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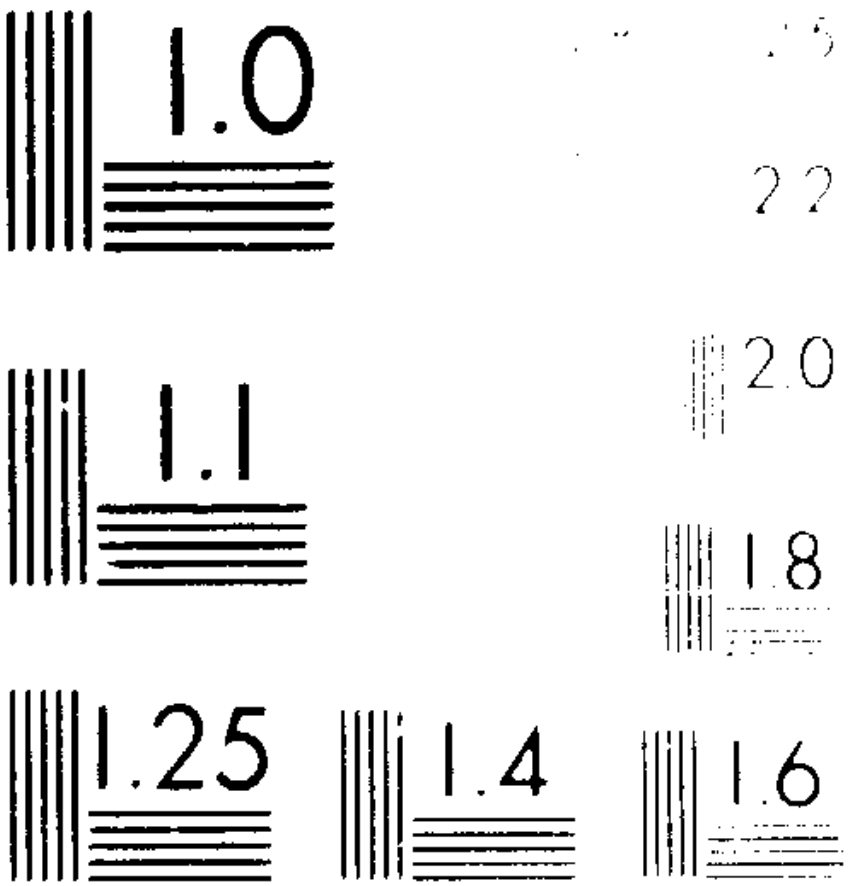
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# Tropical Products Institute

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FINAL REPORT ON AN ASSIGNMENT AT BALAI BESAR INDUSTRI HASIL PERTANIAN (FORMERLY THE CHEMICAL RESEARCH INSTITUTE), BOGOR, INDONESIA

September-October 1981

DP/INS/76/001

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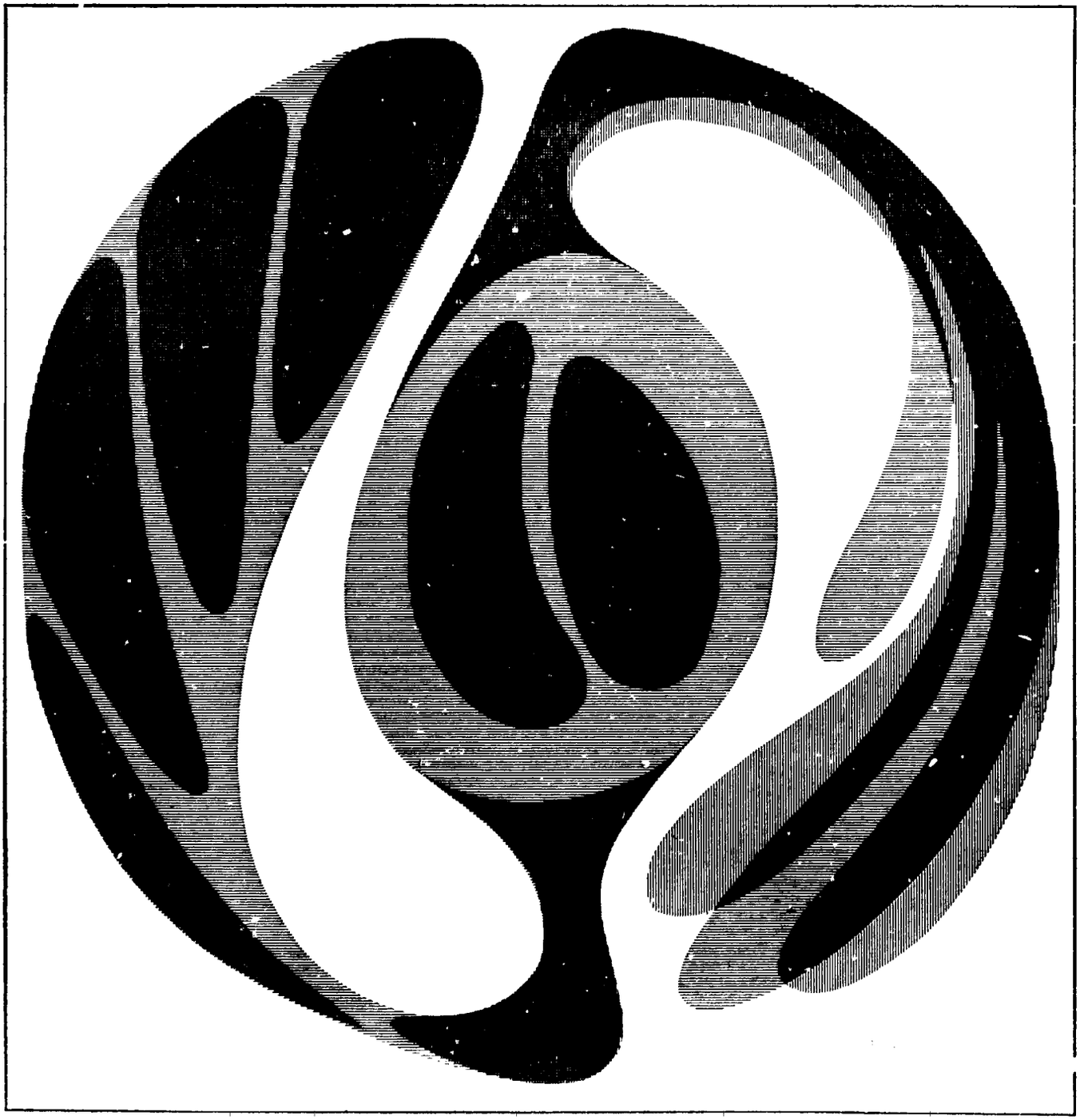

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R1025(C)

# FINAL REPORT ON AN ASSIGNMENT AT BALAI BESAR INDUSTRI HASIL PERTANIAN (FORMERLY THE CHEMICAL RESEARCH INSTITUTE), BOGOR, INDONESIA

September-October 1981

by G E HOWARD and JULIET BAINTON

TPI Contract No C72

Tropical Products Institute
Overseas Development Administration
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## 1. Summary and Recommendations

Two members of the staff of the Tropical Products Institute, namely Dr G E Howard and Mrs S J Bainton, experts in instrumental methods of analysis and general food analysis respectively, spent two months in Indonesia at the Balai Besar Industri Hasil Pertanian (formerly the Chemical Research Institute) on a training mission in accordance with UNIDO contract no. T81/04 with the Tropical Products Institute. Current research projects were used as vehicles for training in high performance liquid chromatography, gas chromatography, fluorimetry, atomic absorption spectrometry and amino-acid analysis.

A satisfactory course of training in each of these subjects was carried out and good progress was made towards achieving the objectives of the projects concerned. The advantages and disadvantages of this mode of training and of training by visiting experts are discussed.

During the course of our work we found that the work of BBIHP was being impeded by the lack of good library facilities and by difficulties in getting apparatus serviced and we make recommendations on these topics.

We attempted to ascertain the remaining need for staff training and we discuss this and make recommendations. The purchase of certain books and items of equipment is recommended.

### 1.2 Recommendations

#### 1.2.1 Training

T(1) Higher education and training overseas should be given to a small number of selected individuals who have the BSc Anal. Chem. and some graduates with more advanced first degrees should be supported in working for research degrees.

T(2) An expert in one or more of the most important groups of commodities should be provided for at least one year to improve the level of expertise in commodities other than essential oils.

T(3) Staff who have received specialist training overseas should train some of their colleagues as soon as possible after their return. This is especially necessary in the case of those who have been transferred to other work on their return from overseas.

1.2.2 Library

L(1) Improvements in the library are needed urgently. The advisory visit by the TPI Librarian which is under discussion should be expedited.

L(2) The organisation of a co-operative photo-copy service within Java should be considered and discussed during the visit mentioned in recommendation L(1) above.

L(3) Short term measures such as an arrangement with TPI for the supply of information should be discussed urgently and should be given such financial support as might be necessary.

L(4) Staff should be instructed in the effective use of the library.

L(5) Books listed in the appendix should be acquired as soon as possible.

1.2.3 Equipment

E(1) Industrial grade gasses supplied for use with the atomic absorption spectrophotometer and gas chromatograph are not nearly pure enough for these purposes. Pure gasses should be imported and an effective device for the purification of nitrogen should be purchased for the Instrumental Analysis Laboratory.

E(2) An amide bonded-phase HPLC column should be purchased for the analysis of sugars.

E(3) The apparatus listed in the appendix should be purchased.

E(4) A more adequate supply of common laboratory glassware and other simple apparatus is needed to permit staff to conduct more than one or two experiments simultaneously.

1.2.4 Miscellaneous

M(1) The organisation and planning of the purchase of consumable stores at laboratory level (eg chemicals and glassware) needs to be improved. At present there seem to be excessively large stocks of some items and no stocks of others. In view of the long delivery times experienced in Bogor and the inevitable financial constraints, this is a matter of considerable importance to the smooth running of the laboratory.

