教育部高等学校轻工与食品学科教学指导委员会推荐教材

食品化学实验双语教程

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FOOD CHEMISTRY LABORATORY MANUAL

中国轻工业出版社

教育部高等学校轻工与食品学科
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前　言

食品化学是研究食品的化学组成、理化性质及其在生产、加工、贮运过程中的化学变化的一门科学，它是食品科学与工程、食品质量与安全专业的专业基础课之一。为了满足部分院校双语教学的需要，我们编写了这本《食品化学实验双语教程》。本教材设计了23个实验，使学生有机会对理论课所学的知识进行实验研究，以加深对理论知识的理解，并具备从事科学研究的能力。

此前，学生应该已经修读过几门化学类课程的实验课，而食品化学实验课将能训练学生从事应用科学研究的技能，并加强对后续专业课程的学习能力。食品化学实验课不像我们已经学过的基础化学实验课，它使用的是组成复杂、含量多变的实验材料，而非纯净的化学试剂，某些情况下你得不出你想要的“正确”结论，而是得到一些难以解释的结果。因此，本课程将培养学生解决食品研究问题，并从一些看起来是“杂乱无章”的结果中做出正确判断的能力。要获得这些科研素养，必须满足如下要求：

（1）实验前的预习：了解你需要做什么和可能获得的结果。

（2）做个细致的观察者：如观察样品的颜色、气味、形状，分析所采取的实验方法能否获得有意义的结果等。

（3）详细记录：不仅记录实验课程要求的数据，而且要记录其它观察结果。

（4）丰富的想象力：对你预期的实验结果和发生的其它实验现象都能进行科学解释。

要圆满地完成本课程，你还必须达到以下要求：

（1）了解食品化学的重要化学反应及其产生的结果（如主要产物，颜色、气味变化等）。

（2）熟悉相关实验方法及其原理。

（3）能以合适的格式报告你的实验结果。

（4）能进行实验设计，研究一些简单的食品化学问题。

PREFACE

Food chemistry, which deals with the composition and properties of food and chemical changes during handling, processing and storage, is an important subject for students majoring in Food Science and Engineering or Food Safety and Quality. And food chemistry experiments are essential part of this subject. The goal of this course for students is to make scientific measurements of some important chemical reactions occurring in foods, thus 23 experiments have been designed in this manual to illustrate some of chemical and physical principles discussed in lectures. To satisfy the requirements of bi－linguistic teaching, this *Food Chemistry Laboratory Manual* is edited in English.

This manual is intended for students who have previously taken laboratory courses in chemistry and biochemistry. This course provides opportunities to improve experimental design and practical laboratory skills and can also be a useful reinforcement for other specialty courses. More importantly, food chemistry experiments very often do not work according to plan. In chemistry laboratories the chemicals are pure, the conditions are controlled and a "right answer" in theory can be expected. However, in food chemistry we often have more complex starting materials and many side－reactions can occur in parallel under non－ideal conditions. Not surprisingly, the data obtained can be confusing and hard to interpret. This course is intended to develop students' skills to solve food research problems and to conduct critical analysis of the data in a complex food matrix. When attending this course, students are required to:

(1) Come to the lab prepared: read the experiment in advance and have an idea of the results you expect.

(2) Make good scientific observations: what is the appearance of your samples before, du ring and after the reaction? What are their sensory characteristics? Do you think your measurement technique is capable of giving meaningful results from the samples?

(3) Thoroughly record your observations: rigorously record the data required and also note your other observations. Remember that your experimental notebook is not just a record of the experiment, but also must be a complete guide for another scientist to repeat your results and can be a legal document in a court of law or patent application.

(4) Be imaginative and creative: with your detailed observations you can formulate or refine your scientific theory based on what you expected and what actually occured in the experiments.

To successfully complete this course you will be also required to:

(1) Understand the important reactions in food chemistry and their consequences in food systems.

(2) Develop an expertise with the analytical methods to used measure these reactions.

(3) Report data and observations in an appropriate format.

(4) Design and conduct an experiment to study simple food chemistry systems.

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LABORATORY REGULATIONS

(1) Consuming food or beverages is not allowed in Food Chemistry Laboratory Classes.

(2) Do not pipette any liquid by mouth.

(3) Untied long hair and inappropriate footwear such as sandals are not allowed.

(4) All sharp objects, such as broken glasses, must be discarded into designated container.

