APPLIED DAIRY CHEMISTRY AND TECHNOLOGY

by

Vernal S. Packard

Department of Food Science and Nutrition

Minnesota Extension Service

Minnesota/South Dakota Dairy Food Research Center

University of Minnesota

St. Paul, Minnesota 55108

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Why did the use of whey proteins in a concentrated, liquid, special dietary food cause the product to set up in a gel? How might the proteins been handled/processed to prevent gelation?

These are the kind of questions this course seeks to answer through a better knowledge of the chemistry of milk. It will consider factors that influence composition and quality of milk from the cow through processing and storage of milk and dairy products. The emphasis is on chemistry - but solely as this fundamental aspect of all matter can be related to process technologies that best serve the dairy and food industry.

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CHAPTER I

BASIC CONCEPTS AND TERMS

molecule can be further broken down without losing its characteristic identity. Putting it positively, all molecules of lactose will possess the same chemical fingerprints as all other molecules of lactose.

An example may help explain the significance of molecular identity. Lactose can serve that purpose well. For many years, science held that milk of mammals was the only source of lactose. It could be found nowhere else. And indeed, rarely is it found elsewhere. Nevertheless, its chemical imprint was observed in certain plants long after it was known to be a constituent of milk. And the scientists who made the discovery could be certain that they were dealing with lactose and no other molecule because of the unique chemical and physical properties this "unknown" compound was found to possess. No matter how complex a mixture of compounds, molecules of lactose can be isolated and identified by various chemical means. You can be as sure that you are "seeing" lactose as you are in recognition of a parent or friend.

It is by chemical and physical characteristics that molecules are known. It is equally true that unlike molecules will behave differently in some one or more specific ways.

Organic and inorganic chemistry

Another distinguishing aspect of molecules should be mentioned. It is the feature that separates two major fields of chemical science. With but few exceptions, the difference lies in the presence or absence of the element carbon. More specifically, **organic** chemistry, as distinguished from **inorganic** chemistry, is the study of **hydrocarbons** or their derivatives (compounds derived from them).

The word "organic" originates from the early premise that compounds of this kind were thought to be present in or manufactured only by living things. This premise is not true, and today thousands of organic compounds are recognized as being apart from life processes. Still, the carbon atom and the hydrocarbon (meaning carbon/hydrogen) linkage remain as telltale signs of an organic compound—this as distinguished from the vast majority of mineral or mineral-like compounds classified as "inorganic."

Organic compounds/molecules can be generally separated into two major categories. There are those that occur in chain-like structures which are referred to as **aliphatic** (from the Greek meaning oil or fat) and those whose chemical structure is cyclical (or diamond-shaped). The latter are termed **aromatic**, a word implying the presence of an odor or perfume, as is often associated with these compounds.

The three major organic compounds found in milk are **fat**, **protein** and **carbohydrate**. The latter is another term for sugar or sugar-like compounds. As already indicated, the major carbohydrate of milk is **lactose**.

Depicting elements and molecules

It is possible to draw a picture or create a three-dimensional object that gives to molecules a specific shape and identity. In doing so, different

relative weight that it is possible to titrate acidity or alkalinity, i.e. to know the strength of a given acid or alkali. Titratable acidity of milk can be determined only upon the basis of the relative weights of the alkali (sodium hydroxide) used in the titration and the acid (lactic acid) equivalent in which the titration is expressed.

Relative size of a molecule can only be assessed by comparing molecular weights of the molecules. In this way, a molecule of casein can be shown to be gigantic compared to a molecule of lactose. In this way one can determine whether or not a given molecule will pass through or be retained by a given membrane in an ultrafiltration unit.

Weights of molecules are determined by the weights and number of the various elements that make up the molecule. Weights of the elements have been calculated relative to the weight of oxygen which has arbitrarily been assigned a weight of 16.0.

Atomic weight, therefore, is the relative weight (mass) of an element compared to the weight of oxygen.

The following chart provides a list of major elements encountered in dairy and other biochemical products:

Element	Symbol	Atomic Weight	Element	Symbol	Atomic Weight
Aluminum	Al	26.97	Manganese	Mn	54.93
Arsenic	As	74.91	Mercury	Hg	200.61
Barium	Ba	137.36	Molybdenum	Mo	95.95
Boron	B	10.82	Nickel	Ni	58.69
Bromine	Br	79.92	Nitrogen	N	14.008
Calcium	Ca	40.08	Osmium	Os	190.2
Carbon	C	12.01	Oxygen	O	16.00
Chlorine	Cl	35.457	Phosphorus	P	30.98
Chromium	Cr	52.01	Platinum	Pt	195.23
Cobalt	Co	58.94	Potassium	K	39.096
Copper	Cu	63.54	Selenium	Se	78.96
Fluorine	F	19.00	Silicon	Si	28.06
Gold	Au	197.2	Silver	Ag	107.88
Helium	He	4.003	Sodium	Na	22.997
Hydrogen	H	1.008	Sulfur	S	32.066
Iodine	I	126.92	Tin	Sn	118.70
Iron	Fe	55.85	Tungsten	W	183.92
Lead	Pb	207.21	Urarium	U	238.07
Lithium	Li	6.94	Vanadium	V	50.95
Magnesium	Mg	24.32	Zinc	Zn	65.38

In analytical work, atomic and molecular weights are usually expressed in grams. But any unit of measure is acceptable, recognizing that relative differences are being addressed.

the concept of moles, but is standardized in terms of the amount of solution involved. The unit of measure in this case is the liter.

A molar solution, therefore, is the amount of a substance, in gram molecular weight, found in one liter of the solution.

Using 58.454 as the gram molecular weight of salt (sodium chloride), a one molar solution would contain 58.454 grams of salt per liter. In the same way, one millimolar solution of salt would contain 58.454/1000 = 0.058454 grams of salt per liter.

By similar reasoning, a solution that contains 29.2 grams of salt per liter is a one-half (0.5) molar solution. That is, one-half of 58.454 is 29.2. That amount in grams per liter is a half-molar solution.

The term molarity or molar solution, therefore, is an expression of the amount of a given substance found in one liter of solution.

Molality

Because the term is used in the scientific literature, it is well to know that molality differs from molarity in that weight rather than volume is the unit of expression. Molality is the gram molecular weight of a given substance in 1000 grams of solvent, solvent being the medium in which the substance is dissolved. A 1.0 molal solution of salt is, therefore, 58.454 grams of sodium chloride per 1000 grams of solution. Molal expressions are usually deemed more convenient when properties of both the solvent and solute (substance dissolved in the solvent) are under consideration.

Mole-Fraction

Often, a solution will consist of two or more dissolved substances. If the moles of each substance are known, it is possible to calculate the mole-fraction of each. Assume, for example, that a solution contains N_a moles of substance "a," N_b moles of substance "b" and N_c moles of substance c. Then:

$$\text{Mole-fraction of "a"} = \frac{N_a}{N_a + N_b + N_c} = X_a$$

$$\text{Mole-fraction of "b"} = \frac{N_b}{N_a + N_b + N_c} = X_b$$

$$\text{Mole-fraction of "c"} = \frac{N_c}{N_a + N_b + N_c} = X_c$$

Assume that the solution contained 0.5 moles of sodium chloride, 2.0 moles of lactose and 0.3 moles of lactic acid. Then:

In milk, for example, sodium chloride exists primarily, though not totally, as sodium ions (Na+) and chloride ions (Cl-).

Ions have important characteristics. They will allow passage of an electric current, for one. For another, those ions that carry a positive charge will have a natural attraction--an affinity--for those that bear a negative charge. The reverse is also true. Ions of like charge will repel each other; they will seek to remain apart.

The concept of moles and molarity are convenient expressions for ions in solution. Take sodium chloride as example. If it is completely ionized and present as a 1.0 molar solution (i.e. 58.454 grams per liter), then it is appropriate to indicate that the solution is 1.0 molar in sodium ion content and 1.0 molar in chloride ion content.

Or consider sodium sulfate--Na_2SO_4. A one molar solution of this compound, if completely ionized, may be said to be one molar in sulfate ion (SO_4^{--}), but 2.0 molar in sodium ions (Na^+); i.e. there are two sodium atoms present in the molecule. For a good example of the application of these concepts to dairy product processing and analysis, refer to the chapter on **Minerals/Salts of Milk.**

Perhaps it is appropriate that the nature of ions should serve as an introduction to one of the more recent processing techniques to be applied within the dairy industry. More will be said about this technology later. For now it will suffice to point out that **electrodialysis**, the process by which certain mineral components are removed from whey, depends for its usefulness on the presence of minerals in their ionic--their charged--state. By the same technology, **lactic acid** ions can be removed as well as some of the ions from the group of milk components generally referred to as **nonprotein nitrogen.**

Ion exchange, another technology for removing mineral components, also operates on the basis of attraction/affinity for each other of electrically charged components.

Electrochemical equivalent

It is often desirable to know how many moles of a substance of given electrical charge are required to neutralize the opposite electric charge of another substance.

The question, for example, is: How many moles of Na+ ions from sodium hydroxide (NaOH) are required to exactly neutralize the Cl- ions in a solution of hydrochloric acid (HCl)? Because NaOH provides one positively charged sodium ion and HCl one negatively charged chloride ion, one "mole" of Na+ can be expected to neutralize one "mole" of Cl-. In other words, one mole of Na+ is equivalent to one mole of Cl-.

By this reasoning, 1.0 liter of 1.0 molar NaOH would exactly neutralize 1.0 liter of 1.0 molar HCl. Or 1/1000 of a liter--a "milli" liter--of the alkali would exactly neutralize a milliliter of the acid.

Elements like iron and copper that ionize in solution undergo oxidation as a result of loss of electrons. They are reduced by gain of electrons. Because these two ions readily take up electrons, both serve as strong oxidizing agents, i.e. promoters of oxidation.

Chemical compounds are often classified as either oxidizing or reducing agents depending upon their propensity to promote oxidation or reduction.

Oxidation via loss of electrons can be depicted as follows:

$$Fe^{+++} + e^{-} \longrightarrow Fe^{++}$$

Ferric ion electron Ferrous ion

The higher of two oxidation products usually carries the suffix "ic", the lower oxide the suffix "ous". In this context, Fe_2O_3 is termed ferric oxide, FeO, ferrous oxide. Similarly, Cu_2O is named cuprous oxide and CuO, cupric oxide.

Oxidation-reduction "potential" can be measured as fractions of a volt of electricity. This potential is designated E_h. As such, it provides an indication of the oxidizing or reducing properties of a compound or solution.

A (+) potential is indicative of oxidizing properties, a (-) potential, reducing properties. The E_h of fresh milk ranges from + 0.25 to + 0.35 volt at normal pH (6.6 - 6.7).

As a general reaction, redox potential decreases with increase in pH.

Redox potential of milk is determined mainly by presence of (1) oxygen, (2) ascorbate, and (3) riboflavin (vitamin B_2). Ascorbate is the salt of ascorbic acid (vitamin C). Ascorbate in milk as it comes from the cow is in the reduced form. Through loss of hydrogen, reversible oxidation occurs between ascorbate and dehydroascorbate.

Riboflavin plays a key role in oxidation-reduction reactions mainly through its activity as an oxidizing agent when activated (excited) by rays of visible light. The process is called photooxidation. Exposure of milk to light results in "activated" riboflavin which, in turn, may oxidize compounds such as ascorbate, methionine, cysteine and other similar milk components. The latter two compounds are amino acids. Amino acids other than these two may also be oxidized by riboflavin.

In particular, oxidation of methionine by riboflavin leads to production of methional, a compound thought to be largely responsible for the off-flavor that occurs when milk is exposed to light.

Extensive heat treatment of milk activates reducing agents in proteins, thereby inhibiting oxidation. These reducing agents consist of sulfur and hydrogen--SH groups--and are usually referred to as sulfhydryl or thiol compounds.

* reaction with acids to produce salts

* ability to dissolve proteins in cleaning processing equipment

* ability to saponify fats, i.e. to produce soaps

pH

pH is a measure of the intensity of an acid or alkaline reaction. Because acidity depends upon presence of H+ ions and alkalinity on OH- ions, the proportion or the ratio of H+ to OH- ions determines the pH. When these two ions are present in equal amounts, the reaction is neutral, neither acidic nor alkaline.

The pH at neutrality is designated as 7.0. When H+ ions exceed OH- ions in number, the reaction is acidic. pH values in this case fall below 7.0. They range from just under 7.0 all the way down to 0.0, depending on the relative strength of the reaction. The stronger the reaction, the lower the value.

When OH- ions exceed H+ ions in number, the reaction is considered to be alkaline (or basic). pH values of alkaline reactions range from just over 7.0 on up to 14.0, depending upon the degree of alkalinity. The higher the value, the more alkaline the reaction.

In mathematical terms, pH is designated as follows: $\frac{1}{pH} = \log [H+]$ or

$pH = -\log [H+]$. Because a log scale is involved, each unit change in pH reflects a 10-fold change in acidity or alkalinity. For example, a pH of 5.0 is ten times as acid as a pH of 6.0, 100 times more acid than pH 7.0 etc.

For all practical purposes, therefore, the pH scale runs from 0.0 to 14.0. This scale can be represented in terms of the excess of either H+ or OH- ions. Figure 1 may help explain the concept of pH.