M(2) Log books should be kept for each instrument, especially the amino-acid analyser, gas chromatograph and atomic absorption spectrophotometer, as an aid to routine maintenance, replenishment of reagents and ordering of gasses, replacement lamps etc., in good time.

M(3) One person should be made responsible for laboratory "housekeeping" so that cupboards and drawers are kept tidy, water stills and distilled water containers are kept clean and adequate stocks of common apparatus and reagents are maintained.

## 2. Introduction

This assignment was undertaken by the authors, who are members of the staff of the Tropical Products Institute (TPI), in accordance with UNIDO contract No. T81/04 which required TPI to provide inter alia one expert in instrumental methods of analysis and one in general food analysis for a period of two calender months each exclusive of briefing an de-briefing in Vienna.

The purpose of the contract which formed part of UNDP project DP/INS/76/001 entitled Food Processing Research in the Chemical Research Institute Bogor, was to strengthen and upgrade the analytical and testing capabilities of the Chemical Research Institute for foodstuffs in general and processed foods in particular since their food analysis and quality control practices were in urgent need of modernisation. The contract required that a number of members of the staff of CRI should receive training at TPI and that the experts specified above should visit Indonesia to reinforce this training by guiding and working with some of the trainees on specific projects.

Several years elapsed between the conception of the project and its completion and a number of changes took place during this time. While most of the trainees were in Britain, the CRI was transformed into the Balai Besar Industri Hasil Pertanian (BBIHP) (Major Institute for Research and Development of Agro-based Industry) the structure of which is shown below.

BBIHP

| | | | |
|---|---|---|---|
| Inst. for Research in Food and Beverages | Inst. for Development of Food and Beverages | Inst. for Research in Chemurgy and Miscellaneous Industries | Inst. for Development of Chemurgy and Miscellaneous Industries |

This necessitated the promotion of one trainee to a senior position and the transfer of others to posts different from those held when their training began. Consequently, when our assignment began it was clear to us that it could not be undertaken in the manner envisaged originally. We raised this point during our briefing in Vienna and it was agreed that we should use our own judgement in consultation with the UNDP Project Leader and proceed as nearly as possible in accordance with the general intentions of the Project and of our contract. This was also discussed with Mr F M Iqbal the UNIDO Field Representative in Jakarta.

On our arrival at BBIHP in Bogor, we were met by Mr Salya Sait, Head of the Institute for Research in Chemurgy and Miscellaneous Industries, Mr G B Ciptadi, Head of the Institute for Development of Chemurgy and Miscellaneous Industries, and Dr Latif Rasulpuri, UNDP Project Leader. The Director of BBIHP, Dr Dardjo Somaatmadja, was absent on sick leave. At this meeting, a list of projects was presented to us as the basis of our programme.

We considered that it would be most beneficial if we were to work on projects that involved the use of techniques that could be applied to a wide range of projects. For that reason we selected the analysis of syrups which seemed to be suitable for high performance liquid chromatography (HPLC), the determination of thiamine in wheat flour which required the use of fluorimetry, the determination of calcium and iron in wheat flour which required atomic absorption spectrophotometry and the analysis of chocolate pastes which required the analysis of oils and fats by gas chromatography (GC). HPLC and GC would be dealt with by Dr Howard while spectrofluorimetry and atomic absorption spectrophotometry would be dealt with by Mrs Bainton.

It was decided some time before our arrival that we would not cover amino-acid analysis because the amino-acid analyser at BBIHP was out of order, obsolete and beyond repair, but when we arrived in the laboratories we found that an HPLC instrument specially equipped for amino-acid analysis had been delivered three months previously and work on bringing it into use had just begun. We decided therefore, Mrs Bainton should provide training in this technique in addition to the items mentioned above.

## 3. Training Programme

### 3.1 Analysis of Syrups

#### 3.1.1 High Performance Liquid Chromatography (HPLC)

In this context, syrups were fruit flavoured cordials with a very high sugar content. Some of these products were in fact sweetened entirely with synthetic sweeteners (saccharine and/or cyclamate) and carboxymethyl cellulose was added to increase the viscosity and thus to give the appearance of a high sugar content. Since other soft drinks were also sweetened with synthetic sweeteners, they were also within the scope of the project.

In order to show that a syrup is not what it purports to be, it is necessary to demonstrate the absence of sugar and the presence of thickener, to identify and estimate the sweetener and to identify the colouring matter. As ther is a comprehensive scheme for the analysis of food dyes in "Official Methods of Analysis of the AOAC" 13th ed. 1980 that topic was left aside, but no methods were given for the identification or determination of carboxymethy cellulose and the methods for cyclamate and saccharine were cumbersome. Consequently it was considered to be justifiable to develop an HPLC method for the sweeteners as a training exercise if none already existed. An attempt to carry out a literature search in the BBIHP library had to be abandoned and work was begun on the separation of saccharine and cyclamate. Meanwhile a literature search carried out at TPI at the authors' request revealed no previous work on the subject.

It was known that saccharine could be separated from other constituents of soft drinks that did not contain cyclamate, so the mobile phase and column used for that purpose were used as a starting point but saccharine could not be separated from cyclamate. Other mobile phases were selected on the basis of solvent strength, dipole moment, and proton donor and acceptor capacity. These concepts were explained as work proceeded. Only a partial separation was achieved so ion-pair chromatography was tried but without success. Two different columns were also tried.

At this point, it was decided that enough time had been spent on the separation and attention was transferred to the quantitative aspects of the analysis. On one occasion while quantitative tests were in progress, however, it was noticed that the degree of separation had increased when a new batch of mobile phase was brought into use. It seemed likely that the separation was extremely sensitive to small changes in the very small proportion of acid present in the mobile phase, so this was altered systematically in very small steps until a complete separation was achieved.

Since saccharine is fluorescent under ultra-violet light, the fluorescence detector seemed to offer a method for the estimation of saccharine in the presence of cyclamate without separating them chromatographically. Calibration experiments including tests for possible quenching by cyclamate were carried out but it was found that the fluorescence of saccharine was too weak to be easily used for the analysis of soft drinks.

It was noticed in earlier experiments that the response of the ultra-violet detector to cyclamate was very much weaker than it was to saccharine and the refractive index detector was adopted for use in the qualitative work. This difference in response would make it possible to determine one sweetener in the presence of the other, even if they could not be separated by using the ultra-violet and refractive index detectors together. The mathematics of this use of two detectors was explained and formulae were calculated. The cyclamate available when work began was known to be impure and a pure specimen was obtained in order to calibrate the detectors. It was then found that the apparent response of the ultra-violet detector to cyclamate had been due entirely to the impurities. This simplified the mathematics of the differential use of two detectors but at this point a complete separation of cyclamate from saccharine was achieved which made this approach unnecessary.

Since soft drinks often contain caffein as a stimulant and benzoate as a preservative, exercises in determining these compounds together with saccharine were also carried out.

3.1.2 While training in HPLC was in progress, an investigation into the properties of carboxymethyl cellulose was carried out. It was found that solutions containing 1.5 per cent were far too viscous for use as syrups but at that concentration the refractive index and specific gravity of the solutions were similar to those of sugar solutions of similar concentration but negligible viscosity. It is clear, therefore, that the presence of carboxymethyl cellulose would not give seriously misleading results in the determination of sugars by either refractive index or specific gravity. Since saccharine and cyclamate are used at concentrations of less than 0.5 per cent, they would also make a negligible contribution to refractive index and specific gravity.

The thin-layer chromatography method for non-nutritive sweeteners given in "Official Methods of Analysis of the AOAC" begins with a series of solvent extractions to separate sweeteners from dyes, sugars and other flavouring constituents. This seemed equally suitable as a prelude to HPLC and was adopted for the analysis of some purchased samples.

A syrup and three soft drinks were analysed by the procedure developed during this work. The syrup contained 0.3 per cent of saccharine and a diet drink contained 0.06 per cent. Cyclamate was absent from both of these samples and the other two contained no synthetic sweeteners.

It is known that some dietetic drinks are sweetened with appreciable amounts of sorbitol which would interfere with the determination of sugar by the refractive index and specific gravity methods. If BBIHP wishes to detect and/or determine sorbitol it will be necessary for them to purchase an "amide" bonded-phase column for the purpose.

3.2 Amino Acid Analysis by High Performance Liquid Chromatography

3.2.1 Equipment: Shimadzu LC-3A with Fluorescence Detector FLD-1.

The HPLC system was recently by the Shimadzu engineer who gave Ms Ingrianni one day of training. This appears to have been very superficial and because of this she had very little depth of understanding of the instrument and was understandably running into difficulties for which she was not responsible. There are difficulties inherent in any of the current methods of amino acid analysis whether it is the more usual liquid chromatography, gas chromatography or high performance liquid chromatography, the latter still being relatively new and having more technical difficulties than the others. The instructor found it necessary to undertake a considerable amount of practical work on this project herself and by the end of the two month period the major difficulties had been overcome and the instrument was producing fairly reliable responses. Sample analysis had been demonstrated and a complete assay procedure and specific notes are included in the appendix 4. Although the equipment is functioning adequately, time must now be alloted to testing it's reproducibility and accuracy, initially with standard solutions and then with samples. Little time was available for instruction in sample preparation, and the estimation of cystine and available lysine were not covered.

Mr Janaka, who was trained at TPI earlier this year, has had experience with these methods and it would be most beneficial if he were to use this aspect of his amino acid analysis training to instruct Ms Ingrianni and perhaps to undertake a joint project to analyse amino acids in foods and/or feeds.

3.3 Analysis of Chocolate Paste

The purpose of this project was to devise a method for the analysis of chocolate spreads in order to determine the identity of the fats used in them. It was selected because it clearly provided a good vehicle for training in both gas chromatography and the analysis of oils and fats which is usually accomplished by converting the glycerides of which fats are composed, into the corresponding methyl esters which are then analysed by gas chromatography.

