Determination of Moisture in Cheese and Cheese Products

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Variables related to oven-drying samples of cheese and cheese products to determine moisture content were examined to provide more efficient and reproducible methods. Over 6500 samples of cheese were analyzed in an effort to modify the current AOAC procedure. The gravity atmospheric oven was unsuitable for use in accurate moisture analysis because of wide temperature differentials within the oven cavity. Use of this for oven moisture determination resulted in higher variance, which corresponded to the high temperature variation within the oven. Cheese sample preparation using an Oster blender yielded consistently lower variance in final moisture content than did preparation of cheese samples with a hand grater, rotary grater, and plug and plunger. Sample size of 3 ± 0.25 g maximized surface area-to-volume ratios and yielded a lower error in final moisture content because of better control of ambient weight loss rates. Use of combination of disposable 5.5 cm diameter aluminum sample pans with 5.5 cm diameter glass fiber filter pads for covers produced a smaller standard deviation for moisture analysis than did the AOAC pan and insert cover and filter paper covers. All pans must be pre-dried for at least 3 h at 100°C, and the glass fiber covers should be pre-dried for 1 h under the same conditions. All dried pans and covers must be stored in a desiccator with active desiccant. Equipment upgrades from the existing AOAC standard methods provide safer more efficient methods of analysis.

Obtaining accurate and reproducible results from cheese moisture determinations is important from legal and economic viewpoints in the dairy industry. However, the ability to achieve accuracy from current standard methods is questionable. In 1982, 1984, and 1985, Kraft, Inc. (Glenview, IL) conducted national collaborative studies for the determination of moisture, fat, salt, and pH in Cheddar cheese samples. In 1985, 70 laboratories from the United States and Canada participated. Only 54% of those participating laboratories submitted values within the acceptable range of ± 0.25% moisture. Moreover, one-third of the laboratories could not produce a testing standard deviation (σn=1) below 0.4000.

In response to these outcomes, the objectives of this research are threefold: (1) to study the variables affecting moisture determinations in cheese and cheese products; (2) to identify procedures and equipment that will allow trained analysts to obtain consistently accurate results; (3) to identify a procedure or procedures to be used as reference for all other moisture determinations for cheese and cheese products.

Guidelines set by the National Cheese Institute subcommittee on cheese moisture analysis include limiting the testing standard deviation for analysis of moisture to 0.1500, and defining what will be considered as “moisture,” or “solids,” for the purpose of this research: “Solids are derived by the method that gives the most reproducible loss of weight and generates the least amount of decomposition.”

Review of Literature

Nature of Water in Foods

The water molecule forms a nearly perfect tetrahedron. However, because the oxygen atom is more electronegative than the hydrogen atom, bonding electrons are more attracted to oxygen, causing an imbalance of molecular charge. This results in an unusually high dipole moment, which explains the ease with which water molecules associate (1).

Most water in foods is immobilized because of the presence of hydrogen bonds and electrostatic forces. “Bound water” occurs to differing degrees in food. King (2) defined 4 forms of water binding in foods: (1) Free water.—Which constitutes the majority of water present in fresh foods and which exerts the full vapor pressure of water at a given temperature. (2) Water of crystallization.—Which is incorporated into solid crystal structures of simple molecules as water of hydration, e.g., crystalline copper sulfate, CuSO4·5H2O, and lactose monohydrate, C12H22O11·H2O. (3) Water of constitution.—Which is incorporated into macromolecules, such as DNA, in much the same way that water of hydration is incorporated for simple molecules. (4) Sorbed water.—Which may be further divided into: (a) Water of interposition.—Located in more or less mobile films, causing a vapor pressure depression; (b) Multilayer absorbed water.—Where water molecules are located near to, but not primarily at, strong polar absorption sites; and (c) Monolayer absorbed water.—Which is primarily absorbed on a one-to-one basis at strong polar absorption sites.

The amount of moisture in foods varies greatly as does the type and degree of free and bound water. Therefore, methods
of moisture analysis for different food types must be investigated separately and thoroughly. Because there is no simplistic and accurate way of determining absolute moisture content, standardization of any method of analysis can be difficult. Volatilization of nonaqueous constituents, for example, can result in erroneously high moisture values. The water of hydration of α-lactose can be removed only in part by oven-drying (3). With the aid of water sorption isotherms, Labuza (4) determined that energy requirements to drive off the last amount of water increased greatly as forces which hold the remaining water become stronger. There is, therefore, a fine line between removing as much water as possible and applying too much energy, which can cause decomposition and oxidation of the sample.

**Drying of Foodstuffs**

Drying a wet solid involves simultaneous heat and mass transfer. The primary mechanism for movement of liquid to the surface of the drying solid is capillary action of diffusion, which is highly dependent upon the nature of the substance being dried (5). The rate at which drying occurs is a complicated function of exposed surface area of the solid, temperature differential between the solid and heating medium relative humidity, and the heat and mass transfer coefficients of the material being dried. However, typical drying curves (Figures 1 and 2) describe types of drying rates which may be encountered in the drying of food material (6). The interval from A to B is the period during which unsteady state conditions approach steady state and the surface of the drying particle warms. At point B, the magnitude of the drying rate is solely governed by the rate of moisture evaporation from the surface of the food product. Conditions necessary to sustain a constant drying rate must be such that the rate of migration of moisture within the solid to surface is at least equal to the rate of evaporation of moisture from the surface. Therefore, the surface remains saturated with moisture and simulates wet-bulb temperature conditions. Movement of moisture to the surface during this period is primarily by capillary action. These conditions continue until the characteristic critical moisture content is reached at point C. Here, moisture from within the drying solid can no longer reach the surface as fast as it is evaporated. The surface begins to dry and the drying rate falls. Also, surface temperature of the food product increases as it is no longer at wet bulb conditions. The rate of drying is now dependent upon the rate of moisture migration to the surface. During this period, capillary action yields to diffusion.
Some materials may exhibit more than one falling drying rate phase. The transition of phase I to phase II is marked by the total drying of the product surface. At this point, evaporation moves below the surface of the drying material where drying rate depends upon diffusion of heat into the product and mass (vapor) out of the product. This combination results in a slow drying rate. Dependency of drying rate on the factors mentioned above is important to consider when analytical methodology to measure moisture content is being developed.

Maillard Browning and the Drying of Cheese

Of the 3 mechanisms by which browning can occur in foods, nonenzymatic (Maillard browning) is the one of most concern when assaying for moisture in dairy products, particularly moisture content caused by the evolution of water that occurs during the browning reaction. This action may influence the overall detection of moisture (7). However, studies on the possible pathways which the reaction of lactose and amino groups can take, show that the evolution of a molecule

Table 1. Composition of cheese (%)

<table>
<thead>
<tr>
<th></th>
<th>Mild Cheddar cheese</th>
<th>Mozzarella cheese</th>
<th>Process cheese</th>
<th>Process cheese spread</th>
<th>Vac-Proc cheese spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>36.09</td>
<td>50.13</td>
<td>40.17</td>
<td>45.51</td>
<td>46.27</td>
</tr>
<tr>
<td>Protein(^a)</td>
<td>27.19</td>
<td>29.19</td>
<td>22.07</td>
<td>19.22</td>
<td>19.93</td>
</tr>
<tr>
<td>Fat(^b)</td>
<td>34.70</td>
<td>16.80</td>
<td>30.35</td>
<td>20.50</td>
<td>21.00</td>
</tr>
<tr>
<td>Salt(^c)</td>
<td>1.62</td>
<td>1.21</td>
<td>2.30</td>
<td>1.87</td>
<td>1.98</td>
</tr>
<tr>
<td>Lactose/galactose(^d)</td>
<td>0.29</td>
<td>0.04</td>
<td>0.36</td>
<td>7.34</td>
<td>7.84</td>
</tr>
<tr>
<td>pH(^e)</td>
<td>5.08</td>
<td>5.12</td>
<td>5.54</td>
<td>5.68</td>
<td>5.70</td>
</tr>
</tbody>
</table>

\(^a\) Determined by Kjeldahl method.  
\(^b\) Determined by Babcock method.  
\(^c\) Determined by Volhard method.  
\(^d\) Determined by UV method.  
\(^e\) Determined by quinhydrone golf electrode method.

