HANDBOOK OF FOOD ANALYTICAL CHEMISTRY

WATER, PROTEINS, ENZYMES, LIPIDS, AND CARBOHYDRATES

HANDBOOK OF FOOD ANALYTICAL CHEMISTRY

PIGMENTS, COLORANTS, FLAVORS, TEXTURE, AND BIOACTIVE FOOD COMPONENTS

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Edited by

Ronald E. Wrolstad
Terry E. Acree
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PREFACE

Accurate and state-of-the-art analysis of food composition is of interest and concern to a divergent clientele including research workers in academic, government and industrial settings, regulatory scientists, analysts in private commercial laboratories, and quality control professionals in small and large companies. Some methods are empirical, some commodity specific, and many have been widely accepted as standard methods for years. Others are at the cutting edge of new analytical methodology and are rapidly changing. A common denominator within this diverse group of methods is the desire for detailed descriptions of how to carry out analytical procedures. A frustration of many authors and readers of peer-reviewed journals is the brevity of most Materials and Methods sections. There is editorial pressure to minimize description of experimental details and eliminate advisory comments. When one needs to undertake an analytical procedure with which one is unfamiliar, it is prudent to communicate first-hand with one experienced with the methodology. This may require a personal visit to another laboratory and/or electronic or phone communication with someone who has expertise in the procedure. An objective of *the Handbook of Food Analytical Chemistry* is to provide exactly this kind of detailed information which personal contact would provide. Authors are instructed to present the kind of details and advisory comments they would give to a graduate student or technician who has competent laboratory skills and who has come to them to learn how to carry out an analytical procedure for which the author has expertise.

Some basic food analytical methods such as determination of °brix, pH, titratable acidity, total proteins and total lipids are basic to food analysis and grounded in procedures which have had wide-spread acceptance for a long time. Others such as analysis of cell-wall polysaccharides, analysis of aroma volatiles, and compressive measurement of solids and semi-solids, require use of advanced chemical and physical methods and sophisticated instrumentation. In organizing *the Handbook of Food Analytical Chemistry* we chose to categorize on a disciplinary rather than a commodity basis. Included are chapters on water, proteins, enzymes, lipids, carbohydrates, colors, flavors texture/rheology and bioactive food components. We have made an effort to select methods that are applicable to all commodities. However, it is impossible to address the unique and special criteria required for analysis of all commodities and all processed forms. There are several professional and trade organizations which focus on their specific commodities, e.g., cereals, wines, lipids, fisheries, and meats. Their methods manuals and professional journals should be consulted, particularly for specialized, commodity-specific analyses.

This two-volume handbook is derived from another John Wiley & Sons publication, *Current Protocol in Food Analytical Chemistry*. That manual was published from January 2001–December 2003 in loose-leaf and CD-Rom format. That design permitted addition of new and revised units on a quarterly basis. The two-year compilation of these units makes for a very complete reference on food analytical methods.

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FOREWORD TO CURRENT PROTOCOLS IN FOOD ANALYTICAL CHEMISTRY

Accurate, precise, sensitive, and rapid analytical determinations are as essential in food science and technology as in chemistry, biochemistry, and other physical and biological sciences. In many cases, the same methodologies are used. How does one, especially a young scientist, select the best methods to use? A review of original publications in a given field indicates that some methods are cited repeatedly by many noted researchers and analysts, but with some modifications adapting them to the specific material analyzed. Official analytical methods have been adopted by some professional societies, such as the Official Methods of Analysis (Association of Official Analytical Chemists), Official Methods and Recommendation Practices (American Oil Chemists' Society), and Official Methods of Analysis (American Association of Cereal Chemists).

The objective of *Current Protocols in Food Analytical Chemistry* is to provide the type of detailed instructions and comments that an expert would pass on to a competent technician or graduate student who needs to learn and use an unfamiliar analytical procedure, but one that is routine in the lab of an expert or in the field.

What factors can be used to predetermine the quality and utility of a method? An analyst must consider the following questions: Do I need a proximate analytical method that will determine all the protein, or carbohydrate, or lipid, or nucleic acid in a biological material? Or do I need to determine one specific chemical compound among the thousands of compounds found in a food? Do I need to determine one or more physical properties of a food? How do I obtain a representative sample? What size sample should I collect? How do I store my samples until analysis? What is the precision (reproducibility) and accuracy of the method or what other compounds and conditions could interfere with the analysis? How do I determine whether the results are correct, as well as the precision and accuracy of a method? How do I know that my standard curves are correct? What blanks, controls and internal standards must be used? How do I convert instrumental values (such as absorbance) to molar concentrations? How many times should I repeat the analysis? And how do I report my results with appropriate standard deviation and to the correct number of significant digits? Is a rate of change method (i.e., velocity as in enzymatic assays) or a static method (independent of time) needed?