Practical training in the technique of gas chromatography was begun with the preparation of column packing materials, packing columns by two methods and conditioning them for use. The reasons for the procedures demonstrated were explained and the consequences of departing from them were described. The procedure for determining the optimum conditions for any given separation were demonstrated using the analysis of cocoa butter as an example.

Training in the analysis of oils and fats began with practice in the preparation of methyl esters of fatty acids from coconut oil and cocoa butter using the standard methods prescribed by the Union of Pure and Applied Chemistry.

When this initial training had been completed successfully, samples of commercial cocoa butter and cocoa butter prepared in the laboratory by solvent extraction of cocoa beans were analysed. It was considered that the most likely adulterant of cocoa butter would be tengkawa oil (Shorea spp), also known as illipe oil and Borneo tallow, which has a fatty acid composition similar to that of cocoa butter. An authentic specimen of this oil was not available so one was prepared by extraction of tengkawa seeds and it was found to have the composition expected. Because of the similarity in the composition of the two oils there is no satisfactory method for the detection of Shorea oils in cocoa butter.

While this work was in progress, the fatty constituents of three brands of chocolate spreads (one imported and two Indonesian) were extracted and analysed. It was immediately obvious that the imported paste contained mainly coconut oil while the Indonesian products appeared to contain mainly cocoa butter (or perhaps tengkawa oil) with some coconut oil.

Since the adulteration of coconut oil with palm oil is also a matter of concern in Indonesia, mixtures of these two oils were prepared and analysed to show how the degree of adulteration can be determined quantitatively.

This work was seriously delayed by the extremely rapid deterioration of the column packings caused by presence of impurities in the carrier gases, nitrogen and helium, in spite of the use of molecular sieves to purify them. This demonstrated the need for the purchase of an effective gas purification system such as the "Supelco" furnace. It also rendered it impossible to continue to use most suitable stationary phase, diethyleneglycol succinate, and we were compelled to use the less satisfactory, but more stable, Apiezon L which could not separate all of the constituents of the oils in question. Fortunately, the separation of the major constituents was sufficient to permit the demonstration of methods for the calculation of the quantity of one oil mixed with another and an explanation of the limitations of such calculations.

3.4 Thiamine Content of Wheat Flour by Spectrofluorimetry

3.4.1 Equipment: Turner Spectrofluorometer Model 430

The instrument was not being used routinely, primarily because the operating instructions in the handbook were almost incomprehensible and gave little explanation of the mode of operation. It had also developed a fault which needed attention by the agent in Jakarta. This was rectified and the instrument was succesfully calibrated.

The thiamine determination was carried out by the method described in Appendix (6). The method suffers from inherent difficulties arising from the chemical properties of the substances involved and the greater part of the training period was spent on overcoming these problems. Most of them were successfully rectified but work still needs to be continued in order to improve the reproducibility and reliability of the results. It is essential that the estimation should be carried out immediately after the completion of the sample preparation. Consequently, samples can not be prepared by one section to be run on the fluorimeter by the Instrumental Analysis Section some time later. Once reproducible results have been achieved, surveys of a wider range of commodities could be initiated as

well as commencing Vitamin C determination, also by spectrofluorimetry. Ms Sumarsi is due to visit TPI in the near future, to train in vitamin analysis and this initial work in Bogor has been extremely useful as a preparation for this as well as forming part of her overall training. It would be beneficial if she could continue working on vitamin analysis in order to have as much experience as possible before coming to London.

3.5 Calcium and Iron Determination by Atomic Absorption Spectrophotometry

3.5.1 Equipment: Varian Techtron Model 1000

The instrument was in working order and was being used routinely but the calcium lamp necessary for this project was not available at the beginning of the training period.

The trainees already knew how to operate the instrument so that training was devoted to improving their existing techniques rather than instruction in a completely new procedure. There were two aspects to this (1) optimising and speeding-up the sample preparation and (2) improving the technique for using the spectrophotometer.

(1) The preparation of samples involves ashing at a high temperature (550°C) in a muffle furnace and dissolving the resulting ash in dilute acid. Previously this had taken up to three working days, partly because it was thought essential to heat the sample until the ash was completely white and partly because it was considered to be unwise to leave the furnaces on overnight because of the fire risk. There are two Indonesian standards of which (SP-SMP57-1975), after experimentation, seemed more suitable than either the other Indonesian standard or the method used at TPI.

(2) For the determination of some elements, such as calcium using an air/acetylene flame the sensitivity was only adequate. One reason for this was the basic model of the spectrophotometer and the other was the industrial grade of gasses used. Normally nitrous oxide/acetylene would be used in this situation but this was considered to be dangerous since the instrument had no safety devices. However, optimisation and calibration of the instrument were carried out although at the low sensitivity it was merely a demonstration and would have to be repeated when purer gases were supplied.

It would be worth considering the purchase of a modern Atomic Absorption Spectrophotometer incorporating automatic safety devices and with greater sensitivity for trace metal analysis.

4. Evaluation of Training Methods

The training of laboratory staff in analytical techniques or in dealing with groups of commodities can be carried out either in a suitable institution overseas, such as TPI, or by visiting experts. Overseas training has the advantage that in addition to the specific training it also demonstrates the high standards set in well-run laboratories. It is also possible to mount a more intensive course of training in such laboratories than would be the case in less advanced laboratories. There is, however, a serious disadvantage in that trainees are taught how to do things in the absence of the difficulties that exist in their home laboratories. They might even be taught methods that cannot be used at home because they lack facilities that are taken for granted elsewhere. Trainees are often unaware of the need for improvements in long-standing practices until the matter is brought to their attention by visitors and even then they are sometimes reluctant to do anything about it.

The experience of working overseas should also discourage experts from advocating the use of equipment that cannot be maintained properly in the country concerned.

As there are marked advantages in both methods of training, we consider that overseas training coupled with visits by foreign experts should be arranged whenever possible.

In the present instance, our presence in the BBIHP laboratories enabled us to give simultaneous training to seven people working on four different projects and using five different instrumental techniques. We were able to advise on incidental matters such as the purity of compressed gasses, the storage of gas cylinders, the presence of algae in distilled water, and the purchase of relatively minor pieces of apparatus. We were also able to assist in overcoming teething troubles peculiar to a particular model of HPLC which we do not have in our own laboratories. If we had not worked for some time at BBIHP we would not have become aware of certain serious difficulties which we discuss later in this report.

The original intention of the UNDP project was that we should assist people who had been trained at TPI to apply their training to specific research projects, but for reasons explained in the Introduction, this was not possible although one of the trainees on the HPLC project had been well trained elsewhere in the operation of that instrument. In order to adhere as closely as possible to the original intention of the

project and to conform with the wishes of BBIHP we used reseach projects as vehicles for basic training in certain techniques and as a result of this experience we consider that this method of training has both advantages and disadvantages the balance of which depends entirely upon the nature of the project.

The principal advantage is that the trainee learns something of the expert's approach to problem-solving while undergoing basic training. A further advantage is that the training is directly related to a genuine problem rather than to artificial exercises of little intrinsic interest. Training on a project also gives some experience in the commodities concerned.

The disadvantage is that the training tends to be biased towards those aspects of the technique that are applicable to the project and it is possible that some equally important aspects of the technique could be neglected. Experts who are experienced intructors will avoid this extreme by diverging from the project if necessary but the project might still be less satisfactory than a specially designed demonstration as a means of illustrating some effects.

These disadvantages will not arise in every case, and they will not always be severe when they do occur, especially if the instructor is aware of them. Consequently when time is short we consider that the advantages predominate, especially if the trainee continues to work on the same project or on the same group of commodities for some time after training ends.

5. Requirements for Further Training

Most of the more experienced members of the staff who have a formal qualification, have the BSc in Analytical Chemistry awarded by the Academy of Analytical Chemistry in Bogor which is not recognised as a sufficient qualification for direct entry to a higher degree course. We came to the conclusion that in spite of the good potential of some individuals this relatively weak academic background limited their ability to plan and execute research projects without a good deal of supervision which at present can be provided only by the heads of the constituent institutes of BBIHP in addition to their administrative duties.

The Director is well aware of this weakness at middle management level and has a long term plan to obtain further education for suitable members of his staff and is supplementing this by recruiting graduates at Doctorandum and Ingeneur levels, but these people will not be ready to lead research teams for some years after graduation. We recommend, therefore, that funds should be provided for the further training of selected individuals with the BSc Anal. Chem. to fill this gap and that support should be provided for a small number of Doctorandum graduates to proceed to higher degrees.

Training in the present programme has been concentrated quite properly on the improvement of analytical procedures, but we consider that there is a marked lack of expertise in commodities other than essential oils in which BBIHP has considerable expert knowledge.

The small size of BBIHP makes it inevitable that each member of the staff will have to cover a wider range of commodities than one would wish. Expertise in a commodity area is acquired largely by extensive reading accompanied by practical experience under the guidance of an expert over a period of several years. It is difficult, therefore, to teach much more than quality control in a short period of training such as might be suitable for teaching an analytical technique. We consider that the best way of transferring this kind of expertise is by the presence of a visiting expert for a period of at least a year. We recommend therefore that the most important areas of work should be identified by BBIHP and an expert recruited.

6. Other Requirements

In the course of our work we realised that BBIHP, like many other institutes in non-industrialised countries, labours under certain disabilities, none of which can be remedied quickly. The ones that affected our own work directly where the inadequacy of the library and the difficulty of maintaining the more complicated scientific instruments.

6.1 Library

We found that no survey of the literature concerning the projects had been undertaken. The two trainees alloted to the analysis of syrups were shown how to use Chemical Abstracts but we found that this journal had not been received since 1975, none of the volumes received since 1966 had been bound and some of the unbound issues could not be found. Consequently, our attempt to obtain information about earlier work relevant to our project had to be abandoned.

There are few scientific journals in the library, the runs are short and often incomplete. A very large proportion of the text-books and reference books are out of date and there are few recent books other than those on food processing provided by UNIDO. The library was poorly organised although some improvements were made during our visit.

