Analysis of Oilseeds, Fats and Fatty Foods

Edited by J.B. Rossell
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ANALYSIS OF OILSEEDS, FATS AND FATTY FOODS

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ANALYSIS OF OILSEEDS, FATS AND FATTY FOODS

Foreword

This book is concerned with analysis.

The products covered are essential parts of the human diet and provide essential nutrients. However, they must be of good quality if they are to fulfil their proper role in the human diet. Analysis is the tool to ensure that they are of good quality. As Mr. D. Pocklington says in the first chapter '. . . the use of dependable and validated methods is essential if the required quality in terms of accuracy is to be reached'. He mentions the circumstances in which it is essential to use a nationally or internationally validated method.

Oilseeds are an important source of oils and fats (together with animal and marine sources) and of animal feed. In the second chapter, Mr. J. L. R. Pritchard deals with the analysis and properties of oilseeds. He addresses the main analytical indices specified for trading purposes and reinforces the point made in Chapter 1 about the use of nationally or internationally validated methods. He also describes the properties of common commercial oilseeds and touches on new oilseeds.

In his chapter on oilseed residues, Mr. Pritchard deals not only with their analysis and properties but with government controls, nutritional value, protein denaturation and anti-nutritional factors.

Two major anti-nutritional factors—mycotoxins and glucosinolates—are treated in the next two chapters.

The chapter on mycotoxins classifies them, dealing with the type of food at risk and mentioning regulations relating to mycotoxins in food and foodstuffs designed to protect the consumer. It touches on legislation for animal feeds and feeding-stuffs and treats environmental

factors affecting the production of mycotoxins. There is a section on methods for the detection and determination of mycotoxins including the important question of sampling. Finally, the author of this chapter deals with more recent developments in mycotoxin methodology.

The presence of glucosinolates in cruciferous oilseeds has hindered the use of their meals in animal feed. Seeds with low glucosinolate levels have been developed which has brought with it a great deal of interest in glucosinolate analysis. The fifth chapter presents information on the current state of glucosinolate analysis and recommends those methods best for particular purposes including methods for the analysis of individual glucosinolates and for the determination of total glucosinolate content of oilseeds crops and commodities.

Chapter 6 covers the extraction of fats from fatty foods and the determination of fat content, discussing the use of standard methods for the determination of fat content. There is a discussion of standard methods for:

meat and meat products;
milk and dairy products;
oilseeds and residues;
animal feedingstuffs, and
cereal products.

The chapter also deals with non-standard, routine procedures and physical methods for the determination of fat (such as near infra-red reflectance).

Dr. Rossell is concerned with crude vegetable oils and fats in the work which he has contributed. In particular, he discusses analyses for oil quality and oil composition, including fatty acid composition. In his chapter he deals with tests for oil quality (including the identity characteristics of vegetable oils and fats) and sterols and tocopherols.

In Chapter 8, Animal Carcass Fats and Fish Oils, Dr. Enser deals first with animal fats including their composition. He discusses extraction procedures and covers their application to sheep, beef and pig carcases. So far as fish oil is concerned, he deals with extraction procedures, the fatty acid composition of fish oil, its triacylglycerol structure and unsaponifiable matter.

Butters, margarines and other spreads are covered in Chapter 9. The author discusses the nature and properties of milk fats and associated methods of analysis and margarine, including its manufacture and routine analysis.

He finishes with a discussion on reduced and low fat spreads. He also considers their routine examination.

Quality control is an essential tool for oils and fats processors world-wide. In Chapter 10, Mr. Mcginley deals with this subject in great detail. He suggests the components of a quality control system, covering feedstock quality, refining, analysis for feedstock quality, bleaching and testing methods to control quality. He deals with modification processes such as hydrogenation and process control in these procedures. Finally, he discusses the applications of processed oils and fats.

Last, but not least, is sampling. It needs to be carried out properly because the analysis of an unsatisfactory sample is of no use. The late Dr. A. Thomas deals with sampling in the final chapter in which he covers the subject from the taking of the sample increments from a parcel of goods through to the test portion on which the analysis is performed.

The majority of the authors are actively involved in national and international standardisation of methods through the BSI, the AOCS, ISO, IUPAC and FOSFA International Committees. This, together with the international origins of the contributions, broadens the scope and usefulness of the text in support of current trade developments.

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1

Precision and Accuracy of Analysis; Standardisation of Analytical Methods

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1.1. INTRODUCTION

A surgeon may have the highest of qualifications, he may have at his disposal one of the most well-equipped operating theatres in the country, but if he were to undertake major surgery using an unproved technique, the results could be quite disastrous. This is perhaps a statement of the obvious, yet in some analytical laboratories it is not always fully appreciated that the expertise of analysts, and the quality of equipment and reagents, cannot compensate for any deficiencies in the methods used for analysis.

