rtech laboratories
from Land O'Lakes

Analytical Laboratory
P. O. Box 64101, St. Paul, MN 55164-0101
Phone: 1.800.328.9687 or 651.375.2207
Fax: 651.486.0837

ACCREDITED
ISO 17025
Testing Certificate Nos.
1765.01 and 1765.02

Chemistry Test Description Summaries

Foods are tested for physical and chemical properties for various reasons including quality assurance, new product development, solving functionality issues, learning about competitor products, and preparing accurate nutritional information. The analytical laboratory uses documented and approved test methods and procedures for all standard laboratory activities and current standard reference methods for test methods and procedures, whenever possible.

When a client requests a special analysis, the validation process may be modified with agreement from the client and laboratory management. The resulting analysis would be documented fully through the project or customer supplied methods processes. Validation is required for modified standard methods, nonstandard methods, and laboratory developed methods.

See test descriptions below:

Acid Degree Value
This method is used to measure the Acid Degree Value (ADV) in dairy products. ADV is a measure of hydrolytic rancidity. It is defined as the milliliters (ml) of 1N base required to neutralize the acids in 100g of fat. This test is very similar to Free Fatty Acids and is typically performed on fat extracted from dairy products.

Ash
Ash is an estimate of the total mineral content of foods. Minerals in ash are in the form of metal oxides, sulfates, phosphates, nitrates, chlorides and other halides therefore ash content overestimates total mineral content since oxygen is present in many of the anions. Ash provides a crude idea of mineral content and is required when total carbohydrates are determined by calculation. The sample is typically heated in an oven at 550°C for 5 hours and then weighed as a percent of the original sample to give percent ash. Some samples may require other temperatures or times.

Brix
Brix is the sugar content of an aqueous solution. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by weight (% w/w). Brix is traditionally used in the wine, sugar, fruit juice, and honey industries. Brix is determined by refractive index of a solution as compared to a set of sucrose standards.

Calcium
See **Minerals**

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Calories
Calories are calculated after obtaining results for protein, fat and carbohydrates. The calculation is as follows:

Calories per 100 grams of sample = 4(%Carbohydrate by difference) + 4(%Protein) + 9(%Fat)

Carbohydrates
For nutritional labeling purposes, carbohydrates are calculated by difference after determination of ash, protein, moisture and fat. Specific carbohydrate types are determined by assaying for specific sugars.

Carbohydrate by difference = 100% - % ash - % fat - % moisture - % protein

Casein or (Insoluble Protein at pH 4.6)
Casein is the predominant protein in milk. It can be precipitated from dairy products by lowering the pH to 4.6. . After precipitation and filtration, the filtrate is assayed by the Kjeldahl method. The filtrate value subtracted from the total protein value gives the casein value. Some heat denatured whey proteins and other proteins can behave like casein and precipitate at pH 4.6. Results are reported as protein precipitated at pH 4.6.

Chloride
Chloride in food products and ingredients is determined by a **direct titration** with silver nitrate to a potentiometric endpoint.
From the chloride measurement, salt content can be determined, assuming that sodium chloride is the only source of salt.

Cholesterol
For nutritional labeling purposes, cholesterol is determined by first saponifying the sample followed by subsequent extraction into an ether phase and then reduced to dryness. The residue is dissolved into N'N' dimethylformamide where cholesterol is derivatized. The resulting trimethylsilyl ether derivative is extracted into heptane and is analyzed by capillary GC.

Color, Hunter L.a.b.
Color is determined using the Hunter L.a.b. scale in order to quantify color. The three axes in the color scale are black to white (L), green to red (a), and blue to yellow (b).

Copper
See **Minerals**

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Dropping Point
The dropping point of a fat or oil is the temperature at which the sample will change from a semi-solid to a liquid and become fluid under conditions of the test. This method is applicable to hydrogenated and non-hydrogenated fats and oils that solidify sufficiently when held at –5° C or lower for the allotted time.