The pH of fresh milk is usually given as 6.6, i.e. slightly acidic. The end point--the point at which a color change takes place--of the indicator (phenolphthalein) used in determining titratable acidity of milk is about 8.3. For comparison purposes it may be of interest to note that the pH of apples is about 3.0, of eggs 7.6 to 8.0, of soft drinks 2.0 to 4.0, and of drinking water 6.5 to 8.0.

pH is a most important concept. It provides a measure which can be used to determine whether or not disease-causing bacteria will grow or not grow. It provides target values for determining when cultured milk products have reached their most flavorful endpoint. By establishing precise patterns of processing, pH can be used to automate cheesemaking and other systems. pH provides a basis for knowing whether a given enzyme--a compound that hastens biological processes--will function at an optimal rate. Knowing the pH of any food system can provide major insights into its general quality, including color, texture, flavor and microbial status.

$$\% \text{ lactic acid} = \frac{\text{weight of lactic acid (grams)}}{\text{weight of sample (grams)}} \times 100$$

Weight of lactic acid = milliliters (ml) of 0.1 N NaOH x 0.009

In the above equation, the value 0.009 derives from the fact that the molecular weight of lactic acid is 90. One milliequivalent is 90 divided by 1000 = 0.009.

If 9.0 grams of sample are used, the equation becomes:

$$\% \text{ lactic acid} = \frac{\text{ml of 0.1 N NaOH x 0.009}}{9.0} \times 100$$

$$= \text{ml of 0.1 NaOH x 0.1}$$

The titratable acidity reaction of fresh milk is due to the natural presence of carbon dioxide (which reacts as carbonic acid), proteins (which are amphoteric in nature, hence react as acids), phosphates and citrates. The contribution of these components to titratable acidity of fresh milk is about as follows:

Component	% Acidity
Carbon dioxide	0.15
Citrates	0.1
Proteins	0.9
Phosphates	0.6
Total	0.175

From the above information, it is apparent that fresh milk will produce a titratable acidity reaction of about 0.175% (as lactic acid) even though no lactic acid is present. This is referred to as apparent acidity, and should be distinguished from developed acidity, the true lactic acid produced by bacterial action.

Titratable acidity was long used as a measure of milk quality. The assumption was made that apparent acidity would remain fairly stable at about 0.16 to 0.17%. Values above this level were considered to result from lactic acid produced by bacterial action, which, in turn, reflected a milk supply of poor quality. Over the years, however, two conditions have altered the original premise.

First, the base "apparent" acidity level has increased as a result of breeding practices leading to higher levels of protein. The contribution of protein to apparent acidity is high. Milk supplies of high protein content are likewise high in apparent acidity, though little or no lactic acid is present. The base level of acidity of fresh milk may be as high as 0.18 - 0.20. Milk of some

pH	Grams of H+/Liter	General Relationships
14	1/100,000,000,000,000	pH of 1.0 normal NaOH
13	1/10,000,000,000,000	Strongly alkaline cleaning compounds: pH of 0.1 normal NaOH
12	1/1,000,000,000,000	pH of 0.01 normal NaOH, KOH
11	1/100,000,000,000	
10	1/10,000,000,000	Weakly alkaline cleaning compounds
9	1/1,000,000,000	
8	1/ 100,000,000	pH 8.3 = phenolphthalein end-point
7	1/10,000,000	neutral pH
6	1/1,000,000	pH 6.6 - 6.7 = pH of fresh milk
5	1/100,000	
4	1/10,000	pH 4.6 = isoelectric point of casein pH 4.1 - 4.3 = ending acidity, S. lactis
3	1/1,000	pH 3.4 - 3.6 = ending acidity, L. bulgaricus
2	1/100	0.1 normal lactic acid = pH 2.4
1	1/10	0.1 normal HCl = pH 1.1
0	1	1.0 normal sulfuric acid = pH 0.3

Figure 2. Hydrogen ion concentration and certain related factors

Buffers

When acid or alkali is added to pure water at pH 7.0, an immediate change in pH occurs. If, however, the water contains a mixture of sodium bicarbonate and sodium carbonate, addition of some small amount of acid or alkali would be found to cause little or no change in pH. Indeed, the addition of small amounts of acid or alkali to milk do not cause measurable differences in pH. In both cases it could be said that the fluids have been buffered against a change in pH. The word buffer, then, in this context, is taken to mean any substance that serves to resist a change in pH.

Several components of milk serve as buffers. These are carbon dioxide, proteins, phosphate, citrate, and certain minor constituents.

Any substance that functions as a base in the presence of acid and vice versa is defined as amphoteric. This is a buffering type of reaction, or course, and

Table 1. Kinds of dispersions/solutions as reflected in various milk components

Kind of Dispersion/solution	Characteristic/Milk Component
1. Coarse dispersion	suspension or emulsion; fat in milk; globules 0.1 - 22 microns in diameter; oil-in-water
2. Colloidal solution	particle size ranging from 0.001 to 0.1 microns in diameter; casein and some amount of certain minerals (calcium and phosphorus)
3. True solution	particle size less than 0.001 micron; lactose, some level of certain minerals
4. Ionic solution	electrically charged particles of molecular size; certain mineral components, citrates, phosphates etc.

* Note: a micron is 0.001 millimeter or 39.37 millionths of an inch.

Milkfat droplets/globules are an example of suspended solids that float ultimately to the surface. Milkfat, of course, can be stabilized to prevent the creaming effect. **Homogenization** is one technology used for this purpose. In homogenization, size of fat globules/particles are greatly reduced. The fat in this case remains dispersed. Such a mixture—of two liquids that do not dissolve in each other—is called an **emulsion.**

Fat in milk is an **oil-in-water** emulsion. The oil—fat—is dispersed in the aqueous phase. The reverse—water-in-oil—emulsions also occur. Butter is a prime example of this type of emulsion.

Homogenization is not the only way in which emulsions are stabilized, i.e. prevented from separating into discrete layers of fat and water. This process is also brought about by presence or use of **emulsifying agents.**

Any compound that has, by chemical nature, an attraction for—a tendency to dissolve in—both water and fat/oil, tends to serve as an emulsifier. A compound of this kind has a split chemical nature. Part of it has an affinity for water and part is attracted to fat. In a mixture of fat and water, compounds of this kind tend to align themselves such that the water-loving portion is oriented toward water, the fat-loving portion toward fat.

Under certain conditions, some solutions, or perhaps more precisely, certain kinds of dispersed matter, form a jelly-like mass. Gelatin was one of the early substances found to function in this manner. From that observation stemmed the word gelation, now used to indicate the process by which all similar structures are formed, and gel, the generic term applying to the structure itself.

In milk it is casein that is most commonly known for its ability to form a gel. In some situations, as in manufacture of cheese, the gel structure is essential and desirable. But when a gel forms in a dairy product that is supposed to remain liquid, it is rightfully seen as a defect, and a major defect at that. Age-thickening of evaporated milk and ultra-high-temperature(UHT) milk are good examples.

Whey proteins also enter into the gelation process. These proteins, both as they are found in natural whey and also in various concentrated forms (whey protein concentrates) most often find markets as ingredients in other foods. Gelling properties directly determine their utility and, thereby, their market potential.

Lastly, and primarily because casein enters so significantly into the manufacture and finished characteristics of so many dairy products, the term micelle should be explained.

The scientific literature is replete with references to the casein micelle. In this usage, the word derives from the Latin "mica," meaning grain or crumb. It is indicative of a particle that tends to move or function as a unit, although in fact consisting of smaller, likewise discrete particles. Casein, along with the identifiable fractions of which it is constructed, often responds in this manner.

Many of the above terms and concepts will be further treated in accompanying chapters.

Selected references:

1. Alpha-Laval AB. Dairy Handbook. Alfa-Laval, Inc., Lund, Sweden.

2. Clark, W. 1952. Topics in Physical Chemistry, 2nd ed., The Williams and Wilkins Co., Baltimore MD.

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Chapter II

Milk Composition and Related Factors

For study purposes, the components of milk can be divided into two groups: (1) major components (those constituents or groups of constituents present in relatively large amounts) and minor components (those constituents present in relatively small amounts). The latter, namely vitamins, enzymes and gases, should be considered minor only in terms of level, not significance in milk. Other minor components are also of importance and will be discussed as deemed appropriate.

MAJOR COMPONENTS

The major solid components of milk are fat, protein, lactose, and minerals. Because price of milk is based on fat alone or fat and one or more of these components, they are of special significance.

Often, for pricing and other purposes, some of these components are grouped together. Protein, lactose, and minerals, for example, are the major components of solids-not-fat (SNF). As implied, SNF refers to all solid constituents in milk except fat. Fat, together with SNF, make up total solids, i.e. all of the solid matter of milk. These relationships may be expressed as follows:

COMPOSITION OF MILK

		% by weight	
	Water	87.60	
Total Solids (12.41%)	Fat	3.70	
	Protein	3.24	Solids-not-fat (8.71%)
	Lactose	4.81	
	Minerals (as ash)	0.66	

In the above data, mineral content is expressed as ash. Ash is not mineral matter as it exists in milk. Rather, it is the material yet remaining after milk is incinerated. Although ash is often taken to mean mineral matter, it is in fact different from true minerals both in content and amount. Ash includes certain mineral elements found in proteins of milk and excludes organic ions such as citrate. On balance, ash level in milk is perhaps 0.2% lower than level of mineral salts.

The preceding information is of more than academic interest. For analytical work in which lactose content is derived by difference (lactose = SNF - (% protein + % ash), lactose content will exceed the true level by the amount that ash is lower than true mineral content. Certainly this is valid in theory. In practice, however, any difference between a measured value and a value derived by difference will depend not solely upon the chemical relationships involved, but equally as well upon the relative accuracy of the method used to determine lactose content. Hence, data contrary to theory are to be found in the scientific literature.

Table 2. Upper Midwest Regional Differences in Milk Composition

	PERCENT (by weight)				
Region	Fat	Protein	Lactose	SNF	Total Solids
NE Minn., NW Wis.	3.74	3.24	4.81	8.71	12.45
West Central Wis.	3.76	3.26	4.81	8.73	12.49
NE Iowa, SW Wis.	3.71	3.23	4.84	8.73	12.44
E and Central Minn.	3.67	3.23	4.81	8.70	12.37
S Minn., N Iowa	3.60	3.23	4.82	8.71	12.31
W and NW Minn.	3.60	3.19	4.81	8.64	12.24
N. Dak., S. Dak.	3.60	3.22	4.80	8.68	12.28

Adapted from Halverson and Kyburz

Within the region reflected in Table 2, fat content varies by over 0.1% and total solids by 0.17%. These are relatively small but nonetheless significant differences. Lower levels of fat and protein suggest less potential yield of cheese from a given amount of milk.

Even greater differences in milk composition will be found between more widespread sections of the United States.

Relationship between components

A general relationship exists between the major components of milk: An increase or decrease in one component is followed by a like, though proportional change in others. Interest most frequently lies in the relationship between fat and SNF or fat and protein. Although the change in lactose content with given change in level of fat or protein may be very small, the relationship yet holds true.

Because of the interrelationship between milk components, it is possible, knowing the percentage of one, to estimate by calculation the percentage of another. This can be a valuable tool when, for example, direct analytical measurement is not possible. It is also of value when a given method of analysis is not too precise. In such case, a calculated estimate may be as valid or more valid than a measured value.

The relationship between components is determined by a statistical calculation called a **regression analysis.** The common form of the equation that results is as follows:

$$Y = a + b(x)$$

In the equation, Y is the component whose level is to be estimated; "a" and "b" are constants (technically the intercept and slope, respectively); "x" is the percentage of the known component.

These equations suggest that, at any given level of fat, milk supplies of later years have provided proportionately higher levels of SNF.

Table 3 provides equations and regression constants for calculating level of selected milk components given known levels of one or more other components. It should be noted that the equations can be solved in either direction, making it possible to derive values of components enclosed within the parentheses. For example, to calculate level of fat given the percentage of protein, use equation number 4. Assume a protein test of 3.2%. Then:

$$\% \text{ Protein} = a + b(F)$$

$$3.2 = 1.74 + 0.4042(F)$$

$$3.2 - 1.74 = 0.4042(F)$$

$$1.46 = 0.4042(F)$$

$$1.46/0.4042 = 3.61\% \text{ fat}$$

Table 3. Equations for calculating level of selected components of milk

	Constants			
Equation	a	b	c	95% Conf. Limits of estimated value
1. SNF = a + b(Fat)	6.97	0.4640	—	0.38
2. SNF = a + b(Prot)	5.08	1.1138	—	0.26
3. SNF = a + b(P) + c(L)	0.13	1.0115	1.1026	0.06
4. Protein = a + b(Fat)	1.74	0.4042	—	0.24

Adapted from Halverson and Kyburz.
Note: In equation number 3, P is percent protein, L is percent lactose.

Considerations in determining price of milk

For many years the purchase price of milk was based upon the percentage of milkfat alone. The base level, above and below which a standard monetary value (called the uniform price differential) was added or deducted, respectively, was 3.50%. This base is still used. In the meantime, though, base levels of other components have been applied in more complex pricing schemes which perhaps better reflect the true value of milk. Not only is level of fat used to determine price of milk, but fat and protein or fat and SNF (i.e. total solids). For this reason, it is of some interest to know the level of protein and/or SNF associated with milk of 3.5% fat. These relationships have been determined for milk in the upper midwest. They are shown in Table 4.

$$\% \text{ skimmilk solids of cream} = \frac{(100 - 40) \times 8.70}{100}$$

$$= \frac{60 \times 8.70}{100}$$

$$= 5.22$$

It is apparent that the skimmilk solids content of cream is lower than that of the skimmilk or the original milk from which the cream was separated. At any given level of skimmilk solids, the amount associated with cream goes down as percentage of fat in cream increases.

Factors influencing component levels and relationships

A number of factors are known to be associated with changes either in level or interrelationship of various milk components. Most important among these are: (1) stage of lactation, (2) season of the year, (3) age of the cow, (4) mastitis/disease, and (5) nutritional status (either with or without treatment with bovine growth hormone). The latter compound(s), whether derived from cows or produced by bacteria or yeasts altered by bioengineering processes, has the potential to significantly increase the productivity of cows.

The five factors given above will be treated separately, with important aspects considered.

