Pak. j. sci. ind. res., vol. 34, nos. 7-8, July-August 1991

OXALIC ACIDS AS A TRAPPING AGENT IN CONWAY MICR–DIFFUSION AND STEAM DISTILLATION METHODS FOR TOTAL VOLATILE BASIC NITROGEN (TVB-N) AND TRIMETHYLAMINE NITROGEN (TMA-N) ANALYSIS AS QUALITY INDICATORS OF FISHERY PRODUCTS

RIAZ FATIMA, KHER-UN-NISA AND R. B. QADRI

PCSIR Laboratories Complex, Off University Road, Karachi - 39, Pakistan

(Received September 12, 1990; revised September 21, 1991)

A new trapping agent has been tested in steam distillation and Conway microdiffusion methods to estimate the TVB-N and TMA-N. Similar results were obtained when oxalic acid (0.004%) was compared with boric acid (2–3%). Merits of oxalic acid as trapping agent in steam distillation and Conway microdiffusion methods are discussed.

Key words: Total volatile basic nitrogen (TVB-N), Trimethylamine nitrogen (TMA-N), Oxalic acid, Boric acid.

Introduction

One of the most characteristic features of the chemical and microbiological changes occuring in fish, shrimp and other seafoods is the production of volatile bases which comprise of ammonia and trimethylamine nitrogen [1]. The measurement of total basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) has been reported to provide a good indication of the deterioration of fishery products [2]. Quantitative determination of total volatile basic nitrogen (TVB-N) developed by Boury [3] is popular among the fisheries researchers as a quality index of fish, shrimp and other seafoods [2].

Trimethylamine, a degradative product of trimethylamine oxide (TMAO) present in fish, shrimp and other seafoods, may be a very good indicator of level of bacterial contamination during postmortem handling [2]. Trimethylamine nitrogen (TMA-N) has been considered as the main objective criterion for assessing the quality of fish and shrimp in international trade. TMA-N has also been proposed by Codex Alimentarius sub-committee on Fish and Fishery Products, as an objective index of quality assessment technique.

As a quality indicator of fishery products, the measurement of TMA-N and TVB-N are insufficient when considered alone. The simultaneous measurement of both parameters provide a better indication of spoilage or quality of fishery products [2]. Various methods have been proposed for TVB-N and TMA-N analysis [4-15]. Most of the methods are in use for fish and shrimp quality assessment to provide their quality status in national and international markets.

The paper is concerned with the assessment of oxalic acid as trapping agent and comparing with results obtained on the same samples using boric acid.

Materials and Methods

Freshly caught shrimp (*Penaeus merguiensis*) used for this study were obtained from commercial sources. Samples were immediately packed in ice and transported to the laboratory within 1–2 hrs where they were used for analysis. For storage, the shrimp were kept in ice. Deheaded shrimp were mixed with crushed ice in the ratio 1:1 (w/w). They were held in plastic container with holes in the bottom for drainage which were put into another plastic container and kept in a refrigerator maintained at 4°. The shrimp were constantly covered with ice during the storage period by re-icing. Samples of shrimp were removed at 4, 8, 12 and 16 days for analysis. For repeated experiments different lots of shrimp collected from commercial sources were used.

Estimation of total volatile basic nitrogen (TVB-N) trimethylamine nitrogen (TMA-N). Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) concentrations in various fresh and stored shrimp samples were determined on a 7% trichloroacetic acid extract prepared from 20gm shrimp muscle by (a) steam distillation method as described by Tompson *et al.* [4] for TVB-N and Tao *et al.* [2] for TMA-N (b) by Conway microdiffusion method as described by Matches [14] for TVB-N and by Thompson *et al.* [4] for TMA-N. These methods use boric acid as a trapping agent. Simultaneously, in the methods boric acid was replaced by 0.004% oxalic acid. Results obtained using boric acid and oxalic acid as trapping agents were compared. Comparison was also made between TMA-N using oxalic acid with picrate method of Dyer as modified by Hoogland [11].

Optimum concentration of oxalic acid that may be used as trapping agent was established by using different concentrations of the acid. Calibration curve prepared of TMA-N and TVB-N (TMA-N-HCl + $(NH_4)_2$ SO_4 in ratio 1:6) was prepared [8].