Fat
Most food products are analyzed for fat by **Acid Hydrolysis** or **Base Hydrolysis**. A sample is first digested in acid or base (hydrochloric acid or ammonium hydroxide) followed by extraction with both petroleum and ethyl ether. Foods insoluble in water, such as cheese are digested in acid, while foods that are soluble in water like whey powders are digested in base before extraction. After the extract is dried, the lipid is weighed and expressed as a percent of the original sample weight. This technique is also known as the **Mojonnier** method.
The Soxhlet (by Soxtec) method is used to determine crude fat. It is a continuous ether extraction method typically used for meats, grain and feed products or foods with added fat. This technique is also referred to as the **Ether Extraction** method.

Fatty Acid Profile
For foods, fat is extracted from a sample, saponified, derivatized, and then analyzed by gas chromatography (GC) to determine which fatty acids are contained in the sample, as outlined in the AOAC Official Method 996.06. Results are reported in percent weight of the total fat. This test is also used to calculate and report amounts of saturated, unsaturated, and trans fats as fatty acids, as required for nutritional labeling of products. Total fat is calculated as triglyceride.
For fats and oils, extraction is not necessary. Results are reported as weight percent of the total fat.

Fiber
Dietary Fiber. Consisting of both soluble and insoluble materials, dietary fiber is a nutritional term defined by the method used to measure it. Insoluble fiber is composed of plant cell wall materials, primarily cellulose and lignin, while water soluble polysaccharides such as gums and β-glucan make up soluble dietary fiber. What all have in common is that they are not digestible by the human gut. Testing for total, soluble and insoluble dietary fiber is done by sequential enzymatic digestion. Total dietary fiber is required for nutritional labeling of products. Soluble and insoluble dietary fiber may be declared voluntarily.

The following tests are applicable to grains, feeds, etc.:
Acid Detergent Fiber is a method used to determine the remaining residue after digestion with sulfuric acid and the detergent cetyl trimethylammonium bromide (CTAB). The fiber residues are primarily cellulose and lignin.

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Crude Fiber is the residue remaining after digestion with sulfuric acid and sodium hydroxide. The compounds that are predominantly removed are protein, sugar, starch, lipids, and portions of the structural carbohydrates and lignin.
Neutral Detergent Fiber (NDF) is the most common measure of fiber used for animal feed analysis, but it does not represent a unique class of chemical compounds. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin.

Free Fatty Acids and Acid Value
Free fatty acids indicate the amount of hydrolytic rancidity that has occurred in a fat. Hydrolytic rancidity is caused by enzyme hydrolysis of fats into free fatty acids and glycerol. The test involves dissolving a fat sample in organic solvent and titrating with sodium hydroxide. Free fatty acid is commonly expressed as percent oleic acid. It can also be expressed in terms of acid value instead of percent free fatty acids. The acid value is defined as mg of potassium hydroxide (KOH) necessary to neutralize one gram of sample. To convert percent free fatty acids (as oleic) to acid value, multiply the percent free fatty acids by 1.99.

Iodine value
The iodine value is a measure of the unsaturation of fats and oils and is expressed in terms of the number of grams of iodine absorbed per 100 grams of oil; % iodine absorbed. It is applicable to all normal fats and oils with iodine values in the range of 18 – 165, that do not contain conjugated double bonds.

Iron
See **Minerals**

Kohman, Butter Composition
For butter and margarine, Kohman is a group of tests consisting of fat, moisture, salt and curd. The weighed sample is heated to evaporate the moisture and re-weighed to measure the moisture lost. The fat is then extracted with petroleum ether and the remaining solids are re-weighed to determine the amount of fat. The solids are then dissolved in hot water and the salt is determined by titration with silver nitrate. The moisture, fat, and salt are subtracted from 100% to determine the amount of curd.

Magnesium
See **Minerals**

Manganese
See **Minerals**

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Minerals
A variety of minerals in a sample may be determined using atomic emission spectroscopy. Atoms from mineral components both absorb and emit unique wavelengths of light when extreme heat is introduced (i.e. ICP). The minerals determined by this method are **calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc.**

Moisture / Loss on Drying / Total Solids
Oven Methods
The amount of water in food products affects its various functional properties. Moisture is measured by different methods depending on product type. Most foods are ground and then dried in a vacuum or forced air oven for a specified time and temperature and the loss in moisture is measured and expressed as a percent of the original sample weight. For samples high in moisture, results are typically reported as total solids (where % Total solids = 100% - % Moisture).

