Analytical Considerations in Nutrition Labelling

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SUMMARY

Nutrition labelling is aimed at providing a means for conveying information of the nutrient content on the label of a food product, thereby assisting the consumer in the wise choice of food. To the food industry, nutrition labelling is important as it provides a means for manufacturers and retailers to be more aware of the nutritional properties of their products. There is currently no mandatory nutrition labelling of foods in Malaysia, except for regulations pertaining to the labelling of "special purpose foods" and fortified or enriched foods. With a rapidly developing food industry and increased awareness amongst consumers of their dietary intake, there is a need to consider the implementation of nutrition labelling for a wider variety of foods. Any proposed scheme for nutrition labelling would necessarily have to take into consideration the stage of development, culture and technical resources available, and should not impose great costs on manufacturers. In addition, consideration has to be given to the analysis of nutrients to be declared on the label. This paper highlights the nutritional significance of selected nutrients and discusses the analytical methods currently used in this laboratory. Methods for the analysis of the macronutrients are generally satisfactory except for individual carbohydrates and dietary fibre which require further development. The common major minerals, calcium, phosphorus, iron, sodium and potassium are being analysed satisfactorily using calorimetric, titrimetric or atomic absorption spectrophotometric methods. The analysis of vitamins faces greater problems, because of the instability of these nutrients. Efforts are being made to develop improved methods for the analysis of several vitamins. There are presently few laboratories in the country experienced in the analysis of the full range of nutrients. Technical expertise needs to be developed through a series of planned training programmes.

INTRODUCTION

The primary objective of nutrition labelling is to describe the nutritional qualities of a food product factually and informatively. It is aimed at providing a means for conveying information of the nutrient content on the label, thereby assisting the consumer in the wise choice of food (FAO/WHO, 1988). Although nutrition education is not the primary aim of nutrition labelling, it does provide support to nutrition education activities as it encourages the use of sound nutrition principles in the formulation of foods, which would be of benefit to public health. Nutrition information on food products helps the population respond effectively to nutrition education initiatives (WHO, 1990).

Nutrition labelling is equally important to the food industry, as labelling provides a means for food manufacturers and retailers to become more aware of the nutritional properties of their products and emphasise these properties to consumers. Food manufacturers have a responsibility to provide accurate, relevant information on the nutritional value of their products to consumers.

NUTRITION LABELLING IN MALAYSIA

There is currently no mandatory nutrition labelling of foods in Malaysia, except for regulations pertaining to the labelling of "special purpose foods". Regulations 388 to 393 of the Malaysian Food Regulations 1985 (Ministry of Health, 1985) provide for obligatory nutrition labelling of foods such as infant formulae and cereal-based foods for infants and young children. These foods are to be labelled with the energy, protein, carbohydrate, fat, vitamin and mineral contents. In addition, under Regulation 26, foods enriched or fortified with permitted vitamins, minerals, essential amino acids or essential fatty acids shall be labelled with the type and quantity of the nutrient.

In a rapidly developing country like Malaysia, the need for food preservation, packaging, and processing is likely to continue to increase. The food industries in the country are indeed developing at a rapid pace. Malaysian consumers are becoming more aware of their dietary intake, not only in terms of food additives but also the nutrients contained therein. There is increased awareness of the importance of nutrition to health and fitness. There is therefore a need to consider the implementation of nutrition labelling.

The proposed scheme would necessarily have to take into consideration the stage of development, culture and technical resources available, and should not impose great costs on manufacturers. There would

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necessarily have to be a great deal of planning and discussion for a feasible scheme for the country.

Consideration has to be given to the analysis of the nutrients to be declared on the label, which is the focus of this paper. The significance of selected nutrients to nutritional well-being is highlighted and the methods used in this laboratory for the analysis of these nutrients are discussed.

DECLARATION OF NUTRIENTS

The nutrient content of foods declared on the label can be obtained in several ways (Sanders and Jee, 1991), including:

a. Analysis of the food by the manufacturers;
b. Calculation from the ingredients used (based on values given in food composition tables); and
c. Calculation from generally established and accepted data.