(5) Solid food waste should be wrapped with plastic garbage bags before being disposed into the wastebin.

(6) Work on volatile chemicals must be performed in ventilating fume hood.

LABORATORY REPORTS

The following documentation is requested for all laboratory reports:

1. Title Page

Must include: course title, subject title, experiment title, author's name, group number & group members, date (performing experiment and submitting report).

2. Introduction and Aims

Describe the basic science relevant to the work and your goals/hypotheses for the study. Cite references to support your basic science (e. g. lab manual, textbooks and journals) and list your references at the end of the text. Do not copy section contents from this manual or other materials, plagiarism will not be tolerated.

3. Experimental Procedure

Describe what you did and how you did it. Very often this section can be abbreviated to: "The experiment was conducted as described in the *Food Chemistry Laboratory Manual* (Ou et al, 2007)." But be sure to list any changes or expanded descriptions to procedures. The method section should be written in a past impersonal form with passive voice (e. g. "A standard protein solution was prepared by weighing 20. 0g of soy powder…" not "I prepared a standard protein solution by weighing 20. 0g of soy powder…").

4. Results

Experimental data can be presented in tabular or graphical form or both. All tables, graphs and figures should be numbered in sequence and titled accurately. Data and title that appear in tables, graphs and figures should be complete and self-explanatory so that a reader can then interpret the results without referring to the text for necessary information.

5. Discussion

The discussion section may include a statement of the results reported in the tables and figures. Students need to: (1) Account for and interpret observations, results and their implications referred to each table and figure in your discussion; (2) Compare the actual results to those predicted by theory; (3) Discuss other observations and experimental errors; and (4) When questions are included in experiments, incorporate answers to these questions in the discussion.

6. Conclusion

Summarize your observations, results, and conclusions previously presented in the discussion section. The conclusions may be presented as a series of numbered statements, which include only pertinent information based solely on data within the report.

CHAPTER 1

WATER ACTIVITY

1.1 INTRODUCTION

When water interacts with solutes and surfaces, it is unavailable for other hydration interactions. The term "water activity" (a_w) describes the (equilibrium) amount of water available for hydration of materials: a value of unity indicates pure water whereas zero indicates the total absence of "free" water molecules, and addition of solutes always lowering the water activity. a_w is the effective mole fraction of water, defined as $a_w = \lambda_w x_w = p/p_0$ where λ_w is the activity coefficient of water, x_w is the mole fraction of water in the aqueous fraction, p is the partial pressure of water above the material and p_0 is the partial pressure of pure water at the same temperature (i. e. the water activity is equal to the equilibrium relative humidity (ERH), expressed as a fraction).

a_w is a particular relevance in food chemistry and preservation. It is a critical factor that determines the shelf life of food. While temperature, pH and several other factors can influence organisms growth in a product, a_w may be the most important factor in controlling spoilage. Most bacteria, for example, do not grow at $a_w < 0.91$, and most molds cease to grow at $a_w < 0.80$. By measuring a_w, it is possible to predict which microorganisms will or will not be potential sources of spoilage. Water activity——not water content——determines the lower limit of available water for microbial growth. In addition to influencing microbial spoilage, a_w can play a significant role in determining the activity of enzymes and vitamins in foods and can have a major impact on their color, taste, and aroma. It also controls the textural characteristics such as crispness and crunchiness (e. g. the sound produced by "crunching" breakfast cereal disappears when $a_w \geqslant 0.65$).

1.2 OBJECTIVE

The objective of this experiment is to learn how to determine a_w and to demonstrate the impact of various a_w on the texture and visual sensory quality attributes for foods.

1.3 APPARATUS AND INSTRUMENTS

(1) Desiccators
(2) Balance
(3) Water activity system meter
(4) Texture analyzer
(5) Souffle cups

1.4 REAGENTS AND MATERIALS

1.4.1 Saturated standard solutions: Prepare 3 ~5 different saturated standard solutions (Table 1.1 ~Table 1.2) in humidity chambers (desiccators).

Table 1.1 a_w for saturated standard solutions

Compound	a_w	Compound	a_w
NaOH	0.07	$NaBr \cdot 2H_2O$	0.58
CH_3COOK	0.23	NaCl	0.75
$MgCl_2 \cdot 6H_2O$	0.33	$(NH_4)_2SO_4$	0.82
K_2CO_3	0.43	KBr	0.83
$Mg(NO_3)_2 \cdot 6H_2O$	0.52	$BaCl_2$	0.90
$Ca(NO_3)_2$	0.56	$Pb(NO_3)_2$	0.924

Note: Use distilled or deionized water to prepare solutions. Solutions may be prepared with hot water; however, cool them down sufficiently prior to placing them in chambers.