Figure 3. Temperature differentials while drying process cheese samples in gravity (GR), forced-air (FA), and vacuum (VAC) ovens. Sample pan load: STK = half-full, stacked; STG = half-full, staggered; FULL = full.
of water per molecule of lactose actually can range from 5 molecules to none (8, 9). The necessary components are a reducing sugar and an amino group from a protein (10). Ashoor and Zent (11) found that α-lactose produced a level of browning higher than that produced by D-glucose, D-fructose, and D-ribose. Among the amino acids, lysine is particularly susceptible because of its basic pH and free ε-amino group. The level of moisture is important also with respect to Maillard browning reaction rate. At water activity levels between 0.6 and 0.7, maximum reaction rate occurs (12).

Browning of process cheese and Mozzarella cheese has resulted in a number of studies (13–15). Streptococcus thermophilus, used in Mozzarella cheese cultures, would survive the heat treatment in making process cheese. If the particular strain of S. thermophilus does not ferment galactose, then the reducing sugar would cause browning in process cheese. This is an important consideration for moisture assays and may explain variations of results between certain brands of cheese.

**Cheese Moisture Determination Methods**

Incorrect sampling procedures of any cheese may introduce significant error if the sample is not representative of the lot, vat, or block. Potential variations of moisture within blocks of cheese must be considered in the sampling of blocks, wheels, or barrels of cheese (16, 17). Variation of moisture in 290 kg blocks of Cheddar cheese, for example, is a result of temperature differences within the blocks of cheese during cooling. Non-uniform cooling induces moisture diffusion from high- to low-temperature areas (18).

Moisture can be separated from cheese and cheese products in 4 ways: direct heating; distillation; microwave energy; and freeze-drying. Examples of such methods are presented in AOAC Official Methods of Analysis (OMA; 19) and Standard Methods for the Examination of Dairy Products (SMEDP; 20). Moisture determination involving the use of direct heat is the most common method in the food industry (21). Historically, concern has been directed at analytical reproducibility and repeatability. Helmke et al. (22) conducted an exhaustive study on variables involved in moisture analysis of cheese, including length of time in which cooled, dried cheese samples remain desiccated before weight gain occurs; uniformity of temperatures within drying ovens; and sample size. Moisture may be removed from cheese samples by direct heating in 2 ways: by heating samples under vacuum conditions or under atmospheric conditions. Both methods are of prime importance for this research.

The AOAC official final action method for removing moisture from cheese using the vacuum oven method follows:

Use clean and dry wide-mouth, cylindrical receptacles of suitable waterproof, greaseproof material (glass, stainless metal, suitable plastic material) of quality suitable for sterilization, if necessary, and of capacity suited to size of sample to be taken.

When cheese can be cut, take narrow wedge reaching from outer edge to center. When not permissible to cut cheese, take sample with cheese trier. If only one plug can be obtained, take it perpendicular to surface of cheese at a point ⅛ distance from edge to center and extending either entirely or half-way through. When >1 plug can be taken, draw 3 plugs, one from...
center, one near edge, and one midway between other 2. Use ca 2 cm (3/4 in.) of rind portion of core to reseal hole.

Cut wedge sample into strips and pass 3 times through food chopper. Grind plugs in food chopper (preferable method), or cut or shred very finely and mix thoroughly.

Weigh 2–3 g prepared sample into weighed round, flat-bottom metal dish, $5$ cm diameter and provided with tight-fit, slip-in cover. In case of soft cheese and process cheese of high moisture content, weigh 1–2 g and partially dry on steam bath. Place loosely covered dish on metal shelf (dish resting directly on shelf) in vacuum oven, kept at 100°C. Dry to constant weight (ca 4 h) under pressure $\# 100$ mm Hg (13.3 kPa). During drying, admit into oven slow current of air (ca 2 bubbles/s) dried by passing through $\text{H}_2\text{SO}_4$. Stop vacuum pump and carefully admit air into oven. Press cover tightly into dish, remove dish from oven, cool, and weigh. Express loss in weight as moisture (19).

Similarly, Standard Methods for the Examination of Dairy Products (SMEDP; 20) has also defined a standard method for analysis of cheese moisture using a vacuum oven:

**Apparatus and Reagents**

- **Aluminum moisture dishes.**—AOAC specifications, round, flat-bottomed, at least 5 cm in diameter and provided with close fitting slop-in cover, Cenco No. 12715 or equivalent.
- **Balance.**—0.1 mg sensitivity.
- **Blender.**—Oster, Waring, or equivalent.
- **Crucible tongs.**
- **Desiccator.**—With efficient desiccant.
- **Spatula.**
- **Steam bath.**
- **Thermometer.**—110°C.
- **Sulfuric acid.**—Technical or CP.
- **Vacuum oven.**—Equipped with gas washing bottles containing sulfuric acid. These bottles should be arranged so that moisture is removed from air entering the oven from the outside. Use Corning glassware No. 31760 or equivalent.

**Vacuum pump.**—Welch-distillation type, capable of maintaining a minimum vacuum of 66 cm, preferably 74 cm in oven.

**Collection and Preparation of Sample**

- (a) **Natural cheese.**—When natural cheese can be cut, take a narrow, wedge-shaped segment reaching from outer edge to center and place in container with a tight-fitting lid. When cheese cannot be cut, take sample with cheese trier. Three plugs of cheese should be taken, one from the center, one from a point near the outer edge, and one from a point halfway between the other 2. If barreled cheese is to be sampled, take a 25 to 30 cm plug about 7 cm from edge of barrel and angle at 11° to the outside. Use portion of plug 10 cm below surface for test. Cheese plugs should be placed immediately in container with tight-fitting lid. Before taking sample for analysis, the wedge-shaped segment should be cut very finely with the aid of a grater, knife, or spatula, and minced well. If plugs are taken, mix plugs with the aid of a blender for a total of about 15 s. Turn blender off and shake samples and jar manually to help move larger particles toward blade-side of mixer. Avoid prolonged mixing, as generated heat will cause fat separation and mashing of product.

- (b) **Process cheese, cheese food, and spreads.**—With aid of a spatula, cut cheese into small segments and place in 120 or 240 mL sample jar. (Avoid taking outer surface of cheese for sample use.)

- (c) **Regular cheese spreads.**—With aid of a spatula, thoroughly mix ca 150 g of sample cheese in 240 mL sample jar.

**Preparation of Moisture Dishes**

Wash aluminum moisture dishes thoroughly, rinse, and dry in oven for several hours at 100°C. Store in clean desiccator until used.
Procedure

(a) Weigh 3 ± 0.5 g sample into preweighed dish on analytical balance. Where possible, distribute sample evenly over bottom of dish. Some samples require predrying. Place dish with cover placed beneath it on metal shelf in vacuum oven for 5 h. The oven should be at temperature specified for the particular sample being tested, with minimum vacuum of 66 cm, 74 cm is preferred. During drying, admit into oven a slow current of air (about 2 bubbles per s) dried by passage through sulfuric acid washing bottles.

(b) After 5 h, shut off vacuum pump and slowly readmit dry air into oven. Using tongs, press cover lightly into dish and remove dish from oven. Cool in desiccator until dish has reached room temperature. Weigh dish.

Calculations

\[
\% \text{ Moisture} = \frac{\text{loss in weight} \times 100}{\text{weight of sample}}
\]

\[
\% \text{ Solids} = 100 - \% \text{ moisture}
\]
Notes

(a) Temperature should be measured under vacuum by a thermometer in contact with the shelf or wall of oven. Temperature in open space of oven will be different.