Stage of lactation:

For regulatory (legal) reasons, milk cannot be shipped to market until five days following calving. First milk (colostrum) is of significantly different composition than milk produced later in lactation. It is generally high in its content of fat, protein (including casein and whey proteins), calcium, phosphorus, chloride and SNF in general. The composition, though important in meeting nutritional and disease-fighting needs of the newborn calf, differs from later milk in both flavor and other esthetic qualities.

Data in Figure 1 reflect the trends occurring in major milk components over a single lactation. Both fat and protein show marked decreases in level over the first four or five weeks, followed by a gradual increase throughout the remainder of lactation. Not uncommonly, the highest levels of these two components are found in milk produced at or near the end of lactation.

As already mentioned, lactose content of milk is quite stable. This fact holds generally true even in the face of advancing lactation. However, a gradual decline in level, though slight, does tend to occur starting at about the fourth month and continuing through much of the remainder of any given lactation period.

Seasonal variations in milk composition:

Seasonal variations in milk components are due at least in part to changes in temperature. Fat, protein, and SNF all tend to increase during colder months

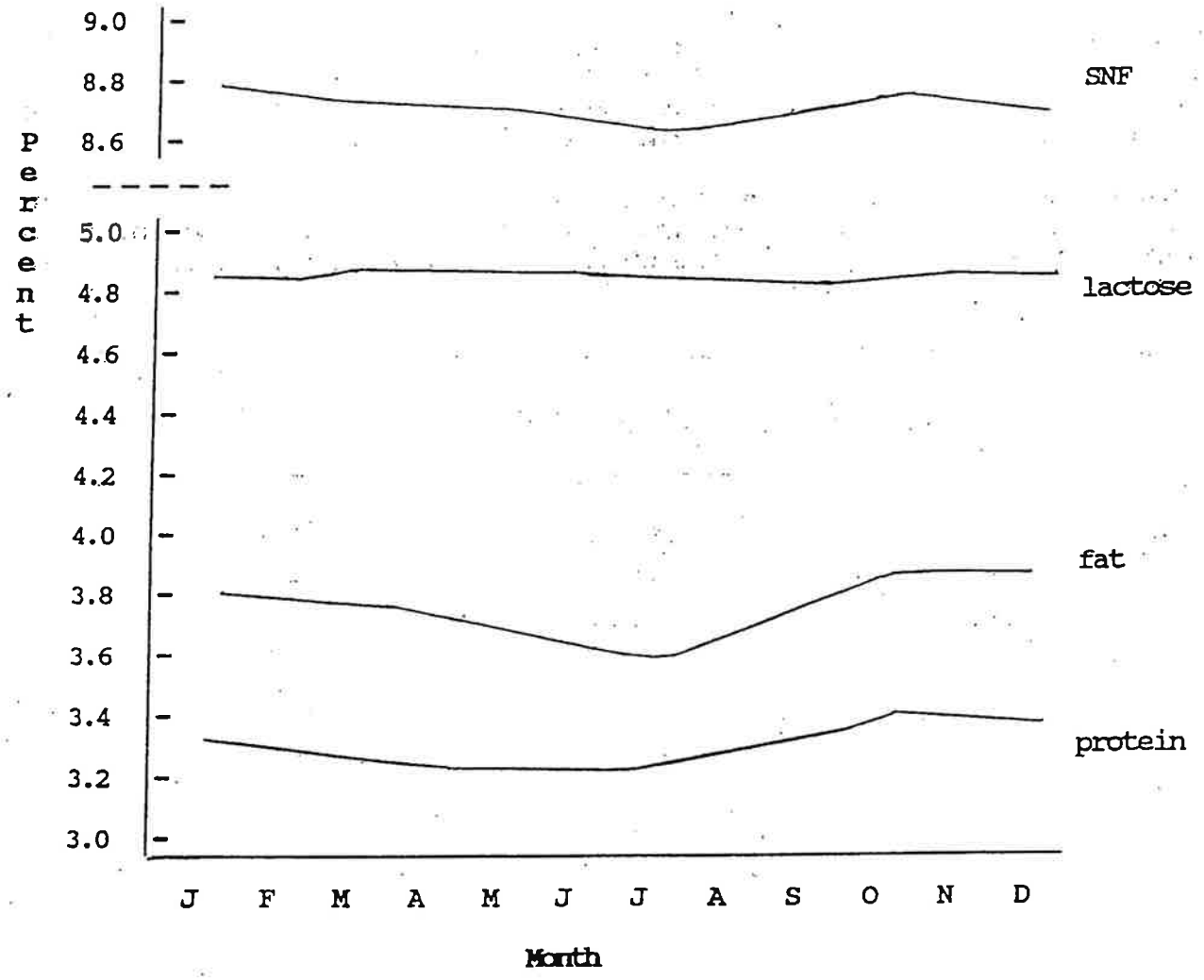


Fig. 2. Seasonal variations in selected milk components. Adapted from Halverson and Kyburz

Age of the cow:

As a general rule, level of both fat and SNF of milk decline with advance in age (number of lactations). From six years of age through remaining lactations, percentage of fat appears to decrease somewhat faster than that of protein. The extent of decrease in fat may be no more than 0.2% over the life of a cow.

Studies indicate that casein content decreases while level of total protein (crude protein) remains relatively constant over successive lactations. This being the case, it can be assumed that the aging process results in an increase in percentage of whey proteins and/or nonprotein nitrogen. The issue is complicated by possible progressive injury to the udder from the milking process itself or from prior or continuing exposure to mastitis or other infections.

the udder to blood and, thereby, various components carried by blood. The former could result in loss of milk constituents synthesized by the udder, including casein and lactose. Leakage into milk of materials found in blood could account for increased levels of sodium, chloride, and immunoglobulins (one of a group of proteins that are considered a part of the whey proteins of milk), and an increase in pH.

In addition, any condition that results in an increase in body temperature of the cow will more than likely cause a temporary drop in yield of milk and, in turn, an increase in percentage of fat, assuming that synthesis of fat is not greatly hampered. Extended states of disease, as with severe cases of mastitis, not only reduce yield, but may do such violence to milk synthesis that levels of both fat and SNF decrease.

Data in Table 6 summarize the overall effects of relatively severe states of mastitis on the composition of milk. All of the data were derived from experimental work done on individual quarters of cows. As such, the data cannot be extrapolated directly to pooled herd milk or pooled milk supplies in dairy plants. However, the data give evidence of the kind of changes that indeed take place, though less pronounced in combined lots of milk even at similar levels of somatic cell count.

Both total solids and SNF levels fall in milk from mastitic cows. To some extent, compensatory changes take place, with gains in percentage of one component making up for losses that take place in another. This is one reason why percentage change of groups of components, as represented by both total solids and SNF, may belie the significance of changes occurring in the individual components of which they consist. This is certainly true of total proteins. In this latter instance, increases in whey proteins generally compensate for decreases in casein content. Data in Table 7 provide more detailed evidence of this phenomenon.

Data in Table 6 also suggest that loss in percentage of fat contributes significantly to the observed lowering of total solids content. As mentioned previously, the initial response to mastitis may result in a loss in milk yield and, because fat synthesis continues relatively unabated, an increase in fat percentage.

Mastitis infections cause mixed changes in levels of mineral elements, as data in Table 6 reflect. Overall levels of calcium, phosphorus (inorganic) and potassium appear to decrease. Content of magnesium, sodium, iron, copper and zinc tend to decrease.

The former changes, particularly the drop in calcium and phosphorus, are considered to reflect losses <u>not</u> so much in dissolved or colloidal forms of these elements, but rather in protein components--casein, particularly--to which some portion of each is complexed. It is thought that increases in sodium and chloride content stem from "leakage" of the elements from blood to milk. Potassium losses may perhaps be explained as a flow in the opposite direction, i.e. from milk to blood.

indicating the nature of these latter considerations. Once again it is important to emphasize that the data reflect experiments done on milk from individual quarters, not pooled herd or plant milk, where overall component relationships likely would be quite different.

From data in Table 7, it may be noted that total protein level remains fairly constant, but due mainly to compensatory increases in whey protein to losses in casein.

Table 7. Effect of mastitis (as reflected by somatic cell count) on percentage of protein and protein fractions of milk

Component or Fraction	Estimated Somatic Cell Counts [1] (thousands)		
	<250	500-1000	>1000
Total protein	3.61	3.59	3.56
Casein	2.79	2.65	2.25
Casein:total protein ratio	0.77	0.71	0.69
Casein fractions			
alpha $_{s1}$-casein	1.33	1.09	0.85
beta-casein	1.06	0.92	0.65
kappa-casein	0.16	0.20	0.19
Whey protein (total)	0.82	1.10	1.31
alpha-lactalbumin	0.28	0.30	0.23
beta-lactoglobulin	0.25	0.31	0.22
serum albumin	0.15	0.23	0.35
Immunoglobulin	0.14	0.26	0.51

Adapted from Haenlein et al.
[1] Estimated by Wisconsin Mastitis Test (WMT)

Ratio of casein to total protein decreases as the mastitic condition worsens. The significance of this fact concerns methods of estimating casein content from a measure of total protein. Accurate, inexpensive, direct methods for determining casein content of milk are not readily available, although advances have been made. Older, indirect procedures are both slow and relatively imprecise. For the preceding reasons, casein content is often estimated as a percentage of total protein. Generally, level of casein in milk produced in the upper midwest is taken as 78% of total protein. This estimate is then used to calculate a theoretical/ideal yield of cheese from a given milk supply. Cheesemaking processes can then be assessed for efficiency based upon actual vs theoretical yield. To the extent that mastitis alters the ratio of casein to total protein, calculated values vary from true potential; the mechanism for assessing processing efficiency breaks down.

Table 8. Influence of mastitis on lipase activity and related aspects

Component or Property	Wisconsin Mastitis Test Score: Less than 10	Over 20	Change
Fat (%)	3.45	3.20	-
Phospholipid (mg/g fat)	4.78	3.55	-
Lipase activity	1.49	1.73	+
Free fatty acids (mg/g fat)	23.27	34.10	+
Acid Degree Value	0.64	1.17	+

Adapted from Randolph et al. (1974)

Nutritional status:

Although nutrition of cows has been studied extensively, two dietary factors have come under major scrutiny in later years. These are level of energy intake and level of protein intake.

If energy intake is lowered 30% below requirements, milk yield decreases as does percentage of SNF and protein. Declines in the latter two components are in the order of 0.3-0.5% and 5.0-6.0%, respectively. In general, energy restrictions are of greater significance to milk yield than to percentage loss of components.

Feeding energy levels well above requirements will produce only relatively small increases in milk components, and then only up to the genetic potential of the cow(s).

In practice, however, the influence of any changes in feed can only be assessed against the present nutritional status of lactating animals. An increase in level of energy intake may indeed cause substantial increases in components of milk of underfed cows. It is for this reason that contradictory effects have been noted when cows are turned out on lush spring pasture after several months of winter feeding. For cows surviving at 75% of nutritional requirements, increased energy intake from pastureland raises SNF level about 0.33%. Milk of cows winter-feeding at 130% of requirements, under similar spring pasture conditions, will show a decline in SNF of about 0.12%.

In one study in which three rations, one at 1.1 kg less, one at 100%, and one at 2.3 kg energy above standard requirements, protein percentage of the milk supplies were 3.08, 3.19, and 3.30, respectively. Most of the increase in protein came as casein, although with minor increases in whey proteins as well. The maintenance of energy requirements, therefore, has a direct impact on the potential yield of cheese from a given supply of milk. This has to be seen as possibly the most important basis for assuring adequate levels of energy intake.

appropriate, considering the current state of scientific knowledge, to state that minimal changes in composition occur as long as cows are fed commensurate with increases in milk yield, and then only if cows are able and willing to consume the required levels. In this respect, energy intake appears to be the most critical factor. Data in Table 9 clearly illustrate this fact.

It should be pointed out that data in Table 9 were derived over short term trials. The cows in the study were fed a total mixed diet ad libitum. That is, the diet was balanced and the cows were allowed to consume as much as they would eat.

At the level of BGH cited, milk production increased 28.6%. The significant changes in milk composition include an increase in fat percentage, a decrease in total crude protein level and an increase in content of alpha-lactalbumin. It is also important to note that protein percentage declined in face of increasing fat content. This is contrary to the normal relationship between these two components. For milk such as this, a fat test alone would be inadequate for assessing value; commonly used regression equations for calculating level of components from a known level of one component would not serve accurately. Hence, these are major compositional variations from the norm.

In addition to the increase in percentage of fat, the composition of the fat changes, apparently because some small portion is drawn from body stores of the cow rather than the process of synthesis inside the udder. Such fat is more unsaturated than normal fat, with possible implications to flavor of cheese (where fat breakdown products contribute to flavor) and development of oxidized flavors in milk and dairy products.

Because total protein level declines and level of alpha-lactalbumin, a whey protein, increases, the net result would imply a lowered content of casein the major protein of cheese. The cheese yield potential of such milk would thereby be significantly lowered. These are most important considerations and suggest an essential need to properly feed cows to which BGH is being administered.

Neither lactose nor calcium levels appear to be altered by BGH treatment of cows, the former possibly affirming the biological necessity to maintain proper osmotic balance. Magnesium level increases in milk of this kind and, depending upon the balance of alterations in mineral content overall, some slight change in freezing point of milk might be anticipated. Similarly, other changes in milk characteristics could take place. To date, studies on susceptibility of BGH milk to hydrolytic rancidity seem to indicate no problem under appropriate feeding conditions. In practice, under the varied conditions that exist in the field, this and other quality issues yet remain possible concerns and areas for further investigation.