During food processing, slight variations in recipes between batches can occur. Furthermore, nutritional changes, such as thermal degradation of vitamins, may occur as a result of processing. Therefore, analysis of representative samples of the foodstuffs would give the most accurate nutrient composition.

NUTRIENTS AND ANALYSES

Fat, energy and health

The amount of energy in fat (9 kcal/g) is $2^1/_4$ times that in protein or carbohydrate (4 kcal/g). The consumption of high fat foods is thus more likely to lead to an excess of calories in the diet. High fat intake has been associated with increased risk of coronary heart disease (CHD) and certain cancers (e.g. breast cancer). There is thus a great deal of interest in the fat content of foods. WHO (1990) has recommended limiting total fat intake to 30% of total energy intake.

Fat in most foods is a mixture of triglycerides, phospholipids, sterols and related compounds. Triglycerides, in turn, consist of fatty acids bound to glycerol. There are three types of fatty acids, with different nutritional implications.

Saturated fatty acids.

Saturated fatty acids contain no double bonds and can be considered purely as energy sources. A dietary excess has been implicated with increased risk of CHD. These fatty acids are particularly high in animal fats and artificially hardened fats.

Polyunsaturated fatty acids (PUFA)

These fatty acids contain two or more double bonds and can be further divided into n-6 PUFA, usually present in vegetable oils and n-3 PUFA, which are mainly found in fish oils. These two types of PUFA have double bonds positioned at six carbons (n-6) and three carbons (n-3) from the methyl end of the molecule. They are energy sources, but, in addition, are thought to be able to reduce the risk of CHD, probably because of their involvement in the production of certain prostaglandins in the body.

Monounsaturated fatty acids

These acids contain only one double bond, and were at one time thought to be neutral in their effect on CHD risk. Recent work has however suggested that a diet high in cis-monounsaturated fatty acids may reduce the risk to CHD, and may even be preferable to a diet high in polyunsaturates. Olive oil is particularly rich in these fatty acids.

Cholesterol is a trace constituent of animal fats, but is also manufactured in the body. It has been shown that individuals with high total cholesterol in their blood are at greater risk of CHD. These individuals are often advised to reduce intake of cholesterol-rich foods.

Total fat content is commonly determined by a direct solvent extraction method, using a light petroleum fraction or diethyl ether in a continuous extraction apparatus of the Soxhlet type (Tee, Siti Mizura, Kuladevan, Young, Khor and Chin, 1987). The fat extract is then weighed. The food sample is always dried and ground prior to placing it in a thimble. The method extracts the free lipid content, consisting essentially of neutral fats (triglycerides) and free fatty acids. The efficiency of the method relies a great deal on sample pre-treatment and choice of solvent. The procedure requires a long extraction time, and the extracted fat remains exposed to elevated temperatures throughout this time.

For certain types of foods, e.g. dairy products, the solubilisation extraction method is preferred. The food is first completely dissolved by acid (Werner-Schmid process) or alkaline hydrolysis (Rose-Gottlieb method) prior to extraction with polar solvents. In this process, bound lipids are made free. Separation of solvent containing the extracted fat from the sample solubilised by acid or alkali can be facilitated by a suitable extraction apparatus such as tubes with a siphon or wash bottle type fitting or by tubes specially designed to assist decanting, e.g. Majonnier tubes. This laboratory has adopted a method which is a slight modification of these procedures.

Fatty acids in foods are determined by a gas-liquid chromatographic (GLC) method. Fat is first extracted from the food and the acids methylated prior to separation by the GLC method. The various

components (saturated, mono and polyunsaturated fatty acids) can then be identified and summed (Tee, Ng and Chong, 1979).

Cholesterol in foods can also be determined by a GLC method as has been reported by Tee *et al.* (1979) for various local foods. Various colourimetric methods have also been used in this laboratory.

