

TECHNIQUES IN THE ANALYSIS OF NUTRIENTS IN MALAYSIAN FOODS - A REVIEW

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SUMMARY

Reports on the analyses of nutrients in Malaysian foods, dating from the beginning of the century uptill 1979 were reviewed. All the nutrients that had been reported were examined. For each group of nutrients, the methodologies used were traced. Interesting and/or outstanding features of these reports were discussed. Areas in which data are lacking were pointed out. Finally, the review outlined a proposal to compile all available data into an updated Preliminary Food Table for use in the country. Further work can then be carried out to fill in the gaps or verify certain data to arrive at a comprehensive and up-dated Malaysian Food Composition Table.

INTRODUCTION

Interest in the nutrient contents of local foods was already evident in the early part of the century, as indicated by the report of Stanton in 1923. Most of the work on the subject, however, were carried out in the 1930s and 1940s. There were a few Food Composition Tables published, and a number of reports on the analyses of selected food items and nutrients, particularly some vitamins and minerals. From the 50s, reports on the subject were scarce and far in between. Since no comprehensive review has been done in this field, it was felt essential to examine all the available data on the subject in order that investigators have a clear idea of what had been achieved and what remains to be carried out.

For this review, publications from 1900 uptill 1979 were obtained and scrutinised. This has been presented earlier in the year as 'A Review of the Analysis of Nutrients in Malaysian Foods' (Tee, 1981). For this particular seminar/workshop, those aspects dealing with the analytical techniques used by investigators will be presented.

All the nutrients that had been studied by investigators in the country will be examined with regards to the analytical methods used. Interesting and/or outstanding features of the reports will be discussed. Although the emphasis will be on methodologies, for each group of nutrients areas in which data are lacking will be pointed out.

TECHNIQUES USED IN THE ANALYSIS OF NUTRIENTS

The methodologies used in the analysis of each group of nutrients will be separately discussed. Particular attention will be paid to those reports giving details of techniques used. The analysis of vitamins in local foods will be first discussed - they are perhaps the most fascinating group of nutrients studied.

In the Analysis of Vitamins

The first report on the vitamin content of local foods was that of Stanton in 1923. The presence or absence of vitamins A, B and C in some 30 foods was indicated in a simple table. From then on, various elaborate reports were published on the analyses of vitamins in local foods.

Turning first to vitamin C, the first report of the study of this antiscorbutic factor was from Oliveiro in 1932. He reported some preliminary results of attempts at determining the antiscorbutic value of foods using a biological assay method, where the histological changes of the teeth of guinea pigs were observed. He was able to use this biological method successfully and reported the antiscorbutic value of some 80 local fruits and vegetables two years later (Oliveiro, 1934). Results were expressed as "the minimum protective dose or the least amount necessary to maintain a normal histological picture of the teeth of guinea pigs".

Some years later, in 1939, Leong reported the vitamin C content of over 150 Malaysian foods (Leong, 1939a). A chemical method, by titration with 2, 6-dichlorophenol indophenol dye, was employed. Vitamin C content was reported in international units as well as milligrams. Presence or absence of reversibly oxidised ascorbic acid in the foods was also indicated. A year later, in their study of the nutrient content of over 60 types of vegetables, Rosedale and Milsum (1940) also reported the vitamin C content using the same dye titration method.

In a more recent report, Abdullah and Ragab (1970) had determined the vitamin C content of 26 fruits grown in the state of Selangor. The indophenol dye method was again used. Whereas Leong (1939a) had only indicated the presence or absence of reversibly oxidised ascorbic acid, these investigators had determined the total ascorbic acid content after reducing the extract with hydrogen sulphide.

In another study of the local vegetables, Caldwell (1972) had reported the vitamin C content (reduced form only) of 50 types of this foodstuff. The same dye titration method was used; in fact, this method remains the most commonly used procedure for estimating vitamin C content of foods.

Another well studied vitamin in local foods was vitamin A (and carotene). Aside from the initial report of Stanton in 1923 (discussed earlier in this paper), the first detailed report on this nutrient was probably that of Rosedale

and Oliveiro in 1934. Vitamin A potency of several animal and plant fats and oils were determined by their ability to prevent xerophthalmia in experimental rats. No quantitative values were however given in this biological assay study.

In the same year, Leong (1934) reported his experience in using a colorimetric method for the estimation of vitamin A in oils. The vitamin was said to react with antimony trichloride to produce a blue colour which was then read in a tintometer. Results were expressed as Blue or Carr-Price units.

A year later, vitamin A was reported by Rosedale (1935) for some 100 foodstuffs. Although no details of method were given, it was mentioned that vitamin A (expressed as blue units) and carotene (given in yellow units) were estimated by means of the tintometer. During this transition period of changing over to the colorimetric method, the author had also remarked that the values obtained had "agreed absolutely" with feeding tests in which xerophthalmia had been taken as the criterion.