Caution: The NaOH solution is especially hazardous. Treat all solutions with extreme care.

Table 1.2 Food products and methods for their evaluation after storage at various humidities

Food Product	Evaluation Techniques
Soda crackers	Shortometer; texture analyzer – knife probe
Ripened cheddar cheese	Penetrometer – needle point + 50g weight; texture analyzer – puncture probe
Cream cheese	Penetrometer – cone; texture analyzer – cone probe
Carrots or celery	Warner Bratzler shear press; texture analyzer – knife probe
Hard candies	Word description; texture analyzer – cylinder probe, tension mode

1.4.2 Materials

(1) Apple
(2) Cake
(3) Biscuit
(4) Foil

1.5 PROCEDURE

1.5.1 Procedure 1: Determination of water activity

1.5.1.1 Method of equilibrium relative humidity

A piece of food will gain or lose moisture to the environment but not necessarily in a linear manner. At a given level of atmospheric moisture, one food may bind more or less water than the other. The relationship between food water content and atmospheric moisture content is given by a moisture sorption isotherm. We can measure moisture sorption by the mass gained by a piece of dry food on coming to equilibrium with a known humidity. The easiest way to make a known humidity is to use a saturated salt solution. Any solution has some tendency to bind water and thus there is an equilibrium moisture content above it (the water activity a_w). Typically the more concentrated a solution, the lower the equilibrium moisture above it. If the moisture is constantly removed, for example leaving a pan of salt water out on a sunny windy day, the solution will dry out. If the solution is left in a moist environment it will gain water until it has dried out the environment and come to equilibrium again. We use a supersaturated solution (i. e. with crystals left at the bottom) so that when it absorbs moisture from the environment, more crystals dissolve but the solution concentration remains the same. Similarly if the solution is dried by the environment, more crystals form but the solution concentration remains the same. Some examples of the water activities provided by different saturated solutions at 25℃ are given in Table 1.1.

If we put a piece of food in a container with (not in) a solution, the food will gain or lose moisture until it is in equilibrium with solution. Water activity could be measured by placing samples in equilibrium with a number of salt solutions and calculating the one where there is no mass change.

The determination procedures were as follows:

(1) Prepare 6 desiccators containing a saturated solution with the water – activity modifying salt and a support for the samples.

(2) Each student group selects one product and weighs pieces of food product (4 figure precision) onto labeled aluminum foil, carefully place into the desiccators. Three samples per desiccator.

(3) Store for approximately 2 weeks to allow the samples to come to moisture equilibrium. Be careful to keep the jars somewhere safe where they won't be knocked over.

(4) Reweigh each sample. Because the samples will gain or lose moisture rapidly in contact with the lab atmosphere, you will need to weight them quickly. Once completed, return the samples to the desiccator as quickly as possible. Note that you will need the mass of the foil from two weeks ago to make your calculation.

(5) Calculation of a_w. Calculate weight gain and weight loss of the samples and draw the plot using a_w of the saturated solutions as abscissa, weight gain or loss as Y – coordinate (Fig. 1.1), the intersection point of the line with the abscissa is the a_w value of the sample.

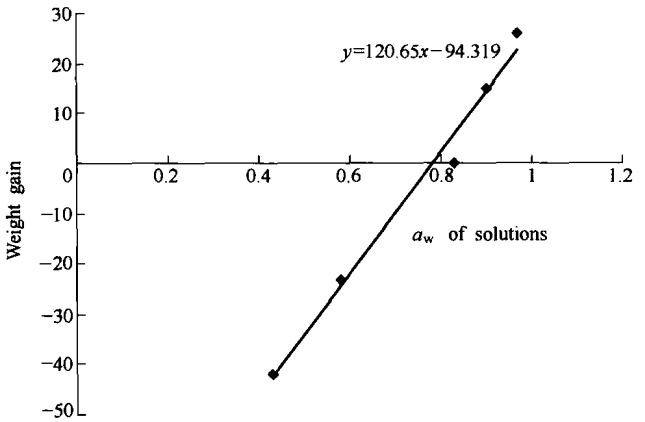

Fig. 1. 1 Relationship between Weight Gain and a_w of the Sample.