Summary of factors influencing the composition of milk

As a quick guide to the influence of various factors on level, or general trend in level, of a variety of major milk components, Table 10 may prove useful. A positive value implies an upward trend or increase in level, a negative sign

Table 10. Summary of the effect of various factors on milk composition

Factor:	Fat	Tot. Prot.	Casein	Whey Prot.	Non Prot. Nit.	Lactose	Sol. not fat	Tot. Solids	Tot. Minerals	Calcium	Chlorides
Stage of lactation:											
Colostrum	+	+	+	+		+	+	+	+	+	
Mid lactation						-					
Late lactation	+	+				-	+	+			+
Season of year:											
Spring/summer			-	+	+	-/+			-	-	
Winter	+	+	+	-	-	+/-	+		+	+	
Temperature:											
85 - 105 F	+[1]	-	-			-	-				+
5 - 40 F	+	+					+				
Age of cow:											
7 years and over	-	+/-	-	+/-	-	-					
Mastitis:	-	+/-	-	+		-	-			-	-
Feed:											
Low energy		-					-				
High energy		+	+		-						
Low protein		-					-				
Excess protein		+/-			+						
Finely chopped hay and low forage	-						-				
Growth Hormone:	+	-	-								

[1] Prolonged exposure to these temperatures will cause a lowering of the percentage of fat.

Table 12. Vitamin losses under various processing conditions

Process	Percent Loss [1]						
	Vit. B1	Vit. B2	Vit. C	Pyridoxine	Biotin	Vit. B12	Folic Acid
Past., 145 F, 30 min.	10	0	20	20	0	10?	10
HTST Pasteurization	10	0	10	0	0	10	10
Ultra High Temp. (UHT)	10	10	10	20	0	20	<10
Evap. Milk Process	40	0	60	?	10	90	?
Spray Drying	10	0	20	0	10	20	?
Roller Drying	15	0	30	0	10	30	?

Adapted from Causeret

[1] A question mark indicates the possibility of some loss by light-induced changes.

Exposure of wholemilk and lowfat milk to light also results in losses of certain vitamins. In particular are Vitamins A, B2 and C vulnerable to light-induced changes and then primarily in clear or translucent containers. The latter vitamin is not present in milk to significant levels and losses do not pose a significant nutritional issue. However, milk is an important source of both vitamins A and B2 (riboflavin) in the American diet; losses in these vitamins must be viewed with concern. In addition, exposure of milk to light may result in development of an off-flavor(s).

Research suggests that the chemical form(s), commonly retinol palmitate, of vitamin A added for fortification purposes is less stable to light than the form found naturally occurring in milk. In any event, lowfat and skimmilk products appear to be more vulnerable to loss than wholemilk.

Several factors are important in determining rate and extent of light-induced vitamin loss. These are as follows:

1. Intensity of light: In this respect, exposure to sunlight is more detrimental than fluorescent light; fluorescent light is potentially more damaging than incandescent light. The more intense the source of light, the greater the rate and extent of vitamin loss.

2. Proximity of light source: In a storage area either at the dairy plant or in a refrigerated cabinet in the marketplace, milk in containers closest to a source of light is most subject to vitamin loss and/or off-flavor development. In this respect, as much as 42% loss in riboflavin may take place in milk at the top of a container and only 8% loss at the bottom of the same container over five days or storage.

3. Surface area exposed: The larger the surface area exposed to light, the greater the risk of loss of vitamins. Thin films of milk, as, for example,

Lastly, it should be pointed out that light not only results in an absolute loss of a certain amount of vitamin A, it also converts the trans form (a specific configuration of the vitamin) to one or more cis forms. The latter are less potent than the former, the net result being a lessening of the vitamin A potency of milk.

Enzymes

Enzymes are organic catalysts. That is, they are compounds that cause biological reactions to proceed at much faster rates than would otherwise occur. At the same time they are organic rather than inorganic in chemical nature. In fact, all enzymes are protein. This fact is important to keep in mind because responses to processing and other treatments follow patterns common to all proteins. For example, enzymes can be inactivated (denatured) by heat treatment, acids and alkalis. Their resistance to such treatments vary, but all enzymes ultimately respond as all proteins respond. But because they enter into biochemical reactions, temperature and pH become important determinants in their activity.

Names of enzymes are often, but not always characterized by ending with the suffix "ase." Lipase, lactase and phosphatase are examples. Chymosin and plasmin are examples of enzyme names not ending in the typical ase suffix.

Well over forty enzymes occur naturally in bovine milk. Some serve useful functions, others are the cause off-flavors and/or undesirable changes in body and texture of milk products. Many are present simply as biochemical compounds remaining from the milk-synthesis process and play no significant role in dairy processing.

Other enzymes enter the milk supply as living matter of (1) bacterial contaminants, and (2) somatic cells, and also from (3) blood arising from badly infected or injured udders.

Enzymes from bacterial contaminants may be generated as a result of growth and multiplication of the organisms or simply occur as residual matter in dead cells. The important point to recognize is that high bacteria counts, even of dead cells, pose potential problems for the processor. Living organisms, of course, produce enzymes to the extent that conditions allow the organisms to grow.

<u>Milk of high quality is milk that is low in numbers of both living and dead bacteria.</u>

Some enzymes reach the milk supply as constituents of somatic cells. Catalase is one example; proteases, enzymes that break down protein, are another. The latter are responsible for bitter, putrid and other off-flavors; they also break down casein, thereby lowering cheese yield potential of milk. Perhaps the most significant enzyme carried by blood is plasmin. This enzyme is also proteolytic (breaks down protein), and is thought to be responsible in major part for loss in cheese yield capacity of milk of high somatic cell

Lipase enzymes occur naturally in milk and also enter milk through bacterial contaminants. Bacteria with the potential to grow at cold temperatures (psychrotrophs) are often sources of lipase enzymes of differing characteristics than lipases found naturally occurring in milk.

Differences in lipase enzymes center not only in their response to temperature and pH, but also in the selectivity with which they attack fatty substances. More details on this characteristic are presented in Chapter III. Here it suffices to say that lipases show preferences for certain fatty acids or certain arrangements of fatty acids on the fat molecule. All lipases attack the ester linkage of fats, but the end result in terms of flavor(s) produced will depend on which of a large number of different fatty acids are released--freed--by the action of any given lipase enzyme.

It is also important to note here that lipases, like other enzymes, differ in their sensitivity to heat. Hence, a heat treatment that may destroy lipases that occur naturally in milk may be inadequate to destroy lipases generated by bacteria. Again, the importance of high quality milk--milk of low bacteria count--is emphasized.

Two different lipases are thought to occur in milk, one is found in colostrum only, the other (lipoprotein lipase) is associated with casein. The latter is the one involved in off-flavor development. It is known to be completely inactivated at pH 4.6, one hour treatment.

The above facts account for the confusion that exists about lipases and the processing issues posed by them. The rancid flavor that they produce may differ depending upon the enzyme(s) involved. A heat treatment considered adequate to destroy lipase(s) may or may not serve the purpose, and for the same reason. Therefore, consistency in processing and in process control is best served by reducing to a minimum all sources of lipases other than those found in milk as it comes from the cow. See Chapter III for more information.

Proteinases: Alkaline serine proteinase is the main proteinase of milk, but is found only at very low levels. The majority may in fact originate in bacterial contaminants. It is also possible that current breeding practices are increasing the level of this naturally occurring proteinase in milk.

This above enzyme is concentrated in the casein micelle and can be extracted only at pH 2.0. It has an optimum pH of activity of about 8.0; temperature optimum is 98.6 F (37 C). It will fully survive pasteurization treatment and even heat treatment of 176 F (80 C) for 10 minutes. Some level of activity may survive ultra high temperature treatment and be the cause of bitter flavors and/or age-thickening. It is more stable at low than high pH levels.

The activity of proteinase is inhibited by presence of beta-lactoglobulin (a whey protein). In fact, its activity ranges considerably higher in milk from which all whey proteins have been removed. It is also inhibited by mercury, zinc, copper and soy bean trypsin.

Present information suggests that this enzyme is identical to plasmin, the enzyme associated with psychrotrophs (cold-growing bacteria). It is known to

in immobilized enzyme technology. This approach has been applied to UHT milk as a method of removing cooked flavor components.

Phosphatases: A number of different phosphatases occur naturally in cows' milk. They may be classified as monoesterases, diesterases, and pyrophosphatases. They differ in the substrates—the chemical compounds or bonds—with which they react to catalyze a breakdown. Both alkaline and acid phosphatases are present in milk. As the words imply, the difference between them lies in the pH at which they find optimum activity. Acid phosphatase responds best at about pH 4.0, alkaline phosphatase at pH 9.5-10.5. It is the latter that finds major application in the dairy industry as a way of determining whether or not milk has been properly pasteurized.

In the application of the phosphatase test to milk, some few facts should be kept in mind. First, the test serves both to determine if milk has received full pasteurization treatment and also to detect possible addition of raw to pasteurized milk.

However, a negative phosphatase test is not an absolute assurance of proper pasteurization. The uncertainty comes about as a result of the way in which the method must be run, with the endpoint taken not as total lack of phosphatase activity, but rather no more than a given base level of activity. It is possible, therefore, that critical mixtures of over- and underheated milk, or some minute level of raw milk in pasteurized milk could go undetected.

Other factors to keep in mind are the necessity of running both negative and positive controls during a phosphatase test. Control tests of this kind simply ensure that a negative test is detected as such and that any improperly formulated or functioning reagent will not result in a negative assessment of a sample that should be found positive. A positive control consists of a sample of milk heated to 194 F (90 C) for one minute i.e. a sample known to be "pasteurized."

In addition to the above, it is known that certain compounds interfere with the normal phosphatase reaction. For example, false positive results may occur in a product that contains vanillic acid, the oxidized derivative of vanillin, an artificial flavoring compound. Certain compounds associated with chocolate milk tend to reduce the phosphatase activity of this product.

Phosphatase enzymes are also produced by certain microbes, again leading to false positive results unless taken into consideration and specifically discounted by further testing. Microbial phosphatase can be ruled out by testing a sample of the milk after a specified heat treatment. The heat treatment is known to inactivate normal alkaline phosphatase, but not the more resistant enzyme produced by bacteria.

Another phenomenon associated with phosphatase and also certain other enzymes is reactivation. This is a process or a treatment that should be more than adequate to inactivate the enzyme, but which in fact restores its activity. Very high, short-term heating may cause reactivation. The temperature range involved runs from about 190 F (88.3 C) for one second on up to 212 F (100 C).

Lysozyme of cows' milk has a molecular weight of 15-18,000, a pH optimum ranging between 6.5 and 8.0, and a temperature optimum of 98.6 F (37 C).

Inactivation of enzymes

Enzymes are protein in nature. They may be inactivated by any treatment known to denature protein. Although they show great variation in sensitivity, heat (temperature treatment), and acid or alkaline conditions (if severe enough) are the two conditions most often causing enzyme inactivation. In most dairy processes, heat treatment alone is a significant factor.

It should be kept in mind, however, that any given heat treatment may only partially inactivate a given enzyme. Any remaining level of active enzyme, however small, may, under the right conditions, continue to carry out the biological process for which it is designed. Only complete inactivation ensures no further enzymatic reaction. It is also important to note that some enzymes under some conditions can reactivate—return from a state of inactivity to a form in which activity once again occurs.

Data in Table 13 summarize significant factors of some of the major enzymes of milk.

Table 13. Major enzymes of milk and significant factors associated with each

Enzyme	pH Optimum	Temperature Optimum (°F)	Molecular Weight	Signif. Aspects/ Characteristics
Catalase	7.0	77-86	200,000	Inhib. at low pH; 84% inactivation by pasteurization; found in skimmilk and FGM[1]
Lactoperoxidase	8.0	95	77,500	indicator of HTST pasteurization; used in cold steril. (LPO: H_2O_2:SCN
Xanthine oxidase	8.3-8.9	98.6	300,000	lipid oxid.; found in FGM[1]; isolate from buttermilk
Sulfhydryl oxidase	6.8-7.0	95	90,000	isolate from whey, UF; oxidizes SH to S-S
Superoxide dismutase	?	?	32,000	acts as antioxidant; counteracts xanthine oxid. and lactoperox.; rather heat stable
Lysozyme	6.5-8.0	98.6	15-18,000	bactericidal agent

devices find application in measuring the amount of milk in bulk tanks and in tank trucks delivering milk to dairy plants.

The level of gases in the original raw milk is generally lower in pooled herd milk as a result of losses during handling and transportation. Gases are forced out of milk to more or less extent by agitation, temperature increases, and by vacuum treatment. Some of these treatments have already been considered as factors that influence titratable acidity of milk.

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Chapter III

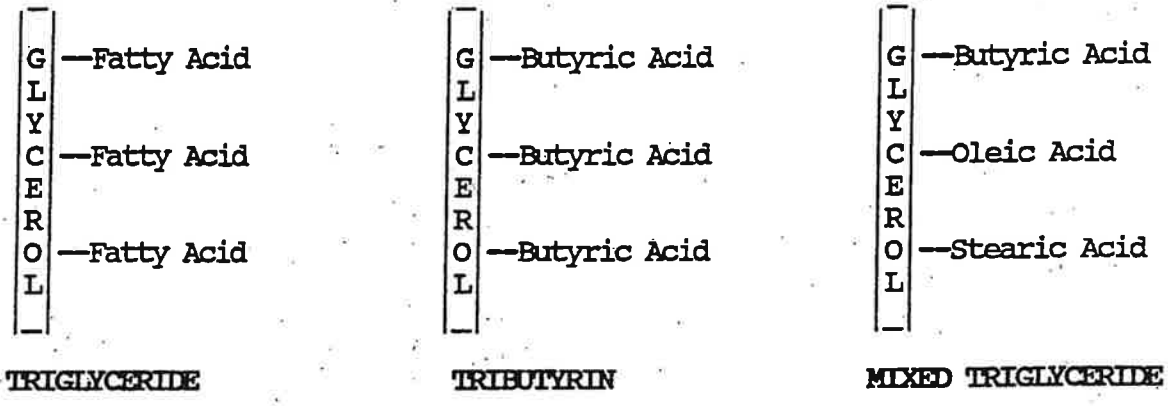
The Chemistry and Properties of Milkfat

The fat of milk provides **richness** in flavor and adds significantly to the **body** of milk and dairy products. It is unique in its chemical make-up, a fact that also contributes to uniquely flavored products like **blue cheese**. At the same time, fat is also the source of two off-flavors. Both **oxidized** flavors and the flavor and pungent odor of **hydrolytic rancidity** originate in milkfat.