These few years seemed to see a number of reports on vitamin A analyses. Simpson (1936) studied the carotene content of palm oils using three different methods: biological assay using experimental rats, direct measurement of yellow colour of chloroform extracts in a tintometer, and using the antimony trichloride method. Results was now expressed in milligram rather than in blue or yellow units.

A major study of the carotene and vitamin A content of Malaysia foods was reported by Leong in 1939 (Leong, 1939b). Carotenoids were determined in over 110 foods and total vitamin A in 50 foods. The precursor of the vitamin was determined by reading the colour in a tintometer directly, whilst vitamin A was estimated after reaction with antimony trichloride. Unlike the report of Rosedale (1935), results were expressed in international units. Later in the year (Leong, 1939c) the same author reported the carotene content of 17 varieties of Malaysian bananas. The colorimetric method was used and results were given as international units of vitamin A. A biological assay was also carried out for two varieties of the fruit for comparison.

Vitamin A was also one of the nutrients reported by Rosedale and Milsum (1940) in their study of nutrient composition of vegetables. After this period of rapid publications in vitamin A study in the 30s, there was a lapse of about thirty years before the next study was reported.

From then on, investigators had used the colorimetric method for carotene and vitamin A analyses (e.g. Chandrasekharan, 1969 and Soh, 1970). More recent investigators had however slightly modified preliminary treatment procedures to give more accurate determinations of carotene content in foods possible. For example, Chong and Soh (1969) in their study of the carotene content of over 30 types of vegetables and fruits, had used a more refined version of the method than that described by Leong (1939b). An initial adsorption chromatography was carried out by which chlorophylls and other unwanted carotenoid pigments were first removed before measuring the cartone content.

Carotene concentration was expressed in microgram and also converted to vitamin A equivalents in international units.

Reports on vitamin B analyses of local foods appeared a few years after those dealing with vitamins C and A. However, when the first report was published (Leong, 1940a), it was a major study of vitamin B1 (thiamine) in over 400 types of local foods. As for the other two vitamins previously discussed, initial studies were carried out using a biological assay method. In this case, the ability of thiamine to "cure" bradycardia in experimental rats was used. Results were expressed in international units.

Subsequent reports on this anti-beri-beri factor saw a total change in the method used (Simpson et al, 1951; Leong, 1953; Simpson and Chow, 1956a, 1956b; and Soh, 1970). A chemical method, known as the thiochrome method was used in favour of the biological assay procedure. This method, which involves a preliminary incubation of the food with takadiastase followed by separation of the vitamin by adsorption and subsequent elution from decalco, has remained as the standard method for estimating vitamin B1 in foods. Results were expressed in the more familiar milligram.

Leong (1940b) also reported the contents of another B vitamin in local foods. Nicotinic acid content was studied in 100 foods using the cyanogen bromide-aniline method, based on the Koenig reaction. This was the only major report on the study of nicotinic acid content of Malaysian foods. Other reports were those unpublished but documented in the Nutrition Division of the Institute for Medical Research (1961, 1962, 1971 and 1972).

Riboflavin was another B vitamin studied in the country. The first Malaysian report was that of Leong (1941), in which a biological method, based on the growth response of rats, was used for determining riboflavin content of over 50 types of local foods. After a number of years, in another report from this investigator (Leong, 1953), this vitamin B2 was estimated using a totally different method, namely by direct fluorometry after removal of interfering substance in the extracts using potassium permanganate.

After this, there were a few reports on nutrient analyses in foods, where riboflavin was one of the nutrients studied (Simpson and Soh, 1956; Nutrition Division, Institute for Medical Research, 1961, 1962, 1971 and 1972; and Sedky et al, 1972). None of these reports, however, had discussed the method used for determining this vitamin.

The next report devoted solely to riboflavin determination in local foods was published some 20 years after the last report of Leong in 1953. Caldwell and Enoch (1972) reported the content of this vitamin in some 50 types of local leafy vegetables. The fluorometry method, essentially the same as that described by Leong (1953), was used. This is also currently the method that is being used by this laboratory.

From this review, it is clearly seen that there had been some interesting developments in vitamin analyses of local foods. In keeping with international developments, there had been a definite change in methodologies. Generally, biological assay methods had given way to the more convenient and rapid, though not necessarily more accurate, colorimetric and fluorometric methods. There had also been an obvious change in the units in which results were expressed, alongside this change in methodology. From the initial report of Stanton (1923), in which there was only an indication of either the presence or absence of vitamin C, it had moved onto "minimum protective dose" by Oliveiro (1934), thence onto international units by Leong (1939a) and finally as milligram in subsequent reports.