The quality performance of laboratories is therefore directly related to the use of validated or standardised analytical methods. In this context the terms *validated* and *standardised,* when applied to an analytical method, should be understood to indicate that the performance of the method has been demonstrated to meet a required degree of precision and, where appropriate, its *accuracy* (or rather its *trueness*—see Table 1.1 in which the revised ISO (International Organisation for Standardisation) definitions of accuracy, precision and trueness are given) has been shown to be satisfactory.

The vital role that the analytical method plays in the quality performance of analytical laboratories cannot be over-emphasised. Whilst analyst expertise, quality reagents and equipment all contribute to the quality of results produced by a laboratory, the use of dependable and validated methods is essential if the required quality in terms of accuracy is to be reached.

TABLE 1.1
ISO definitions of accuracy, trueness, etc

Accuracy
Total displacement of a result from a reference value
This displacement is due to:
random errors—related to precision, and
systematic errors—related to trueness

Trueness
Can only be measured if a reference value is available
May be defined as 'the closeness of agreement between the average value obtained from a large set of observations and the accepted reference value'

Precision
As a measure of random error is usually expressed in terms of repeatability[a] and reproducibility,[a] these being computed as standard deviations of the set of observations
May be defined as 'the closeness of agreement between independent test results obtained under prescribed conditions'
It is not related to the true value

Bias
Is a measure of the displacement from a true or reference value; this displacement, which is a systematic error, may be attributed to:

(a) bias of the test method, and
(b) bias of the laboratory using that test method

It may be defined as 'the difference between the expectation of the observed values or test results and the accepted reference value'

[a] See Table 1.14 for the ISO definitions of these terms.
Reference: ISO 5725 (draft revision) *Accuracy (trueness and precision) of measurement methods and results. Part I: General principles and definitions.*

Laboratory in-house quality control obviously relies on validated methods. Such methods may have been developed by the laboratory itself or have been published by standards organisations. For many purposes a laboratory may find it satisfactory to use methods it has developed and validated, but when results are to be considered by other laboratories or organisations, the use of a nationally or internationally validated method will normally be essential.

The AOAC (Association of Offical Analytical Chemists) has a well-deserved excellent reputation for publishing in its Journal methods which have been validated by collaborative study, and simultaneously, a full report on the study. The final texts of the methods are subsequently reproduced in the AOAC's book of *Official Methods of Analysis*.[1] This book of methods was first published in 1920 and is

constantly being revised and expanded—the last edition (15th) appearing in 1989. Supplements containing newly validated methods are issued each year and incorporated into a new edition of the methods book at about 5-yearly intervals.

One might be justified in assuming that all methods published by ISO, and national standardising bodies such as the British Standards Institution have been validated by collaborative study. However, it was in 1981 that the ISO Technical Committee 34 (Agricultural food products) first drew the attention of its sub-committees to the necessity of ensuring that all methods being considered for adoption as ISO standards were first to be thoroughly validated for their performance in terms of precision before being published as definitive standards. It was also emphasised by this ISO Committee that this necessary validation could only be accomplished by properly conducted collaborative studies, and it may therefore come as a surprise to learn that, until relatively recently, few standards organisations (the AOAC, as indicated above, is a notable exception) stipulated that a method must be validated by collaborative study *before* it could be adopted as a standard.

An ISO TC 34 committee document[2] circulated in 1986 indicated that the number of ISO methods (for the analysis of food and agricultural materials) supplied with precision data is relatively low in the case of general methods, and that very few of the reference methods have such data. The reason for this lack of precision data would appear to be that the methods had not been validated by collaborative study and hence no precision data were available for inclusion in the standard methods.

If we examine the *British Standard 684* (methods of analysis of fats and fatty oils)[3] we find that the situation is only marginally better. Although some information as regards precision will be found in 14 of the 50 or so methods in the Standard, it would appear that in many cases arbitrary repeatability values have been cited, rather than values derived from the statistical evaluation of collaborative study results.

In the Foreword to the 6th Edition (1979) of the *IUPAC Standard Methods for the Analysis of Oils, Fats and Derivatives*[4] it is stated that 'all the methods have been studied in collaborative tests, and adopted only when concordant results have been obtained'. This may well be the case, but unfortunately it has not been the policy, until relatively recently, for this organisation to publish results of the collaborative studies which led to standardisation of the methods. Such a claim does

not appear in the Foreword to the 7th Edition (1987)[5] of this work and here again it will be found that the citation of precision characteristics is the exception rather than the rule.

A consequence of this lack of information regarding the precision and accuracy of standard methods is, understandably, that a government department may be inclined to recommended AOAC methods, rather than methods published by its own national standards body (or even international standard methods), to be used in connection with the enforcement of legislation such as labelling directives. This is because, as indicated above, the AOAC has adhered to its policy of publishing precision data and reports on the collaborative studies conducted for the validation of its standard methods, thereby making this data readily accessible for scrutiny by legislative bodies.

