A MODIFIED COLORIMETRIC METHOD BASED ON McCARTHY AND PAILLE PROCEDURE FOR ESTIMATION OF METHIONINE

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A rapid determination of methionine in crude proteins has been suggested by McCarthy and Paille[1]. We in this laboratory were interested to try this method but we could not procure heptaflouro butyric acid from abroad due to explosive nature of the chemical. We have therefore standardized the McCarthy and Paille method in terms of the quantities of reagents to be added and the time of reaction using hydrochloric acid instead of heptafluoro butyric acid. This note describes such modification in the method.

Small pieces of fish muscle were dehydrated and defatted using acetone or absolute ethanol and 0.2 gm of this material in fine powder condition was transferred to a 50 ml conical flask fitted with a B-14 socket. To this 2 ml distilled water and 6 ml of 10% NaOH were added. Air-condensor of 16" length and fitted with B-14 cone was fitted in the flask. These flasks (six in number) were arranged in stable circular condition by using two plates of thicker gauge. One of the plates sits on the neck of the flask while the other plate just could not pass through these flasks. The contents were digested on an oil-bath at 115°C for 30 minutes (Figure 1). With this arrangement the initial volume of 8 ml remained almost constant and prevented the foam to come out during digestion.

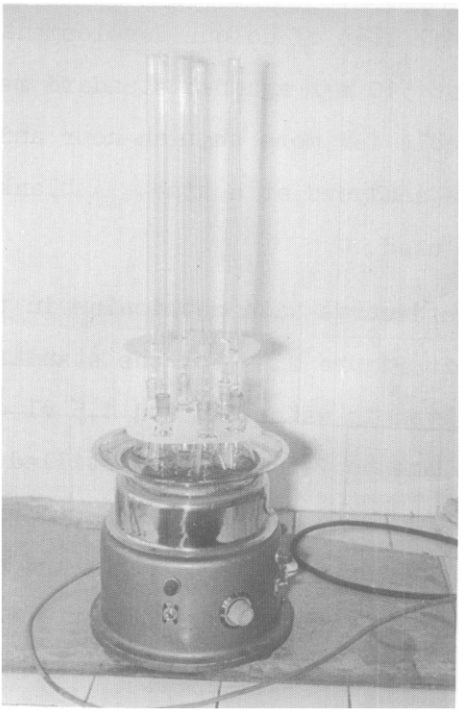

Fig.1. Digestion of samples with alkali at 115°C using conical flasks fitted with air condensors and using two circular plates

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After digestion, the volume of the hydrolysate was adjusted to 10.0 ml using distilled water. 2.5 ml of the hydrolysate were taken in a 50 ml conical flask and to this 5 ml of distilled water were added. The color forming reagents were run in the following order after mild shaking for 10 minutes after addition of each of the reagents.

0.3 ml of 10% sodium nitroprusside

2.0 ml of 3% glycine

3.0 ml of 6N HCl

All the reagents used were freshly prepared. Ten minutes after the addition of the acid, the solutions were centrifuged

for 10 minutes at about 2500 r.p.m. to remove any precipitate formed in the final step of colour development. The red colour formed was read at 510 mμ against standard methionine solution. The colour is stable for more than an hour and as such a number of samples can be analysed at a time. A blank was run with water and the reagents used.

The standard curve with methionine in the range of 0.2 to 1.6 mg is shown in Figure 2. For the standards take 0.2 to 1.6 mg methionine dissolved in water and add 1.5 ml of 10% NaOH. After making up the volume to 7.5 ml with distilled water, add colour forming reagents. With the standards, no precipitate is formed after adding the colour forming reagents and as such these could be taken for colorimetric readings without centrifuging.

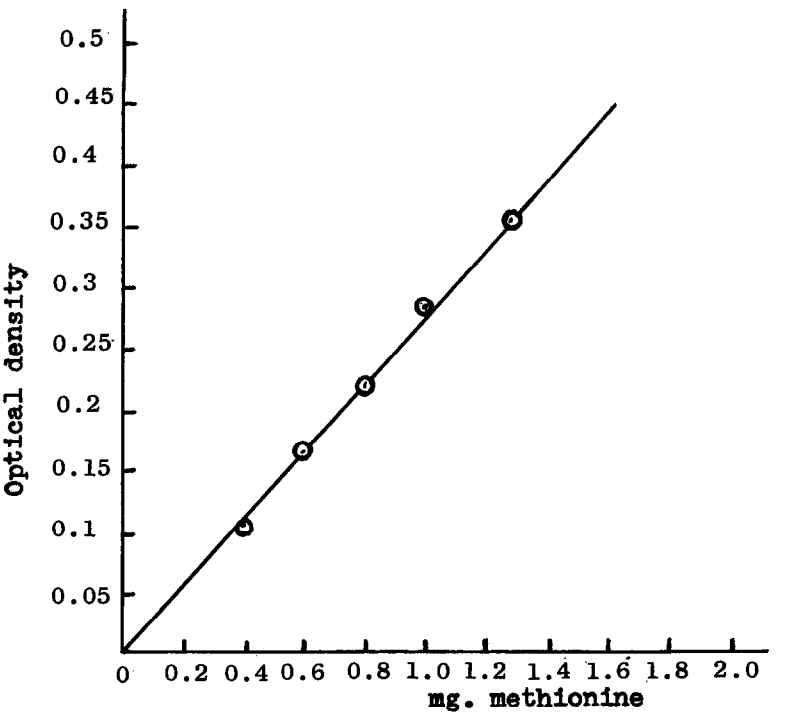

Fig.2. Standard calibration curve for methionine.

The values obtained for four samples of fish, each run in triplicate, are confirmed by microbiological assay method (Table 1). The recovery of the added methionine is in the range of 92-96% (Table 2).

The main points of modification are: the use of 6N HCl instead of heptafluoro butyric acid (which is of explosive nature); removal of final precipitate after addition of colour forming reagents; time od reacting and the quantities od reagents

Table 1. Methionine content of samples by the colorimetric and microbiological assay method.

Sample	Colorimetric method (modification)	Microbiological assay
1	2.70	2.80
2	2.98	3.08
3	3.80	3.67
4	3.05	3.02

added. Also digestion of the samples (defatted and dehydrated) with sodium hydroxide under refluxing using just two circular plates is suggested. The agreement of these results with those of microbiological method and the attainment of consistently high recoveries of methionine encouraged us to have confidence in this modified method.

Table 2. Recovery of added methionine.

Methionine content of the sample* mg	Methionine added to the sample* mg	Expected theoretical value after addition of methionine mg	Experimental value mg	Percentage recovery
2.8	2.5	5.3	4.88	92.1
2.8	5.0	7.8	7.12	91.3
3.56	2.5	6.06	5.92	97.7
3.56	5.0	8.56	8.28	96.7

* 0.2 gm of the material taken for digestion.

REFERENCE

1. McCarthy, T.E. and Paille, M.M. (Sr.)., A rapid determination of methionine in crude proteins., Biochemical and Biophysical Research Communications., 1959, 1, 29.