and other side reaction.

There has lately been a good deal of rethinking on the advisability of repeating or restricting the use of the compound even in countries where the use of CTC in fishery products has been legalised.

Shri Madhavan Nayar pointed out that all through the different processing stages in the factory ice is used, but even in spite of that certain definite cases of bacterial contamination are found to occur particularly in the cooked peeled prawns. Whether the material is cooked in shell or cooked after peeling, the partially sterilized material has been found to take on heavy loads of bacteria during subsequent processes, starting with the stage when cooked prawns are chilled in an ice-bath. The desirability of using an antibiotic at that stage to prevent bacterial prolification should be examined.

Dr. A. N. Bose pointed out that the experiments carried out at CIFT laboratories have indicated that the fish material will take on some of the colours of the antibiotic and this might stand in the way of recommending its use under commercial conditions. The need for more exact information on the qualitative nature of the bacteria in the prawn product after icing was emphasised.

It was recognised that the reduction in the time lag after cooking and until quick-freezing will generally retard the multiplication of bacteria.

Dr. Pillai further explained that the problem of high bacterial count in cooked frozen prawn has been investigated thoroughly by the CIFT. The results of these studies indicated that it is caused by external contamination from surfaces in the vessels, tables, etc, and from the water and ice used in the post-cooking operations. The initial cooking of the material effects a partial sterilization, the average count at this stage being about 1000 colonies/g. This residual bacteria also multiply during the interval between cooking and final freezing, if the temperature is not kept low. However as the interval is generally very short, such a multiplication cannot result in very high counts in the frozen product. The CIFT has recommended positive steps to overcome this defect of high counts in cooked

frozen prawns, viz. cooking and drying of the cooked prawn to be effected in a box dryer — the two processes together not taking more than 10 to 15 minutes, and packing the material immediately into trays, using well-cooled chlorinated water for glazing purposes and immediate freezing.

Shri G. N. Mitra enquired whether the ice used by the processors is manufactured by themselves or by other concerns. Shri Madhavan Nayar stated most of the large processing factories have their own ice production. Two or three ice-factories outside the industry cater to the requirements of many processors. He added that owing to the inadequacy of municipal water supply, water from tube wells are used in the production of ice, and its quality cannot therefore be above reproach.

Dr. Pillai then pointed out that where chilling in ice bath is resorted to, care should be taken to use chlorinated water and ice prepared from chlorinated water. He also pointed out that in factories where the above suggestions have been implemented more than 90% of the products are found to be well winthin the limits set for such products. On enquiry Dr. Pillai informed that a limit 200,000 colonies/g. for cooked frozen prawns is set by most of the importing countries.