It is a cause for considerable concern that perhaps the majority of published standards for oils and fats analysis are enjoying a status to which they may not be entitled, i.e. being viewed as definitive validated methods. Furthermore, even where some reference is made in the text of a standard method to its precision, such information is often of little practical value to the analyst, and may be intelligible only to a statistician. The lack of uniformity in presenting statistical data, as it relates to the precision of the method, certainly warrants the attention of those who are directly responsible for the drafting of standard methods of analysis.

Accordingly it is proposed to consider in this chapter:

(1) what has been achieved in the way of harmonisation in protocols for the organisation of collaborative studies designed for the validation and standardisation of methods of analysis;
(2) the need to make the best possible application of data derived from statistical analysis of collaborative study results;
(3) how precision and other performance data can be effectively drafted for analytical quality control purposes; and
(4) the progress made internationally towards harmonisation in the presentation of statistical parameters in standardised methods.

1.2. HARMONISATION OF COLLABORATIVE STUDY PROTOCOLS

A standards organisation considering for 'adoption' a method that has already been published by another organisation, would normally want

to satisfy itself that the method had been validated by a properly organised collaborative study. Furthermore it would require to know whether the precision of the method had been evaluated by an accepted method of statistical analysis of the results obtained during the collaborative study. The considerable variation in the approach by standards organisations to method validation has impeded the adoption process and in certain cases has resulted in the duplication of analytical work. The latter has sometimes taken the form of actually repeating a collaborative study, generally following a much more clearly defined protocol.

Fortunately the need for harmonisation in the approach to collaborative studies of methods has now been recognised by standards organisations and it is satisfying to see the successful outcome of the symposia organised by IUPAC (International Union for Pure and Applied Chemistry) to achieve this harmonisation.

The IUPAC Working Party on the Harmonisation of Collaborative Study Protocols, at its meeting in Boston (USA) during August 1987, put the finishing touches to a document which was subsequently published under the title *IUPAC-1987 Protocol for the Design, Conduct, and Interpretation of Collaborative Studies*.[6] This document provides standards organisations with specific recommendations for meeting certain minimum requirements for collaborative studies. If a collaborative study is to be indicated as complying with these requirements then it must be in conformity with the minimum rules outlined in the document. A summary of the main recommendations will be found in Table 1.2, but three of what may be viewed as the most important recommendations can be mentioned here:

(a) not less than five materials should normally be provided for analysis by participants in the collaborative study;
(b) a required minimum of eight laboratories reporting valid data for each material analysed;
(c) two analyses to be carried out on each material, this replication being produced by blind duplicates or split-levels.

1.2.1 Models for Collaborative Studies

Studies organised over the last 5 years or so have generally been based on one of the five models illustrated in Table 1.3. The first model (uniform-level without 'blind duplicates') has been extensively used but is not now regarded as being very satisfactory for accurately

TABLE 1.2
1987-IUPAC harmonised protocol for the design, conduct, and interpretation of collaborative studies

Note: Only the main recommendations agreed at the joint ISO/IUPAC/AOAC Harmonization Workshop (held in Geneva, May 1987 and confirmed at the meeting of the IUPAC Working Party on the Harmonization of Collaborative Analytical Studies at Boston (USA) in August 1987) have been reproduced

(1) The results of collaborative studies should be analysed by one-way analysis of variance ('material-by-material'), but more complex analyses are not precluded
(2) The absolute minimum number of materials to be used in a collaborative study is five. However, when a single level specification is involved this may be reduced to an absolute minimum of three
(3) The most important objective of a collaborative study should be attaining a reliable estimate of reproducibility[a] parameters. To the extent that the performance of known (parallel) replicate analyses detracts from this objective, a requirement for the use of this type of replicate analysis should be discouraged
(4) The best estimate of repeatability[a] parameters is obtained by the following procedures (listed approximately in order of desirability):
    (a) the use of a split-level design (single values from the analysis of each of two closely related materials);
    (b) the use of both split-levels and blind duplicate analyses in the same study;
    (c) the use of blind duplicate analyses;
    (d) the use of known duplicate analyses (two repeat analyses from the same test sample), but only when it is not practical to use one of the preceding designs;
(5) The minimum number of participating laboratories in a collaborative study is eight. Only when it is impossible to obtain this number may the study be conducted with less, but with an absolute minimum of five laboratories. (Although it is desirable to have more than eight laboratories participating, studies containing more than about fifteen laboratories become unwieldy)
(6) The precision estimates are to be calculated both with no outliers removed and with outliers removed, using the Cochran and Grubbs outlier tests. The Grubbs tests should be applied only to laboratory means, not to individual values of replicated designs. Outlier removal should be stopped when more than 22% (i.e. more than two out of nine laboratories) would be removed as a result of the sequential application of the outlier tests

[a] As defined in ISO 5725-1986—see Table 1.14.