Protein

Protein determination in foods has great nutritional significance. It is an important nutrient required for growth and repair of tissues. Millions of children in the world suffer from protein-energy malnutrition, and providing adequate protein for an expanding world population remains a continuous challenge. Frequently, the food analyst is interested to know the total protein content of a food, although it may be made up of a complex mixture of proteins. This is most commonly estimated from the organic nitrogen content determined by the Kjeldahl procedure. Although it has been subject to modification over the years, the basic Kjeldahl procedure remains the most reliable technique for organic nitrogen determination (Egan, Kirk and Sawyer, 1981).

The Kjeldahl procedure is based on the wet digestion of the food sample by heating with concentrated sulphuric acid in the presence of metallic and other catalysts to effect the reduction of organic nitrogen in the sample to ammonia, which is retained in solution as ammonium sulphate. The digest, having been made alkaline, is distilled to release the ammonia which is trapped and titrated. This is the method in use in this laboratory, and good results have been obtained with recovery tests (Tee *et al.*, 1987).

Apparatus for the distillation of ammonia has been conventionally the Markham still. In recent years, the Kjeldahl procedure has been mechanised and automated. Several such systems are now increasing in popularity in Malaysia.

From the nitrogen content determined, total protein is estimated by calculation using specific factors, based on the average nitrogen content of the food. The factors recommended by FAO/WHO (1973) are being used by this laboratory. Values so obtained suffer from two major inaccuracies: the inclusion of non-protein nitrogen in the Kjeldahl procedure, and the inaccuracies of the factors used.

Carbohydrates

Available carbohydrates

Carbohydrates are the most abundant and widely distributed food component. These include a diverse range of carbohydrates, ranging from monosaccharides to complex hetero-polysaccharides. Thus, the determination of carbohydrate in foods has presented special problems to food analysts. Present carbohydrate values are frequently calculated by difference, i.e. by subtracting the measured moisture, protein, fat, ash and fibre from 100.

However, owing to the increasing awareness that specific carbohydrates play significant metabolic and functional roles, and the availability of analytical tools to determine individual carbohydrate components, many laboratories are now moving towards the determination of their distribution in foods. For the purpose of nutrition labelling, individual sugars would also be more useful.

Methods for the direct determination of individual carbohydrates are now fairly well established. Essentially the methods require the separation of free sugars from the insoluble polysaccharides by extraction procedures. The former, mainly monosaccharides, disaccharides and other oligosaccharides are then determined as total free sugars or each individual sugar is determined separately after further separation procedures. The residues remaining after the initial extraction procedures are measured for starch and the non-starch polysaccharides. The book by Southgate (1976) gives a comprehensive treatment on the determination of food carbohydrates.

Unavailable carbohydrates: crude fibre and dietary fibre

Crude fibre in food analysis is taken to mean the insoluble and combustible organic residue that is left after the removal of carbohydrate, fat and proteins under prescribed conditions. This residue is largely cellulose with a proportion of lignin and hemicelluloses from the food sample.

In this laboratory, crude fibre is determined routinely by defatting by ether treatment, followed by successive treatments with acid and alkali to remove the carbohydrates and proteins (Tee *et al.*, 1987). The residue remaining after this, is dried and weighed. This is taken to be the weight of the inorganic matter and crude fibre. This residue is ashed, and the weight of the ash determined, from which the weight of the crude fibre can be determined by difference.

The empirical methods for the determination of crude fibre as described above are of little use as they do not measure any specific carbohydrate or group of carbohydrates, and considerable losses of cellulose can occur (Southgate, 1976). Various workers have urged the more accurate determination of fibre in foods as dietary fibre, perhaps due to the increasing attention being given to the role of indigestible fibre or roughage in the diet in clinical nutrition.

Dietary fibre includes the polysaccharides that are not hydrolysed by human alimentary tract enzymes, and

lignin. These are the unavailable carbohydrates, and include hemicelluloses, pectic substances, cellulose, gums, mucilages, algal polysaccharides, and chemically modified polysaccharides. A number of methods for the estimation of dietary fibre have been developed (Southgate, 1976; Selvendran and DuPont, 1984). This is a relatively new area of study in Malaysia, and this laboratory has recently carried out the analysis of dietary fibre in a variety of foods.