The Director is well aware of the deficiencies of the library and arrangements are being made to obtain training in Britain for the librarian and an advisory visit by the librarian of TPI has been proposed, but the high cost of bringing the library up to date and of maintaining it at a high standard in perpetuity will certainly prevent any rapid improvement.

Until such time as they do have easy access to a good library, there is a risk that the staff will waste their time by working on problems to which there are published solutions and by using out of date methods. Adequate project formulation and planning will also be impossible. Short term arrangements for providing information are therefore needed urgently.

We are informed that scientific libraries exist in Bandung and Jakarta but the time and expense that would be involved in BBIHP staff visiting these libraries frequently would be prohibitive. If BBIHP were provided with sufficient abstract journals and reference books, a loan or photocopy service provided by these libraries would go some way towards meeting their requirements.

Even this would take time to organise and the information needs of BBIHP are urgent. We recommend that the possibility of obtaining assistance with information retrieval from TPI should be explored. Under such a scheme, literature searches would be undertaken for BBIHP by TPI scientific staff, using TPI library, and relevant references and photocopies would be provided. Under present economic conditions financial support from a body such as UNIDO might be necessary if the arrangement were to involve a substantial amount of work.

The inadequacy of the library, coupled with the fact that the books are inevitably in a foreign language discourages the use of the few really useful books that they have. Consequently, at least some members of the staff do not appreciate the value of a library and do not know how to make full use of one. The use of a library should feature in future training programmes and could well form part of the duties of a visiting expert.

We recommend that the books listed in the appendix should be purchased immediately.

6.2 Equipment

BBIHP is well equipped with modern analytical instruments but too many of them are out of action for months at a time, some have been virtually written off, because of the bad service provided in Java by the manufacturers and/or their agents. This is probably due in part to a shortage of good service engineers, but the purchase of spare parts that can be obtained in Britain within a few days takes months in Bogor and the prices paid are more than twice those quoted in Europe.

BBIHP does try to assess the effectiveness of local agents when considering the purchase of instruments but agencies change hands and frequently deteriorate. We recommend that in selecting instruments they should favour those with no unnecessary refinements. For example a meter (ie a needle and dial) is usually more reliable than a digital readout and manually-operated valves are less likely to fail than electronically controlled ones. There is obviously no simple remedy for this state of affairs but we recommend that the staff should be much more persistent in making complaints which should be addressed not only to the agents but also to the head offices of the manufacturers concerned.

Our work was also delayed by the poor quality of the compressed gasses available in BBIHP. Gasses of good quality can be imported and if they make the difference between success and failure they are well worth while since the quantities used are not large.

We recommend that pure acetylene should be imported for the atomic absorption spectrophotometer since it cannot easily be purified. Nitrogen and helium for the gas chromatograph can be purified by means of a "Supelco furnace" such as is already in use in the Essential Oils Laboratory and we recommend that one should be purchased for the Instrumental Analysis Laboratory. Until this is done we recommend oxygen-free nitrogen should be imported.

7. General Conclusions

It seemed likely, for reasons given in the Introduction, that we would be compelled to depart from the original purpose of our visit but in the event we were able to devise and execute a useful programme of training that was entirely in the spirit of the original plan, albeit with different trainees.

The programme suggested to us by BBIHP was too ambitious but the reduced version that we adopted was well suited to the time available. We were able to cover all of the important features of the techniques in which instruction was given and useful progress was made in the projects used for instruction.

The fact that our trainees were not those who had been trained previously at TPI as originally intended, was counteracted to some extent by the fact that the trainee primarily concerned with HPLC had attended a comprehensive course on the operation of the instrument given by the manufacturer of the apparatus, one of the others had an elementary knowledge of the atomic absorbtion spectrophotometer and one of the former TPI trainees had given some instruction in the operation of the gas chromatograph to those who were to work on that instrument.

The trainees varied greatly in their facility in English but at least one member of each group had an adequate knowledge of the language. We always found it possible to make ourselves understood, although care was needed to ensure that the words used were known to the trainees.

Since most of the people trained at TPI are not now engaged in the work for which they were trained, we recommend that they should now train their successors to minimise loss of expertise in those techniques.

We consider that the training of staff overseas followed by visits from foreign experts has considerable advantages over either method used alone and should be continued as necessary. Some progress has now been made towards satisfying the needs of BBIHP for training in analytical methods, but there is a need for greater expertise in some other areas including major commodities other than essential oils. The areas of expertise that are likely to be most useful in the future should be identified and put in order of priority so that a definite programme of training can be drawn up in harmony with the long-term objectives of BBIHP.

If BBIHP is to achieve its full potential, however, staff training must be accompanied by major improvements in the library facilties.

## 8. Persons Contacted in Connection with the Project

### 8.1 UNIDO

Vienna - Mr Gardellin (Contracts Branch)
Mr Ghelardoni
Mr Ross (Finance Officer)
Mr Konstantinov (Project Officer)

Jakarta - Mr Rana (UNDP Resident Representative)
Mr Iqbal (UNIDO Field Representative)

Bogor - Dr Rasulpuri (UNIDO Project Manager)

### 8.2 BBIHP

Dr Dardjo Somaatmadja (Director)

Mr Salya Sait (Head of Institute for Research in Chemurgy and Miscellaneous Indstries)

Mr G B Ciptadi (Head of Institute for Development of Chemurgy and Miscellaneous Industries)

Mrs Djoearni Ali (Head of Institute for Research in Food and Phytochemistry)

Mrs Atti Hermann (Head of Institute for Development of Food and Phytochemistry)

#### 8.2.2 Trainees

| | |
|---|---|
| Mr M Slamut P | (HPLC) |
| Mr M Junus | (HPLC) |
| Mr Dedi Mahdar | (Gas Chromatography of Oils and Fats) |
| Miss Nia S | (Gas Chromatography of Oils and Fats) |
| Miss Ingrianni S | (Amino-acid Analysis and Atomic Absorption Spectrophotometry) |
| Mrs N Sumarsi | (Determination of Thiamine by Fluorimetry) |
| Miss Rochimah | (Atomic Absorption Spectrophotometry) |

## 9. Acknowledgements

We wish to express our gratitude to Dr Dardjo, Mr Salya, Mrs Djoearni and all of their colleagues at BBIHP who did so much to make our stay in Indonesia both pleasant and productive.

We thank Dr Latif Rasulpuri, UNIDO Project Manager, for his assistance, advice and support throughout our assignment.

We also thank our colleague Mr A Dann whose advice and liaison with BBIHP together with the preliminary visit by Dr H Parr were extremely helpful in preparing for our visit.

APPENDIX 1

Books suggested for immediate purchase

1) A.J. MacLeod, "Instrumental Methods of Food Analysis", 1973
Paul Elek (Scientific Books) Ltd., London.

2) R. Lees, "Food Analysis: Analytical and Quality Control Methods for the Food Manufacturer and Buyer", 1975
Leonard Hill Books, London.

3) L.R. Snyder and J.J. Kirkland, "Introduction to Modern Liquid Chromatography" 2nd edition. John Wiley and Sons Inc. New York, Chichester, Brisbane and Toronto, 1979.

4) H.P. Burchfield and E.E. Storrs, "Biochemical Applications of Gas Chromatography", Academic Press, New York and London, 1962.

APPENDIX 2

Equipment

We consider that the work of BBIHP would be facilitated by the purchase of the following items of equipment.

1) All-glass water still for the Instrumental Analysis Laboratory where a supply of high grade distilled water is essential.

2) Water de-ioniser. De-ionised water (as distinct from distilled water) is required for amino-acid analysis, atomic absorbtion spectrophotometry and fluorimetry. Consequently, if the existing de-ioniser cannot be restored to working order, a new one should be obtained making sure that cartridges of ion-exchange resin, batteries and any other consumable items for the model chosen are readily available in Indonesia.

3) A top-pan balance capable of weighing to two decimal places. At present the only top-pan balance available to the Instrumental Analysis Laboratory is kept in another laboratory some considerable distance away.

4) One analytical balance weighing to at least four decimal places. The balance used by staff of the Instrumental Laboratory is in another laboratory and it is bad practice to carry accurately weighed substances for long distances before they are used.

5) Heating mantle block, 6 flask capacity. The even heat provided by a mantle is essential for some purposes such as the hydrolysis of proteins for amino-acid analysis. Since amino-acid analysis and many other determinations require samples to be heated for many hours it is absolutely necessary that several samples and their duplicates should be processed simultaneously if gross waste of time is to be avoided. Consequently, at least one block of six mantles are required.

6) Accessories to permit the use of small tubes in the "Heraeus" centrifuge. If such accessories are not available, a small bench-top centrifuge capable of speeds up to 5000 rpm would be useful.

7) pH Meter reading to two decimal places. The existing pH meter is accurate to only one decimal place which is adequate for most purposes, but if maximum reproducibility is to be obtained in amino-acid analysis the pH of buffers must be adjusted to the second decimal place.

8) There is a shortage of glassware and other small items of apparatus such as retort stands, clamps and rings. Consequently experiments that should be carried out simultaneously are done consecutively which is a waste of time. Experiments are interrupted to wash individual flasks or test tubes, and dangerously chipped or cracked glassware is retained in use long after it should have been thrown away.

APPENDIX 3

## Analysis of Syrups

### Physical Properties

The refractive index, specific gravity and viscosity (Redwood) of a series of solutions of sugar and carboxymethyl cellulose (CMC) were determined at 27°C with the following results.

| | Concentration % | Refractive Index | Specific gravity | Viscosity |
|---|---|---|---|---|
| | 5 | 1.3397 | 1.017 | 34.5 |
| | 10 | 1.3472 | 1.037 | 35.5 |
| sugar | 15 | 1.3551 | 1.056 | 35.7 |
| | 25 | 1.3717 | 1.102 | 38.8 |
| | 0.5 | 1.3334 | 1.001 | 80.7 |
| CMC | 1.0 | 1.3337 | 1.002 | 372 |
| | 1.5 | 1.3343 | 1.008 | - |

The viscosity of the solution containing 0.5 per cent CMC was more than twice that of a 25 per cent sugar syrup while its refractive index and specific gravity are hardly distinguishable from those of water. The viscosity of 1.5 per cent CMC was too high to be determined. It is clear, therefore, that the presence of a useable quantity of CMC will not interfere with the determination of sugar in the field by means of refractive index or specific gravity as determined by a sugar hydrometer.