The fat of milk falls into a general category of fat and fat-like substances given the chemical name **lipid**. Lipids include both true fats and a group of compounds that are similar in nature or, in fact, partial components of true fats.

Chemical nature of fat

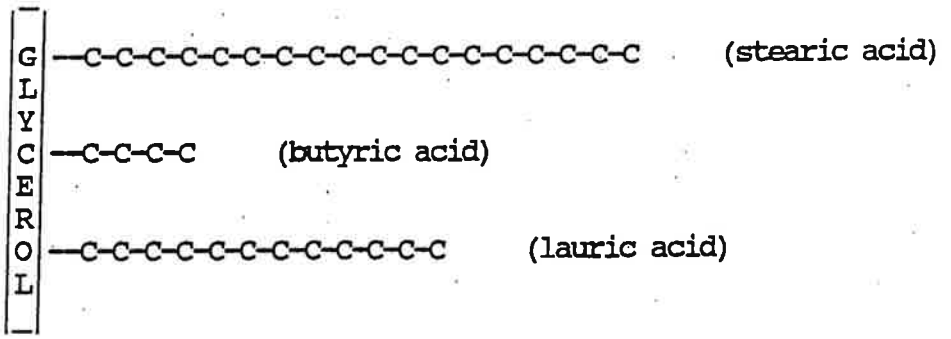
Fat consists of two different kinds of molecules joined together by a unique kind of chemical bond. One of these molecules is **glycerol**. The other is called a **fatty acid**. A true fat consists of **one** molecule of glycerol to which is attached **three** molecules of fatty acids. The chemical bond that joins glycerol with fatty acids is called an **ester** linkage. Some simplified diagrams may help illustrate the point.



The left diagram above pictures a molecule of true fat. Three fatty acids are attached to glycerol, hence the name **triglyceride**. All true fats are triglycerides.

The middle diagram depicts a true fat in which all three fatty acids are the same i.e. **butyric acid**. This molecule, therefore, is appropriately named **tributyrin**.

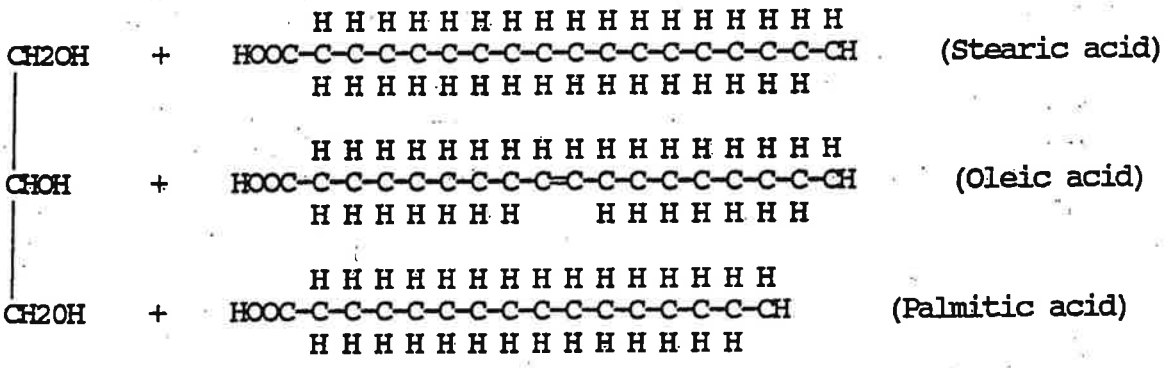
The figure on the right is indicative of a molecule of fat in which three different fatty acids make up the triglyceride. It is appropriately named a **mixed triglyceride**, implying a mixture of fatty acids. Over 250 fatty acids make up the pool from which milkfat triglycerides can be formed. A very large number of different molecules can be and are in fact synthesized by the udder.



In the above diagram, the connecting link in the chains of fatty acids is presented as a C. The letter C is also the symbol for carbon. Indeed, carbon is the element that forms the linkage in fatty acid molecules.

It may be noted that stearic acid contains eighteen carbon units, butyric acid four and lauric acid twelve. Fatty acids of over twenty carbon units exist as components of fats. Most milkfat fatty acids consist of chains even-numbered in length. Few fats contain significant quantities of butyric and other short-chain fatty acids. Chain length is usually designated C (carbon) followed by the appropriate number, i.e. C4 (butyric), C18 (stearic). C18:1 is the designation for oleic acid, a fatty acid that is 18 carbon atoms in length, but which also has one double bond (unit of unsaturation). In this case, the number following C18: indicates the number of double bonds in the fatty acid.

The manner in which fatty acids join with glycerol to form a molecule of fat can also be illustrated by a structural formula:

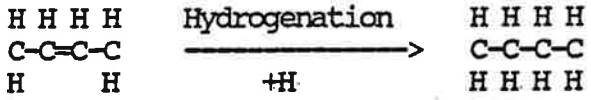


GLYCEROL + THREE FATTY ACIDS COMBINE TO FORM:

Hydrogenation

Hydrogenation is a chemical process of adding hydrogen to unsaturated fatty acids of fat. More or less hydrogen can be added, thus varying the degree of saturation (or unsaturation). Not uncommonly, vegetable fats are to some extent hydrogenated prior to formulation into food products such as margarine. Such fats may yet carry a significant level of unsaturation, but in the process of hydrogenation are stabilized (to a degree) against development of oxidative rancidity.

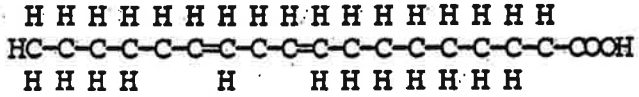
Hydrogenation of fats may be illustrated as follows:



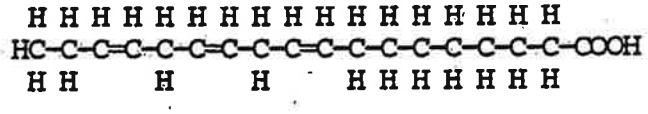
Unsaturated linkage Saturated linkage

Polyunsaturation

Fatty acids that carry unsaturation at only one linkage in the chain are termed **monounsaturated.** Some fatty acids carry two or more points of unsaturation. These are called **polyunsaturated.** Fats consisting of polyunsaturated fatty acids are softer (have lower melting points) than either saturated fats or fats bearing mainly monounsaturated fatty acids. Such fats are also more susceptible to oxidative rancidity—are more unstable—than monounsaturated fats. It is for this reason that some level of hydrogenation is often applied to polyunsaturated fats. Structural formulas for two polyunsaturated fatty acids follow:



LINOLEIC ACID



LINOLENIC ACID

These facts should be kept in mind as dairy farmers begin to seek out high density sources of energy as feedstuffs for cows treated with bovine growth hormone. Depending upon the source of fat in feed, a softer or harder fat may be produced, with possible implications to processes such as continuous buttermaking.

Even under current feeding practices, seasonal changes in milkfat composition require adjustments in pre-treatment of cream prior to buttermaking. Cooler cream temperatures are usually the norm for butter processed in summer months.

On a seasonal basis, Iodine Values of milkfat may range from 28 to 40, the latter found during summer months when and if cows are fed succulent green feeds.

Although feed can be a cause of some changes in milkfat composition, it is not a cause of major changes in milkfat composition. And feeding feedstuffs high in level of unsaturated fatty acids will not result in major changes in milkfat composition unless the unsaturated fats are protected from digestive processes that take place in the rumen, processes that tend to hydrogenate unsaturated fats.

Fatty acid composition of milkfat

Milkfat is known to contain upwards to 400 different fatty acids. Both even- and odd-numbered chains exist as well as branched-chain fatty acids. The former make up about 3.7 percent of the total fatty acid content of milkfat, the latter (which includes a high level of odd-numbered fatty acid chains) about 1.5 percent. Many fatty acids are found only in trace amounts.

Chain length of fatty acids of milkfat vary from 4 to 26 carbon atoms. The most significant difference between milkfat and other animal and vegetable fats is its relatively high content of short-chain fatty acids.

The major fatty acids of milkfat are shown in Table 1. It is important to note that butyric acid makes up 2.5 - 3.5% of the total fatty acids of milkfat. This is a relatively small amount. Nevertheless, the odor and flavor of this acid, when released from fat, is so intense that it is readily detectable at very low concentrations.

Saturated fatty acids found at highest levels in milkfat include myristic, a 14-carbon chain, palmitic, a 16-carbon chain and stearic, an 18-carbon chain. These three fatty acids account for 37% on up to 63% of the total fatty acids in milkfat.

Oleic acid is the major monounsaturated fatty acid of milkfat, ranging from 25% to 35% of the total. This is a significant amount and may well have nutritional implications in human health.

Milkfat carries a small amount of polyunsaturated fatty acids, with linoleic showing highest levels (2% to 3% of the total). Although this level must be considered relatively low, some few other fats carry even lower levels.

Distribution of fatty acids of cows' milkfat

The fatty acids given in Table 1 are generally distributed on the glycerol portion of the molecule as follows:

Fatty acids	Position on the glycerol molecule
C4:0 to C10:0 [1]	mainly at position no. 3
C12:0 to C16:0	mainly at position no. 2
C18:0	found at position nos. 1 and 2
C18:1	found at position nos. 1 and 3

[1] The number following the colon indicates the number of of double bonds, i.e. C18:1 denotes a fatty acid of 18 carbon atoms and one double bond.

Significance of fatty acid distribution

In the body, the lipase enzyme that breaks down fat originates mainly in the pancreas. Pancreatic lipase shows specificity for positions 1 and 3 of fat. Hence, short chain fatty acids are readily split off the triglyceride molecule. Being water-soluble, they are quickly absorbed by the body.

Human infants digest human milkfat more readily than cow milkfat because of the presence of rather large amounts of C16:0 at the number 2 position.

Distribution of fatty acids also influences the **melting point** of fat. Melting point is directly related to **hardness** and **spreadability**. It is possible to **rearrange** fatty acids about the glycerol molecule in a **random** fashion and, thereby, to produce a somewhat more spreadable butter. The process can be done either by chemical or enzymatic means, but is costly and, therefore, not used to any great extent.

Physical and chemical constants of certain fats

The fatty acid composition of a fat determines its chemical and physical characteristics and properties. To some extent, variations in fatty acid content occur within a given fat. However, differences between different fats and oils are usually of greater magnitude. It is these differences that account for variations in quality characteristics of foods and food products processed from given kinds of fat.

A variety of analytical methods can be used to asses various compositional differences between fats. A number of the more important ones follow.

Iodine Value (Number): Iodine Value is a measure of unsaturation, as mentioned previously (see page 5). It may be defined as the number of grams of iodine taken up by 100 grams of fat under specific test conditions. In lieu of

Various methods are used in determining Acid Degree Value. In the method of Thomas et al., this value is expressed as the milliliters of 1N (1.0 normal) base required to titrate 100 grams of fat.

Fresh milk yields an Acid Degree Value of 0.2 to 0.6. An objectionable flavor may be detected at values starting at 1.0.

Specific Gravity: Specific gravity is a measure of the weight of a given volume of substance in comparison to water. It is a form of measure of density. Density is defined as weight per unit volume. Specific gravity may then be defined as the density of a substance in relation to water (density of the substance divided by density of water). Because density varies with temperature, it is essential in expressing results to indicate the temperature(s) at which the measurement was made.

The specific gravity of milkfat is 0.918 at 68 F (20 C). This may be compared to the specific gravity of wholemilk, which ranges from 1.030 to 1.035 (20C/20C).

Table 2 provides a comparison of values of common chemical and physical constants of several fats.

Table 2. Select physical and chemical constants of several fats

Fat	Iodine Number	Refractive Index (40 C)	Melting Point (C)	Saponification Number
Vegetable Fats:				
Cocoa butter	32-43	1.4537-1.4580	28-33	192-198
Coconut	6-12	1.4477-1.4495	20-28	245-262
Corn	120-128	1.4640-1.4680	-18 to -10	187-196
Cottonseed	103-114	1.4645-1.4655	-----	190-198
Palm	45-57	1.4530-1.4590	104-110	196-205
Palm kernel	15-20	1.4492-1.4543	23-30	243-255
Peanut	84-110	1.4605-1.4645	-5.0	183-195
Rapeseed	95-108	1.4720-1.4752 [1]	-----	170-177
Safflower	127-150	1.4690-1.4692	-----	185-195
Soybean	125-140	1.4710-1.4750 [2]	-----	188-195
Sunflower	120-140	-----	-18.0	188-195
Animal Fats:				
Beef tallow	35-50	1.4566-1.4596	42-48	194-200
Lard	56-80	1.4580-1.4620	36-45	193-203
Milkfat	25-35	1.4538-1.4578	30-41	210-240
Mutton tallow	35-46	-----	-----	192-197

Adapted from Handbook of Chemistry and Physics
[1] Measured at 20 C
[2] Measured at 25 C

From the preceding illustration, it may be seen that lecithin carries both water- and fat-soluble characteristics, the fatty acid end of the molecule providing the latter. As such, lecithin concentrates around the fat globules of milk where it serves as an emulsifier.

During separation of cream from skimmilk, lecithin stays with the fat. When cream is churned into butter, much of the lecithin carries into the buttermilk. Buttermilk is usually dried and serves as an ingredient in various food systems. Because of its emulsification properties, buttermilk finds use as an ingredient in ice cream.

Level of lecithin in select dairy products is shown in Table 3.