Results and Discussion

Table 1 shows the amount of TVB-N and TMA-N in shrimp muscle obtained by the steam distillation and Conway microdiffusion methods using increasing concentrations of oxalic acid as trapping agent. The results show that 0.004%

oxalic acid is suitable to trap the TVB-N and TMA-N in the form of nitrogen by the either method.

Calibration curve. In order to see the efficiency of the methods using oxalic acid as a trapping agent, a calibration curve of TVB-N and TMA-N was prepared. The purpose of this curve is to know, how much volume of 0.004% oxalic acid is capable to trap a certain amount of TVB-N and TMA-N in the form of nitrogen to compare it with the same.

Figure 1 depicts the calibration curve for TVB-N and TMA-N prepared by steam distillation and Conway micro diffusion methods using oxalic acid and boric acid as trapping agents. From the curve it is apparent that one milliliter volume of both acids can trap 115 to 120 mg of TVB-N and TMA-N in the form of nitrogen by using steam distillation and Conway microdiffusion methods. This is clear that the capacity of both the acids is not significantly different (r=0.97) and the use of oxalic acid as trapping agent is satisfactory in both the methods.

Comparison of the values of total volatile basic nitrogen (TVB-N) in shrimp muscle using oxalic and boric acid as trapping agents in steam distillation and Conway microdiffusion methods. Table 2 presents the concentrations of total volatile basic nitrogen (TVB-N) in twelve samples of shrimp muscle extract obtained by steam distillation and Conway microdiffusion methods using oxalic and boric acid as trapping agents.

The values of TVB-N acquired using oxalic acid as trapping agent were compared with those obtained by the boric acid. The results show a strong positive relationship (r=0.997) between the two methods. There is no significant difference between the values of TVB-N obtained. Similarly, when oxalic and boric acid used as trapping agents in Conway microdiffusion methods the same trend was obtained between the results (r=0.999).

Comparison of the values of trimethylamine nitrogen (TMA-N) in shrimp muscle using oxalic and boric acid as trapping agents in steam distillation and Conway microdiffusion methods. Table 3 illustrates the results of TMA-N obtained on twelve samples of shrimp muscle extract using oxalic and boric acid as trapping agents in steam distillation and Conway microdiffusion methods. Similar to TVB-N, no significant difference was obtained in the results irrespective of the trapping agent used. The values of (r) were found to be 0.995 and 0.985 for steam distillation and Conway micro diffusion methods respectively.

Comparison of the values of trimethylamine nitrogen (TMA-N) in shrimp muscle as measured by steam distillation and Conway microdiffusion methods using oxalic acid as trapping agent and picric acid method. A comparison of the results (Table 3) obtained by steam distillation and Conway

TABLE 1. AMOUNT OF TOTAL VOLATILE BASIC NITROGEN (TVB-N) AND TRIMETHYLAMINE NITROGEN (TMA-N) IN SHRIMP OBTAINED BY STEAM DISTILLATION AND CONWAY MICRODIFFUSION METHODS USING INCREASING CONCENTRATIONS OF OXALIC ACID.

Trapping agent	Steam distillation method		Conway microdiffusion method	
% of oxalic acid	TMA-N mg/100gm	TVB-N mg/100gm	TMA-N mg/100gm	TVB-N mg/100gm
0.001	1.3	20.0	0.8	17.0
0.002	2.5	27.0	1.2	20.5
0.004	2.8	32.0	1.7	23.5
0.008	2.8	32.0	1.9	25.0
0.010	2.8	32.5	1.8	25.5
0.020	2.8	31.0	1.9	25.5

Note: All concentrations of oxalic acid were standardized with known concentration of sodium hydroxide.

TABLE 2. COMPARISON OF THE VALUES OF TOTAL VOLATILE BASIC NITROGEN (TVB-N) IN SHRIMP MUSCLE AS MEASURED BY STEAM-DISTILLATION AND CONWAY MICRODIFFUSION METHODS USING OXALIC ACID AND BORIC ACID AS TRAPPING AGENTS.