Current Protocols in Food Analytical Chemistry will provide answers to these questions. Analytical instrumentation has evolved very rapidly during the last 20 years as physicists, chemists, and engineers have invented highly sensitive spectrophotometers, polarometers, balances, etc. Chemical analyses can now be made using milligram, microgram, nanogram, or picogram amounts of materials within a few minutes, rather than previously when grams or kilograms of materials were required by multistep methods requiring hours or days of preparation and analysis. *Current Protocols in Food Analytical Chemistry* provides state-of-the-art methods to take advantage of the major advances in sensitivity, precision, and accuracy of current instrumentation.

How do chemical analyses of foods differ from analyses used in chemistry, biochemistry and biology? The same methods and techniques are often used; only the purpose of the analysis may differ. But foods are to be used by people. Therefore, methodology to determine safety (presence of dangerous microbes, pesticides, and toxicants), acceptability (flavor, odor, color, texture), and nutritional quality (essential vitamins, minerals, amino acids, and lipids) are essential analyses. *Current Protocols in Food Analytical Chemistry* is designed to meet all these requirements.

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A WATER

INTRODUCTION

A1 Gravimetric Measurements of Water

A1.1 Gravimetric Determination of Water by Drying and Weighing

A1.2 Karl Fischer Titration

A1.3 Application of Low-Resolution NMR for Simultaneous Moisture and Oil Determination in Food (Oilseeds)

A1.4 Traditional Indirect Methods for Estimation of Water Content: Measurement of °Brix

A2 Vapor Pressure Measurements of Water

A2.1 Factors to Consider When Estimating Water Vapor Pressure

A2.2 Dew-Point Method for the Determination of Water Activity

A2.3 Measurement of Water Activity Using Isopiestic Method

A2.4 Direct Manometric Determination of Vapor Pressure

A2.5 Measurement of Water Activity by Electronic Sensors

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SECTION A
Water

INTRODUCTION

Water determination in foods is a deceptively simple theme. Defining the quantity to be measured identifies the inherent complexity. Three separate types of measure may be appropriate: (a) a gravimetric measure, (b) a measure related to vapor pressure, and (c) a measure of the mobilities of water molecules. The ubiquitous nature of water in our environment provides additional complexity in the challenge of preventing transfer of water between sample and environment. The earliest measures of amount of water were all gravimetric, determining the weight fraction of water in the food. These methods range from simple direct weighing, using a difference technique, to more complex methods where the amount of water is determined by spectroscopic methods or by chemical assay. A wide range of methods have been developed and are in daily use, since gravimetric water content is important for formulation and for labeling purposes. This measure, however, is of little value for the prediction of the stability of a food, even though water plays a critical role in determining the stability characteristics of foods.

For a measure of amount of water relevant to stability concerns, vapor pressure, or its related thermodynamic parameters, is more relevant. Determination of vapor pressure uses methods developed from thermodynamic roots, though if the product is not at true equilibrium, the measured quantity is not a thermodynamic descriptor of the product, although it is still a measure of a product characteristic. Water mobilities are often inferred from spectroscopic measurements of relaxational phenomena. Many workers attempt to identify different "classes" of water characteristic of different ranges of water content and water partial vapor pressure. Spectroscopic measurements, too, are often interpreted in terms of populations of water molecules with similar characteristics.

The objective of this section is to provide clear descriptions of the alternative methods for the determination of gravimetric water content (Chapter A1) and of the range of methods available for the estimation of vapor pressure or its related parameters (Chapter A2).

GRAVIMETRIC MEASUREMENTS OF WATER

UNIT A1.1 describes the direct determination of gravimetric water by drying and weighing, surveying a range of well established techniques. *UNIT A1.2* describes the use of the Karl Fischer titration for the chemical determination of the amount of water contained in a sample. *UNIT A1.3* describes a particular use of nuclear magnetic resonance spectroscopy (NMR) to determine the water content and the oil content of seeds. *UNIT A1.4* provides an overview of some indirect methods for the estimation of water content. It considers the pros and cons of the measurement of physical characteristics (density and refractive index) that may be correlated with the water content of specific systems, and identifies the critical assumptions associated with the use of such indirect methods.