Table 3. Percent (by weight) of lecithin in select dairy products

Dairy Product	Percent
Wholemilk	0.027–0.040
Skimmilk	0.013–0.035
Cream	0.12–0.20
Butter	0.17–0.22
Buttermilk (sweet cream) ...	0.14–0.16

Lecithin is soluble in most organic solvents. Its solubility in acetone is used as a means of separating it from other glycerides.

Because lecithin is soluble in both ether and alcohol, tests methods for fat that use one or both of these compounds as reagents may produce slightly high test results. This becomes a potential problem mainly in analysis of products naturally low in fat content, such as true buttermilk. Both the Mojonnier analysis and the American Association test (using n-butyl alcohol) exaggerate the level of fat (triglyceride) of this product.

Cholesterol

For many years, cholesterol has been a major issue in relation to heart disease. The issue yet remains and is yet associated with much research activity. It is beyond the scope of this text to evaluate the status of these research efforts, but some discussion of this compound is essential.

When fat is treated with alkali, the fat saponifies i.e. soaps of fatty acids are formed. When the fat and fat-like compounds of milk are caused to undergo saponification, some small amount of material does not react. Collectively these unreactive components are termed unsaponifiable matter. As a group, they represent about 0.25–0.45% of the ether-extractable substances in milk. Chief among them is cholesterol.

Table 4. Cholesterol content of various dairy products (milligrams/100 grams)

Dairy Product	Amount
Skimmilk	2
Wholemilk (3.25% fat)	14
Cottage cheese, dry curd	4
Cottage cheese (1% fat)	6
Cottage cheese (creamed, 4% fat)	15
Ice cream	45
Cream, Half and Half	37
Cream, light	66
Cream, medium (25% fat)	88
Cream, light whipping	111
Cream, heavy	137
Cheese, Swiss	92
Cheese, Pasteurized Process American	94
Cheese, Cheddar	105
Cheese, Cream	110
Butter, regular	219
Butter, whipped	219

Adapted from U.S.Department of Ag., Handbook 8-1

Overall level of fat and fat-like substances in milk

At this point, it seems appropriate to briefly summarize the content in milk of the fatty substances discussed thus far. These compounds and their percent by weight in milk are shown in Table 5.

Table 5. Percentage of fat and fat-like substances in milk

Component	Percent in Milk
Triglycerides (true fats)	97-98
Diglycerides	0.25-0.48
Monoglycerides	0.016-0.038
Free fatty acids	0.10-0.44
Phospholipids	0.2-1.0
Sterols	0.22-0.41
Fat-soluble vitamins	0.0031-0.0035

membrane system and alter the physical state of milkfat. Following are some of the more important of these factors/processes.

Temperature fluctuations:

Relatively slight fluctuations in temperature of milk alter the arrangement or orientation of components of the fat globule membrane. **Cooling** of raw milk, for example, followed by **warming** to room temperature (or slightly above) and re-**cooling** to refrigeration temperatures, causes significant changes. Hydrolytic **rancidity** is induced by such mild treatment, likely because the lipase enzyme is allowed to re-position itself directly on the fat globules or because some small amount of free fat is released from the globules and thereby rendered open to attack by lipase.

Cooling of milk as it comes from the cow is in itself a process that causes significant changes in the fat globule membrane. A group of proteins, including certain **immunoglobulins**, attach to the membrane upon cooling. As a group, they are called **cryoglobulins**. These proteins cause the membrane to become "sticky." As a result, fat globules come together in clusters. Clusters tend to rise faster than individual globules, hence cream rises to the surface of milk and in a much shorter time span than would otherwise occur.

Pasteurization, particularly at temperatures well above minimal, causes partial denaturation (destruction) of certain whey proteins. **Immunoglobulins**—antibodies—are particularly sensitive to heat. As mentioned above, some of them, perhaps along with certain other proteins, have the ability to cause fat globules to come together in clusters. This process is called **agglutination**; the proteins that cause it are referred to as **agglutinins**.

The importance of agglutinins/cryoglobulins lie in their affect on creaming of milk. To the extent that agglutinin or proteins of similar nature are denatured, clustering is inhibited; rate of creaming is slowed; amount of cream line is reduced.

The above phenomenon relates directly to a natural law, which is named after the individual who first proposed it. In essence, **Stokes' law** states that the rise or fall of a spherical particle in a liquid varies as the square of its radius. In other words, a fat globule twice as large as another globule will rise <u>four</u> times as fast.

Clusters of globules, being significantly larger than individual fat globules, will rise that much faster. The fact that a cream line appears in quiescent milk in a relatively short time is due mainly to the influence of clustering.

Mechanical separation:

Cream separators operate under the influence of Stokes' Law, with rate of separation being greatly enhanced by increasing the force of gravity. In this respect, large globules of fat or clumps of fat separate that much faster and more efficiently than small ones. Any factor that reduces or inhibits clumping of fat will in turn decrease the efficiency of separation. On the other hand,

to which it is added. It is due entirely to presence of free fat and is referred to as the oiling-off defect.

The critical factor in breaking the emulsion through agitation is presence of air. It is air that provides for incorporation of bubbles that eventually lead to churning. In absence of air--a full pipeline of milk, as example--considerable agitation can take place without significant churning.

Buttermaking, of course, emphasizes factors that cause the original emulsion to be broken down. The membrane is intentionally "scrubbed" from the surface of fat globules which then come together in larger and larger clumps. Clumping is initiated and clumps are held together by presence of free fat. In both batch and continuous churns, air is whipped into cream in the process of churning. The former requires thirty to thirty-five minutes for butter granules to form, the latter only a few seconds.

In buttermaking, advantage is taken not only of incorporation of air but in the temperature of the cream. Milkfat churns most readily and efficiently at temperatures between 50 and 60 F (10 and 15.6 C). However, temperatures as low as 41-43 F (5-6 C) may be used in continuous buttermaking to enhance spreadability of butter made from soft fat of the kind produced by cows on summer feeding regimens.

In order to achieve the appropriate temperature in cream destined for churning in continuous buttermaking equipment, account must often be taken of the latent heat of crystallization (or latent heat of fusion) of fat. As melted fat is cooled, crystals of fat form; in the process heat is generated. The amount of heat produced by this affect is enough to raise the temperature of cream in a storage tank by two to three degrees Fahrenheit. To churn at a temperature of 41 to 42 F may require cooling of cream to 39 to 40 F (3.9-4.5 C).

The overall process for continuous buttermaking usually involves separation of cream followed by pasteurization at 192 F (89 C) for 20 seconds (or similar temperature treatment), cooling to 40 F (4.5 C) or thereabouts and storage prior to buttermaking. During storage, the temperature of the cream slowly rises the two or three degrees anticipated from latent heat of crystallization.

Homogenization:

As might be expected, homogenization causes major disruption of the membrane system. Homogenization also stabilizes the emulsion. It does so by extensively reducing the size of fat globules, so much so that they no longer coalesce and rise. Fat globules originally averaging several microns in diameter are uniformly suspended as particles of 0.5 to 2.0 microns in diameter following homogenization.

Homogenization increases surface area of fat globules four to six times. This is perhaps better understood in the following context: When a fat globule 4.0 microns in diameter is converted to globules averaging 0.8 micron in diameter, 125 new globules are formed; the total surface is increased 5-fold.

* is less heat stable

* is more bland in flavor

Homogenization efficiency—the degree to which rising of cream is inhibited—can be measured rather precisely. This is done by centrifuging 50 milliliters of homogenized milk under controlled conditions and then determining the fat content of the top 10 milliliters. A **Homogenization Efficiency Index** (H.E.I.) is then calculated as follows:

$$H.E.I. = \frac{Fep - Fa}{Fa} \times 100$$

where Fep = fat content of the enriched portion
Fa = fat content of the milk sample

Another method of determining homogenization efficiency requires counting of the number and size of fat globules in five adjacent fields of a microscope. This procedure, called the Farrall Index, is a measure of the size distribution of fat globules.

Summary of factors that influence creaming of milk

The following factors are important in determining rate and extent of creaming:

* Size of fat globules: Large globules rise faster than small ones.

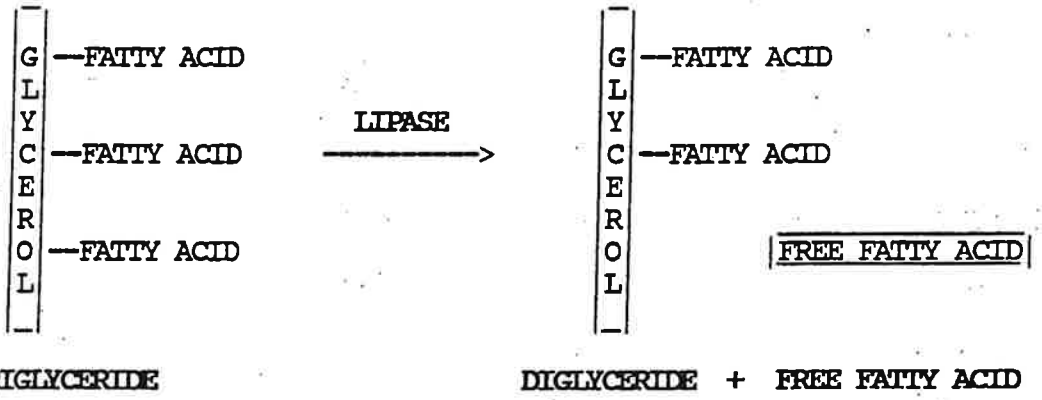
* Agglutination by cryoglobulins: Clusters of fat globules rise faster than individual globules.

* Agitation during early creaming: Even mild agitation will disrupt and/or slow the clustering process, thereby slowing creaming. Clustering can to some extent be re-induced by warming to 104 F (40 C) and re-cooling to refrigeration temperatures. However, this is also a mechanism by which lipase is activated and rancidity development initiated.

* Heating to 158 F (70 C) for 30 minutes or 171 F (77 C) for 20 seconds will denature/inactivate the agglutinins that cause clustering and thereby inhibit creaming.

* Homogenization: denatures/inactivates agglutinins, one of the factors that helps prevent creaming

Milkfat globules of goat, sheep and buffalo milk do not cluster, a natural phenomenon that slows creaming of milk from these animals.



In the above reaction, one fatty acid has been split from the triglyceride molecule. In this case, a diglyceride remains. However, lipase might also free two fatty acids from the fat, in which case a monoglyceride would remain. Both di- and monoglycerides are surface active. They tend to collect at the interface between fat and skimmilk and cause some re-orientation of membrane components, which in turn may trigger further lipase action.

The off-flavors caused by lipase action on milkfat are variously described as bitter, unclean, or soapy. A "barny" odor also accompanies the defect.

Under most situations, hydrolytic rancidity is considered a "defect." However, there are products in which the rancid flavor is not only acceptable, but desired. Blue (roquefort) cheese is a prime example. In addition, rancid milk is often used as an ingredient in milk chocolate, where the rancid flavor actually enhances the flavor of the chocolate product.

The flavors that develop in milk from action of lipase are most often produced by the release of short-chain fatty acids, specifically butyric, caproic, caprylic, capric, and lauric (C4 through C12). The specific flavor that develops depends upon (1) the composition of the fat and (2) the source(s) of lipase.

The composition of fat can vary to some extent as previously described. Not only do lipases show preference to certain fatty acids, but also to the position any given fatty acid holds on the triglyceride molecule. As noted on page two, fatty acids reside either at the top, middle, or bottom of the glycerol molecule (positions number 1, 2, and 3, respectively). Lipase(s) may seek out short-chain fatty acids, but preferentially those that specifically reside at one of the three positions.

by the living organisms and also by lipase of dead cells that originated in the raw milk.

Lipase response to pH and temperature:

As do all enzymes, lipases vary in response to pH and temperature. In general, lipases are active between pH 5.0 and 9.0. The enzymes are usually inactive below pH 3.5 and above pH 9.5. Natural milk lipases show optimal activity at pH 8.0 to 9.0.

The response of lipases to temperature varies. Optimal activity occurs generally between 80 and 113 F (26.6 and 40.5 C). Some lipase activity has been observed at temperatures as low as -20 F.

Inhibition of lipase(s):

Lipase enzymes are inhibited and/or destroyed by heat treatment. Not all lipases respond similarly, however. Perhaps this is one reason why the scientific literature carries a certain amount of conflicting information regarding the influence of heat on lipase.

For most practical purposes, naturally occurring milk lipase can be considered to be destroyed by minimal pasteurization. It is for this reason that milk can be homogenized following pasteurization without threat of rancid flavor development. Although milk is often homogenized prior to pasteurization and at temperatures substantially under those required in pasteurization, it is impossible to state unequivocally that this process will not result in some rancidity development. In any event, reasonable assurance of complete inactivation of all lipase enzymes in milk requires either pasteurization or near-pasteurization treatment.

The following data provides some information about the relative loss of lipase activity under various heat treatments.

Temperature and Treatment		Percent Inhibition of Lipase
135 F (57 C)	10 seconds	42
165 F (74 C)	10 seconds	98
190 F (88 C)	17.6 seconds	some activity remaining

In addition to the above data, the scientific literature shows some survival of active lipase after temperature treatments of 161, 185, and 200 F (71.1, 85, and 93.3 C, respectively) for 18 seconds holding time.

