

Determination of Boric Acid in Foods: Comparative Study of Three Methods

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ABSTRACT

Three methods for the determination of boric acid in foods were studied in detail, namely the titrimetric method using mannitol, and two colorimetric procedures using carminic acid or curcumin. Agar-agar strips, pickled mango, noodles and prawns were analysed and the repeatability, sensitivity and recovery of the methods compared. The titrimetric and curcumin methods gave mean values for boric acid which were significantly ($P < 0.05$) higher than those of the carminic acid method. Results with the titrimetric method did not differ from those with the curcumin method; the latter method gave good recoveries ($\sim 100\%$) for all four foods at all levels of addition. Analyses carried out on NBS Standard Material showed that the curcumin method gave the most accurate results. This method was also found to show the least internal variation both in terms of mean boric acid content and recovery. Furthermore, the method possessed practical advantages over the other two techniques. Based on the results obtained from the comparative studies, the curcumin method was found to be the most reliable and hence would be the method of choice for boric acid determination in foods.

Key words: Boric acid, titrimetric acid method, carminic acid method, curcumin method, Malaysian foods.

INTRODUCTION

Boric acid and borax, in view of their cumulative toxicity, have been declared unsafe as food additives by an FAO/WHO Expert Committee (Davidson *et al* 1975). Although Malaysian regulations (Ministry of Health Malaysia 1985) do

not permit the use of boric acid as a food additive, boric acid has been reported in foods here (Normah *et al* 1984). Boric acid is sometimes used to disguise and mask incipient putrefaction and therefore render marketable, food that has deteriorated (Egan *et al* 1981; Monsereenusorn 1982), however, it is also present in the environment and is a natural constituent of many plant foods (Egan *et al* 1981).

The use of borax and boric acid as food preservatives was considered as early as 1904 (Pfeiffer *et al* 1945) and since then there have been reports on the determination of these preservatives in foods (Alcock 1937; Furlong 1948), plant materials (Basson *et al* 1969; Pickett and Franklin 1977; Melton *et al* 1978) and cosmetics (Crisp *et al* 1985). A review of the literature showed that three methods have been commonly used for the determination of boric acid in foods; the titrimetric method using mannitol and colorimetric procedures based on reactions with carminic acid or curcumin.

A study of the repeatability, sensitivity, recovery and accuracy of these methods is reported here together with experiences in using them.

MATERIALS AND METHODS

Materials

Examples of foods from four different types, namely wet noodle, agar-agar strips, pickled mango and fresh prawns, were selected. These foods were thought to contain considerable amounts of boric acid, either intentionally added or naturally occurring.

All four samples were ground finely using a grinder. The prawns were not shelled prior to grinding since whole prawns are often cooked unshelled. Several test portions were taken from the four homogenised food samples for analysis.

To avoid contamination, borosilicate glassware and containers were not used in the study. Water used for sample preparation and dilution of standard and test solutions was double-distilled and deionised.

Methods

Titrimetric method

The titrimetric method used was that of the Association of Official Analytical Chemists (AOAC) Official Methods of Analysis (Williams 1984) except for the dry ashing procedure where 2 × 8 h ashing (on successive days) was carried out instead of 16 h direct ashing. Food samples of about 10 g were used. Subsequent treatment of the ash solution with lime water, 2.5 M NaOH and 0.5 M H₂SO₄ converted the boric acid into its free state. Mannitol was then added to convert boric acid into a relatively strong monobasic acid which was then titrated with 0.2 M NaOH, in the presence of phenolphthalein, until a permanent pink colour was obtained. The volume of alkali required for the titration was used to calculate the amount of boric acid present, using the relationship 1 ml 0.2 M NaOH = 0.0116 g H₃BO₃. This relationship was determined by repeatedly titrating a 5.7 g litre⁻¹ solution of boric acid with the 0.2 M NaOH solution prepared.

Carminic acid-spectrophotometric method

Carminic acid changes colour from red to blue in the presence of boric acid in concentrated sulphuric acid. This reaction has been used for the determination of boron in water, soil extracts and plant materials (Hatcher and Wilcox 1950; Callicoat and Wolszon 1959; Fries and Getrost 1977). Interference by nitrate and nitrite can be eliminated by addition of a few drops of hydrochloric acid. These methods have been adapted for use in this study.