The refractive index of solutions containing 0.5 per cent CMC and 0.8 per cent cyclamate, which is much higher than would be used in practice, was only 1.3339 at 28.5°C, while the refractive index of a solution containing 0.2 per cent of CMC together with 0.2 per cent of saccharine was 1.3336. It is evident therefore that a "syrup" thickened with CMC and sweetened with saccharine and/or cyclamate could be detected immediately by means of refractive index or specific gravity.

### HPLC

It was found that saccharine and cyclamate could be separated from each other and from benzoic acid and caffeine by HPLC on a C 18 bonded-phase column using a mixture of methanol, water and perchloric acid (30:70;0.5) as the mobile phase.

Calibration curves for saccharine using fluorescence and ultra-violet absorption detectors were prepared as training exercises, but as saccharine and cyclamate have now been separated from each other these are no longer needed. Work will now be concentrated on the use of the refractive index detector.

The sweeteners were extracted from the samples before chromatographic analysis by the method described in "Official Methods of Analysis of the AOAC" 13th ed. 1980 section 20.159 in order to protect the column from contamination. Unfortunately, the extraction process proved to be very slow because of a marked tendency to form emulsions. It is suggested, therefore, that experiments should be carried out with "SepPak" cartridges which will probably remove the sweeteners from the solution together with preservatives and fruit flavourings all of which could then be recovered from the cartridge with methanol or other suitable solvent. It is possible, however, that the other constituents will produce peaks that will interfere with the determination of the sweeteners.

If the experiments with "SepPak" are not successful, it might be possible to prevent the formation of persistent emulsions by diluting the samples heavily. It might also be possible to overcome the problem by clarifying the solutions with lead acetate as described in "Official Methods of Analysis of the AOAC" section 31.021.

Whatever method of sample preparation is finally adopted, it will be necessary to determine the recovery of the sweeteners or other substances that are to be determined by "spiking". That is to say, by the analysis of samples to which known amounts of sweetener etc have been added.

APPENDIX 4

## AMINO ACID ANALYSIS

### Acid Hydrolysis Method For Amino Acid Analysis

**Reagents:**

a. Hydrochloric acid, 6N

- in a fume cupboard and wearing gloves and eye protection, carefully add 534ml of conc. HCl (sp.gr. 1.18) to 400ml of de-ionised water and dilute to 1 litre.

b. Buffer solution, pH 2.2

- dissolve 21g of Citric acid 'AR' and 8.4g of NaOH 'AR' in de-ionized water. Add 16ml of conc. HCl (sp. gr. 1.18) and dilute to 1 litre. Adjust the pH to 2.2 $\pm$ 0.01 with the aid of a pH meter. Filter the solution through a sintered-glass funnel under slight vacuum.

**Procedure**

a. Defat the sample using petroleum spirit or use the material remaining from a previous fat analysis if available. Grind the sample to pass a B.S. 60 mesh sieve (250 mic.) and allow the moisture content to equilibrate with the air. Carry out a Kjeldahl nitrogen determination on this sample if the results of amino acid analysis are to be expressed as g per 16g of nitrogen. Both these methods are described.

b. Weigh accurately, approximately 100-150mg of the prepared sample into a 250ml round, flat-bottomed flask. Add 170ml of 6N hydrochloric acid and a few anti-bumping granules.

c. Connect the flask to a "Quickfit" CX7/05 condenser and reflux for 24 hours (in a fume cupboard) using an electric heating mantle.

d. Wash down the condenser with a little de-ionised water and allow to cool. Transfer the hydrolysate to a 250ml volumetric flask and dilute to the mark. Mix well.

e. Filter about 75ml through a Whatman No. 40 filter paper. Pipette a 5.0mL aliquot of the filtrate into a rotary evaporator flask and evaporate to dryness while rotating the flask in a water bath at 40°C. Add a little water and evaporate to dryness again, to remove residual HCl.

f. Pipette a quantity of pH 2.2 buffer solution to the residue according to the nitrogen content of the sample, thus:

| % Nitrogen | Volume of pH 2.2 buffer |
|---|---|
| 1 - 3 | 5.0ml |
| 4 - 6 | 10.0ml |
| 7 - 16 | 15.0ml |

Swirl the flask to dissolve the residue and use the resultant solution to determine amino acids by loading a suitable aliquot directly onto the column.

REFERENCES


1. 'Amino Acid Determination' - Methods and Techniques by S. Blackburn, 1968. Edward Arnold Ltd., London.

2. 'Techniques in Amino Acid Analysis', Technicon International, Division S.A., Geneva, Switzerland.

3. 'New Detection and Separation Method for Amino Acids by HPLC', Y. Ishida et al, J. Chromat., 1981, 204, 143.

4. 'Basic Considerations in HPLC', International Laboratory, 1980, May/June.

## THE DETERMINATION OF OIL AND FAT

### Principle:

The sample is extracted with petroleum spirit, the solvent is distilled off and the extract dried and weighed. The method is not suitable for samples containing milk powder.

### Reagents:

Petroleum spirit, boiling point 40–60°C.

### Hazard Warning:

Petroleum spirit - highly flammable, toxic, avoid breathing vapour, avoid contact with skin and eyes. Carry out steps (d)-(f) in a fume cupboard and wear gloves and mask.

### Procedure:

a) Weigh accurately, approximately 10g of sample into 30 x 80mm extraction thimble, using a beaker as support.

b) Press a small ball of cotton wool or glass wool into the top to prevent loss of sample. Insert the thimble into a "Quickfit" plain-body extractor (EX 7/23 + EX 13/26).

c) Weigh accurately, a 250ml round, flat-bottomed, "Quickfit" flask (FF 250/35) containing three anti-bumping granules.

d) Pour 80ml of petroleum spirit into the 250ml flask and assemble the apparatus.

e) Connect to "Quickfit" condenser CX 5/23 with adaptor DA37 and reflux on a waterbath for 3 hours.

f) Disconnect the apparatus and allow the solvent to evaporate (or collect if required for re-use).

g) Heat the flask containing the oil/fat for 30 minutes in an oven at 103°C.

h) Cool to room temperature in a desiccator and weigh accurately.

i) Retain the defatted material for amino acid analysis.

## THE DETERMINATION OF NITROGEN AND CRUDE PROTEIN BY MACRO KJELDAHL

Principle:

The sample is digested in boiling sulphuric acid to break down organic matter and reduce nitrogenous compounds to ammonia. Ammonia is liberated by boiling with sodium hydroxide and steam distilled into boric acid and determined titrimetrically.

Before the digestion, tablets containing potassium sulphate or sodium sulphate and mercuric oxide are added. This raises the temperature of the reaction, while the mercuric oxide acts as a catalyst. Other catalysts are sometimes used, such as copper sulphate and selenium, but mercuric oxide is more efficient. This method does not give complete recovery of nitrates and so a modified Kjeldahl method is used for samples containing significant amounts of nitrate.

As the digestion proceeds, some of the sulphuric acid is reduced to sulphur dioxide which in turn reduces the nitrogenous material to ammonia. The ammonia combines with sulphuric acid to form ammonium sulphate.

An aliquot of the diluted digest is reacted with either sodium thiosulphate or sodium sulphide and then sodium hydroxide. The ammonia released is steam distilled, condensed and absorbed into a solution of boric acid to form ammonium borate. The ammonia is then titrated with standardised sulphuric acid (the ammonia can be titrated directly because the boric acid is too weak an acid to affect the hydrogen ion concentration). The temperature of the boric acid solution must not be allowed to rise above 50°C as ammonium borate is volatile.

In some methods, the ammonia is absorbed into a standardised acid solution and back-titrated with standardised sodium hydroxide. The use of a boric acid solution saves time as only one standardised solution is required. Also the boric acid need only be prepared and measured out approximately.

Sodium thiosulphate or sulphide is added to the distillation to break down a mercury/ammonium complex formed during the digestion. Sodium thiosulphate is preferred as it is pleasanter to use and is available in tablet form.

Reagents:

1. Catalyst tablets
   - Kjeltabs AOAC each tablet containing 5g of potassium sulphate ($K_2SO_4$) and 0.35g of mercuric oxide ($H_2O$). Use two tablets per determination. See Note 1.

2. Sulphuric acid
   - $H_2SO_4$, concentrated (98% w/w, d = 1.84).

3. Sodium thiosulphate
   - Thiotabs, each tablet containing 5g of $Na_2S_2O_3$. See Note 1. Use 1 tablet per determination.

4. Boric acid
   - 2% w/v aqueous solution of $H_2BO_3$, A.R.

5. Sulphuric acid
   - 0.1N (0.05M) solution (containing 10ppm mercuric chloride as a preservative). Standardise by titrating 25.0ml with 0.1N anhydrous sodium carbonate $Na_2CO_3$ (5.300g to 1 litre) using methyl orange indicator, (red/acid - yellow/alkaline).

6. Indicator soln.
   - Screened methyl red. Mix 20ml of 0.05% w/v methyl red solution in 80% methanol, with 4ml of 0.2% w/v methylene blue solution in water. The mixed indicator solution is stable for 1 month.

7. Sodium hydroxide
   - 50% w/v aqueous solution. Dissolve 500g NaOH in water (in a fume cupboard), cool and make up to 1 litre.

8. Paraffin wax
   - Add 0.5g to each digestion to stop frothing only if required. Carry out a blank determination also.

9. Ammonium - nitrogen
   - Standard solution. Dry ammonium sulphate at 103°C for 1 hour and cool in a desiccator. Dissolve 1.321g in water and dilute to 1 litre. (See note 2).