Ash and mineral matter

Ash in a foodstuff is the inorganic residue remaining after the organic matter has been burnt away. Hence ash content can be determined by incinerating (dry ashing) a known quantity of foodstuff until constant weight is obtained. The sample is dried and charred on an electric heating mantle prior to ashing it in a muffle, usually in a silica crucible. Although porcelain (which is cheaper) or platinum (which is the least reactive) may be used, the former is less suitable, whilst platinum dishes are very expensive. Wet ashing, e.g. with concentrated acid, is used primarily for the digestion of samples for determination of trace elements and metallic poisons.

The ash is dissolved in an acidic solution and used for the determination of various minerals, discussed below. Details of the ashing procedure and the analysis of the minerals were given by Tee *et al.* (1987).

Calcium

Calcium is of nutritional significance, particularly in bone formation. The procedure in use is the classical method involving precipitation of the calcium in the ash solution (after prior treatment with ammonia) as the oxalate. After collecting the precipitate and proper dissolution, the warm solution is titrated using potassium permanganate. Good results have generally been obtained, although some problems were encountered for samples with very low concentrations of calcium.

The atomic absorption spectrophotometric method for the determination of calcium has also been used in this laboratory. Since phosphorus interferes in this procedure, lanthanum has been added to the sample (and standard) solutions. The method is less time-consuming than the titration method above.

Iron

Iron is of great nutritional significance in Malaysia, in view of the iron deficiency anaemia problem prevalent among young children and women. The method used is based on the reaction with *o*-phenanthroline or dipyridyl which reacts with the iron present to form a red coloured compound, the intensity of which is read in a spectrophotometer at 510 nm. The atomic absorption spectrophotometric method has also been successfully used in this laboratory. Special precautions to be taken include the use of iron-free glass-ware and reagents.

Phosphorus

A simple colorimetric method for the determination of phosphate based on Misson's reaction is being used in this laboratory. Phosphorus present in the ash solution as orthophosphate reacts with a vanadate-molybdate reagent to produce a stable yellow-orange complex of vandi-molybdiphosphoric acid, the optical density of which is measured at 420 nm.

Sodium and potassium

The atomic absorption spectrophotometry method has been used for the analysis of sodium and potassium in foods without much problem.

Sodium is of nutritional significance, particularly in relation to hypertension. Common salt, sodium chloride, is the source of most of the sodium content of the diet. However, it is the total sodium content of the diet that is important.

Moisture

The determination of moisture in foods is important. Firstly, moisture in foods influences microbiological growth and biochemical reaction, and thus affects the stability and quality of foods. Secondly, since the amount of dry matter in a food is inversely proportional to the amount of moisture it contains, moisture content is of direct economic importance to the processor and the consumer.

The determination of water in foods is complex and difficult. This is related mainly to the complex nature of water in foods, known to occur in three forms: as bound water, adsorbed water, and as free water. This classification is however quite arbitrary, and foods may contain varying quantities of the three types of water since they are mostly heterogeneous mixtures of substances. Various methods for moisture determination have been in use, varying in their involvement with the three types of water. These methods have been grouped as drying methods, distillation procedures, chemical assays and physical procedures.

Drying methods are frequently used, wherein the weight loss due to the evaporation of water at or near the boiling point is determined. Results obtained from such methods may not be a true measure of the water content of the sample, since other substances, such as volatile oil may be lost at the drying temperature, or only a proportion of the water is lost, leaving the bound and adsorbed water undetermined. For foodstuffs where decomposition is likely to occur, the vacuum oven method is used, so that lower

temperatures need to be applied. According to Pomeranz and Meloan (1978), vacuum-oven determinations gave the most accurate results for moisture content of most foods. These conventional methods need long drying times.

Rapid approximate moisture determination procedures, used for example during food manufacture, include those that make use of infra-red drying lamps, and may incorporate a direct reading crude balance. Recently, microwave ovens have also been used for rapid laboratory moisture determinations (Egan, Kirk and Sawyer, 1981).