Whilst vitamin A and ascorbic acid had been fairly well studied, reports on vitamin B analyses in local foods had been less satisfactory. Thiamine content as reported by Leong (1940a) had not been verified by other investigators; niacin and riboflavin had only been studied in a relatively small number of foodstuffs. Vitamin D had been dealt with only in one report (Rosedale and Oliveiro, 1934) and even then only briefly. Hence there is still considerable grounds to be covered in this field of vitamin analyses in local foods.

In the Analysis of Minerals

Moving onto another group of nutrients, the minerals, one sees a less dramatic and interesting picture. Nevertheless, there had also been changes in methodologies, as investigators moved from gravimetric to the less tedious volumetric and colorimetric methods.

Prior to discussing each mineral, it would perhaps be interesting to note that the most complete report on minerals contents of local foods was that by Morris and Rosedale as early as in 1935. A total of twelve minerals were tabulated for over 70 types of foods. Details of the procedure used for determining each mineral were also given.

The best studied mineral was perhaps calcium. Morris and Oliveiro (1933) reported the content of this mineral in about 60 types of foods. Calcium was precipitated as calcium oxalate, converted to calcium oxide, weighed and reported as such. Morris and Rosedale, in their report of 1935 (discussed earlier on) had used a similar method. Rosedale and Milsum (1940) had also reported calcium content of vegetables as calcium oxide, although no details of the procedure used were given.

Some years later, Leong and Morris (1947) had used a different procedure for determining this mineral. Calcium was again precipitated as oxalates, but instead of using the more cumbersome gravimetric procedure, calcium present was next titrated with potassium permanganate and results expressed as milligram calcium. Subsequent reports on nutrient analyses of local foods had used this volumetric method for estimating calcium and results were all given as milligram calcium (e.g. Simpson et al, 1951 and Leong, 1953).

Iron determination also started off using a gravimetric procedure. Morris and Rosedale (1935) had precipitated the iron in foods with "cupferron" (ammonium-nitrosophenyl hydroxylamine), separated and weighed as ferric oxide, although the results were expressed in Fe.

It is not clear when the change over to colorimetric procedure took place, but the first report on this was by Simpson et al in 1951. These investigators had estimated iron based on the colour development with thioglycolic acid. In 1953, Leong reported the use of orthophenanthroline for colour development. Subsequent reports did not specify the method used. However, the colorimetric procedure using orthophenanthroline is still one of the most commonly used method.

The first report on phosphorus determination was also by Morris and Rosedale (1935). They had estimated phosphorus (results expressed as P₂O₅) by titration with uranium acetate using potassium ferrocyanide as an external indicator. There had not been many reports discussing on phosphorus determination after this. Some years later, Simpson et al (1951) had described using a colorimetric procedure, based on reaction using molybdate-stannous chloride. Results were given in milligram phosphorus.

Aside from these three elements discussed above, there were hardly any other studies on the other minerals. The only available report was that of Morris and Rosedale (1935) (discussed earlier on in this section) who had also estimated sodium, potassium, magnesium, chloride and sulphur.

The importance of trace elements in human nutrition was soon realized. There were no active publications in this field in the country, but some attention had been given. These were mostly early reports in the 1930s. Copper, manganese and iodine were reported by Morris and Rosedale (1935) for over 70 foodstuffs; zinc was reported by Morris (1940a) for over 100 foods, and the same author (Morris, 1940b) had determined over 150 food items for their cobalt contents.

Mineral analysis had always been neglected compared with the more "glamorous" vitamins. Glaringly lacking in published reports on mineral analyses in local foods were those studies on the content of iron, phosphorus, sodium and potassium. Except for the report of Morris and Rosedale (1935), there had been no other major studies dealing with these important minerals in human nutrition. It is hoped that the availability now of atomic absorption spectrophotometers, the systematic analyses of minerals in local foods would receive more attention.

In the Analysis of Lipids

In this section, only those reports dealing with the analyses of the various lipid fractions will be considered. The analyses of fat content of foods will be discussed later, in the section on proximate composition.

The analyses of fatty acids and/or cholesterol in local foods were all very recent studies. Interest in these lipids had come about due to the world wide attention and concern towards the relationship between dietary fats and the development of coronary heart diseases.

The first of such studies was reported by Chong and Mills (1966). Various types of cooking oils and fats were determined for their fatty acid compositions. Using a gas-liquid chromatographic procedure (carried out by a laboratory abroad), the percentages of individual fatty acids were analysed. The percentages of saturated and polyunsaturated fatty acids in the oils were then determined. Ng and Chong reported a similar study in 1979. More types of oils were being studied, and gas-liquid chromatography was carried out in this laboratory. In the same year, also from this laboratory, Tee et al (1979) extended the study to determining fatty acid composition in some 20 foodstuffs and also in 23 types of cooked, ready-to-eat Malaysian meals. Using the same starting food sample, cholesterol was also determined simultaneously, both using a gas-liquid chromatographic procedure.