Factors protecting lipase from heat treatment:

Lipase enzymes are protected from heat at least to some extent by milkfat, solids-not-fat, and ferric forms of iron. That is, the higher the level of fat

Among factors known to cause aeration and foaming of milk on the farm are:

* Milk lines partially filled with milk
* Cracked or leaky milking inflations (rubbers)
* High-line milking systems (where milk is raised above the heads of cows). This is not to say that all such pipeline systems cause rancidity to develop but that they are generally more problematical in this respect than low-line milking systems.
* In-line milk strainers. Strainers are best placed on the pressure side of the milk pipeline.
* Presence of risers in the pipeline. Risers are sections of the pipeline milking system where milk is raised from a low to a higher level. To the extent possible, risers should be eliminated from pipeline systems.
* Continuously running pumps and/or releaser systems
* Air leaks at pipeline connecting joints
* Air leaks when milking equipment is removed from cows
* Excessive agitation of milk in the bulk tank. This is usually a problem mainly when level of milk is low i.e. first milking

Aeration and foaming is but one way in which lipase action is triggered. This and other activating procedures or processes are as follows:

* Aeration and foaming
* Temperature fluctuations (cooling, re-warming to 80 or 90 F and re-cooling)
* Homogenization
* Mixing raw milk with pasteurized, homogenized milk
* Contamination of milk with surface active agents such as those found in cleaning compounds and certain sanitizers
* Freezing and thawing of milk or cream

In all of the above inducing mechanisms, it is assumed that raw milk is involved. As already mentioned, pasteurization generally destroys lipase enzymes and any rancidity following this process must derive from bacterial contamination or the blending of raw and pasteurized products.

of oxidized dry whole milk and butteroil differs from that of fluid milk products.

There are two major sources of unsaturated fatty substances in milk, true fats (triglycerides) and phospholipids. It is essential to keep these two sources in mind in dealing with off-flavor development in dairy products.

Phospholipids are thought to be mainly responsible for oxidized flavors in fluid milk products. Phospholipids, it will be recalled, are generally associated with the fat globule membrane. Depending upon conditions that may alter or disrupt the membrane, phospholipids will be dispersed more or less into skimmilk. In this way, off-flavors derived from this source can, in a sense, spill over into skimmilk products.

Typically, flavors associated with oxidized phospholipids are the kind referred to as cardboardy or cappy. Oily or tallowy flavors seem more often to be derived from oxidation of true fat.

In overview, oxygen attacks the unsaturated linkage of the fatty acids of fats and phospholipids. C18 fatty acids of three and two double bonds (points of unsaturation) are attacked more readily than the C18 fatty acid with one double bond (oleic acid). Free radicals (R^-), peroxides and hydroperoxides are formed initially. Being unstable, these compounds rapidly break down; the fatty acid chain may in fact break apart, the fragment then becoming responsible for an off-flavor.

Unsaturated lipids (certain phospholipids, for example) that reside near the outer surface of the fat globule and, therefore, near xanthine oxidase enzyme and other components (copper and iron) of the fat globule membrane are among the first to undergo the oxidation reaction.

A large number of different chemical compounds form as a result of oxidation. Of these, certain aldehydes can impart a detectable off-flavor at levels less than 1.0 part per million. Other compounds, as well, contribute to flavor development at very low levels.

To the extent that phospholipids may be found in skimmilk, most dairy products must be considered subject to oxidative deterioration. In addition, oxidation occurs in raw milk, in pasteurized fluid milk products, and in various manufactured products i.e. nonfat dry milk, ice cream, butter, buttermilk (a rich source of phospholipids) derived from buttermaking etc.

One fact should be emphasized. Oxygen is essential for oxidation to take place. Milk or dairy products from which oxygen has been removed do not undergo the oxidation reaction. Deaeration, therefore, is one method of controlling this defect. To preserve dry whole milk during long-term storage, the product is often packaged under nitrogen i.e. oxygen is forced out and replaced by nitrogen.

Milk supplies may differ markedly in susceptibility to oxidation. More often than not, the most susceptible milk is produced during late winter, early spring, and by cows feeding on dry feedstuffs. Differences in susceptibility

Ascorbic acid (vitamin C) also acts as a promoter of oxidation, at least in part by removing an electron from Cu^{++} to yield Cu^{+}, the latter being a stronger oxidizing agent than the former.

Although ascorbic acid promotes oxidation at the concentration found in milk, it acts to retard oxidation at higher concentrations.

* Direct absorption of sunlight or strong artificial light. Although the chemical reaction may differ from other modes of oxidation, certain light rays will cause production of flavor compounds usually characterized as oxidized. Presence of riboflavin (vitamin B_2) enhances the reaction--and riboflavin potency is lost in the process.

* Acidity (low pH). Acidity generally promotes oxidation. Butter made from cultured (ripened) cream is more susceptible to oxidation than butter processed from sweet cream.

* A number of metalloenzymes (enzymes that contain metals) promote oxidation. These include lactoperoxidase, catalase, and cytochromes.

Factors that retard/inhibit oxidation:

* Presence of antioxidants. As the name implies, antioxidants are compounds that retard or inhibit oxidation. The most significant source of antioxidants in dairy products are those that develop upon relatively high heat treatment. At high temperatures, proteins undergo denaturation. In the process, they unfold and certain of them free up reactive chemical fragments called sulfhydryl groups. A "cooked" flavor results. At the same time, sulfhydryl groups act as reducing agents and inhibit oxidation. During storage, sulfhydryl groups are depleted, the cooked flavor slowly disappears and, eventually, the oxidation process may once again be favored.

  Because sulfhydryl groups are such effective antioxidants, fluid milk products, ice cream mix, and cream for buttermaking are often pasteurized at temperatures sufficiently high to ensure some level of sulfhydryl activation.

* Homogenization. The process of homogenization tends to retard oxidation.

* Cows' rations. Oxidation is typically a winter problem associated with a lack of green feed in the ration. Supplementing feed with tocopherol (vitamin E) and/or ethoxyquin will often significantly delay onset of oxidized flavors. However, the transfer of tocopherols to the milk supply is not very efficient and relatively large amounts must be fed to serve a useful purpose.

* Bacteria. It is generally true that oxidation is more of a problem in milk of high quality (low bacteria count) than milk of poor quality. The reason may be twofold. First, some bacteria may utilize a significant level of available oxygen. Without--or at a lower level of

In the past, the most significant source of copper in milk was copper-containing milk-handling equipment, whether pipelines on the farm or processing lines and equipment in the dairy plant. Copper is present in several alloys, among them bronze, brass, "white metal" and nickel silver.

In years past, white metal was commonly used in manufacture of dairy processing lines and equipment. Even a single connecting joint in a pipeline could be a source of sufficient copper to cause oxidation. As mentioned previously, as little as 1.0 part per million or less of this metal can induce oxidation. This much can readily be absorbed from a single white metal surface.

In addition, cleaning and sanitizing solutions in clean-in-place systems pick up copper from white metal, transport the metal throughout the lines and equipment being cleaned, and deposit it over the entire surface. Some amount of this copper is then absorbed the next time product is transferred or processed in that equipment.

The prevention of oxidation, therefore, requires that no copper-bearing alloys be used in milk-handling equipment. Only stainless steel, glass or suitable (food-use) plastics should be used.

It should also be kept in mind that copper is often a contaminant of water supplies. Water is often mixed intentionally with certain dairy foods. It is often an unintentional contaminant. If necessary, water should be treated for removal of copper.

4. Pasteurize or heat-treat products at temperatures high enough to produce some level of sulfhydryl antioxidants. These chemical groups are detectable following heating to 158 F (70 C) for 30 minutes or 169-172 F (76-78 C) for a few seconds. Higher temperatures and/or longer holding times will evolve that many more active sulfhydryls. However, cooked flavors may become objectionable to some consumers at some point; processors may have to settle on a compromise of sorts.

5. Deaerate--remove oxygen from the product. Although not necessary for the most part, vacuum treatment of fluid dairy products may afford some protection against oxidation. Some small level of volatile off-flavors may also be removed in this way. Deaeration and gas-packing (with an inert gas, like nitrogen) of dry whole milk is necessary for long-term storage, especially at high temperatures.

6. Homogenize products to the extent possible. Although the mechanism by which homogenization protects against oxidation is not known, that it does in fact offer protection is well established.

7. Although generally illegal in dairy processing in the United States, it is possible and also effective to use one of several chemical antioxidants as an intentional additive. Such compounds are approved for a number of foods other than dairy products.

different temperature. For this reason, milkfat does not have a single melting point. Instead, it reflects a range in melting point.

All the fat in milk is liquid at 104 F (40 C). Some fat of milk remains liquid at temperatures as low as -100 F.

Some of the factors that determine the hardness/softness of fat are as follows:

* Fatty acid profile: As chain-length of fatty acids increase, melting point increases; melting point decreases with increasing unsaturation; Cis forms of fatty acids have a lower melting point than trans forms. Cis and trans are specific kinds of configurations, with the cis form being somewhat more chunky--compressed--than the trans form.

* Nature of the lipid (fatty) substance:

  For lipids consisting of saturated fatty acids, melting point decreases in the following order: monoglycerides, diglycerides, triglycerides, free fatty acids.

  For lipids with unsaturated fatty acids, melting point decreases in the following order: monoglycerides, diglycerides, free fatty acids, triglycerides.

* Distribution of fatty acids about the glycerol molecule:

  A given fatty acid occurring in the 1 position will lend to the triglyceride a higher melting point than the same acid in the 2 position.

  A symmetrical triglyceride will have a higher melting point than one that is assymetrical. For example, a triglyceride consisting of two C14 and one C16 fatty acids will have a higher melting point when the C16 fatty acid resides in the 2 position (between the two C16 fatty acids.

  A random distribution of milkfat fatty acids on glycerol will result in a more spreadable fat than the original, somewhat ordered arrangement. Fatty acids can be caused to take up random positioning by either chemical or enzymatic methods. In the future, as more is learned about the latter, they will likely see much greater application as fat-softening technologies.

* Polymorphism: When milkfat crystallizes, it does so in more than one crystalline form. This happens as a result of different patterns of molecular packing. At least three different crystalline forms occur.

  This phenomenon--of varied crystal size and shape--is termed polymorphism--poly meaning "more than one," morphism meaning "form" or "shape." The polymorphism expressed by milkfat is thought to

that set up during storage. The Microfix method is perhaps best known. It is most effective following storage at 41 F (5 C) for at least 7 days.

It should be kept in mind, however, that any treatment of cream or butter to produce a spreadable structure can be rendered useless by heating and/or cooling of the product after the process has been completed. Whether these conditions occur in industry/government or home storage, the result is the same—a harder, less spreadable butter.

Blending butter with vegetable oils

Butter/margarine blends have become increasingly popular in recent years. Butter has a unique flavor that margarines cannot match and margarine oils can produce a softness otherwise impossible in butter.

Proportion of butter to margarine may vary significantly. Common blends include butter/margarine ratios of 80:20, 50:50, and 60:40. In all cases the product can be processed by blending the vegetable oil(s) or emulsified oil(s) with cream prior to buttermaking or to granules of butter following churning.

The advantages of this method of enhancing spreading characteristics of butter include low cost and reliable control of finished product consistency. It is also possible to produce a harder product, possibly for shortening use, by blending in hard fats such as beef tallow.

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CHAPTER IV

THE CHEMISTRY AND PROPERTIES OF MILK PROTEINS

excess hydrogen ions, above pH 6.6, the carboxyl groups yield H+ ions. This is a buffering reaction in which pH is stabilized by proteins.

When amino acids come together to form proteins or fragments of proteins, the amino group of one amino acid joins with the carboxyl group of another. The resulting connecting linkage is referred to as a **peptide** bond.

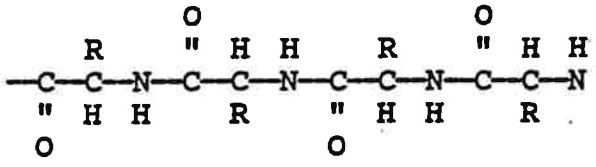
The structure of the peptide bond—the linkage that binds all amino acids of protein molecules—may be depicted as follows:



AMINO ACID 1 + AMINO ACID 2 produces DIPEPTIDE

In the above illustration, two different amino acids have been joined by a peptide bond to produce a **dipeptide**, "di" implying two units. The artificial sweetener, **aspartame**, is a dipeptide. It consists of a combination of the amino acids **aspartic acid** and **phenylalanine**. Aspartame is 180-200 times sweeter than sucrose (table sugar).

In the way two amino acids may be joined together, several of the same or different amino acids may also combine. Relatively small fragments of such combinations are called **polypeptides**. The prefix "poly" implies several amino acids linked together. A configuration of this kind of structure follows:



POLYPEPTIDE

The repetitive nature of the amino acid to amino acid linkage is perhaps more readily observed in the following structural adaptation:

CO—RCH—NH—CO—RCH—NH—CO—RCH—NH—CO—RCH—NH

It may be seen that the hydrogen of the carboxyl group has been lost as the amino group of one amino acid connects to another amino acid at this point. This results in the formation of the C=O or **carbonyl** grouping variously shown as CO or C=O in the above illustrations.

Two other features are common to all naturally occurring amino acids: (1) all are "alpha" in structure, and (2) all are of the L-configuration. The former

Table 1. Common amino acids and various protein sources of milk (grams per 100 grams of protein)

	Total Milk Proteins	Casein	Alpha-Lact-albumin	Beta-Lacto-globulin
Alanine	3.6	3.1	2.1	6.8
Arginine	3.5	4.2	1.2	2.9
Aspartic acid	7.5	6.5	18.7	11.7
Cystine	0.9	0.4	6.4	3.0
Glutamic acid	21.7	23.6	12.9	19.8
Glycine	2.1	2.1	3.2	1.7
Histidine *	2.7	3.0	2.9	1.7
Isoleucine *	6.5	6.6	6.8	7.4
Leucine *	9.9	10.1	11.5	15.0
Lysine *	8.0	8.2	11.5	11.9
Methionine *	2.4	3.3	1.0	3.3
Phenylalanine *	5.1	5.8	4.5	3.8
Proline	9.2	12.3	1.5	5.2
Serine	5.2	6.3	4.8	4.3
Threonine *	4.7	4.5	5.5	5.2
Tryptophan *	1.3	1.5	7.0	2.3
Tyrosine	4.9	6.3	5.4	4.0
Valine *	6.7	7.4	4.7	5.8

* Essential amino acids
Adapted from Dairy Council Digest, vol. 34, No. 3.