(b) The vacuum oven moisture method using 100 ± 2°C can be applied to cheese: natural, process, food, spread (20).

Alternatively, atmospheric drying ovens may be used to determine moisture content. The AOAC has defined a First Action rapid screening method for moisture determination in cheese using a forced-draft atmospheric oven:

Weigh 2–3 g prepared sample into moisture dish with tight-fit cover. Partially dry on steam bath with lid removed and then insert in forced-draft oven that has come to equilibrium at 130 ± 1°C. Dry 1.25 h (with cover entirely off), cover tightly, remove from oven, cool, and weigh (19).

SMEDP also gives a method for determination of moisture in cheese using an atmospheric oven. However, this method applies to a gravity (i.e., no forced air currents) oven as opposed to the forced-air oven stipulated in methods of the AOAC. The SMEDP gravity oven method follows:

Apparatus and Reagents

(Same as required for vacuum oven method except that sulfuric acid and vacuum oven requirements are replaced with gravity oven.)

Collection and Preparation of Sample

(Same as required for vacuum oven method.)

Preparation of Moisture Dishes

Aluminum moisture dishes should be washed thoroughly, rinsed, and dried in oven for several hours at 100°C. Store in clean desiccator until used.

Procedure

(a) Weigh ca 2–3 g sample into preweighed dish on analytical balance. Where possible, distribute sample evenly over bottom of dish. Place dish with cover under it on metal shelf in atmospheric oven. (Natural type cheese: dry at 105 ± 2°C for 16–18 h.)

(b) After specified time in oven, use tongs to press cover tightly into dish and remove dish from oven. Cool in desiccator for at least 30 min or until dish has reached room temperature.

Calculations

(Same as required for vacuum oven moisture method; 20.)

A variation of the vacuum oven method, the sand pan method, a fine sand and small glass rod are incorporated in the procedure (20). The sand is mixed thoroughly with the cheese sample to provide greater surface area per unit volume of cheese. This technique also provides greater chance for erroneous results because of lost sand particles. Sand aids in more

Table 3. Time required to change cheese moisture by 0.01%

<table>
<thead>
<tr>
<th>Cheese</th>
<th>Preparation method</th>
<th>Time to change moisture by 0.01%, s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2–3</td>
</tr>
<tr>
<td>Mild Cheddar&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Blender</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>Hand grater</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>Rotary grater</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>Plug and plunger</td>
<td>9.4</td>
</tr>
<tr>
<td>Mozzarella&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Blender</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>Hand grater</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>Rotary grater</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>Plug and plunger</td>
<td>10.8</td>
</tr>
<tr>
<td>Process&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Blender</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>Hand grater</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>Rotary grater</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>Plug and plunger</td>
<td>14.3</td>
</tr>
<tr>
<td>Spread&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Blender</td>
<td>15.2</td>
</tr>
<tr>
<td>Spread-Vac&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Blender</td>
<td>12.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculations based on 36% moisture.
<sup>b</sup> Calculations based on 50% moisture.
<sup>c</sup> Calculations based on 40% moisture.
<sup>d</sup> Calculations based on 46% moisture.
rapid and efficient drying of the sample. In addition glass rod can be used to spread the sand-cheese mixture evenly across the bottom of the sample pan to provide even and efficient drying conditions.

The second way to separate moisture from food is by distillation. Solvents lighter than water such as toluene and xylene are used to distill water from samples for moisture determinations. Although results are accurate, practicality suffers in terms of routine analysis and safety. The equipment is benchtop-mounted, the procedure is labor-intensive, and the solvents are flammable.

Some food types, however, warrant the use of this analytical procedure on a regular basis. Foods with high volatile constituents, such as blue cheese, favor the use of this method. Consequently, errors caused by volatilization of components other than water may be eliminated in moisture values. AOAC defines a final action standard procedure for use of the distillation method on cheeses with significant amounts of volatiles other than water (19).

The third approach in separating moisture from food is the use of microwave energy. Electromagnetic energy with wavelengths of 1 m to 1 mm are defined as microwaves (23). Frequencies of ca 2450 MHz (million cycles/s) are commonly used in microwave moisture ovens, resulting in wavelengths of ca 0.12 m. These waves of energy are used to excite dipoles (primarily water) within food samples. Friction results as these dipoles move in an attempt to align themselves with an ever-changing electric field. Thus, heat is generated throughout the sample causing evaporation of moisture.

The user should be aware of sources of variability in this technique that may affect the reliability of moisture results obtained with microwave heating. Variables to consider include: (1) differences in sample positioning within the oven; (2) geometry of the sample; (3) sample preparation method; (4) differences in wave patterns generated by different ovens; (5) influence of age or heavy usage on power output from the magnetron; (6) sample thermoconductivity; (7) sample density; (8) specific heat of the sample; and (9) dielectric constants of samples (a function of salt content). Microwave absorptivity increases with higher dielectric constants (24–26).

The final action method covering the use of microwave heating for moisture analysis is described in OMA. Details describe a microwave moisture oven which is no longer available commercially (10 g sample, 74 units power, 2.25 min; 19).

The fourth method for separation of moisture from food is freeze-drying. With this method, samples are frozen slowly to promote large crystal growth. These large ice crystals produce greater porosity within the sample to aid in the eventual escape of moisture. Sublimation is promoted under vacuum conditions and requires about 16 h to complete, depending on sample size, thickness, and composition. Final drying in a forced air or vacuum oven ensures removal of moisture. Emmons et al. (27) found that standard deviations of moisture values were lower in creamed cottage cheese, uncreamed cottage cheese, fluid milk, and goose meat with freeze-drying than with the AOAC vacuum oven method. However, time requirements (ca 25 h) may limit the practical use of this method.

**Chemical Reactivity of Water To Determine Moisture Content**

The Karl Fischer titration is a common chemical method used to determine small amounts of moisture in protein concentrates, oils, and chocolates. Generally, a small sample is used (ca 1 g) to which is added excess Karl Fischer reagent, a
mixture of iodine, pyridine, and sulfur dioxide in methanol (28). Results from a back titration of standardized water and a quick calculation determine the moisture content.

Although moisture analysis can be conducted more rapidly than oven-drying methods, a number of drawbacks must be considered: (1) cost of solvents and reagents; (2) higher coefficients of variance than obtained from vacuum oven method; (3) requirement of small sample size with concomitant variation in moisture; and (4) compounds with reactive groups which interfere with the titration results (i.e., carbonyls such as aldehydes and ketones) may be present in cheese (29–31).

**Measurement of Physical Properties of Product To Determine Moisture Content**

Methods that rely on physical properties of the product to obtain moisture content include gas chromatography, nuclear magnetic resonance, and near infrared reflectance spectroscopy.

The gas chromatographic (GC) method involves extracting moisture from product sample by first blending with a suitable solvent (i.e., 2-propanol or methanol). The extract is filtered, sealed in an air-tight container, and equilibrated to a uniform temperature. A subsample is injected into a gas chromatograph. Subsequent peaks on a strip chart are analyzed by comparison with an internal standard or a standard curve to determine moisture content. GC techniques produced satisfactory results when compared to the AOAC vacuum oven-drying method for meat and cheese moisture (31, 32). However, a limit of practical importance is the high initial investment for equipment and sample preparation requirements.

Nuclear magnetic resonance spectrometry (NMR) relies on the physical properties of the product. The underlying principals of this technique include subjecting a product sample to a strong magnetic field. Magnetic moments of nuclei within the sample begin to align with the applied magnetic field. As radio waves (wavelength: $\lambda = 1–5 \text{ m}$) are transmitted to the sample, nuclei of certain types absorb electromagnetic radiation. Absorption of these radio waves depends upon radio wave frequency, magnetic field strength, and environment of the nucleus. In this way, the structure of the molecule can be identified (33).