Sample No.	Steam distillation method oxalic acid TVB-N mg/100gm	Steam distillation method boric acid TVB-N mg/100gm
1	14.10	15.40
2	19.10	19.59
3	22.50	25.20
4	31.80	32.70
5	37.27	41.00
6	22.10	25.20
7	56.20	56.40
8	19.30	20.90
9	43.19	44.50
10	47.90	48.20
11	42.60	43.70
12	52.90	54.20
	Conway microdiffusion method oxalic acid TVB-N mg/100gm	Conway microdiffusion method boric acid TVB-N mg/100gm
1	11.50	12.30
2	14.28	15.27
3	16.37	17.95
4	24.90	27.60
5	37.40	38.89
6	44.40	46.60
7	37.20	39.30
8	46.40	48.09
9	26.63	28.10
10	15.50	17.70
11	47.00	48.50
12	15.70	17.40

TABLE 3. COMPARISON OF THE VALUES OF TRIMETHYLAMINE NITROGEN (TMA-N) IN SHRIMP MUSCLE AS MEASURED BY STEAM DISTILLATION, CONWAY MICRODIFFUSION METODS USING OXALIC ACID AND BORIC ACID AS TRAPPING AGENTS AND PICRATE METHOD.

Sample No.	Steam distillation method oxalic acid TMA-N mg/100gm	Steam distillation method boric acid TMA-N mg/100gm	Picrate method TMA-N mg/100gm
1	1.22	1.33	1.00
2	1.38	1.60	1.10
3	2.00	2.30	1.80
4	2.00	2.20	1.68
5	2.20	2.30	1.95
6	2.15	2.40	2.00
7	4.88	5.80	4.01
8	1.00	1.20	0.82
9	3.70	3.90	2.60
10	3.70	3.90	2.90
11	3.30	3.60	2.50
12	5.30	5.90	4.20

	Conway microdiffusion method, oxalic acid TMA-N mg/100gm	Conway microdiffusion method boric acid TMA-N mg/100gm
1	0.83	0.88
2	0.81	0.91
3	1.34	1.41
4	1.33	1.43
5	1.66	1.73
6	1.65	1.70
7	4.10	4.30
8	0.61	0.67
9	2.00	2.50
10	2.38	2.50
11	2.46	2.90
12	4.11	4.20

microdiffusion methods using oxalic acid as trapping agent was also made with picric acid method (Table 3), the results obtained correlated significantly (r=0.989 for steam distillation method and 0.987 for Conway microdiffusion method) with those obtained by the picric acid method.

Ice storage. In order to further assess the merits of oxalic acid as trapping agent, samples of shrimp were stored in ice and the TVB-N and TMA-N were estimated by steam distillation and Conway microdiffusion methods using oxalic and boric acids as trapping agents. Figure 2 depicts the analysis of TVB-N and TMA-N in TCA extract of shrimp muscle during ice storage. The results show a definite increase in TVB-N and TMA-N in TCA extract of shrimp muscles during ice storage upto 16 days when either of the method used. Irrespective of the trapping agent, the values of TVB-N and TMA-N obtained by steam distillation method were slightly higher than those obtained by Conway microdiffusion method. Higher values of TVB-N and TMA-N by steam distillation using boric acid as trapping agent have also been reported [2 - 4]. It is, therefore, clear that oxalic acid can be safely used in place of boric acid as a trapping agent in steam distillation and Conway microdiffusion methods to determine TVB-N and TMA-N as spoilage indicator for fishery products.

We found that the cost of oxalic acid is slightly higher than boric acid but the concentration of oxalic acid required to trap the TVB-N and TMA-N in the form of nitrogen is very low i.e. 0.004% whereas minimum concentration of boric acid used in these methods is 2%. Therefore, the cost of oxalic acid becomes lesser than boric acid.