The sample (about 1–5 g) was charred and ignited at approximately 500°C until a whitish residue was obtained. Prior to the ashing process, an amount of calcium oxide (CaO), approximately 1% of the weight of the test portion, was added as an ashing aid. After cooling, the ash was moistened with water, 15 ml 6 M HCl added and the solution diluted to 25 ml with deionised water.

Colour development was carried out by adding 5 ml conc H_2SO_4 to 1 ml of the ash solution, followed by 5 ml of carminic acid. After 45 min the absorbance was determined at 610 nm against the blank. The boron concentration was read from a calibration curve prepared from standard boric acid solutions ranging from 0 to $57 \mu\text{g ml}^{-1} \text{H}_3\text{BO}_3$.

Curcumin-spectrophotometric method

This method is based on the formation of a coloured product upon reaction of boric acid with curcumin in the presence of an acetic acid/sulphuric acid mixture. After extracting the coloured complex into ethanol, the intensity was measured at 555 nm (Kuemmel and Mellon 1957; Williams 1984). The method has a detection limit of $1 \text{ mg H}_3\text{BO}_3 \text{ kg}^{-1}$ (Egan *et al* 1981). The procedure adopted in this study is based essentially on that given by the AOAC (Williams 1984) with some minor changes. The food sample (*c* 0.3 g) was digested with 1 ml conc H_2SO_4 and 2 ml 300 g litre^{-1} hydrogen peroxide in a 50 ml Kjeldahl flask and the solution transferred quantitatively, using deionised water, to a 50 ml volumetric flask and made up to volume.

The volumes of the test solution, standard solution and reagent were reduced to half the amounts specified in the AOAC procedure. In the preparation of the standard graph, instead of using different volumes of a single concentration of boric acid solution, 0.5 ml each of 1, 2, 3, 4 and $5 \mu\text{g H}_3\text{BO}_3 \text{ ml}^{-1}$ standard solutions was used. Treatment of all test and standard solutions were as given in the AOAC procedure. Absorbance of the solutions was measured only at 555 nm as it was found that negligible readings were obtained at 700 nm. The concentrations of boric acid present in the test solutions were calculated using the standard curve.

Comparative study

For each of the four foods selected, 15–20 analyses were carried out using each of the three methods. Mean values and standard deviations of the three methods were then compared. For comparisons of the accuracy of the methods, the levels of boric acid in National Bureau of Standards (NBS) Standard reference material tomato leaves 1573 were determined.

Several preliminary studies were carried out using the carminic acid and

curcumin methods to select suitable concentrations of boric acid to be added to the foods for recovery studies. It was observed that with less than 172 μg of added boric acid there were wide variations in recovery values, since the methods are insufficiently sensitive to detect such low levels. Thus for the carminic acid and curcumin methods, recovery studies were carried out after spiking foods with 172, 343, 515 and 686 μg of boric acid. Since the titrimetric method was found to be relatively insensitive and required much larger quantities of food for analysis, ten-fold higher concentrations of boric acid were added in testing this method.

Statistical analyses

One-way analysis of variance, Student's *t*-test, and the variance ratios or *F*-ratios method were performed (Wernimont 1985).

RESULTS AND DISCUSSION

Comments on practical aspects of methods

During charring of the test portions in the titrimetric method, application of intense red heat should be avoided as it causes loss of boric acid. The titration of the ash solution with 0.2 M NaOH requires extra care as the colour change is quite abrupt.

In the carminic acid-spectrophotometric method, the base added to prevent boron loss (Melton *et al* 1970) was calcium oxide because it gave a smoother ash and occupied less volume. The addition of H_2SO_4 to the ash solution during colour development was carried out in an ice-bath to reduce effervescence and to assist cooling.

In the curcumin-spectrophotometric method, during the preparation of the final solutions it was necessary to stopper the tubes immediately after filtration so as to avoid boric acid loss in the form of volatile ethyl borate (Robinson 1939).