Hester and Quine (34, 35) identified a procedure using NMR for moisture determinations of skim milk powder and cottage cheese. Standard deviations were higher than those obtained for the vacuum oven-drying method (0.2–0.3%). However, less time was required to conduct the test. Techniques have also been described for determining moisture in margarine with a high degree of reproducibility (36).

NMR moisture determination techniques for which the laboratory technician is responsible are less stringent than oven-drying methods. This may reduce variability of results obtained by different technicians and different laboratories. However, the capital investment needed to conduct NMR analysis may preclude the average laboratory from using this technique.

Near infrared reflectance (NIR) relies upon physical properties of the product to determine moisture content. Complex motions of atoms such as vibrating, twisting, and bending within a molecule are characteristic of the functional groups which comprise the molecule, and are characteristic of overall

![Figure 8. Standard deviations of results of moisture analysis versus sample size for process using 4 sample preparation methods.](image-url)
molecular configuration. These atomic motions are responsible for absorption of infrared light, and produce an infrared absorption spectrum. The identity of the molecule can be ascertained because each spectrum is unique to a particular molecule (37).

Wehling and Pierce (38) determined moisture in Cheddar cheese with NIR; wavelengths below 1700 nm were used because of the presence of very strong water absorption bands near 1940 nm. NIR is considered a rapid method for moisture analysis. However, differences in percentage of moisture of Cheddar cheese samples determined by NIR, as compared to oven-drying, ranged from ca 0.7–1.0%. In addition, the spectral response was sensitive to temperature changes and smoothness of the top of the sample when placed in the sample cup for analysis.

Materials and Methods

Cheeses

Five varieties of cheese were used, and cheese from the same lot was used for each experiment. Mild Cheddar cheese was supplied by the University of Wisconsin Dairy Plant, Madison, WI. Mozzarella cheese was supplied by N. Dorman & Co., Inc., Monroe, WI. Process cheese, process cheese spread and vacuum process cheese spread were supplied by Kraft, Inc., Glenview, IL. Composition of each cheese is shown in Table 1. Cheeses were stored in a forced air walk-in cooler held at 6°C.

Drying Ovens

Three types of drying ovens from Lab-Line Instruments, Inc. (Melrose Park, IL) were used in this study: (1) vacuum oven (Model 3623; chamber dimensions: 30.5 × 34.3 × 30.5 cm); (2) forced-air oven (Model 3485M; chamber dimensions: 45.7 × 45.7 × 49.5 cm); and (3) gravity oven (Model 3475; chamber dimensions: 50.8 × 45.7 × 49.5 cm). Ovens 1 and 2 had air distribution manifolds and all were microprocessor controlled. Air distribution manifolds minimized uneven temperature and moisture during drying. The manifold design was specific for each type of oven (Figures 3 and 4).

Table 4. Sample pan and cover combinations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Pan, cm</th>
<th>Cover, cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>AOAC Al 5.5</td>
<td>AOAC Al, 5.5</td>
</tr>
<tr>
<td>AG</td>
<td>AOAC Al 5.5</td>
<td>Glass fiber, 5.5</td>
</tr>
<tr>
<td>AP</td>
<td>AOAC Al 5.5</td>
<td>Paper filter, 5.5</td>
</tr>
<tr>
<td>DG</td>
<td>Disposable, 5.5</td>
<td>Glass fiber, 5.5</td>
</tr>
<tr>
<td>DP</td>
<td>Disposable, 5.5</td>
<td>Paper filter, 5.5</td>
</tr>
</tbody>
</table>
The vacuum oven was evacuated using a one-half horsepower Duo-Seal® vacuum pump (Welch Scientific Co., Chicago, IL). A vacuum of 72.5 ± 1.5 cm Hg was achieved for drying cheese samples. Air allowed into the vacuum oven first passed through a 6.7 × 29 cm laboratory gas and air-drying unit containing indicating desiccant (W.A. Hammond Drierite Co., Xenia, OH). Air flow rate was controlled by a manometer (Gilmont Instruments, Inc., Great Neck, NY; size No. 11, 1–280 mL/min). The air inlet control was used to adjust air flow rate through the manometer. This rate was set at 120 mL/min during drying. This replaced the equipment needed to bubble air at 2 bubbles/s through sulfuric acid.

The vacuum oven was also equipped with 3 long sheathed type K thermocouples to monitor temperature during drying. These were arranged so that each was positioned directly below and in contact with one of the 3 shelves within the drying chamber. Routine checks ensured contact between the thermocouples and the shelves, and the shelves and wall brackets.

**Balance and Computer Data Collection**

A semi-micro digital balance (Mettler Instrument Corp., Hightstown, NJ; Model AE163) was interfaced to a Macintosh SE computer (Apple Computer, Inc., Cupertino, CA) using a standard RS232 bus and Basic software (Microsoft Corp., Redmond, WA) for specific data collection.

**Temperature Sensors and Data Acquisition**

To check the range of temperatures within the gravity and forced draft ovens, 8 T-type thermocouples were placed on each of 4 shelves. Thermocouple wires were attached to the shelves so that the tips were not touching any surface to allow accurate measurement of oven air temperatures. The Apple IlE Computer was interfaced with an ISAACS multiplexer (Cyborg Corp., Inc., Newton, MA) and a cold junction compensator (Omega Engineering, Stamford, CT, Model Omega-CJ) to collect the temperature data from the thermocouples. Temperature readings were monitored throughout the entire drying period.

The design of the vacuum oven restricted access of thermocouple leads into the drying chamber without sacrificing vacuum. A thermocouple digital datalogger (Omega, Engineering, Inc., Stamford, CT, Model Series OM-302) was connected to the 3 existing type K thermocouples located in the vacuum oven. The unit was programmed to read temperature from each thermocouple at any desired time interval.

Both schemes allowed recording of temperature within the ovens to assess temperature differentials during drying of cheese samples. In addition, temperature recovery time required of each oven after the door had been opened and shut could be determined. All thermocouples were calibrated against ice made with deionized water and boiling deionized water before use.

**Pans and Covers**

Two types of pans and 3 types of covers were used. The pans were (1) a flat-bottomed aluminum pan (AOAC, and 5.5 cm diameter), and (2) a disposable, flat-bottomed aluminum moisture pan (Dyn-A-Med Products, Barrington, IL, Model Dyn-A-Dish, No. 80065, 5.5 cm diameter). The covers used were a tight-fitting aluminum slip-in cover (AOAC) to fit pan “a”; round filter paper disks (Whatman International Ltd., Maidstone, UK, No. 1, 5.5 cm diameter); and round glass fiber disks (Whatman, Model GF/A Glass Microfibre Filters, 5.5 cm diameter).


**Humidity Indicator**

Relative humidity was monitored each day with a wet bulb/dry bulb thermometer apparatus. Mercury thermometers were calibrated against ice made with deionized water and boiling deionized water before use. A small electric fan provided the required air flow of 180–230 m/min past each thermometer bulb. cheesecloth was used for the wet wick on the wet bulb thermometer.