Moisture by Karl Fischer Titration
Because other constituents besides moisture may volatilize in an oven giving inaccurate results, moisture in products like fats, oils, whey, and samples that contain alcohol are determined by the Karl Fischer method. The Karl Fischer Volumetric Method electrochemically determines the water content of a sample. The Karl Fischer reagent (the titrant) contains sulfur dioxide which in the presence of water is oxidized by the iodine present in the reagent. When all of the water is consumed, excess iodine depolarizes and causes the current or voltage (measured with an electrode) to change, indicating the endpoint. This method can be used for the determination of moisture in products with anywhere from 0.01% to 100% moisture, but typically Karl Fisher is used in products with 10% moisture or less.

Nitrite and Nitrate
Nitrite and nitrate in dairy powders can be determined by using ion chromatography with a conductivity detector. Limits of detection are 0.5 ppm nitrite and 5 ppm nitrate.

NPN (Non-Protein Nitrogen)
Non-Protein Nitrogen (NPN) is a measure of urea, ammonia, and other low molecular weight nitrogen containing compounds. Samples are prepared by precipitation of the protein with trichloroacetic acid, filtered and then the filtrate is assayed by the Kjeldahl method. This test is usually run in conjunction with protein since that method actually measures all of the nitrogen in a sample and then calculates the total protein based on this result. Subtracting NPN from total protein gives **true protein**.

Particle size, Rotap
This method determines particle size of a powder sample by placing a specific amount of sample onto a sieve, shaking it on a Ro-tap sieve shaker and calculating to determine the amount of

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sample left on the sieve or passed through the sieve. Only sieves certified by the American Society for Testing and Materials are used.

Peroxide Value
Oxidation occurs when a fat molecule chemically reacts with oxygen to form peroxides. Oxidation is catalyzed by heat, light and metals, particularly copper and iron. Although any fat can become rancid, unsaturated fats are more susceptible to oxidative rancidity than are saturated fats.
Oxidized or rancid fats have an objectionable taste and smell. Peroxide value measures oxidative rancidity in fats and oils, and is determined by quantifying the amount of iodine released from a potassium iodide solution, expressed in milliequivalents of peroxide-oxygen per kilogram of fat.

pH
Reflecting the acidity or alkalinity of a substance, pH is described as the measurement of hydrogen ion activity. It is determined by measuring the electric potential of the sample using a pH meter and glass combination pH electrode. A pH value of 7 is considered neutral. A pH value less than 7 is acidic, and a pH value greater than 7 is alkaline (basic). For most samples, pH is determined directly. **Powders** are rehydrated to an appropriate solids level before measurement. The range of this method is pH 2 to pH 11.For high fat samples, such as butter and spreads, the pH is determined on the **serum** phase.

Phosphorus
See **Minerals**

Potassium
See **Minerals**

Protein
The **Kjeldahl method** is used to measure the amount of nitrogen in a sample with an added calculation to determine protein amount. The test is carried out by digesting a sample at 420°C in the presence of a catalyst and sulfuric acid. It is then neutralized, distilled and the ammonia nitrogen is titrated to give a total nitrogen result. The nitrogen is then multiplied by a protein factor to give results in total protein. Typical protein factors are 5.70 for wheat products, 6.38 for dairy products and 6.25 for meat and cereal products. Factors for specific products can be found in USDA's Ag Handbook.
Combustion can also be used to determine protein content. Samples are completely oxidized in the combustion chamber. Then, carbon dioxide and moisture are removed from the resulting gas and any nitrogen oxides are reduced to nitrogen. The amount of nitrogen is then determined. Protein content results can be obtained in a similar manner to the Kjeldahl method where a protein factor is used to convert nitrogen results to protein.

Protein, Denatured
Denatured protein is a calculated value as follows:

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% Denatured Protein = % Protein – % WPN - % NPN – Insoluble Protein at pH 4.6

Proximates
Proximate testing includes ash, fat, moisture (or total solids), and protein. These are the most basic tests in the compositional analysis of food. In the food industry, along with the request for proximates, customers may also request the following calculations using these 4 results:

%Carbohydrate by difference = 100% - %ash - %fat - %moisture - %protein

Calories per 100 grams of sample = 4(%Carbohydrate by difference) + 4(%Protein) + 9(%Fat)

Salt
See **Chloride**

Scorched Particles
This method is used to measure the amount of scorched or burnt particles in a dry dairy powder. The powder is rehydrated and passed through a filter to collect any scorched or burnt particles.