In addition to the thermal drying methods described above, distillation methods and the Karl Fischer titration procedure, an example of a chemical method, have also been used to determine the water content of some foods. In the former, the food is distilled with an immiscible solvent having a higher boiling point and a lower specific gravity than water, e.g. toluene, heptane or xylene. The volume of water distilled is measured. Low results have been known to be obtained with these methods, but less decomposition of foods than drying at elevated temperatures may occur (Pomeranz and Meloan, 1978). The Karl Fischer titration method is based on the non-stoichiometric reaction of water with iodine and sulphur dioxide in pyridine-methanol solution. The method is employed mainly for foodstuffs with fairly low moisture content, e.g. sugar confectionery, chocolate, molasses and dried vegetables (Egan, Kirk and Sawyer, 1981).

Analysis of vitamins

Vitamins are important to human nutrition, participating in metabolic processes. Although the biological functions of various vitamins in the human body are not fully understood, the importance of these nutrients has been much emphasised. In many cases, the importance of vitamins has been over-emphasised and misuse of these nutrients may result in detrimental effects.

Interesting and significant developments in vitamin analysis in the country have been observed (Tee, 1990). In keeping with international developments, there has been a definite change in methodologies. Generally, biological assay methods had given way to the more convenient and rapid colorimetric and fluorimetric methods. More recently, HPLC methods have been more widely used. There has also been a change in the units in which results were expressed.

Current methods for vitamin analysis in this laboratory are detailed in the IMR manual by Tee *et al.* (1987).

Thiamine

Thiamine or vitamin B_1 is determined by the thiochrome procedure based upon the oxidation of thiamine to thiochrome which fluoresces in UV light. Under standard conditions and in the absence of other fluorescing substances, the fluorescence is proportional to the thiochrome present, hence to the thiamine originally in solution.

Several preliminary steps have to be carried out. Thiamine in the foodstuff is first extracted using dilute acid. An enzyme preparation (e.g. takadiastase, Mylase-100 or α-amylase) is then added to convert bound thiamine to its free form. The extract is next purified by passing through a column of Decalso (Fisher Scientific Co.). Thiamine is first adsorbed onto the column, thereby separating it from any substances which might interfere with subsequent steps in the determination. Acid potassium chloride solution is then passed through the column to elute the adsorbed vitamin. The thiamine is then treated with alkaline potassium ferricyanide to oxidise it to thiochrome. The thiochrome is extracted into isobutyl alcohol and its fluorescence measured in a fluorimeter.

Riboflavin

The procedure for riboflavin or vitamin B_2 determination makes use of the distinguishing property of riboflavin to emit a yellow-green fluorescence in neutral solutions.

Since riboflavin may occur combined with proteins, the food has to be treated with enzyme or dilute acid to liberate the vitamin. Protein and other interfering substances are then precipitated. After filtering, the extract is further treated with potassium permanganate to remove interfering fluorescent substances, followed by the addition of hydrogen peroxide to remove excess permanganate. Fluorescence of the extract is then measured in a fluorimeter. A little sodium hydrosulphite is added to quench the fluorescence due to riboflavin and the fluorescence reading again taken. The remaining fluorescence is due to other interfering substances and is subtracted from the initial fluorescent reading.

Since riboflavin is light sensitive and is most readily destroyed by light in the blue and UV region, it is necessary to perform all operations in the absence of strong light, and to use amber glassware. It is also important to ensure that the pH of all solutions containing the vitamin is below 7.0, since riboflavin is unstable in alkaline solution. Recovery tests have to be performed regularly.

Niacin (nicotinic acid)

Niacin, and the amide of niacin, niacinamide, are widely distributed in foods. As with the determination of thiamine and riboflavin, they are usually bound to other chemical compounds and must therefore be freed by hydrolysis with strong acid or

alkali or by enzymatic treatment prior to analysis by chemical methods. Hence niacin derivatives such as coenzymes and niacinamide are converted into free niacin. To eliminate the interference of biologically inactive materials that may be present, purification procedures are required and blank determinations are necessary during colour development. Niacin in the extract is reacted with cyanogen bromide to give a pyridinium compound. The latter undergoes rearrangement yielding derivatives that couple with aromatic amines (sulphanilic acid) to produce coloured compounds. Under proper conditions the absorbance produces is proportional to the amount of niacin present and may be measured in a colourimeter.