Study in this field had not attracted many investigators. This is perhaps because fatty acid analyses has thus far been looked at from the view point of over-nutrition, which affects a small segment of the population. It should be worthwhile to examine fatty acid composition to identify rich sources of essential fatty acids in local foods which may be lacking in the dietaries of the community at large.

In the Analysis of Proximate Composition

There had not been much problems with the laboratory analyses of moisture, protein, ash and fat in foods (from which carbohydrate and calorie may then be calculated). Procedures had remained essentially the same through the years. It is clear that the analytical methods may vary slightly for different groups of foodstuffs, for example the analyses of fat in milk and moisture in certain foods containing high percentages of volatile oils. There are however, no proper documentation of experiences of such analytical procedures by local investigators.

The first major report on the analyses of proximate composition (including fibre) in foodstuffs was that by Rosedale (1935). Subsequently, in the Food Table of Fasal (1941), Willimott (1949a) and Oliveiro (1955), proximate composition (without fibre) were also reported. Aside from these four Food Tables, there were few other individual reports on the analyses of proximate composition of foodstuffs. Those that dealt with a fairly large number of foodstuffs were the reports of Rosedale and Milsum (1940), Simpson et al (1951), the unpublished reports of the Nutrition Division, Institute for Medical Research Result (1961 and 1962), and the study of Tee et al (1979) on cooked meals. Others, such as Willimott (1949b), Leong (1953), Simpson and Soh (1956), Nutrition Division, Institute for Medical Research (1971 and 1972) had reported on the proximate composition of single foodstuffs.

The analyses of fibre need some mention at this point. Fibre content of local foods had been documented only in two major reports: the Food Composition Table of Rosedale (1935), for over 140 foodstuffs and the study of Rosedale and Milsum (1940) of about 60 vegetables commonly eaten by the Malays. Subsequent investigators did not include this "nutrient" in their proximate composition analyses, although the Nutrition Division of this Institute has some unpublished data on this.

In the Evaluation of Protein Quality

A discussion on the evaluation of protein quality will be included in this chapter since this relies essentially on the amino acid composition of foodstuffs.

The study of the protein quality of foods had attracted the attention of many investigators internationally as the problem of protein-calorie malnutrition and the means of combating this "disease" of millions of the less privileged became better understood. Chemical methods, such as by amino-acid composition analyses, as well as biological evaluation methods were widely used.

Locally, the study of amino acid composition of foods was reported as early as in 1935 by Rosedale. About 20 types of foods were analysed and although methodologies available then did not permit computation of all the amino acids present, it was a good start. However, there were no follow-up reports in this field. Uptill today, there is no major report on the amino acid composition of local foods although methodology and instrumentation for these analyses have been fairly well established elsewhere. There are however isolated studies on single foodstuffs, such as the report of Yeoh and Chew (1977), in which the amino acid composition of cassava leaf protein was analysed. In a different approach, Chong and Soh (1966) used a single amino acid, namely available lysine content, as an indicator of protein quality of ikan bilis.

Rosedale in his study of 1935 also attempted at evaluating the protein quality of the foods studied for their amino acid composition by a biological method. Growth of animals fed these foods singly and in combinations for 8 weeks was studied. Biological evaluation of protein quality too did not attract many investigators in this country. One of such reports, by Tee (1976), had described in detail the determination of Nett Protein Utilization of high protein snack foods. Essentially it involved feeding these foods to 30-day old albino rats for ten days and determining nitrogen intake, output and retained in these animals. Nett Protein Utilization, Biological Value and Digestibility were then obtained simultaneously. There may be a few other investigators employing this or the Protein Efficiency Ratio method for biological evaluation of protein quality, but there certainly had not been much enthusiasm.

CONCLUDING REMARKS

From this review, one is able to have a better insight into research and development that had taken place in the field of nutrient analyses of local foods. The interesting features of these reports have been noted. Areas in which future workers might perhaps pay more attention to have been indicated. Another even more important outcome of this review will be the possible uses of all these data that have been collected and scrutinised in the course of the exercise.

As mentioned earlier at the beginning of this paper, this presentation forms part of a comprehensive review of the analysis of nutrients in Malaysian foods, which includes aspects other than the analytical techniques. To utilise all the data that had been collected in the course of the review, an attempt has been made to compile them into an up-dated Preliminary Malaysian Food Composition Table. From this preliminary Table compiled, one can then see clearly what data are available and what further data need to be collected. It will be necessary too to verify certain data. It is hoped that by this process of filling in the gaps and/or making necessary verification of results to this preliminary Table, it will be possible to arrive at a comprehensive, updated Malaysian Food Composition Table.

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