10. Anti-bumping granules

11. Sucrose, A.R.

- 2g can be used in place of the sample to carry out blank determinations.

12. Tryptophan

- Alternative standard, 13.37% N. (See note 2).

Hazard warning:

Mercuric oxide - Toxic, handle the catalyst and tablets with tongs which should be kept specifically for this purpose.

Sulphuric acid - Corrosive, causes severe burns. Wear gloves and face/eye protection,

Sodium hydroxide - Corrosive, causes severe burns. Wear gloves and face/eye protection.

Procedure

a) Accurately weigh approximately 2g of sample onto a 9cm filter paper, fold the paper carefully to contain the sample and transfer to a 300-500ml Kjeldahl digestion flask. Carry out a blank at the same time on the filter paper using 2g of AR sucrose in place of the sample.

b) Add two AOAC Kjeltabs (or 10g $K_2SO_4$ and 0.7g HgO). Then add 30ml of concentrated sulphuric acid.

c) Heat gently; if frothing occurs reduce heat or remove from heat until it subsides. Samples with high fat contents will froth excessively. To control this add 0.5g of paraffin wax (a blank must be carried out likewise).

d) The reaction mixture will turn black due to the dehydrating action of the sulphuric acid and the formation of free carbon. As the reaction proceeds, gradually increase heating. When a clear green or yellow solution is produced, heat strongly for 2 hours. The sulphuric acid should condense in the neck of the flask, if not then adjust heating and fume extraction rate to avoid loss of sulphuric acid. Allow to cool to near room temperature. Do not allow the digest to completely solidify (ie do not leave at this point either overnight or over the weekend) as loss of nitrogen will result.

e) Add approximately 150ml of water to the cooled digest and swirl to dissolve. Warm if necessary to dissolve any crystals of potassium sulphate.

f) Transfer the diluted digest quantitatively to a 250ml volumetric flask and make up to the mark with water.

g) Pipette a suitable aliquot into the distillation flask (refer to table 3 below), add 200ml of water and some anti-bumping granules.

Table 3 - Aliquots to take for crude protein determination

| Approximate % of Crude Protein in the sample | Aliquot required for the distillation (ml) | Approximate quantity of 50% w/v NaOH to add to the distillation |
|---|---|---|
| 0 - 15 | 250.0 | 95 |
| 15 - 30 | 100.0 | 65 |
| 30 - 65 | 50.0 | 35 |
| 65 - 100 | 25.0 | 25 |

h) To a 500ml conical flask add 100ml of 2% boric acid solution and 0.5ml of indicator.

i) Place the flask so the bottom of the condenser is below the surface of the solution, and turn the condenser water on.

j) To the distillation flask, add 1 tablet of sodium thiosulphate and a suitable quantity of sodium hydroxide (see Table 3 above) and immediately connect up to the distillation apparatus. Mix the contents thoroughly by swirling, and start heating. It is important at this point to make absolutely sure that the contents are thoroughly mixed, as if this is not done the flask could explode when heated.

k) When about 200ml of distillate has been collected in the now green boric solution, remove the splash head from the distillation flask and cease heating. Do not cease heating and leave the apparatus connected as the boric solution will suck back into the distillation flask.

l) Titrate the distillate with 0.1N sulphuric acid to the grey-mauve end point.

m) Carry out a blank determination.

Notes:

1. Kjeltabs AOAC and Thiotabs are available from Thompson and Capper Ltd. (Manufacturing Chemists), 3 Goddard Road, Astmoor Industrial Estate, Runcorn, Cheshire, WA7 1NU, England.

2. Standard determinations are only necessary in order to check that the application of the method is correct and need not be done for routine nitrogen determination.

Calculations

$$\% \text{ total nitrogen} = \frac{(\text{Titre} - \text{blank}) \times 0.0014 \times 250 \times 100}{\text{mass of sample (g)} \times \text{aliquot}}$$

This calculation assumes the standard sulphuric acid to be exactly 0.1N (1ml ≡ 0.004904g of $H_2SO_4$ ≡ 0.0014gN).

If the actual normality differs, then multiply the above equation by:

$$\frac{\text{actual normality}}{0.1}$$

The difference between two duplicate determinations should not exceed

(i) 0.03 as an absolute value, for nitrogen contents of less than 3%;
(ii) 1% relative to the mean, for nitrogen contents between 3-6%;
(iii) 0.06 as an absolute value, for nitrogen contents above 6%.

Report the nitrogen result to the nearest 0.01%.

$$\% \text{ crude protein} = \% \text{ nitrogen} \times 6.25$$

Take the result as the arithmetic mean of the two duplicates. Report the crude protein result to the nearest 0.1%. The factor 6.25 is normally used for animal feedingstuffs, the product %N x 6.25, giving 'crude' protein or 'generalised' protein. This factor derives from 100/16 = 6.25, as there is an average 16% nitrogen in protein.

References

(i) The fertiliser and feeding stuffs regulations (1973) HMSO, London.

(ii) R.B. Bradstreet (1965). "The Kjeldahl method for organic nitrogen." Academic Press.

(iii) Fleck, A., and Munro, H.N. (1965). "The determination of organic nitrogen in biological material; a review." Clinica Chimica Acta, 2.

(iv) M.B. Jacobs (1958). "Chemical analysis of foods and food products." 3rd edition. Van Nostrand Princetown N.J.

(v) Hautala, E. and McDonald, G.M. (1978). "Control of mercury from Kjeldahl wastes." J. Assoc. Off. Agr. Chemists 61, 1.

## OPERATION OF THE SHIMADZU HPLC FOR AMINO ACIDS

### Starting Procedure

A. Elimination of air from the buffer leads:

This is not always essential and need only be carried out if the leads appear to have air bubbles or when the buffer reservoirs are changed.

1. Do not switch on instrument.

2. Unstopper the corks from the buffer reservoirs and make sure the lead filters are still well under the surface of the liquid.

3. Using small syringe, remove air from each lead in turn by gently withdrawing liquid/air from the gradient selector. Sometimes it is necessary to push liquid (which had been taken into the syringe) back into tne line to disturb air bubbles stuck to the tubing. This must be done gently as more air may be produced by forceful treatment.

B. Priming the buffer pump:

4. Switch on programmer (SGR-1A) and set to 04,04,04,04,04,00.

i.e. line 1(4 mins) -- pH buffer 3.20
2(4 mins) -- pH buffer 4.20
3(4 mins) -- pH buffer 9.00
4(4 mins) -- NaOH solution
5(4 mins) -- pH buffer 3.20
6(00 mins)-- pH buffer 3.20

5. Set pump (LC-3A) flow rate to 4ml/min.

6. Open drain valve.

7. Set injector to middle position between "inject" and "load".

8. Press LC-3A pump on.

9. Press start button. Each buffer in turn will be pumped through the pump system and out through the drain valve.

10. At the end of the programme (when red light has returned to 1) switch off pump.

C. Regeneration of the column:

11. Close injector port to "inject" position. This will enable buffer to pass through the sample loop and onto the column.

12. Close the drain valve.

13. Alter the flow rate of the pump to 0.3ml/min.

14. Adjust the programmer to 05, 05, 05, 07, 35, 00.

15. Switch on oven to setting of 55°C.

16. Start the buffer pump.

17. Start the programmer.

D. Introduction of OPA reagent to the system:

18. When the programmer has reached "35" (or line 5 - pH 3.2 buffer) place the reagent lead into the OPA reservoir. Draw off air from that lead with a syringe.

19. Start reagent pump.

20. Switch on fluorodetector and recorder.

E. Injection of sample:

21. When regenerative programme is complete, the equipment is ready to start an analysis.

22. Switch off buffer pump and alter the flow rate to 0.5ml/min.

23. Alter the programme to 16, 16, 35, 7, 35, 00.

24. Switch on buffer pump.

25. Turn injector port switch to "load".

26. Carefully load the sample.

27. Close injector port switch to "inject".

28. Press start button on programmer and at the same time, mark the chart paper to show beginning of trace.

F. Other parameters:

29. The flow rate of OPA reagent is set for 0.75ml/min and therefore the total flow rate, out of the detector should be 1.25ml/min.

30. Check the flow rate.

31. Check the pump pressure - it should be between 100-120kg/$cm^2$.

32. With a lul aliquot of standard (suitable detection limit 0.0025umole) the range setting should be 128.

G. Close-down procedure:

33. When the programme reaches line 5 (35 min wash with 3.20 pH buffer) switch off the reagent pump and place the lead in distilled water.

34. Switch on reagent pump again for the duration of the programme.

35. Switch off detector and recorder.

36. On completion of programme, switch oven setting to 25°C, open oven door and allow the fan to cool it. When thermometer shows 25°C, switch off the oven.

37. Switch off buffer pump and reagent pump.

38. Switch off main controls.

39. Stopper the reagents with the rubber bungs provided.

40. Record the 'run' in the log book with the following information:-

1. Date
2. Sample details
3. Trace number
4. Type of analysis
5. Dilution factors
6. State of reagents

APPENDIX 5

## Analysis of Chocolate Pastes

Method

1. If the sample consists of a seed or other solid, reduce it to a coarse powder or meal and extract with petroleum ether in a Soxhlet extractor. If it is a paste, mix or stir it with several portions of solvent.

2. Saponify and esterify the extracted fat or oil by the methods specified in International Union of Pure and Applied Chemistry. "Standard Methods for the Analysis of Oils, Fats and Soaps. 5th edition with supplements. Pergamon Press, Oxford, New York, Sydney, etc, 1977, which are not reproduced here for reasons of copyright.

3. Analyse by gas chromatography using a 10ft (3m) column containing 10 per cent of diethyleneglycol succinate on 80-100 or 100-120 mesh "Chromosorb W" or "Gaschrom Q". The support should preferably be treated with DCMS. The temperature should be about 185°C and the flow rate for columns with an internal diameter less than 2mm should be 10-15ml/min.