Niacin and niacinamide are both stable in the dry form and in aqueous solutions and are unaffected by light and pH. However cyanogen bromide is extremely poisonous. All operations involving this reagent should be carried out in an efficient fume cupboard. Do not breathe any vapour, and if solution comes in contact with skin, wash immediately with water. Due to the high toxicity of the reagent and possible interference by other compounds, we are examining the possibility of using an HPLC method for the determination of this vitamin.

HPLC of B-vitamins

With a view towards simultaneous determination of several B-vitamins in foods, an HPLC method is being developed. Such a method would reduce drastically the time required for analysis. Several existing methods were examined and tested, including reports by Wehling and Wetzel (1984), Wills, Wimalasiri and Greenfield (1985), and Dong, Lepore and Tarumoto (1988). A reversed-phase HPLC system is being studied, using a μBondapak C_{18} column, and a mixture of methanol, glacial acetic acid and water as the mobile phase. Using a multi-channel UV absorbance detector for simultaneous detection at three wavelengths, it was possible to detect and quantitate vitamins B_1, B_2, B_6 and niacin. Work is continuing to improve resolution of the peaks, e.g. by the use of ion-pairing reagents.

Ascorbic acid

Ascorbic acid is determined by the indophenol dye method, which determines the reduced form of the vitamin. It is based on the powerful reducing properties of the vitamin. Ascorbic acid is first extracted from the food using an aqueous solution of metaphosphoric acid-acetic acid mixture as a stabilising agent, then titrated with the oxidation-reduction indicator of 2,6-dichlorophenol indophenol. In this reaction, ascorbic acid is oxidised to dehydroascorbic acid and the indophenol dye is reduced to a colourless compound. The end point of the titration is hence easily detected when excess of the unreduced dye gives a rose pink colour in acid solution.

The value of the indophenol reagent for the determination of ascorbic acid is limited by the presence of reducing substances in the foods such as ferrous iron, stannous tin, cuprous copper, sulphur dioxide, sulphite or thiosulphate. Another limitation of the method arises when the sample to be analysed gives a highly intense coloured solution, such as the reddish-purplish colours of certain fruits. To overcome the problem, the above procedure has been modified to include an ether extraction step to aid in detecting the end-point.

Ascorbic acid is a highly unstable vitamin; it is sensitive to alkalis and to oxidation. The sampling and extraction of the sample material must therefore be carried out with minimum delay. As much of the comminution as possible should be conducted in the presence of stabilising acids, as mentioned above. These acids are able to retard the oxidation of ascorbic acid by inactivating the catalytic effects of ascorbic acid oxidase, copper and iron and maintain the acidity required for the reaction with the indophenol dye. It is also desirable to perform the titration rapidly.

This laboratory has also studied a fluorometric method for the determination of vitamin C. In this procedure, ascorbic acid is oxidized to dehydroascorbic acid with Norit (activated carbon). Aliquots are then reacted with o-phenylenediamine to produce a fluorescent quinoxaline derivative, which on activation at 350 nm fluoresces at 430 nm. In contrast to the dye titration method which determines only reduced ascorbic acid, the fluorometric method estimates total ascorbate derived from ascorbic acid as well as preformed dehydroascorbic acid (DHAA). Differences in values obtained by the two methods are dependent on the amount of DHAA present in the food (Tee, Young, Ho and Siti Mizura, 1988). Either method may be used, depending on the objectives and resources available to the analyst. The important point is that reports of vitamin C values must clearly state what the analytical data represents.

Carotenes in plant materials

Many laboratories use the AOAC method (Deutsch, 1984) for the determination of carotenes in plant materials. In this method, acetone-hexane extracts of fruits and vegetables are chromatographed on a column of a mixture of activated magnesia (Sea Sorb 43) and diatomaceous earth (Hyflo Super Cel) to separate the carotenes. The yellow coloured β-carotene is eluted from the column using 10% (w/v) acetone in hexane and read in a spectrophotometer at 436 nm.