Conclusion

No significant difference between results was obtained

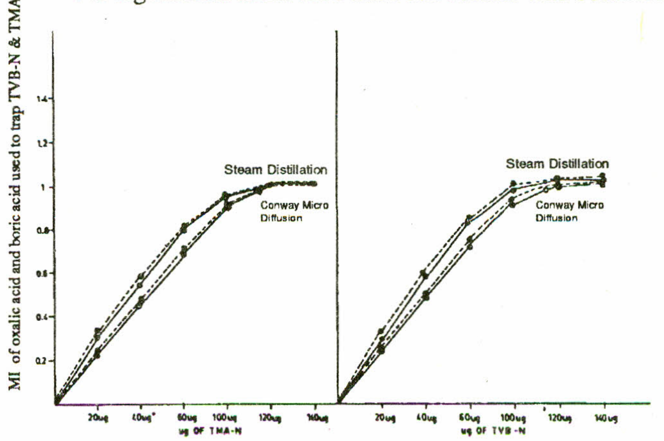

Fig. 1. Calibration curve for total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) by steam distillation and Conway microdiffusion methods using oxalic acid, boric acid as trapping agents. Boricacid • . . . . • and oxalic acid 0. . . . . . .0.

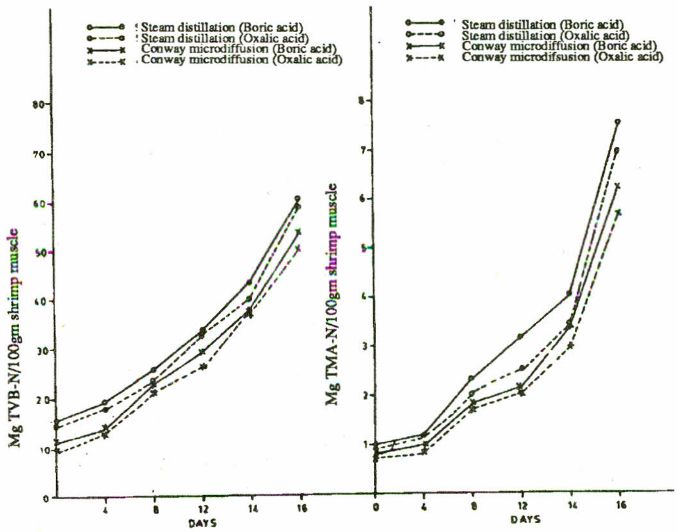

Fig. 2. Mean total volatile basic nitrogen (TVB-N) value and trimethylamine nitrogen (TMA-N) value determined by steam distillation and Conway microdiffusion methods, of shrimp stored in ice for upto 16 days.

by oxalic and boric acid to trap the TVB-N and TMA-N in the form of nitrogen. Oxalic acid can be used instead of boric acid for determination of TVB-N and TMA-N without affecting the accuracy of the results.

References

1. J.M. Shewan, D.M. Gibson and C.K. Murray, Fish Insp. Qual. Contr., **71**, 183 (1971).
2. P. Malle and S.H. Tao, J.Fd. Protection, **50**, 756 (1987).
3. M. Boury, L'alteration du Poisson Rev. Trav. off Peche Mmes., **9**, 401 (1936).
4. B.F. Cobb 111, I. Alaniz and C.A. Thompson, J. Fd. Sci., **38**, 431 (1973).
5. D. Pearson and M. Muslemuddin, J. Assoc. Public Analysts, **6**, 117 (1968).
6. J. Billion, N. Ollieuz and S.H. Tao, Rev. Technique Veterinaire de 1' Alimentation, **18**, 13 (1979).
7. Anon, *Proposed Official Chemical Method Number 49* (Dept. of Fisheries and Oceans, Fish Branch Ottawa, Ontario, 1979).
8. J. R. Botta, J.T. Lauder and M.A. Jewer, J. Fd. Sci., **49**, 734 (1984).
9. S.A. Beatty and N.E. Gibbons. J. Fish. Res. Bd. Can., **3**, 77 (1973).
10. D. Pearson and Muslemuddin, J. Assoc. Public Analysts, **7**, 50 (1969).
11. P.L. Hoogland, Circ. Fish Res. Bd. Can, New Series No. 3 (1956).
12. E.J. Conway, *Microdiffusion Analysis and Volumetric Error* (The Mac-Milliam Company, New York, 1958), pp. 199.
13. G.W. Chang, W.L. Chang and K.B.K. Lew, J. Fd. Sci., **41**, 723 (1976).
14. J.R. Matches, J. Fd. Sci., **47**, 1044 (1982).
15. A. Nicolaus and V. Wilfried, Z. Lebensm-Unters. Forsch., **189**, 309 (1989).