USE OF ANALYTICAL TECHNIQUES IN FOOD RESEARCH IN MALAYSIA

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INTRODUCTION

Food products are produced by various processing methods. At each stage of processing, starting from raw materials till finished products, food analytical techniques are used to ensure that the food produced are sufficiently nutritious, wholesome, free from harmful and hazardous substances and safe for human consumption.

There are several institutions in Malaysia which are directly involved in food analytical techniques under the programmes of ASEAN-protein and ASEAN-Food Technology Research and Development Projects. The main institutions reported in this paper are :

1. Food Technology Division, MARDI
2. Division of Human Nutrition, IMR
3. Faculty of Food Science and Technology, UPM.

These three institutions work closely with each other towards achieving a common end, that is, advance in food analytical research which have direct application in food nutrition for the benefit of the people at large.

This report concentrates on the activities which are presently carried out by the above institution within the context of food analytical techniques. Some methodology, problems in their application and success in getting results are presented in this paper for the purpose of exchanging information.

RESEARCH CONDUCTED AT VARIOUS INSTITUTION

MARDI

Under ASEAN-Protein Project I, MARDI has successfully produced high protein extruded snacks and biscuits. These snacks and biscuits were distributed to school and pre-school children as supplementary feeding. Field acceptability trials were carried out by community development (KEMAS) and Ministry of Health while monitoring activity is done by IMR.

Analytical techniques used in this research is for the purpose of getting the proximate composition of the finished products. Table 1 shows the composition of 'Nutrima' Snack, the brand name of the extruded product.

Table 1. Composition of Nutrima Snack

Nutrima snack	Composition
1. Wt/pack	25 gm
2. Protein	3.0 gm
3. Fat	4.0 gm
4. Calorie/pack	120
5. Enriched with	Vit. A, B_1, B_2 and niacin
6. Made of	Rice, corn, soybean, veg. oil, vit. and Chicken flavour.

Utilization of winged bean was also carried out as a result of an increasing awareness of the potential of winged beans as protein source. Winged bean was analysed and fluor was prepared from the bean for incorporation into high protein food. Table 2 shows the nutrient composition of winged bean as compared to soybean.

Table 2. Nutrient composition of winged bean and soybean

Composition (gm)	per 100 gm	
	Winged Bean	Soy Bean
H_2O	6.7 - 24.6	10.2
Protein	29.8 - 37.4	35.1
Fat	15.0 - 20.4	17.7
CHO	31.6 - 28.0	32.0
Fibre	5.0 - 12.5	4.2
Ash	3.6 - 4.0	5.0
Cal (mg)	204 - 370	226
Phosphorus (mg)	276 - 320	546
Iron (mg)	9.6 - 11.8	8.5
Thiamine (mg)	1.4	0.66
Riboflavin (mg)	0.2	0.22

ASEAN-protein project II deals with processing and utilization of full fat soy flour. Spray dried beverage base was prepared by modification of Illinois and Mustakas processes.

The use of soy flour in the substitution of wheat for 'Instant' noodles was also tried. It was found that substitution at increasing levels up to 35%, had increased the yellowness of the product. The results from the organoleptic evaluation indicated that the overall preference was for 15% soy substitution.

Under ASEAN-protein project III, MARDI focussed on soysauce manufacturing techniques. Research in this aspect started in 1975 with the objectives of (1) studying and improving methods of soysauce production and shelf life (2) assessing the composition and nutritional value during fermentation and in the end products (3) isolating and identifying the microorganisms present and (4) screening for pathogens and mycotoxins.

The chemical analysis on soysauce was carried out on salt content, protein, pH, ash, total sugars acidity, benzoic acid and artificial sweeteners. It was found that soysauce have pH range of 4-5 with acidity of 0.2-0.5% (as acetic acid).

The protein analysis is affected by the addition of monosodium glutamate. Benzoic acid was found to be in the range of 150 - 200 ppm, although the permitted level is only 100 ppm.

Under ASEAN-FTRD projects MARDI is involved in the following research :

Project I - Processing of Underutilized local fruits.

Project II - Improvement and development of dried traditional Malaysian cakes.

Projects under ASEAN-FTRD started in 1982 during which time the focus was on literature reviews and surveys. The actual experimental trials started last year 1983.