Suggestions for the continuation of the project

Analyse as many samples of unadulterated Indonesian coconut oil, palm oil, tengkawa oil and cocoa butter as possible over a period of several months, checking the reproducibility by doing duplicate determinations. Calculate an average composition of each oil.

2. Prepare synthetic mixtures to simulate adulterated oils of commerce and analyse them. Calculate the proportions of the oils in the mixtures using the average composition of each oil as the basis of the calculation. Compare the results of these calculations with the known proportions of the oils in the mixtures.

APPENDIX 6

## Thiamine determination

This method is a hybrid between the AOAC (12th ed) thiamine method, and the method given in "Methods of Vitamin Assay" 3rd edn edited by the Association of Vitamin Chemists.

The determination comprises 3 steps:

1. Extraction of thiamine.
2. Conversion of thiamine to thiochrome.
3. Measurement of fluorescence.

Two further steps, which are not included here, may be necessary after the extraction and before the conversion of thiamine to thiochrome when meat or yeast products are being analysed because the thiamine is then associated with phosphate as the coenzyme thiamine pyrophosphate.

### Reagents

1. NaCl reagent grade.

2. 15% NaOH in water.

3. 1% potassium ferricyanide solution. Dissolve 1g $K_3Fe(CN_6)$ in water; dilute to 100ml.

4. Oxidising agent (alkaline potassium ferricyanide). Dilute 3ml of (3) above, to 100ml with 15% NaOH. Prepare fresh daily and store in brown bottle.

5. 0.1N HCl. Dilute 8.5ml conc HCl to 1 litre with water.

6. 0.1N $H_2SO_4$. Dilute 2.8ml conc $H_2SO_4$ to 1 litre with water.

7. 2.5m sodium acetate solution. Dissolve 205g anhydrous $NaC_2H_3O_2$ or 345g of $NaC_2H_3O_2.3H_2O$ in water and dilute to 1 litre.

8. Isobutyl alcohol (2 methyl-propan-1-ol). Shake with activated charcoal to remove any fluorescence.

9. Enzyme solution - an impure amylase is used, since this will contain phosphatases. Prepare fresh daily. Suspend 6g enzyme in 2.5n sodium acetate and dilute to 100ml.

10. 25% KCl solution. Dilute 250g KCl to 1 litre with water.

11. Acid KCl. Dilute 8.5ml conc HCl to 1 litre with the above 25% KCl solution.

12. Activated Decalso. To 100g Decalso, contained in a Buchner funnel add 200-250ml of hot 3% acetic acid solution, and allow the acid to remain in contact with the Decalso for 10-15 mins. Drain off with mild vacuum. Repeat the acid wash and drain, then wash again using 250ml hot 25% KCl soln. Wash once more with hot acetic acid soln. Drain, and rinse out any traces of KCl with 500-600ml portions of hot water, under mild vacuum. Test that the wash water is free of chlorides by adding a few drops to some 1% $AgNO_3$ soln. A white precipitate indicates presence of Chloride. Dry Decalso at room temperature or in an oven below 100°C.

13. Stock thiamine soln (100µg/ml). Dissolve 100mg thiamine in 25% ethanol and dilute with ethanol to 1 litre. This is stable for several months at 5°C or below.

14. Intermediate thiamine soln (5µg/ml). Dilute 5ml stock thiamine soln (at room temp.) to 100ml with water.

15. Thiamine working soln (0.2µg/ml). Pipette 4ml thiamine intermediate soln into a 100ml flask with 75ml 0.1N $H_2SO_4$ and 5ml sodium acetate soln. Make up to 100ml with water.

16. Stock quinine (100mg/ml). Dissolve 100mg quinine sulphate in 0.1N $H_2SO_4$ and dilute to 1 litre with acid. Store in dark bottle.

17. Working quinine soln. (1.0µg/ml). Dilute 10ml stock quinine to 1 litre with 0.1N $H_2SO_4$.

Procedure

1. Extraction with acid

a) Weigh quantity of sample containing about 10-30µg thiamine (or 5g if the thiamine concentration of the sample is unknown), into a 150ml flat-bottomed

flask. Add 75ml 0.1N HCl (5) and stopper using a bung with a capillary tube in it. Heat for 30 minutes in a boiling water bath with occasional shaking.

b) Cool, to below 50°C and add 5ml enzyme suspension (9) then incubate at 40-50°C for a minimum of 2 hours. If the sample is a cereal product, where most of the thiamine is in the "free form", add 5ml sodium acetate soln (7) instead of 5ml enzyme, and proceed directly to step (C). This was the method used for wheat flour.

c) (i) Cool flasks to room temperature, then transfer contents to 100ml volumetric flasks and make up to volume with water. Filter the solution, discarding the first few ml of filtrate. This filtrate may form the assay sample solution, so proceed to step (H).

(ii) If blanks from previous determinations have shown a high "background" fluorescence (a reading of more than 1.5 times the standard blank value), it is advisable to remove the interfering compounds by running the sample down an ion-exchange resin column. The need for this step can only be determined by trial and error. Proceed to step D.

d) Purification - place a plug of glass wool in the base of an adsorption tube, and fill tube with water. Gently pour in activated Decalso until there is a 4-8mm deep layer in the reservoir. Drain off the water until none is visible above the Decalso.

e) Pipette 25ml of the filtrate from (C) above, into the absorption tube reservoir and allow to drain, then wash down the tube with 3 successive 10ml portions of hot water. Discard all eluate from these washings. Do not let the surface of the Decalso become dry.

f) Place a 25ml vol flask beneath the columnn, and wash the column with 2 successive 10ml portions of hot acid 25% KCl (11) (this should not be boiled). Collect the eluate from these two washings in the volumetric flask, then make up to volume with acid 25% KCl. This forms the assay sample solution (purific).

g) Repeat (D), (E), and (F), but in step (E), in place of the sample filtrate, pipette 25ml of working thiamine standard (15) (0.2ug/ml) on to each of two duplicate columns. The eluate from this is the assay standard solution.

2. Conversion of thiamine to thiochrome

h) Weigh 1.5g NaCl into a 50ml plastic centrifuge tube. Add 5ml of either assay sample solution or assay standard solution. Using an automatic pipette which delivers rapidly, add 3ml oxidising agent (alkaline ferricyanide (4)) to the tube, while swirling to mix. Immediately add 13ml iso-butanol, stopper and shake for 15 seconds. This stage is very important.

i) To a similar tube, add 1.5g NaCl and 5ml sample or standard solution. Follow the procedure in (h) above, but add 3ml alkali (2) instead of oxidising agent. This is a reagent blank and must be prepared for each sample and standard.

j) When all tubes have been prepared, shake again for 2 mins.

k) Centrifuge at 1000rpm for 5 mins to separate the layers. The top layer (iso-butanol) contains the thiochrome, and should be completely clear.

l) Set up the fluorimeter with excitation wavelength 365nm and emission wavelength 435nm. Adjust the fluorimeter settings using quinine sulphate (17) as described.

m) Read the fluorescence of the iso-butanol fraction of the standard (St), then the standard blank (StB) then read the fluorescence of the unknown sample (S) and the sample blank (SB).

$$\text{Calculation: } \frac{S-SB}{St-StB} \times 0.2 \times \frac{25}{V} \times \frac{100}{\text{wt sample}} = \text{Thiamine } \mu g/g$$

100 — original dilution

0.2 — conc of standard µg/mL

25/V — Adjust for volume eluted down column usually 25/25=1

Notes

1. Once the thiamine has been oxidised to thiochrome, speed is essential as the extract is extremely sensitive light. The centrifuge tubes should be protected from the light and determination of the thiochrome concentration carried out immediately.

2. Spectrofluorimetry is a highly sensitive method of detection and it is imperative that all glassware is absolutely clean and chemical reagents very pure. Beware if blanks give an abnormally high reading, as this might be the reason.

References:


1. "Methods of Vitamin Assay" (1961), 3rd edition. Prepared and edited by the Association of Vitamin Chemists. Interscience Publishers, New York.

2. "Vitamins and Other Nutrients", Methods of AOAC, (1980), 12th edition, 43, 740-742.

3. "Principles of fluorescence", Molecular Biology, 2, 2-36.

## OPERATION OF THETURNER SPECTROFLUOROMETER

1. Turn the main switch to "on".

2. Press the Xenon start button to start the lamp.

3. Check that the lamp is alight.

4. Allow the instrument to warm up; 5–10 minutes if the machine has been left in the "warm-up" position or at least an hour if it has been switched off.

### Setting the instrumental zero

5. Turn the sensitivity control fully clockwise and then one complete turn back anti-clockwise.

6. Turn the range switch to zero.

7. Check that the meter is reading zero. It should be, but in unusual cases where it is not, adjust the amplifier zero control with a small screwdriver.

### Checking the sensitivity

8. Turn the blank control clockwise and the blank switch to low.

9. Turn the meter damp switch to 1.

10. Turn the shutter control to off.

11. Turn the range switch to minimum sensitivity.

12. Install a standard in cell 2 (usually 1.0µg/ml quinine sulphate). Compartment number should be visible in back left corner.

13. Depress the shutter control down to read.

14. If the meter reads more than 20 (on the 0-100 scale) turn the sensitivity switch to low.

15. Release the shutter control.

Setting the blank

16. Install a reagent blank, e.g. 0.1N HCl.

17. Depress shutter control to read - it is easier to get a preliminary setting on "record".

18. Increase the setting of the range switch until the meter reads 70 (on the 0-100 scale) or if that is not posible set the range switch to x 1000.

19. Turn the blank completely anti-clockwise until the meter reads zero. If this is not possible, turn the blank switch to high and try again.