Contributed by David S. Reid

VAPOR PRESSURE MEASUREMENTS OF WATER

The initial unit in this chapter (*UNIT A2.1*) discusses the constraints which must be considered when attempting to estimate the vapor pressure above an aqueous system. These constraints are operative whichever technique may be utilized. The use of a dew-point cell to estimate vapor pressure and water activity is described in *UNIT A2.2*. This unit clearly identifies the precautions which are essential to a good dew-point determination. The use of isopiestic techniques, in which a known atmospheric condition is produced and the sample is assumed to have equilibrated with this atmosphere, is the subject of *UNIT A2.3*. The techniques of this unit are frequently employed for the special purpose of determining moisture sorption isotherms. These describe the relationship between the gravimetric water content of a sample and the partial water vapor pressure sustained by the sample. Such relationships can be a useful tool for correlating/estimating moisture content from partial water vapor pressure measurements and vice versa. *UNIT A2.4* describes the direct manometric measurement of water vapor pressure. The method is very demanding of good technique, which is why it is seldom used. All primary vapor pressure data result from the use of this type of apparatus. The primary vapor pressure data for pure water (used as the reference data for all of the indirect methods, including dew point, isopiestic, etc.) were produced by direct manometric evaluation. *UNIT A2.5* describes the use of electronic sensors for vapor pressure measurement. This method is considered the most simple method for measuring water activity. Advantages and limitations of different types of electronic sensors are discussed.

David S. Reid

Gravimetric Measurements of Water

A1

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Gravimetric Determination of Water by Drying and Weighing

Water (moisture) in a sample is measured gravimetrically by determining the weight loss in a sample after it has been placed in an appropriate oven (convection, vacuum, or microwave) for a given time. In addition, there are automatic moisture analyzers available that utilize infrared lamps as a heat source. These types of moisture analyzers are fast but many times are matrix dependent, which requires some trial-and-error testing to determine the correct settings (power and time). Water and moisture are used interchangeably in the description of these protocols. In addition, it is assumed in the gravimetric method that only water is removed in the drying process, when in fact there may be volatile loss in some samples.

Although the measurement of weight loss due to evaporation of water is frequently used to calculate moisture content, it should be pointed out that the value obtained may not be a true measure of water content. In some samples, only a proportion of the water present is lost at the drying temperature. The balance (bound water) is difficult to remove completely. In addition, the water lost may actually increase as the temperature is raised. Some samples with high fat content may exhibit volatile oil loss at drying temperatures of 100°C. Weight loss may also be dependent on such factors as particle size, weight of samples used, type of dish used, and temperature variations in the oven from shelf to shelf. Thus, it is important to compare results obtained using the same drying conditions.

This unit provides three protocols for which there are established procedures for various matrices. The Basic Protocol describes water removal and quantitation after a sample is placed in a convection oven. It is probably the method of choice when one does not know which method to choose when dealing with an unknown matrix, or when one looks at samples that foam excessively in the vacuum oven method or "react," such as popcorn under vacuum. Alternate Protocol 1 describes water removal and quantitation after a sample is placed in a vacuum oven. Because it is at reduced pressure, drying times are slightly reduced compared to the convection method. In addition, drying temperatures <100°C are possible, which is important for samples that may decompose at higher drying temperatures. Alternate Protocol 2 describes water removal using a microwave source where such analyzers measure and calculate loss automatically.

MEASURING MOISTURE USING A CONVECTION OVEN

BASIC PROTOCOL

Water is measured in a sample by determining the loss in weight for the sample after it has been dried in a convection oven. The method requires only a small amount of homogeneous sample and can measure an effective range of 0.01% to 99.99% water.

Materials

Homogeneous sample
Convection oven capable of maintaining a temperature of 103° ± 2°C
Aluminum weighing dishes (with or without covers)
Desiccator with desiccant
Balance capable of measuring ±0.1 mg

1. Set the temperature of a convection oven to 105°C.
2. Dry an aluminum weighing dish (and cover, if used) ≥1 hr at 105°C. Cool and store dried dish in a desiccator. Cool ≥30 min before using.

Covered weighing dishes are useful when analyzing samples that splatter. Weighing dishes without covers may otherwise be preferred, as they are disposable.

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