Project I deals with the processing of four underutilized local fruits namely 'sentul', 'kundang/remia', 'tamarind' and 'rukan masam'. These fruits are processed into dried/salted products, jam, fruit leather, candy, pickle, vinegar, concentrate and jelly. Further improvement and other aspects of study such as sensory evaluation, packaging and shelf life are being conducted.

Project II focussed on the processing of dried traditional cakes with the objectives of promoting the growth of small-scale food industry. Some traditional processing techniques are being improved by using appropriate machines.

The cake chosen is 'baulu' a popular traditional cake among Malaysians. The preliminary trials is on the formulation of recipe, improving the keeping quality, mechanising and sorption isotherm study. Further work is needed.

Institute of Medical Research (IMR)

IMR is involved in research on analysis of the nutrient content of local foods. The ultimate objective is to arrive at a comprehensive Food Composition Table suitable for use in Malaysia. It is irony that in the 1980's with the attention given to food research Malaysia still does not have a proper Food Table. Nevertheless, in 1982, IMR managed to produce a preliminary table of 'Nutrient Composition of Malaysian Foods' which was compiled by Tee E. Siong.

The current research, which is expected to contribute to the comprehensive Food Composition Table, is conducted in collaboration with MARDI and UPM. For the purpose of streamlining the methodology, the analytical technique used for each nutrient has been agreed upon by the three institutions concerned. The analysis on proximate composition, vitamins and minerals of Malaysian food is described in the next section of this report.

As mentioned earlier IMR is also involved in field acceptability trials of extruded snacks.

Faculty of Food Science and Technology, U.P.M.

Under ASEAN-Protein Project, UPM embarked on the produc-

tion of low cost high protein food from Tilapia. Tilapia in Malaysia is an underutilized fresh water fish. Various products were tried such as production of crackers (keropok), fish ball, tilapia in tomato sauce, spiced-minced fish and fish cake, with good acceptance. Table 3 shows the proximate analysis of Tilapia Mossambica.

Table 3. Proximate analysis of T. Moasambica

Composition	%
Moisture	78.20
Ash	1.04
Fat	2.20
Protein	18.60

Another project carried out by UPM is the development of food for special target groups, such as schoolchildren and infant. High protein calories and snacks are produced containing various ingredients. The snack contains between 10-15% protein. The product shows that the protein quality has been improved.

Under ASEAN-FTRD, UPM is involved in two projects, namely : Project I - Use of appropriate Technology in Processing, Preservation and Packaging of Food.

Project II - Studies on dehydration techniques, storage methods and safety aspects of selected food.
Part I - starchy food
Part II - local spices.

Project I, deals with improvement of quality of the army combat ration and storage and processing characteristics of guava. Trials are being conducted to :

a. produce an intermediate moisture food from beef
b. produced canned sliced guava, juice and jam
c. determine the effect of temperature on the physical and chemical properties of fresh guava.

The study is still at preliminary stage and further work need to be carried out.

Project II, deals with the production of powder of flakes from local starchy food, such as, banana, potatoes and rice. These products can be used as a base for infant weaning food, with the addition of other ingredients such as groundnut, mungbean and 'ikan bilis' (anchovies).

Another aspect of project II is the production of local spices through the application of appropriate dehydration techniques. Ginger has been dried and turned into powder, while some trials on candy making are still going on. Table 4 shows the preliminary result of ginger analysis.

Table 4. Analysis of Ginger

Ginger analysis	% composition
Moisture (fresh)	85.2
Moisture (dehydrated)	11.4
Starch	47.8
Crude protein	8.4
Crude fibre	13.3
Total ash	5.3

ANALYTICAL TECHNIQUES IN NUTRIENT ANALYSIS

The most common and frequent analytical techniques used for food research include the proximate composition, vitamin

A and/ or carotene, thiamin, riboflavin, ascorbic acid, and minerals, that is, calcium, phosphorus, iron, sodium and potassium.

The Division of Human Nutrition, IMR is taking the lead in conducting research on the analysis of nutrient contents of local foods. This research project is carried out in collaboration with MARDI and UPM. The analytical techniques used for each nutrient has been agreed upon by the three institutions concerned. The methods used in analysing the proximate composition, vitamins, minerals and the problems encountered are briefly described here.