Mean values and variability of methods

Table 1 shows the mean concentrations of boric acid as obtained from three different methods of determination, carried out on agar-agar strips, pickled mango, wet noodle and fresh prawns. One way analysis of variance showed that for all four foods, there was a significant difference ($P < 0.01$) between the mean values obtained for the three techniques. The mean values obtained by the curcumin method were significantly higher ($P < 0.001$) than those given by the carminic acid method. Although there were differences between the means from the titrimetric and carminic acid methods the former gave significantly ($P < 0.001$) higher results only for agar-agar, mango and noodle. While the mean values for the three foods, excluding prawn, given by the titrimetric method were higher than those obtained by the curcumin method the difference was significant ($P < 0.001$) only for agar-agar.

There was a general trend of mean values given by the three methods, *viz* titrimetric > curcumin > carminic acid, and this was true for all the foods studied except for prawn (Table 1). Results obtained for analysis of prawn by the titrimetric method did not follow the general pattern given by the other three foods. This is

TABLE 1
Concentration of boric acid ($\mu\text{g g}^{-1}$) in four selected foodstuffs as obtained from three different methods of determination

	<i>Titrimetric</i>	<i>Curcumin</i>	<i>Carminic</i>
Agar-agar strips			
Mean	1169	918	788
SD	114	60	72
<i>n</i>	16	16	16
CV (%)	10	7	9
Mango, pickled			
Mean	829	819	590
SD	47	21	70
<i>n</i>	15	16	16
CV (%)	6	3	12
Noodle, wet			
Mean	547	532	412
SD	62	32	41
<i>n</i>	20	20	20
CV (%)	11	6	10
Prawns, fresh			
Mean	313	347	287
SD	60	23	29
<i>n</i>	16	14	16
CV (%)	19	7	10

thought to be due to the relative insensitivity of the method to small amounts of boric acid.

Standard deviations obtained for each of the four foods studied (Table 1) were used for determining and comparing the variabilities of the three procedures using the *F*-ratios method. The variances of the curcumin method were significantly ($P < 0.01$) lower than those of the titrimetric and carminic acid methods, and the variances of the titrimetric method were significantly higher ($P < 0.05$) than those given by the carminic acid method.

Validation of methods

Table 2 gives results of the accuracy of determination by each method when applied to NBS Standard. With the exception of the titrimetric method, all of the values obtained by the other two methods fall around the NBS value of $172 \mu\text{g}$ boric acid g^{-1} . Boric acid in the NBS Standard by the curcumin method varied between 126 and $194 \mu\text{g g}^{-1}$ (mean $166 \pm \text{SD}37$) while those obtained by the carminic acid method varied between 103 and $160 \mu\text{g g}^{-1}$ (mean $141 \pm \text{SD}34$). The curcumin and the carminic acid methods show CV of 22 and 24% respectively. On the other hand, the titrimetric method could not detect the level of boric acid in the NBS Standard.

TABLE 2
Concentration of boric acid in National Bureau of Standards reference material
tomato leaves 1573 by three methods of determination

	<i>Concentration of boric acid ($\mu\text{g g}^{-1}$)</i>			
	<i>NBS value</i>	<i>Titrimetric</i>	<i>Curcumin</i>	<i>Carminic</i>
1st analysis		ND	177	103
2nd analysis		ND	126	160
3rd analysis		ND	194	160
Mean	172	ND	166	141
SD		NA	37	34
<i>n</i>		3	3	3
CV (%)		NA	22	24

ND not detected.

NA not applicable.

Recovery values

Mean recoveries and the associated coefficients of variation, of boric acid from the spiked food samples by the three methods are shown in Table 3. For the mango sample at all levels of addition, the titrimetric method gave good recovery, the mean ranging from 96.5 to 99.5. There were considerable variations in mean recoveries for the other foods and there was no clear trend with increasing level of spiking. It can clearly be seen from the table that at all levels of addition, and for all the foods analysed, the curcumin method gave good mean recoveries (c 100%) except at the lowest level of addition for agar-agar strips. For the carminic acid method, as the level of boric acid addition increased, the recovery seemed to fall for all the foods except agar-agar. Only three were between 90 and 100%.