Sodium
See **Minerals**

Sorbic Acid
Sorbic acid is used as a cheese preservative and is often allowed in a formula at no more than 0.2%. Products are tested for sorbic acid using HPLC. Sorbic acid is reasonably effective in inhibiting mold on high acid foods and less effective with higher pHs. It becomes almost useless at pH levels close to or above 6. Another interesting note about sorbic acid is that it can be metabolized by mold and is decarboxylated to create 1,3 pentadiene. This compound is very obnoxious and has been attributed to flavor complaints and product recalls in the past.

Specific Gravity
Specific gravity is the ratio of the density of a substance (weight per volume) to the density of a reference substance, almost always, water. A pycnometer is used to contain a specific volume of sample so that it can be weighed at a specific temperature. Specific gravity is commonly used in industry as a simple means of obtaining information about the concentration of solutions of various materials such as brines, hydrocarbons, sugar solutions (syrups, juices, honeys, etc.) and acids.

Sugars
Sugars in foods are measured for nutritional labeling purposes and functional issues including enzymatic browning, viscosity, and flavor. A high performance liquid chromatography (HPLC)

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method is used to determine levels of fructose, glucose, sucrose, maltose, and lactose in cheese, milk and other food products using an NH_2 analytical column, a refractive index detector, and an acetonitrile/ water mobile phase. The sum of these five sugars is reported as the total sugars on a Nutrition Facts Panel.

Another method, for specific sugar determination is the enzymatic method. Boehringer Mannheim Enzymatic kits are available for a number of different sugars assays. Assays are performed using a UV spectrophotometer.

Titratable Acidity
Titratable acidity measures the amount of alkali required to neutralize the acidic components of a given quantity of sample, and is expressed as percentage of a specific acid (e.g. lactic, acetic or citric). This is a measure of the total acidity of a sample; different acidic components within a sample cannot be distinguished from one another.

Total Solids
See Moisture

Vitamin A (Retinol)
For nutritional labeling purposes, vitamin A is determined by saponifying the sample followed by neutralization and dilution. The resulting solution is analyzed for vitamin A (retinol) content by employing High Pressure Liquid Chromatography (HPLC) and an UV detector.

Vitamin C
For nutritional labeling purposes, vitamin C is determined by extraction of the sample with a phosphate solution. The final solution is analyzed by using ion pairing and high Pressure Liquid Chromatography (HPLC) equipped with an UV detector. The vitamin C is a sum of both L ascorbic and dehydro ascorbic acid.

Water Activity (a_w)
Water activity is a measure of the energy status of the water in a system. It is defined as the vapor pressure of a liquid divided by that of pure water at the same temperature; therefore, pure distilled water has a water activity of exactly one. It is often used as a measure of the water available for the proliferation of microorganisms. Water activity can be an important tool for predicting shelf life of foods, and also helps determine the type of processing and/or packaging that may be necessary for a particular food.

WPN by Kjeldahl(Whey Protein Nitrogen)
The undenatured whey protein nitrogen is referred to as whey protein nitrogen (WPN) and is often used as an indicator of the heat treatment applied to a product. Casein and the heat denaturated whey proteins are removed by filtration after precipitation of the sample with NaCl. The undenatured whey protein (and nonprotein nitrogen) remains and is measured by Kjeldahl.

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The NPN is measured independently and subtracted to give the WPN result. WPN is expressed as milligrams of nitrogen per gram of product

WPN by Spectrophotometry
This test determines the amount of undenatured whey protein nitrogen (WPN) in nonfat dry milk. Denatured whey proteins are precipitated by concentrated salt solution. Filtrate containing undenatured whey protein is made turbid by addition of hydrochloric acid and read spectrophotometrically. The readings are compared with those on a standard curve for NFDM. For samples other than NFDM, the WPN must be performed using the Kjeldahl method as there is no standard curve for other types of dairy powders or products. These results are expressed in milligrams Nitrogen per gram of sample.

Zinc
See **Minerals**