**Processing**

The following protocol was used to prepare and handle the cheese samples:

(1) Cheese was macerated while its temperature was still low after storage in the cooler. Surgical vinyl gloves were used for handling the cheese. Approximately 1 kg cheese was unwrapped and ca 1–1.5 cm was trimmed from edges. To control moisture evaporation, the trimmed block of cheese was placed in a 15 × 21.5 cm polybag during preparation, and wrapped tightly. Four maceration techniques were used: a blender, a hand grater, a rotary grater, and a plug and plunger. An Oster blender was used (John Oster Mfg. Co., Milwaukee, WI, Model Galaxie VIII) which had a sterilizable blade assembly for easy and thorough cleaning. The chunk of cheese was further cut into small cubes (ca 2 cm) and 4–6 of these cubes were placed in a 1 L Mason jar. The sample was blended for 7–10 s in a pulsating on/off fashion. The ground sample was quickly transferred to a 0.25 L blender jar and sealed with a lid and ring. The process continued until the sample jar was full to within 1.5 cm of the top. When the hand grater was used, the grater was placed directly over the opening of the sample jar and the chunk of cheese was grated. This continued until the jar was full to within 1.5 cm of the top. The jar was then covered and sealed immediately. The rotary grater required cutting the chunk of cheese into smaller pieces to fit the feed end of the grater (ca 3–4 cm²). The rotary grater was also placed over the opening of a sample jar which allowed the grated cheese to fall directly inside. After the jar was filled to within 1.5 cm of the top, it was covered and sealed immediately. Using the opened end of the plug and plunger device, a plug was taken from the chunk of cheese (ca 2 × 5 cm diameter). A wooden plunger then forced the plug through a wire mesh (0.3 cm²) located at the other end of the tube. Again, the cheese was delivered directly into the sample jar. The jar was covered between plugs, and when it was filled to 1.5 cm of the top, the sample jar was sealed.

(2) The prepared cheese was allowed to equilibrate to room temperature before samples were weighed.

(3) Contents of the sample jar were mixed by shaking. Only the cover ring was removed just before moisture samples were weighed. The lid remained in place and covered the jar at all times. It was only momentarily lifted during the weighing process to allow removal of a small sample of cheese.

(4) After the samples were weighed, they were placed in the drying oven within 15 min. This prevented case hardening which could interfere with drying rates and efficiency.

| Table 5. Time and temperature data for Cheddar cheese and vacuum oven |
|---|---|---|---|---|---|
| Time, h | Sample data | Temperature, °C |
| | | 65 | 80 | 90 | 100 | 110 |
| 5.5 | Moisture b | 36.71% | 36.84% | 37.08% | 37.16% | 37.43% |
| | σ (n-1) c | 0.1460 | 0.1046 | 0.1286 | 0.0896 | 0.0781 |
| | ΔE d | 3.42 | 6.34 | 7.22 | 9.37 | 9.34 |
| 5.0 | Moisture | 37.09 | 37.33 | 37.29 |
| | σ (n-1) | 0.1403 | 0.1477 | 0.1322 |
| | ΔE | 11.12 | 8.93 | 12.25 |
| 4.5 | Moisture | 37.01 | 37.26 | 37.30 |
| | σ (n-1) | 0.1019 | 0.1148 | 0.1322 |
| | ΔE | 8.09 | 8.62 | 12.25 |
| 4.0 | Moisture | 37.16 |
| | σ (n-1) | 0.0812 |
| | ΔE | 9.39 |
| 3.5 | Moisture | 37.22 |
| | σ (n-1) | 0.1404 |
| | ΔE | 9.61 |

---

a Sample data = average of 40 replicates.

b Moisture = %.

c σ (n-1) = Sample standard deviation.

d ΔE = Total color difference.
(5) The dried samples were put in a clean desiccator with indicating desiccant and allowed to come to room temperature before being reweighed.

Each trial conducted in this research was repeated a minimum of 10 times. The culmination of experiments throughout this study produced analysis of 6500 samples.

**Colorimetry**

A programmable colorimeter (Beckman Instruments, Inc., Fullerton, CA, Model D-25-9) was used to determine the CIELAB values and total color difference ($\Delta E$) of 2 of the cheeses studied: Cheddar cheese and cheese spread. Total color difference can be calculated by:

$$
\Delta E = \sqrt{\Delta a^*}^2 + \sqrt{\Delta b^*}^2 + \sqrt{\Delta L^*}^2
$$

where the color indices are defined as: $a^*$ ranges from red to green; $b^*$ ranges from yellow to blue; and $L^*$ ranges from white to black.

The instrument was standardized with clean white and black glass plates. A freshly blended product was used for comparison for each cheese studied. (Cheddar: $L^* = 70.85$, $a^* = 15.57$, $b^* = 57.24$; spread: $L^* = 76.61$, $a^* = 12.44$, $b^* = 46.85$).

Twenty dried cheese samples (3 g sample, dried concurrently) were ground with a mortar and pestle to yield a uniform representative sample of dried cheese for a given set of drying conditions. Ground cheese was then split into 2 equal lots. Each lot was placed in a clear optical sample dish (6 × 3 cm) diameter which was put on the light port of the colorimeter. The sample dish was covered to avoid stray light. The CIELAB and $\Delta E$ values were recorded 3 times per lot, once for each 60 degree rotation of the sample dish to avoid light diffraction biases. Finally, for each representative sample, duplicate sets of 3 readings were averaged for the 4 color values measured.

**Results and Discussion**

**Effect of Oven Temperature Profile on Moisture Content**

Eight thermocouples were placed in a grid-like fashion on each of 4 shelves within the gravity and forced-air ovens. However, the vacuum oven was designed so that one thermocouple per shelf was used to record temperature for 3 shelves within the drying chamber. The temperature was monitored to determine come-up time, recovery time, temperature discontinuity, and the effect of temperature on moisture content of cheese samples.

**Determination of Come-Up Time**

Come-up time is that amount of time elapsed from the moment the oven is turned on until the operating temperature is reached in the drying chamber. It is important to ascertain how long this period of time is when warming the oven. The gravity oven required 2 h and 21 min to reach 100°C, whereas the
forced-air oven needed only 24 min to reach the same temperature. This can be attributed to the more efficient heat transfer which resulted from the forced convection in the forced-air oven. The vacuum oven, however, allowed heat transfer only by conduction and required 2.5 h to reach 100°C within the chamber.

**Determination of Recovery Time**

The recovery time was determined by measuring the time required for the oven to regain its operating temperature after the oven door had been opened for 5–10 min, and then closed. This time would allow placing cheese samples in the oven at the onset of drying. The forced-air oven recovered its operating temperature in <10 min, whereas the gravity oven needed at least 1.5 h. The vacuum oven required 1 h to regain its operating temperature. This was expected because heat transfer by conduction is not as efficient as heat transfer by convection.

**Determination of Temperature Discontinuity**

After operating temperature was reached, the temperature profile within the drying chamber was monitored in each oven. Temperatures at 32 locations were recorded per min in the gravity and forced-air ovens. To determine if the number and layout of the samples drying in the oven (fill of oven) affected these readings, the experiment was repeated 4 times to accommodate different oven fill configurations: empty; half-full, stacked; half-full, staggered; and full.

With the oven half-full, 64 processed cheese spread samples (3 g, disposable pan) were distributed throughout the oven, 16 pans per shelf. In the stacked configuration, the sample pans were aligned in a column from shelf to shelf; with the staggered configuration, the pans were shifted over one location on alternate shelves. In the full configuration, 128 processed cheese spread samples, 32 per shelf, were used. The location of the sample pans was measured and marked with an indelible pen to provide placement continuity.

The vacuum oven was tested under 3 conditions: empty; half-full, stacked; and full. Because heat is transferred by conduction under a vacuum, the staggered half-full configuration was not tested. Thirty-six sample pans, 12 per shelf, were used for the half-full layout, and 60 pans were used in the full configuration.

Throughout the duration of the drying period, oven temperature fluctuated about the same for each layout (Table 2). Note, however, that the gravity oven temperature fluctuated twice as much as the vacuum oven and 6–7 times as much as the forced-air oven. Differences in temperature across the oven cavity were also analyzed, and these readings appeared independent of oven fill. Here, the gravity oven differential was 1.5–2 times as great as the vacuum oven and forced-air oven, respectively. The reasons for the disparity of temperature profiles between the gravity and forced-air ovens were (1) the comparative convective currents within the forced-air oven (ca 700 ft/min) and the gravity oven (ca 80 ft/min) con-
tributed to the difference in temperature changes; (2) the air flow manifolds in the forced-air oven directed convective currents evenly throughout the oven, contributing to temperature uniformity; and (3) each oven was equipped with a different thermostat control. Temperature control and monitoring in the gravity oven relied on a hydraulic thermostat design, as opposed to a considerably more accurate microprocessor control device used in the forced-air oven. This feature greatly contributed to temperature accuracy and uniformity within the forced-draft oven.