The titrimetric method (Table 3) gave large variations in recovery at low levels of addition, particularly for prawn at the 172 μg level. At the 515 and 686 μg levels the method was found to be more reproducible with CV < 10% for all the foods. There was thus a trend of decreasing CV as the levels of added boric acid increased. These results confirm the earlier observation that the titrimetric method appears to be less sensitive at low concentrations of boric acid. On the other hand, the curcumin method showed good reproducibility and the least variation in recovery with CV < 10% for all the foods. This was true for all levels of addition except for agar-agar at the 172 μg level. There was no definite trend in the CV values as with the carminic acid method over the four levels of addition. It was noted that the method was found to have larger variations in recoveries for all the foods than the other two methods.

CONCLUSIONS

Among the three analytical techniques studied, the titrimetric method was found to give the highest mean values of boric acid concentration, followed by the curcumin method. Mean values obtained by the carminic acid method were the

TABLE 3

Recovery (%) of boric acid by the titrimetric, curcumin and carminic methods from foods spiked with various concentrations of boric acid

Added boric acid concentrations ^a ($\mu\text{g g}^{-1}$)	Titrimetric		Curcumin		Carminic	
	Mean ^b \pm SD	CV (%)	Mean ^b \pm SD	CV (%)	Mean ^b \pm SD	CV (%)
Agar-agar strips						
172	92 \pm 62	62	84 \pm 13	15	77 \pm 16	21
343	74 \pm 15	20	98 \pm 3	3	88 \pm 18	21
515	85 \pm 5	6	100 \pm 3	3	86 \pm 8	10
686	87 \pm 5	5	98 \pm 3	3	80 \pm 15	19
Mango, pickled						
172	97 \pm 18	19	99 \pm 10	10	100 \pm 18	18
343	97 \pm 6	6	108 \pm 2	2	94 \pm 5	5
515	97 \pm 6	7	99 \pm 3	3	89 \pm 19	21
686	100 \pm 5	5	106 \pm 3	2	87 \pm 12	14
Noodle, wet						
172	99 \pm 7	7	101 \pm 4	4	93 \pm 23	25
343	85 \pm 13	15	97 \pm 2	3	85 \pm 21	25
515	92 \pm 3	3	103 \pm 7	7	87 \pm 21	24
686	85 \pm 4	4	104 \pm 1	1	85 \pm 18	21
Prawns, fresh						
172	79 \pm 17	21	105 \pm 9	8	88 \pm 13	15
343	78 \pm 6	7	99 \pm 5	5	77 \pm 7	10
515	99 \pm 9	9	100 \pm 8	8	81 \pm 8	10
686	88 \pm 5	5	99 \pm 2	2	75 \pm 8	11

^a For the titrimetric method, the concentrations of boric acid used for spiking were ten times those listed.

^b Each value is the mean of four determinations.

lowest. Upon examination of the coefficients of variation and variances obtained, it was found that the curcumin method gave the most reproducible results. The titrimetric method gave the largest variation in mean boric acid content, whilst the carminic acid method was intermediate in this respect. The curcumin method proved to be the most accurate as shown by the accuracy of determination of the NBS Standard. With regard to recovery of added boric acid, the curcumin method was found to show the best and to give the least variation. The titrimetric and carminic acid methods, on the other hand, gave considerable variations in recovery.

Various parameters studied have shown that the curcumin method was superior to the other two. The method is also preferred from the practical point of view. Firstly, only a small amount of food sample (0.5 g) was required for good reproducibility and recovery. On the other hand, at least ten times this amount was needed for the titrimetric method. Secondly, determinations using the curcumin method could be accomplished within three days while the titrimetric method required double that time (two days of 8 h dry ashing being required).

Considering all the findings of the study, it can be said that the curcumin method would be the method of choice and can be expected to give reliable results. The

method has been employed for the second phase of the project, that is, to study the prevalence of contamination of boric acid in various foods available locally. A total of 300 washed and unwashed food samples from nine food groups, including cooked and processed foods, have been analysed for boric acid content, the results will be published later.

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