The a_w value can be calculated from the regression equation. For example, the regression equation in Fig. 1. 1 is $y = 120.65x - 94.319$; when $y = 0$, $x = a_w = 0.78$.

1. 5. 1. 2 Method of using water activity meter

The water activity meter has a small chamber where the headspace is in contact with a mirror. The mirror is chilled and eventually reached the dew point of the air (i. e. the temperature when water starts to condense on surfaces). The mirror "fogs up" and the temperature at which this occurs is measured and the relative humidity of the gas (and hence the food) is calculated automatically.

1. 5. 2 Procedure 2: Effect of varying water activity on the texture and visual sensory quality attributes of foods

(1) Prepare the saturated solutions shown in Table 1. 1 in 5 humidity chambers (desiccators).

(2) Place representative samples (at least five of each) in 2 - ounce plastic souffle cups and store in evacuated humidity chambers for 2 weeks. Compare to fresh controls.

(3) Evaluate the foods listed in Table 1. 2 utilizing the techniques suggested in there.

1. 6 STUDY QUESTIONS

(1) Was the a_w determined on the Water Activity meter close to the value expected from the relative humidity of the saturated solutions? What conditions might account for discrepancies?

(2) Are a_w and product moisture directly related? Discuss.

(3) What are the ramifications of packaging on product shelf life in relationship to a_w?

(4) When a cheese and cracker snack food is made for retail distribution, why do the a_w of the cheese and that of the cracker have to be the same? How does the cheese still maintain its "soft" texture? (Note: consider humectants).

(5) Suggest other tests that could objectively or subjectively evaluate the effect of a_w on

foods.

1.7 VOCABULARY

abscissa *n.* 横坐标
aqueous *adj.* 水的，水成的
aroma *n.* 芳香，香气，气味
coefficient *n.* 系数，常数
condense *vt.* 浓缩
crispness *n.* 脆性，松脆物
crunchiness *n.* 嘎吱嘎吱声
crystal *n.* 晶体
adj. 结晶状的
desiccator *n.* 干燥器
dew point *n.* 露点
equilibrium *n.* 平衡
ERH (equilibrium relative humidity) 平衡相对湿度
foil *n.* 箔，金属薄片
moisture sorption isotherm 吸湿等温曲线
penetrometer *n.* 硬度测量计
saturated *adj.* 饱和的
shortometer *n.* 酥松性能测定计
souffle cup 奶杯
spoilage *n.* 食物变质，食物腐败
supersaturated solution 过饱和溶液
textural *adj.* 质构
water activity 水分活度

CHAPTER 2 LACTOSE

2.1 INTRODUCTION

Lactose (milk sugar) is a disaccharide composed of galactose and glucose linked by a β-galactosidic bond. The hydrolysis of lactose is catalyzed by the enzyme lactase, a β-galactosidase. The principle dietary source of lactose is milk, which contains slightly less than 5% (w/w) of the sugar. Other dairy products contain varying amounts of lactose, depending on processing and storage conditions.

Lactose cannot be absorbed intact from the gastrointestinal tract but must be hydrolyzed to glucose and galactose by intestinal lactase. Most mammals, with the exception of humans whose ancestors came from northern Europe and a few isolated populations in Africa and India, lose the ability to digest lactose when they reach weaning age. As a result, consumption of dairy products may cause gastrointestinal discomfort in some people.

This problem has presented a challenge to food scientists to develop ways for reducing the lactose content in dairy products. Fermentation has been used for centuries for the purpose of milk preservation and, probably coincidentally, lactose reduction. Yogurt is one fermented dairy product that is frequently recommended for persons with low intestinal lactase activity. This recommendation is not without controversy, since the fermentation process used in yogurt manufacture hydrolyzes only about 20% of the lactose. The lactose content of yogurt may be quit variable since many manufactures add nonfat milk solids to their yogurt, thereby increasing the lactose content. Still, many lactose intolerant individuals claim that they can consume yogurt with none of the symptoms they experience when they drink milk. This has led to speculation that enzymes produced by the bacteria used for yogurt fermentation. *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, may be active in the guts of those who consume the yogurt and may aid in the digestion of the lactose after ingestion of the yogurt. Kolars et al. have reported some convincing evidence in support of this hypothesis.