The vacuum compared to the forced-air or gravity oven has minimal convective currents. However, the oven is designed to maximize heat transfer by conduction. In addition, temperature is microprocessor-controlled. These 2 factors explain the lower temperature differentials found in the vacuum oven as compared with the gravity oven.

**Cheese Moisture Versus Drying Ovens**

The same processed cheese spread samples (3 g, disposable pan) described above, were analyzed for moisture content. Standard deviations were then compared with oven temperature differentials.

Significantly higher standard deviations (Figure 3) were obtained from results of samples dried in the gravity oven, which also showed the highest temperature differential within the oven cavity. Samples dried in the forced-air oven yielded lower standard deviations than did those dried in the gravity oven. This corresponds directly to lower temperature differentials discussed above.

Although the temperature discontinuity in the vacuum oven was higher than that in the forced-air oven, analysis of cheese moisture values showed that the vacuum oven yielded lower standard deviations because it combined elevated temperatures at greatly reduced pressures. This combination resulted in more efficient drying conditions than found either in the gravity or forced-air oven. Furthermore, the vacuum oven air manifolds are located in the front bottom of the chamber, which allows a small stream of dry air to sweep through the oven and exit at the other manifold, located in the upper rear of the oven. This manifold configuration sweeps evaporated moisture from the oven chamber. Data collected to determine the influence of extent of oven fill did not show strong correlation with temperature differentials or standard deviations for any of the ovens tested.

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Sample data⁵</th>
<th>Temperature, °C</th>
<th>65</th>
<th>80</th>
<th>90</th>
<th>100</th>
<th>110</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Moisture⁶</td>
<td></td>
<td>44.22%</td>
<td>45.29%</td>
<td>46.95%</td>
<td>47.73%</td>
<td>48.00%</td>
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<tr>
<td></td>
<td>σ (n-1)⁷</td>
<td></td>
<td>0.3185</td>
<td>0.1311</td>
<td>0.1507</td>
<td>0.1101</td>
<td>0.1129</td>
</tr>
<tr>
<td></td>
<td>ΔE⁹</td>
<td></td>
<td>30.74</td>
<td>54.31</td>
<td>59.64</td>
<td>63.22</td>
<td>63.27</td>
</tr>
<tr>
<td>17</td>
<td>Moisture</td>
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<td>46.31</td>
<td>47.31</td>
<td>47.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>σ (n-1)</td>
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<td>0.0843</td>
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<tr>
<td></td>
<td>ΔE</td>
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<td>59.78</td>
<td>67.23</td>
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</tr>
<tr>
<td>16</td>
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<td>46.87</td>
<td>47.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>σ (n-1)</td>
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<td>0.0834</td>
<td>0.0659</td>
<td>0.1853</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ΔE</td>
<td></td>
<td>56.96</td>
<td>63.56</td>
<td>65.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Moisture</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>σ (n-1)</td>
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<td></td>
<td></td>
<td>0.0886</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>ΔE</td>
<td></td>
<td></td>
<td></td>
<td>66.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Moisture</td>
<td></td>
<td></td>
<td></td>
<td>47.48</td>
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<td></td>
<td>σ (n-1)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>ΔE</td>
<td></td>
<td></td>
<td></td>
<td>66.65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁵ Sample data = average of 40 replicates.  
⁶ Moisture = %.  
⁷ σ (n-1) = Sample standard deviation.  
⁹ ΔE = Total color difference.
used were mild Cheddar, Mozzarella, process cheese, process cheese spread, and vacuum process cheese spread. Preparation methods used included a blender, hand grater, plug and plunger, and rotary grater. Sample size categories were 2–3, 6–7, and 10–11 g. Standard AOAC aluminum moisture dishes (5.5 cm) were used with corresponding slip-in aluminum covers. In addition, relative humidity was recorded throughout this experiment.

Combinations of 3 variables were analyzed for rates of weight loss at ambient temperature. Changes in cheese sample weight were recorded immediately after delivery of the proper sample size to a pan tared on an electronic balance where weight was recorded ± 0.1 mg every 5 s for 5 min using computer data acquisition.

**Determination of Weight Loss Rates**

Weight loss rates were obtained by regression analysis, and average rates were obtained from 10 replicates of each combination in the experimental design. Data from cheese samples before drying had a high degree of linearity \( R > 0.997 \). This suggests that for the first 5 min, weight loss from ambient moisture evaporation is due to the constant drying rate phase of the drying curve. Weight loss rates versus sample size are compared for mild Cheddar, Mozzarella, and process cheese in Figures 4, 5, and 6, respectively. The rate for cheese spread was \( 2.44 \times 10^{-5} \) g/s and that for vacuum process cheese spread was \( 3.002 \times 10^{-5} \) g/s. As expected, with a larger sample size, weight loss caused by evaporation was greater. Also, of 4 sample preparation methods, the blender method gave consistently low weight loss rates for each cheese and sample size.

**Ambient Weight Loss Rate and Change in Moisture Content**

It is important to consider the consequences of ambient weight loss rates on final cheese moisture values. The contribution of error to these values will be greater if initial cheese sample weights are not measured as rapidly as possible. For a given sample size, sample preparation method, and corresponding weight loss rate, the amount of time required to change moisture content by 0.01% for each cheese is listed in Table 3. This experiment was repeated with cheese samples which had been dried in a vacuum oven (100°C, 4.5 h) to check for possible hygroscopicity. These data, however, were inconclusive, which mitigates the problem of hygroscopicity in samples of dry cheese.

**Relative Humidity and Ambient Weight Loss Rate**

The rate of drying during the constant-rate period of the drying curve can be characterized by equation 1 (18).

\[
\frac{dw}{dt} = \frac{hA(T_s - T_w)}{L} \quad (1)
\]

---

**Table 9. Extended time and temperature data for Cheddar cheese and vacuum oven**

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Sample data (^a)</th>
<th>Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moisture (^b)</td>
<td>65</td>
</tr>
<tr>
<td>5.5</td>
<td>( \sigma_{(n-1)}^{c} )</td>
<td>35.69</td>
</tr>
<tr>
<td></td>
<td>( \Delta E^{d} )</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>Moisture</td>
<td>35.64</td>
</tr>
<tr>
<td></td>
<td>( \sigma_{(n-1)}^{c} )</td>
<td>0.1184</td>
</tr>
<tr>
<td></td>
<td>( \Delta E^{d} )</td>
<td>5.57</td>
</tr>
<tr>
<td>4.5</td>
<td>Moisture</td>
<td>35.59</td>
</tr>
<tr>
<td></td>
<td>( \sigma_{(n-1)}^{c} )</td>
<td>0.1523</td>
</tr>
<tr>
<td></td>
<td>( \Delta E^{d} )</td>
<td>3.94</td>
</tr>
<tr>
<td>4.0</td>
<td>Moisture</td>
<td>35.54</td>
</tr>
<tr>
<td></td>
<td>( \sigma_{(n-1)}^{c} )</td>
<td>0.1223</td>
</tr>
<tr>
<td></td>
<td>( \Delta E^{d} )</td>
<td>8.47</td>
</tr>
<tr>
<td>3.5</td>
<td>Moisture</td>
<td>35.54</td>
</tr>
<tr>
<td></td>
<td>( \sigma_{(n-1)}^{c} )</td>
<td>0.1223</td>
</tr>
<tr>
<td></td>
<td>( \Delta E^{d} )</td>
<td>8.47</td>
</tr>
</tbody>
</table>

\(^a\) Sample data = average of 40 replicates.
\(^b\) Moisture = %.
\(^c\) \( \sigma_{(n-1)}^{c} \) = Sample standard deviation.
\(^d\) \( \Delta E^{d} \) = Total color difference.
where, $\frac{dw}{dt}$ = change in moisture per unit time; $h$ = heat-transfer coefficient for conditions at the cheese-air interface; $A =$ total surface area; $T_a =$ air dry bulb temperature; $T_w =$ wet bulb temperature ; $L =$ latent heat of vaporization.