Proximate composition

On the whole, there has been relatively little problem with determinations of proximate composition of foodstuffs. Methods used have been fairly well standardized and satisfactory results have been obtainable.

Moisture, although strictly speaking a non-nutrient, is being routinely analysed since it affects the contents of nutrients in the foods. The direct heating or drying method using air-ovens is usually used. For foodstuffs that contain large amounts of volatile substances or when decomposition is likely to occur, the less severe conditions of a vacuum oven is used.

Protein analysis is carried out by the classical semi-micro Kjeldahl method, wherein nitrogen is first determined and converted to crude protein content by appropriate factors. Digestion and distillation is carried out by conventional laboratory apparatus. Fat has been determined in our laboratories by the conventional soxhlet continuous-extraction method. There has been no changing over to the more sophisticated or

automated instruments now available for the analysis of the three components mentioned above. This is mainly because the conventional methods used appear satisfactory, and the number of samples presently analysed do not justify the purchase of such more expensive instruments.

Ashing of the food is carried out in the muffle furnace to obtain the ash content, as well as for the determination of minerals.

Crude fibre analysis has been most unsatisfactory. The method used is the hot acid-alkali digestion procedure, which has been much criticized. We have yet to establish a suitable procedure that gives a more accurate estimation of the component of dietary fibre.

Carbohydrate content has been calculated after determination of the above mentioned components. Calorie content of the food is then calculated using the Atwater factors of 4, 4 and 9 for carbohydrate, protein and fat respectively. The calorimeter is available only to some of us, and is being used for determining energy content of specific foodstuffs.

Several areas that we have not been able to place greater attention are, for example, the determination of amino acid and fatty acid composition of foodstuffs. Some analysis are being carried out for selected foods, but we expect a greater coverage of these nutrients at a later stage of the programme. The component sugar and polysacharides of foodstuffs too have not been routinely estimated.

Vitamins

Vitamin analysis as a group had presented us with the most problems and difficulties. Much time has been spent on finding the best conditions and parameters to give us satis-

factory results. This has contributed a great deal towards slowing down the progress of the food analysis programme.

The method used for vitamin A (and carotene) analysis for animal foodstuffs is essentially that of the AOAC (1980), with some modifications by our laboratories. The food is first saponified and the vitamin A (and carotene) in the unsaponifiable fraction is then extracted into hexane. The extract is then chromatographed on a column of alumina. Carotenoids in the extract will be separated in the process and may be determined colorimetrically (at 436 nm) after elution from the column with 4% acetone in hexane. Vitamin A remaining in the column is next eluted using 15% acetone in hexane. After evaporation and making up in a suitable volume of alcohol, it is read at 325 nm in a UV spectrophotometer. We have experienced considerable problems with the procedure, especially with the recovery. Much time and chemicals have been used on looking into the reasons for the low recovery (<50%) we have always obtained for vitamin A, whereas carotene recovery has often been over 80%.

The method for the determination of carotene in fresh plant materials has also been modified from that of the AOAC (1980). Carotene is first extracted from the fruits and vegetables using a solvent mixture of acetone-hexane. Acetone is then removed from the extract by repeated washing with water. The extract, containing a variety of fat-soluble plant pigments besides carotene, is next chromatographed on a column of a mixture of activated magnesia and diatomaceous earth to separate out the carotene. The yellow coloured carotene eluted from the column using an acetone-hexane (1+9) mixture is then read in a spectrophotometer at 436 nm. After some initial problems, the procedure is now used with greater confidence and

better recovery values have been obtainable.

Thiamin and riboflavin have been determined by fluorimetric methods. An enzyme preparation potent in diastatic and phosphorolytic activity is first added to the food extract to liberate the bound forms of these B vitamins. For thiamin determination, the extract is then purified in a column of decalso. The thiamin eluted using potassium chloride solution is next treated with alkaline potassium ferricyanide to oxidise it to thiochrome. The so-formed thiochrome is extracted into isobutyl alcohol and its fluorescence measured in a fluorescence spectrometer. For the determination of riboflavin, interfering substances in the extract is first oxidised by potassium permanganate, and the resulting solution read in a fluorescence spectrometer. Both methods have been fairly well established and satisfactory recoveries have been obtainable, although the need to minimise the exposure of solutions to light, especially for riboflavin determination, has to be emphasized to the laboratory technologist. Especially for foodstuffs with low amounts of these vitamins, the use of a stable and sensitive instrument is imperative.