Exposure to intense light should be avoided during carotene assays, since the pigments may be destroyed under such conditions. The use of amber glassware or its equivalent will provide further protection.

Vitamin A (and β-carotene) in foods of animal origin

An AOAC method is also widely used for the determination of foods of animal origin containing both retinol (preformed vitamin A) and carotenes (precursors of vitamin A).

The finely ground food is first saponified (hydrolysed in the presence of alkali) thereby removing fat-soluble interfering substances. Vitamin A remaining in the unsaponifiable fraction is then extracted into hexane. The extract is next chromatographed on a column of alumina to separate retinol from the carotenoids present. Carotenes are first eluted from the column using 4% acetone in hexane and quantitated by taking the absorbance reading in a spectrophotometer at 450 nm. Vitamin A is next eluted from the column using 15% acetone in hexane and read in a spectrophotometer at 325 nm.

It is a long procedure, and only a few samples can be comfortably handled at a time. Precautions have to be taken to carry out steps in subdued light as vitamin A is destroyed by UV light and is sensitive to oxidation in air. Low recovery values are not uncommon.

HPLC of retinol and carotenoids

The open-column chromatography methods of the AOAC for the determination of retinol and carotenoids have been under criticism in recent years. The main concern is that the methods are not specific for retinol and the main provitamin A carotenoids. It is thus thought that vitamin A values of foods so obtained may have been unreliable. Secondly, the unmodified method is not able to estimate the amounts of other carotenoids present in the food. Some of these other carotenoids do not possess vitamin A activity, but may occur in higher concentrations than β-carotene, and are now thought to play important roles beyond their classical functions in nutrition and vision. With their highly conjugated double bonds, carotenoids may act as free radical traps or antioxidants, and therefore play important roles in cancer causation and prevention (Peto, Doll, Buckley and Sporn, 1981; Temple and Basu, 1988).

There has thus been greater emphases on obtaining more accurate data on vitamin A values of foods. Rapid advances have taken place in the development of methodologies for more accurate quantitation of various carotenoids and retinoids in foods (Tee and Lim, 1991a). Since the late 1970's, HPLC has become a widely used procedure for these purposes, mainly because of the ability of the technique to effect rapid separation, its non-destructiveness and, more importantly, the better resolution that is achieved.

This laboratory embarked on a systematic study to develop improved methodologies for the separation and quantitation of retinol and carotenoids in foods and biological specimens, especially serum. A simple non-aqueous reversed-phase HPLC method was developed, workable for routine analysis of a wide variety of foods of both plant and animal origin. The method was also applicable to the analysis of blood samples for the assessment of vitamin A status of communities. Such a system would then obviate the use of different and complicated chromatographic conditions for different food samples, as well as for blood.

The HPLC method developed has been applied to the study of carotenoid composition of various Malaysian vegetables and fruits (Tee and Lim 1991b). Carotenoids were separated isocratically on a C_{18} column using a mixture of acetonitrile, methanol and ethyl acetate, and peaks monitored using a fixed-wavelength detector at 436 nm.

The HPLC method developed was applied to the study of retinol and several carotenoids in various foods of animal origin (Tee and Lim, 1992). The same chromatography conditions (e.g. column and mobile phase) as those for plant materials were used. To enable the simultaneous detection and quantitation of retinol and carotenoids in a single chromatographic run, two detectors were connected in series, one set at 313 nm and the second to monitor the eluate at 436 nm.

CONCLUSION

In the development of nutrition labelling, it is important to consider the development of capabilities in nutrient analysis. Depending on the extent of the labelling scheme, the type of nutrients to be analysed can be extensive and complicated, but a wide range of analytical methods will have to be employed.

Expertise is available in Malaysia for the analysis of the major nutrients of nutritional significance. However, there are presently few laboratories experienced in the analysis of the full range of nutrients. Technical expertise will therefore have to be developed through proper training programmes for analysts.

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