For drying rates obtained with different relative humidities, the following may be written:

$$\frac{dw_1}{dt} = h_1A_1(T_a - T_w) \frac{1}{L}$$  \(2\)

$$\frac{dw_2}{dt} = h_2A_2(T_a - T_w) \frac{1}{L}$$  \(3\)

Under constant-drying rate conditions, mass transfer out of the cheese particle is equal to heat transfer in. Therefore, the heat transfer coefficient at the surface of the macerated cheese particulate is solely governed by geometry, with all other variables being constant. Heat transfer coefficients in equations 2 and 3 are equal if the same sample preparation method is used. Similarly, total surface area in this experiment was held constant for the same sample preparation method and sample size.

Latent heat of vaporization is a function of temperature and moisture content only when the original moisture content is <10%. Therefore, in all cases considered in this study, latent heat of vaporization is a constant.

$$h_1 = h_2$$  \(4a\)

$$A_1 = A_2$$  \(4b\)

$$L = constant$$  \(4c\)

Taking ratios of equations 2 and 3 and simplifying, the following is obtained:

$$\frac{\frac{dw_1}{dt}}{\frac{dw_2}{dt}} = \frac{T_a - T_w}{T_a - T_w}$$  \(5\)

If room temperature is held constant, then,

$$T_{a1} = T_{a2}$$  \(6\)

and equation 5 can be simplified further to give

$$\frac{\frac{dw_1}{dt}}{\frac{dw_2}{dt}} = \frac{DT_{1}}{DT_{2}}$$  \(7\)

That is, to determine the effect of relative humidity on ambient weight loss rates, take the ratio of the differences between wet bulb temperature and room temperature.

**Example:** An analysis laboratory is equipped with an accurate thermostat which keeps temperature constant at 70°F, but has no way of controlling humidity. A technician conducts moisture analysis for mild Cheddar cheese samples (2–3 g) using the blender preparation method on a day when relative humidity is at 80%. One week later, the experiment is repeated.
using the same cheese, under the same conditions, except the relative humidity has changed to 50%. If the initial ambient weight loss rate is \(3.0 \times 10^{-5}\) g/s, the weight loss rate for the second analysis conditions can be determined as follows: Initial conditions: \(T_a_1 = 70^\circ F; (80\% \text{ PH})\) \(T_w_1 = 67^\circ F; D_T_1 = 3^\circ F;\)
\[
\frac{d w_w}{d t}_1 = 3.0 \times 10^{-5} \text{ g/s.}
\]
Final conditions: \(T_a_2 = 70^\circ F; (50\% \text{ RH})\) \(T_w_2 = 58^\circ F; D_T_2 = 12^\circ F;\)
\[
\frac{d w_w}{d t}_2 = 7
\]
Using equation 7,
\[
\frac{3.0 \times 10^{-5} \text{ g/s}}{\frac{d w_w}{d t}_1} = \frac{3}{12}
\]
and,
\[
\frac{d w_w}{d t}_2 = 1.2 \times 10^{-4} \text{ g/s}
\]
The rate of weight loss caused by evaporation during ambient, pre-dry conditions is 4 times as fast during the second analysis than during the first. That is, if it takes 10.7 s to change the moisture content by 0.01% during the first analysis, it only takes 2.6 s during the second.

Therefore, prepared cheese for moisture analysis should be weighed as quickly as possible after it is exposed to outside air from the sample jar. This will help minimize variations in final moisture values caused by fluctuations in relative humidity.

**Effect of Cheese, Preparation Method, and Sample Size on Moisture Content**

Knowledge of ambient weight loss rates offers insight on contribution to error during preparation and initial weighing of cheese samples. However, further experimentation was required to determine which preparation method and sample size yielded the least variance in moisture content. A full factorial design was used to determine the effect sample size and sample preparation method had on final moisture values.

Three varieties of cheese were macerated using 4 sample preparation methods and were weighed to 3 sample weights. The cheeses used were mild Cheddar, Mozzarella, and process.

Process cheese spread and vacuum process cheese spread were not tested because of limitations of preparation method and sample size. Preparation methods used included a blender, hand grater, plug and plunger, and rotary grater. The sample size categories were 2–3, 6–7, and 10–22 g. Standard AOAC aluminum moisture dishes were used with corresponding slip-in aluminum covers. In addition, relative humidity was recorded throughout these experiments. Cheese samples were dried in a vacuum oven (100°C, 4.5 h, 73 ± 1 cm Hg vacuum) and reweighed to assay for moisture lost.

**Sample Preparation Method and Sample Size**

Standard deviations from results obtained with the blender sample preparation method were relatively small and more consistent in variation than data calculated from moisture values obtained by processing cheese through the hand grater, ro-
tary grater, or plug and plunger (Figures 7, 8, and 9, respectively). Use of the hand grater can be somewhat awkward and yields large shreds of cheese. The thickness and length of the shred size depends upon 3 factors: the pressure applied while shredding, the distance traveled while stroking, and the surface area of the chunk of cheese being shredded.

The rotary grater produced more uniform shred particulates than did the hand grater. In addition, it yielded the smallest particulate geometry of all methods studied and, therefore, the highest surface area per unit mass. Control of experimental error was difficult because of greater ambient weight loss rates. Consequently, variation in moisture content was unpredictable. Shred geometry obtained from the plug and plunger device was largest. This could interfere with efficient heat and mass transfer during drying. As a result, wide variations in moisture content were observed in cheese samples prepared using this device.

**Sample Size and Moisture Content**

Because the blender is the sample preparation method of choice, analysis of the effects of sample size on the variation in moisture content were restricted to this method.

Results from the 2–3 and 6–7 g samples were acceptable for cheeses used in this study. Standard deviations of results from the 10–11 g samples were almost twice those from the 2–3 and 6–7 g samples. Samples of 10–11 g were cumbersome and often required removing aliquots from the sample jar 2 or 3 times to obtain the correct amount at initial weighing. Thus, error may have resulted from 2 sources: time required to record the weight of an exposed sample, because error increases as moisture evaporates from the sample; with large sample weights, cheese in the sample jar is exposed to outside air 2 or 3 times for every sample weighed. Appreciable dehydration of cheese in the sample jar contributes to error in final moisture values.

The 2–3 g size was favored over the 6–7 g size for 2 reasons: (1) Cheese must be obtained from the sample jar twice to reach the target weight of 6–7 g. Potentially, a greater variance in results occurs with each opening of the sample jar. (2) Uniformity results in the proposed standard method because cheese spread and vacuum process cheese spread both require 2–3 g sample size.

**Effect of Sample Pan and Cover Style on Moisture Content**

Combinations of different sample pan styles and covers were tested to determine any effect on variability in final moisture content values. Two sample pans were used in combination with 3 types of covers (Table 4). Cheddar cheese samples (2–3 g), prepared with the blender method, were dried in a vacuum oven (100°C, 4.5 h, 73 ± cm Hg) for this comparison. Moisture content was assayed, and standard deviations were calculated from these results.