A more recent approach to lactose reduction in dairy foods is the addition of lactase to the product by the consumer. At least one company (McN－PPC, Inc., Ft. Washington, PA; http://www.lactaid.com) currently markets a yeast－derived lactase under the trademark lactaid,

The enzyme preparation comes in a solution, and directions say to add four or to five drops of Lactaid per quart of milk and incubate for 24h in the refrigerator. The manufacturer claims that this will reduce the lactose content of milk by more than 70%.

The concentration of many compounds involved in biochemical reactions may be determined by measuring the optical density (absorbance) of the solution. Of course, this works only if the compound in question has a unique absorption maximum, i. e., it must absorb at a wavelength at which no other substance present absorbs. Lactose and galactose do not have distinct absorption maxima and, therefore, cannot be measured directly. Fortunately, they can be measured indirectly by taking advantage of coupled reactions with other compounds that do have distinct absorption maxima. A coupled reaction with NAD^+ or $NADP^+$ is commonly used in biochemical assays. Reactions of NAD^+ or $NADP^+$ with substrates are enzyme catalyzed and thus allow for highly specific assays if a purified form of a suitable enzyme is available. NAD^+ or $NADP^+$ are oxidizing agents and react stoichiometrically with reduced substrates (in our case, D－galactose) to produce an oxidized substrate and NADH or NADPH. NAD^+ (or $NADP^+$) and NADH (or NADPH) have distinctly different absorption spectra (NADH absorbs at 340nm whereas NAD^+ does not (Fig. 2. 1)).

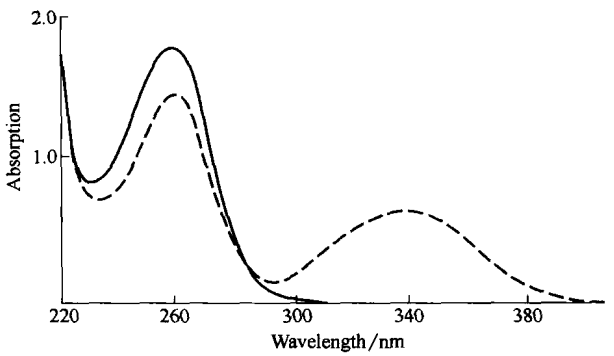

Fig. 2. 1 Absorption spectra of NAD^+ (solid line) and NADH (dashed line)

Lactose cannot be measured directly. It must first be hydrolyzed to glucose and galactose. Galactose is then oxidized to galactonic acid by NAD^+. The reaction is catalyzed by galactose dehydrogenase. The amount of NADH produced in the reaction is stoichiometric with galactose and lactose. See the Boehringer Mannheim kit directions for details.

2. 2 OBJECTIVES

(1) To determine the lactose content of yogurt.
(2) To estimate the lactase activity in yogurt under simulated intestinal conditions.
(3) To assess the effectiveness of a commercial lactase.
(4) To become familiar with a commercially available assay kit.

2.3 APPARATUS AND INSTRUMENTS

(1) Volumetric flasks, two each with 50 and 100mL
(2) Pipettes
(3) Spectronic 1600
(4) Pipeters, 5 - 50, 50 - 250, and 250 - 1000mL
(5) Water bath, 37℃
(6) pH meter
(7) Top - loading balance

2.4 REAGENTS AND MATERIALS

(1) Skim milk
(2) Plain yogurt (containing a live culture)
(3) Kit for the determination of lactose and galactose in foods (catalog No. 176303)
(4) Trichloroacetic acid, 3mol/L
(5) Lactaid
(6) NaOH, 1mol/L
(7) pH buffers
(8) Lactose standard (from kit)
(9) Galactose standard (2.5g/100mL in distilled water)

2.5 PROCEDURE

For this experiment, each student should develop his or her own protocol for determining the lactose and galactose content of milk and yogurt samples using a Boehringer Mannheim lactose - galactose kit. The following samples will be ready to analyze at the beginning of the laboratory period.

(1) Skim milk, pasteurized.
(2) Skim milk, pasteurized, treated with lactaid (a β-galactosidase preparation) for 24h in a refrigerator.
(3) Low - fat plain yogurt containing an active culture.
(4) Low - fat plain yogurt with active culture incubated at 37℃ (pH 7) for 3h.
(5) Lactose standard.
(6) Galactose standard.
(7) Reagent blanks: lactose and galactose.

Your protocol should contain the following.

(1) Step - by - step directions for sample preparation of skim milk and yogurt. If you believe