20. Release the shutter control.

Setting of the sensitivity scale and reading the samples

Suggested arrangement of readings for estimating thiochrome concentration:-

Cell 1. - 0.1N HCl (The solutions in cells 1 and 2 are
2. - 1.0ug/ml quinine sulphate for setting the scale on the instrument)
3. - Standard thiamine reacted
4. - Standard thiamine unreacted
5. - Sample reacted
6. - Sample unreacted

21. Turn cell compartment to No. 2.

22. Depress shutter control.

23. Select range selector setting (normally X300) and sensitivity control setting so that the meter reads full scale deflection.

24. Read on 0-100 scale, if using minimum sensitivity, X10, X100, X1000. Read on 0-33 scale, if using X3, X30, X300.

25. Check the blank (in cell 1) reads zero.

26. Read the sample and standard thiochrome extracts ensuring that the shutter control is depressed for as short a time as possible to avoid over-exposure to UV light and possible degradation of the thiochrome.

27. Frequent readings of the blank and quinine sulphate may be necessary to check the stability of the instrument.

28. If more extracts are to be read, it is convenient to put these into cells 3-6 and keep the blank and quinine sulphate in cells 1 and 2 for reasons stated (27).

29. On completion of the readings, either switch off the machine or turn to "warm up" if it is to be used in the near future.

30. Thoroughly clean and dry the cells.

APPENDIX 7

## THE DETERMINATION OF CALCIUM BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

### Principle:

The sample is ashed and soluble mineral constituents dissolved in hydrochloric acid. The calcium content is determined by atomic absorption spectrophotometry in the presence of lanthanum to supress interference e.g. from phosphorus and alkali metals.

### Reagents:

1. Hydrochloric acid, 50/50 v/v solution.

2. Lanthanum chloride solution, 26.6% w/v $LaCl_3$ (10% w/v La).
   - moisten 117g lanthanum oxide ($La_2O_3$) in a 2 litre beaker with water. Whilst stirring, slowly add 350ml concentrated hydrochloric acid (SG 1.18). Stir until lanthanum oxide has dissolved. Cool, dilute to 1 litre with water. Filter through a 32cm, No. 1 Whatman paper into 1 litre polythene storage bottle.

3. Calcium standard stock solution A.
   - dry calcium carbonate at 103°C for one hour, cool in desiccator. Weigh accurately 2.497g into weighing boat. Transfer to 1 litre volumetric flask with about 100ml deionised water. Whilst swirling, slowly add 60ml IN hydrochloric acid. When carbonate has dissolved make up to mark with deionised water.

     1.0ml contains 1000ug Ca.

4. Calcium intermediate stock solution B.
   - pipette 50ml of stock solution A to 1000ml volumetric flask. Dilute to mark with deionised water.

     1.0ml contains 50ug Ca.

5. Hydrochloric acid, 1N.

Prepare an HCl extract solution of the sample using the following method.

## THE PREPARATION OF ASH EXTRACT SOLUTION

### Principle:

Organic matter is destroyed by dry ashing at 500°C ± 20°C and soluble mineral constituents dissolved in HCl and $HNO_3$. Any silica present is dehydrated and thus rendered insoluble, permitting the extraction of calcium from any orthosilicates present which are decomposed by HCl to give calcium chloride and silicic acid. The silicic acid is made insoluble by heating for 1 hour at 100°C to avoid interference during atomic absorption spectroscopy. The method is suitable for the determination of Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn in most feedingstuffs but not for mineral concentrates as these require dissolution by wet digestion. Incomplete recovery of some elements can occur, especially Cu and P by adsorption into the silica. This is prevented by the use of $HNO_3$ in addition to HCl.

### Reagents:

1. Hydrochloric acid, 50% (approx 6N).
   - in a fume cupboard and wearing gloves and eye protection, carefully add 500ml of conc. HCl (sp.gr. 1.18) to 500ml of water in a 2 litre beaker, stirring constantly. Cool. Store in a polythene aspirator.

2. Nitric acid, 33% (approx 6N).
   - precautions as above for HCl; add 330ml of conc $HNO_3$ sp. gr. 1.42 to 660ml of water in a 2 litre beaker, stirring constantly. Cool. Store as for HCl.

### Hazard Warning:

HCL - harmful vapour, causes burns, avoid breathing vapour, prevent contact with skin and eyes.

$HNO_3$ - harmful vapour, causes severe burns, contact with combustible materials may cause fires.

(These are best dispensed by auto-pipette).

### Procedure:

a) Weigh approximately 5g of sample, accurately, into a silica basin (refer to method for determination of ash), with a watch glass on top.

b) Carbonise over a hot plate or a flame prior to incineration at 550°C ± 15°C for 2-3 hours or until a light grey or reddish ash remains that is free of carbon particles. The dish is re-heated at 550°C ± 15°C for 1 hour periods until a difference of less than 0.002g is obtained between consecutive weights.

Dilute ash extract solution using table below as a guide to bring the calcium level within the instruments range. (Refer to feedstuffs table for an average Ca figure for the sample under test). Use only deionised water (see note 1).

| % Calcium in sample | Sample weight and dilutions | | (5ml $La^{3+}$ per 50ml) |
|---|---|---|---|
| 0.005 - 0.01 | 5g - 100 | | 25 - 50 |
| 0.01 - 0.05 | " | | 10 - 50 |
| 0.05 - 0.1 | " | | 5 - 50 |
| 0.1 - 0.5 | " | 10 - 100 | 10 - 50 |
| 0.5 - 1.5 | " | 10 - 100 | 5 - 50 |
| 1.5 - 2.5 | " | 10 - 100 | 2 - 50 |
| 2.5 - 5.0 | " | 10 - 500 | 5 - 50 |
| 5.0 - 10.0 | " | 5 - 1000 | 10 - 50 |
| 10.0 - 25.0 | " | 5 - 1000 | 5 - 50 |

3. Prepare standard solutions as follows:

Pipette 10.0ml of stock solution B (50µg/ml) to a 50.0ml volumetric flask, dilute to mark with deionised water: Contains 10µg/ml. Prepare suitable standard solutions (at least three) from the dilution guide below (see notes 2, 3 and 4).

| | | | | | |
|---|---|---|---|---|---|
| From 10µg/ml, dilute | 5 - 50 (+ $La^{3+}$) | = | 1.0µg/ml |
| " | " | 10 - 50 | " | = | 2.0 | " |
| " | " | 15 - 50 | " | = | 3.0 | " |
| " | " | 20 - 50 | " | = | 4.0 | " |
| From 50µg/ml(B), | " | 5 - 50 | " | = | 5.0 | " |
| " | " | 10 - 50 | " | = | 10.0 | " |
| " | " | 15 - 50 | " | = | 15.0 | " |

4. Prepare a blank solution (5ml $La^{3+}$/50ml).

5. Set up the atomic absorption according to the instruction manual using the 422.7nm line. Adjust fuel to obtain the highest stable reading whilst aspirating a standard solution.

6. Spray deionised water into the flame and zero the instrument (if many samples are being analysed it will save time if the instrument is zeroed with the blank solution). Spray standard solutions and then samples, washing with deionised water and taking a zero reading between each. Run standards between every 5 or 6 sample solutions and after running samples.

Calculation:

Average the standard readings (which should be nearly identical). Subtract blank absorbance from standards and samples unless the instrument was zeroed using blank. Prepare a calibration curve and read samples off curve to obtain calcium content in µg/ml.

Calculate % calcium in sample:

$$\% \text{ Calcium} = \frac{\mu g/ml \text{ (from graph)} \times 100 \times \text{dilutions*} \times 100}{10^6 \qquad \text{sample wt (g)}}$$

* eg for 10-100, 5-50 dilution (for Ca content between 0.5-1.5):

$$= \frac{\mu g/ml}{10^6} \times \frac{100 \times 100 \times 50 \times 100}{Wt \quad 10 \quad 5}$$

Notes:

1. Deionised water is free from particulate matter (often found in distilled water) which could block the nebulizer.

2. Dilution table for samples is a rough guide only. If sample solutions lie outside the range of the chosen standards then more suitable standards can be prepared rather than diluting the samples further.

3. The 'working' standard solutions (1.0-15.0µg ml) should be prepared fresh before use.

4. The dilution guide for the standards avoids use of small volume pipettes as they can give inaccurate results.

References:

1. The Fertilizer and Feedingstuffs Regulations, 1976, HMSO.

2. "Nutritive value of American Foods", Nov 1975, Agricultural Handbook No 456, Agricultural Research Service, USDA.

3. "Cereal Foods", AOAC Methods, Ch 14, 211-220.

## RAPID CHECK TO ESTIMATE SUITABLE DILUTIONS OF THE ASH SOLUTION FOR ELEMENT ANALYSIS

Expected concentrations of trace metals in foodstuffs can be found in literature and composition tables and dilutions can be calculated from these. There is a quick, practical way of determining suitable dilutions as follows:

1. Take 1ml of concentrated ash solution and place it in a 10ml stoppered graduated test tube.

2. Make up to the 10ml mark with deionised water.

3. Set up the AAS for the element to be tested using recommended standard concentration (see Instrument Handbook) and parameters.

4. Aspirate solution in the test tube. This is a 1ml-10ml dilution.

5. If the meter reading is greater than the standards, take 1ml of the diluted material (2) and place in another stoppered 10ml test tube. Dilute to 10ml, making the serial dilution of 1ml-100ml (i.e. 1ml-10ml and 1ml-10ml).

6. Continue to dilute serially, making use of all the test tube graduations (i.e. 1ml-2ml, 3ml, 4ml ..... 9ml, 10ml) to reach a dilution where the meter reading is similar to the standard readings.

7. Record the serial dilutions and calculate the total dilution e.g.

| | | |
|---|---|---|
| 1ml - | 10ml | X10 |
| 1ml - | 10ml | X10 |
| 1ml - | 5ml | X5 |
| 1ml - | 2.5ml | X2.5 = 1ml-1250ml |

Accurately repeat the dilution process using pipettes and volumetric flasks

| | | |
|---|---|---|
| e.g. | 5ml - | 50ml |
| | 5ml - | 50ml |
| | 5ml - | 25ml |
| | 2ml - | 5ml |