**Cover Style and Variability of Moisture Content**

Results from moisture determination using combinations of glass fiber disks as covers and disposable and AOAC pans had the smallest standard deviations (Figure 10), and, corresponding moisture values from samples in disposable pans were lower. Standard deviations obtained from moisture values using paper filter disks as covers were higher than those obtained when glass fiber disks were used. In separate tests conducted to ascertain the hygroscopicity of paper versus glass fiber disks, paper filter disks displayed a tendency to attract moisture after pre-drying, whereas glass fiber disks showed no such activity. The amount of moisture accumulated per disk was dependent on the relative humidity and time.
required to weigh each disk. If these factors were not carefully controlled, larger standard deviations and higher moisture contents were obtained when paper filter disks were used.

Higher moisture values and greater standard deviations were obtained when cheese was dried in AOAC pans with AOAC covers rather than cheese in AOAC pans with glass fiber disks for covers. To allow moisture to escape during drying, the AOAC cover on the AOAC pan must be removed or slipped to the side, i.e., off-center. Fat in cheese spatters during drying, and pans with covers removed partly or fully allow some fat to escape. The amount of fat lost from spattering depends upon how far off-center the cover is placed. Error incurred from loss of fat leads to erroneously higher moisture values and greater standard deviations.

Pan Style and Variability in Moisture Content

Results obtained with AOAC sample pans with glass fiber covers produced moisture values and standard deviations only slightly different from those obtained when disposable pans and glass fiber covers were used. However, pitting and the subsequent development of pinhole-sized openings in the sample pans may occur over time in AOAC pans which are washed in alkaline detergents. Often, early detection of these holes is difficult. If pan integrity is not properly monitored, substantial error in moisture values will result. In addition, operating costs for disposable pans are lower than those for AOAC pans because disposable pans do not require washing. Therefore, for cheese moisture analysis, disposable sample pans are preferred.

Effect of Drying Time and Temperature on Moisture Content

Length of drying time and oven temperature were varied to determine an optimum combination for moisture determination. Cheese samples were analyzed for moisture content (%), standard deviation of moisture results \( \sigma_{n-1} \), and total color change \( \Delta E \). A high lactose cheese (process cheese spread) and a low lactose cheese (mild Cheddar) were tested to ensure consideration of extent of browning during drying.

Samples were prepared by the blender method, weighed to \( 3 \pm 0.25 \) g in disposable pans, and covered with glass fiber disks. This experiment was repeated for both cheeses in the forced-air and vacuum ovens. Temperature was tested at 5 levels (65, 80, 90, 100, and 110°C) in both ovens. Time ranged in the vacuum oven from 3.5 to 5.5 h in ½ h increments. Time in the forced-air oven ranged from 14 to 18 h in 1 h increments. Combinations of these time and temperature increments enveloped the existing standard method requirements for each oven.

**Figure 13.** Weight change of labeled, disposable sample pans during second hour of drying in vacuum and forced-air ovens.
High Lactose Cheese and the Forced-air Oven

Results from drying process cheese spread in the forced air oven yielded moisture values that were significantly higher than those obtained from drying in the vacuum oven. These samples also browned considerably. Loss in weight from nonenzymatic browning (Maillard) may explain the disparity in color and weight of these samples because process cheese spread contains higher amounts of lactose than mild Cheddar. In addition, conditions for browning are more favorable in the forced-air oven than in the vacuum oven. One requisite for the Maillard reaction is moisture. Because the forced-air oven operates at atmospheric pressure rather than low pressure as in the vacuum oven, moisture requires longer to evaporate. This leaves the cheese samples more moist for longer periods of time at high temperatures. Consequently, more browning may occur in the forced-air oven. For these experiments, it took 3–5 times longer for cheese to dry in the forced-air oven than in the vacuum oven.

Determination of Target Time and Temperature Combinations

Grand means obtained from these experiments were used to target time and temperature conditions for moisture analysis. More than one acceptable time and temperature combination resulted. Therefore, optimum conditions were chosen which least deviated from existing standard methods, yet ensured proper drying. Mild Cheddar cheese and process cheese spread dried at 100°C for 4.5 h in the vacuum oven produced results which most closely corresponded to their respective grand means. Mild Cheddar cheese dried at 100°C for 16–17 h in a forced-air oven also produced moisture values close to its grand mean (limit 16.5 h at 100°C). A target time and temperature for cheese spread dried in the forced-air oven was not determined because of excessive browning and moisture values at all combinations tested.

To more closely define proper time and temperature limits for cheese dried in the vacuum oven, an extension of this experiment was conducted. Mild Cheddar cheese and process cheese spread were dried at 100°C for 4.5 h, 90°C for 4.0 h, 100°C for 4.0 h, and 100°C for 3.5 h (Tables 9 and 10). Different lots of both cheeses were used for these experiments than were used for all previous trials; therefore, tests at 100°C for 4.5 h were repeated for comparison. Upon final analysis, 4.75 ± 0.25 h at 100°C was selected as optimum for cheese dried in the vacuum oven.

Determination of Pre-Dry Conditions for Pans and Covers

Disposable sample pans and glass fiber disks (covers) were dried to constant weight at 100°C in forced-air and vacuum ovens. Disposable sample pans (average weight 1.3 g) were labeled with a permanent marker. Twenty pans were dried in each oven. Pan weights were recorded after 1, 2, 3, and 15 h drying time (Figures 11–15). Drying sample pans in either oven required at least 3 h to maintain weight fluctuations of ±0.2 mg. Similarly, unlabeled glass fiber disks (average weight 0.3 g) were dried. Changes in weight ≤± 0.1 mg were achieved when pans were dried for 1 h. Therefore, labeled, disposable sample pans must be pre-dried for at least 3 h at 100°C in either a forced-air oven or a vacuum oven. Pans must then be kept in a clean, dry desiccator with functioning desiccant until used. Predrying glass fiber disks is less critical; however, it is recommended that they be pre-dried at 100°C for at least 1 h, and kept in a clean, dry desiccator until used.
Conclusions

This research project improved upon the existing AOAC method for cheese moisture. Based on the results from over 6500 samples analyzed, the following recommendations are made for analysis of cheese and cheese products for moisture content:

1. With respect to equipment upgrade:
   a. A drying column moisture trap with indicator desiccant coupled with an air velocity meter (Gilmont Instruments, Inc., Great Neck, NY; size No. 11, or equivalent) should be used. An air flow of 120 mL/min should be used for drying cheese. These conditions should replace the hazardous and corrosive sulfuric acid drying apparatus.
   b. Air distribution manifolds such as those found in Lab-Line Models No. 3623 and 3485M should be installed in vacuum and forced-air ovens to minimize the occurrence of cold spots during drying. In addition, oven temperature should be microprocessor-controlled on all ovens.
   c. The gravity atmospheric oven should not be used for cheese moisture analysis because of high variance of temperature throughout the oven cavity during drying.
   d. A semi-micro, digital electronic balance (Mettler Instrument Corp., Highstown, NJ; Model AE163, or equivalent) with an accuracy of ± 0.1 mg should be used to minimize weighing errors and increase speed of weighing.

2. Cheese samples should be prepared by the blender method to avoid “oiling off” or fat separation.

3. During sample preparation, cheese should remain covered at all times to minimize results caused by ambient weight loss rates. Mason jars with lids and screw rings should be used to store cheese.

4. Sample size of 3 ± 0.25 g should be used to maximize surface area-to-mass ratios; all cheese samples should be allowed to remain inside the sample pan while drying.

5. Sample pans: disposable aluminum moisture dishes, 5.5 cm (Dyn-A-Med Products, Barrington, IL; Model Dyn-A-Dish, No. 80065, or equivalent) should be dried at least 3 h at 100°C in either a forced-air atmospheric oven or a vacuum oven.

6. Glass fiber disks, 5.5 cm diameter (Whatman International Ltd., Maidstone, UK; Model GF/A, or equivalent), should be used as covers for samples when drying.

7. Cheese samples should be dried in a vacuum oven at a temperature of 100°C for 4.75 h ± 15 min.

8. Dry cheese samples should be dried in a forced-air oven at a temperature of 100°C for 16.5 h ± 30 min.

References