For the determination of niacin, the food is also subjected to an initial acid hydrolysis to free the vitamin. Niacin in the extract is reacted with cyanogen bromide to give a pyridinium compound, which then complexed with aromatic amine to produce coloured compounds, read at 450 nm in a spectrophotometer. Though relatively more stable to light and pH, the procedure also calls for careful techniques and a stable spectrophotometer since the timing of reading the coloured solution is important. The laboratory technologist has also to be fully aware of the toxic nature of the cyanogen bromide used.

The titration method using the oxidation-reduction indicator dye 2,6-dichlorophenolindophenol has been used for the determination of ascorbic acid in foods. Only the reduced form of the vitamin is being determined. The procedure has proved to be useful for a large variety of foodstuffs, and good recoveries have been obtained. Every effort however had to be taken to minimize vitamin C loss during the determination. For certain foodstuffs the end point of the titration has been difficult to ascertain, especially for foods yielding coloured solutions. Attempts have been made to overcome this problem encountered for coloured solutions by using extraction with diethyl-ether during titration. The fluorimetric method involving reaction of the ascorbic acid with phenylenediamine, as well as the 2,4-dinitrophenyl hydrazine method have recently been introduced. It is not yet possible to report on their uses.

Some work on vitamin analysis using HPLC has also been carried out. Determination of the water-soluble vitamins appear to be satisfactory although these were not run routinely on the instrument. The analysis of the fat-soluble vitamin (A, D, and E) in pharmaceuticals also did not present much problems. However with foodstuffs of animal origin, we have experienced some difficulties. This mainly involve the preliminary clean-up procedures, resulting in contaminating component(s) in the vitamin peak. Work is still being carried out to resolve the problem.

Other vitamins that have yet to be looked into at a later date are vitamins B12 and folates. These are vitamins of considerable nutritional significance in the country and should deserve some attention.

Minerals

After determining the total ash content of the food, the ash obtained is dissolved in nitric acid and made up to a suitable volume for mineral analysis. Only a few minerals have been determined routinely on all foodstuffs.

Calcium in the foods have been formerly determined by titrating against standard potassium permanganate, and iron is reacted with O-phenanthroline or dipyridyl and the coloured compound formed read in a spectrophotometer at 510 nm. The analysis of these minerals, and including sodium and potassium are now performed using an atomic absorption spectrometer with an air-acetylene flame system. Recoveries do vary somewhat, but this is felt to be due to the instrument which has been giving intermittent problems.

Phosphorus is determined in the ash solution using the vanadate-molybdate reagent to produce a yellow-orange complex which is measured in a spectrophotometer at 420 nm.

CONCLUSION

In food research and development, the importance of analytical techniques is beyond mention. Every food, to start with, must be analysed in terms of its composition. Proximate analysis is the basic information needed before further work can be carried out. Most research projects under ASEAN-Protein and ASEAN-FTRD involve analytical techniques. The authors are wondering whether a common technique for certain analysis can be established and agreed upon by ASEAN food scientists and technologists.

In preparing the food composition table, many methodological problems still exist. Other analysts in ASEAN are probably facing similar problems. In Malaysia, it was observed

that many reported or established methods do not include details which are important to the user. Many of these methods do not work satisfactorily without some modifications. Since a large variety of foodstuffs are involved, the conditions may have to be slightly different for each food. Hence, much time and chemicals have been spent by the analyst in developing countries, where such resources are scarce, in trying out, perfecting and adopting a reported method. Thus in a forum such as this, the analyst will have the opportunity to exchange experiences and learn to avoid the mistakes of the others.

It would also be worthwhile for this forum to consider the standardization of analytical techniques so as to ensure better compatibility and comparability of results. Such standardization does not necessarily mean the adoption of a single method for a particular nutrient. Nor does it necessitate the adoption of modern methods requiring the use of sophisticated instruments with high capital and maintenance costs. Standardization should therefore define the methods in use for a particular nutrient and identify and adopt those that give comparable results.

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