Aside from the amino acids shown in Table 1, milk also contains glutamine, hydroxy proline, asparagine, the sulfur-containing cysteine, and some few others. Cysteine is found at levels of about 1.1 and 0.3 g/100g protein in beta-lactoglobulin and blood serum albumin, respectively.

Essential amino acids

A number of amino acids are not synthesized by the body. One or two others may not be synthesized in quantities adequate to meet human needs. Together, these amino acids are termed "essential" or "indispensable." That is, it becomes necessary for good health that these amino acids be provided in the diet; the body has no other way of obtaining them.

Nine amino acids are considered essential for humans. These are the ones indicated by an asterisk in Table 1. In addition, arginine may not be produced in adequate amounts in some instances. Another amino acid, histidine, is considered essential for infants and possibly for adults.

Biological quality of proteins

That some amino acids must be derived from the diet is an important concept—important because proteins differ in levels of these specific amino acids. In

Of course, daily diets usually include protein from several different sources, some complete, some incomplete in amino acid content. Even plant proteins, most of which fall well below in biological value most animal sources of protein, can provide the necessary amino acids levels if the proper mix is consumed. For example, seeds and legumes serve human needs if taken together. So do wheat and bran products and rice and legume products. In such instances, the amino acid content of the one plant food complements the other; the mixture can be considered complete.

Conjugated proteins

Some proteins form complex associations with non-amino acid elements or compounds. **Phosphoproteins** have already been mentioned. Certain proteins are found complexed with copper, iron or other metals. These are called **metalloproteins.** Proteins also associate with lipids (**lipoproteins**) and carbohydrates (**glycoproteins**). Proteins that contain nucleic acid are called **nucleoproteins.**

The physical structure of proteins

The very long amino acid chain of a protein is coiled around itself in a fashion similar to a ball of yarn. Although the coil thus formed may not be as neat as the analogy suggests, the winding arrangement is highly specific for different proteins and proteins are generally **globular** in shape.

The uniqueness of individual proteins arises from the differences in sequence of amino acids that make it up. A large number of amino acids exist, hence a vast number of possible chains or arrangements of amino acids exist. In recent years, the precise sequence of amino acids in certain proteins has been determined. The first protein to be so characterized was the hormone insulin.

Proteins can therefore be envisioned as long chains of amino acids, fragments of which may be repeated over and over again, the whole being wound around itself in the form of a coil.

Denaturation

It is the unique amino acid chain and the unique way in which that chain is wound around itself that characterizes the native protein. In its natural form, the protein carries out its biological action. That action may be simply to serve as a source of nutrients. Or it may be a more complex function, as in the action of hormones, enzymes and toxins.

But proteins also serve as ingredients in foods. As ingredients, they are also expected to serve a given function— to provide structure/volume to bread and baked goods, to whip into a stable foam, as egg white does, or to absorb grease in a sausage.

Certain proteins will carry out the above functions, but do so only in their native state. Egg white is a good example. It whips into a meringue and does so readily—but not after it has been fried. In the frying/heating process, the clear, translucent protein of egg white quickly turns white and opaque,

after the bacteria have been destroyed, slowly split chemical bonds, break off fragments or otherwise change the physical and chemical structure of the protein; gelation or flavor changes ultimately occur. The break down may begin soon after processing. Product defects, however, may not become apparent for several months.

Isoelectric point of proteins

Suspended or dissolved in a liquid, amino acids of proteins carry an electrical charge, either positive or negative depending upon the pH. Amino acids differ in this respect. At neutral pH, some, like glutamic acid, are negatively charged; others, like lysine are positively charged.

Negative charges arise when the carboxyl group ionizes as follows: COOH----> COO- and H+. Positive charges occur when the amino group, NH_2, picks up a H+ ion to form NH_3+. The addition to milk of an acid, for example, provides H+ ions which in turn join with amino groups to form the positively charged component.

At any given pH, the electrical charge that surrounds a molecule of protein, depends upon the specific amino acids of which it consists. The net charge may be either positive or negative. As such, the protein responds as a positively or negatively charged unit. That is, in an electrical field, positively charged particles move toward the negative pole and vice versa.

It is this property that allows charged particles, like minerals (ions), to be removed from whey in the process of electrodialysis. In the same way, protein particles of like charge tend to repel each other and, thereby, remain suspended in a liquid. Casein in skimmilk is an example. At the pH of fresh milk (6.6-6.7), the casein particles carry a net negative charge, a factor important in stabilizing the suspension.

As pH changes, so does the net charge of proteins. Various proteins respond differently in this respect because of differences in amino acid content. At some point—at some given pH—the number of positive charges exactly equal the number of negative charges (the amount of NH_3+ equals the amount of COO-). That pH value is referred to as the **isoelectric point** of the protein.

The prefix "iso," in this case, means "same: or "similar". When positive and negative forces of a protein are balanced in this respect, the protein carries a neutral charge. It is at that particular point, in that very condition or state, that it is least soluble, therefore most likely to precipitate out of solution/suspension.

The isoelectric point is an important concept in dairy processing. It is the pH at which a given milk protein most cleanly and efficiently separates from solution. It is the point at which casein goes from colloidal particle to curd. It is the point—though at a different pH than for casein—at which alpha-lactalbumin is most unstable and most readily precipitated. The same is true of all proteins.

Fat globule membrane proteins are present to a level of about 0.04%. They are known to include, among others, xanthine oxidase, butyrophilin, glycoprotein B, and alkaline phosphatase. To a great extent the specific proteins found to associate with fat globules depend upon the method of preparation of the fat globules prior to isolation of the proteins. Only recently have steps been taken to define these procedures.

Protein vs nitrogen content of milk

Protein content of milk is measured by the Kjeldahl method. If this method is not used as such, it is yet the sole reference procedure for protein determinations and is the basis, therefore, upon which other methods are calibrated. Hence, infra-red instruments are calibrated and monitored to read levels of protein established by the Kjeldahl method.

This fact has practical implications in that the Kjeldahl method does not measure protein content per se, but rather nitrogen content of milk or other food product.

Nitrogen, it will be remembered, is present in all proteins, along with carbon, hydrogen and oxygen. Nitrogen content varies to some extent from protein to protein, but is nonetheless an element common to all proteins. It can be measured, therefore, and the level related to protein content of various substances.

To convert nitrogen content to protein content, a factor is used. The factor is derived from the average nitrogen content of the amino acids making up a given protein or proteins. The nitrogen content of milk proteins averages 15.67%. The factor for converting nitrogen to percentage of protein in milk becomes 6.38 (100 divided by 15.67 = 6.38).

Not all proteins have the same nitrogen content. Not all proteins are best served by a factor of 6.38. A factor of 6.25 is common to a number of proteins other than those found in milk.

Beyond the preceding considerations, another significant fact needs to be considered. That is that not all nitrogen in milk is found in proteins. There is in fact some nonprotein nitrogen. This nitrogen is also measured by the Kjeldahl method and is a complicating factor in milk protein analysis.

Nonprotein nitrogen—implications to protein analysis

Nitrogen content is used as a gauge of protein content of milk. Indeed, level of protein(s) in milk is often given as percentage of total nitrogen content. Of note is the fact that milk contains both protein and nonprotein nitrogen (NPN). The latter consists of small amounts of organic and trace amounts of inorganic compounds. Creatine, creatinine and urea are examples of nitrogen-containing organic compounds, nitrate an example of a source of inorganic nitrogen.

Unless accounted for and excluded, NPN, as part of total nitrogen analysis by the Kjeldahl method, is included in the calculation of protein content. Amount

Ratio of casein to total protein/nitrogen content of milk

Although tests for casein are available, no simple, direct method of casein analysis is yet in common usage. At the same time, casein content of milk is an essential factor in calculating yield of cheese from a milk supply. In practice, therefore, most cheese processors estimate casein content.

If total protein content is established either by or based upon the Kjeldahl method, casein is taken as 78% of total crude protein content. On a true protein basis, casein is more appropriately estimated as 82% of the measured amount. These relationships are shown in Table 3.

Table 3. Percentage of nitrogen-containing components of milk and milk proteins

	Percentage		
	In Milk	Of True Protein	Of Crude Protein
Crude Protein	3.2	100	100
True Protein	3.04		
Casein	2.5	82.2	78.1
Whey Proteins	0.54	17.8	16.9
NPN	0.16	0.16	5.0

From data in Table 3, percentage of casein to crude protein is determined: 2.5 divided by 3.2 X 100 = 78.1. Nonprotein nitrogen does enter into the calculation. On a true protein basis, the calculation becomes: 2.5 divided by 3.04 X 100 = 82.2. In this case, NPN is discounted. That is, crude protein minus NPN equals true protein, or 3.2 - 0.16 = 3.04%.

Ratio of casein to total crude protein has been found to vary from 74.2 to 81.3% in milk supplies of the upper midwest. However, the value of 78% has been found to be stable enough in these supplies to agree with a measured (analytical) value to within plus or minus 0.09% at 95% confidence.

Calculating cheese yield of milk from fat and total crude protein content

Cheese consists essentially of casein and milkfat. The oldest and perhaps best known formula for estimating yield of cheese is one developed by and referred to as the Van slyke and Price Formula. It is given as follows:

Table 4. Approximate percentage and isoelectric point (pI) of proteins and protein fractions of milk

Protein or Fraction	Percentage	Isoelectric Point
Total protein	3.34	
Total casein	2.66	4.6
alpha-s_1 casein	1.0	5.1
alpha-s_2 casein	0.26	
beta-casein	0.94	5.3
kappa-casein	0.40	3.7-4.2
Total whey proteins	0.68	
beta-lactoglobulin	0.35	5.3
alpha-lactalbumin	0.16	5.1
immune globulins	0.06	4.6-6.0
blood serum albumin	0.04	4.7
Proteose-peptone	0.07	3.7

Adapted from Whitney et al. (1976) and Eigel et al. (1984)

Note: A gamma-casein fraction was formerly thought to exist, but was found to be a part of beta-casein. Data in this table reflect that fact.

Casein

Casein is the major protein of milk, varying in amount from a low of about 2.2% to as much as 4.0% in certain breeds of cows. On average, casein content of milk ranges near 2.5 to 2.6%.

Casein consists of the usual elements found in all proteins, i.e. carbon, hydrogen, oxygen, and nitrogen. In addition, casein is known to contain a small amount of magnesium and citrate and larger levels of phosphorus, sulfur and calcium. The whole is bound together in a calcium-phospho-caseinate complex. Casein is dispersed in skimmilk mostly as colloidal particles (2.40%). A small amount (0.26%) of soluble serum casein is also present.

Casein contains about 0.85% phosphorous. Whey proteins contain no phosphorus. This dissimilarity results in significant differences in the way these proteins react to processing conditions.

Phosphorus in casein is esterified to the protein mainly through condensation with hydroxyl groups of serine. This type of bond is represented:

It is important to note that the calcium of casein remains with casein in the curd produced by rennet action. Cheeses produced by rennet action, therefore, remain good sources of this nutrient. Most hard cheeses (cheddar, swiss, colby etc.) fall in this category.

On the other hand, acid coagulation of casein results in a loss of much of the calcium originally associated with the protein. For this reason cottage cheese curd is a poorer source of calcium than cheese products processed with rennet/chymosin.

Data in Table 4 indicate the four major fractions of casein. These fractions are identified by the rate of speed of migration in an electric field by a method called gel electrophoresis. Each fragment is a discrete, readily identifiable piece of the casein molecule.

The four fractions of casein are: alpha-s_1, alpha-s_2, beta and kappa. It is important to note, particularly in terms of cheese manufacture, that alpha-s and beta caseins are stabilized against precipitation—against curd formation—by kappa casein.

Hence, of the four fractions, kappa casein perhaps looms most important in practical terms. Only to the extent that rennet/chymosin and rennet-like enzymes are able to break down kappa casein is efficiency in cheese manufacture possible.

Kappa casein is known to vary in sensitivity to rennet in milk of different species of mammals. It may vary to some extent even within milk of cows. Genetic variants are known to exist, and it is possible that breeding practices could result in milk supplies more or less readily attacked by rennet.

The cheesemaking process, then, proceeds along rather well defined chemical lines. Rennet action is closely linked to the first step in casein coagulation, and calcium to the second and final stage. The reaction is described as follows:

Step 1. $$\text{k-casein} \xrightarrow{\text{rennet}} \text{para-k-casein} + \text{glycomacropeptide}$$

Step 2. $$\text{para-k-casein} \xrightarrow[\text{pH 6.4 - 6.0}]{\text{Ca ++}} \text{dicalcium para-k-casein}$$

In the above sequence of events, kappa-casein (k-casein) is attacked by rennet, resulting in formation of para-k-casein and a whey-soluble glycopeptide fragment. The latter consists of some amount of carbohydrate and a small fragment of protein.

The end result of step one is to disrupt the protective influence of k-casein on alpha-s and beta-casein. Both para-k-casein and the other caseins exposed in this reaction are sensitive to presence of calcium ions.

The maximum rate of coagulation of casein by chymosin occurs at temperatures between 104 F (40 C) and 107.6 F (42 C). Coagulation does not occur below 50 F (10 C) nor above 149 F (65 C).

* Enhances the body of cultured milk products. Various cultured milk products can be processed to yield a more viscous/thick body by heating milk to a minimum of 195 F (90.6 C) for 10 minutes. The effect is due mainly to an increase in the viscosity of casein.

* Influences the potential for gelation of Ultra-High-Temperature (UHT)/sterilized milk. The problem of gel formation in UHT milk is best avoided by very high heat, short-time treatment i.e. 300 F (148.9 C), 3-4 seconds.

The following treatments are listed in order of <u>increasing</u> risk of gelation: 300 F (148.9 C), 6.9 seconds; 289 F (142.8 C), 6.9 seconds; 280 F (137.8 C), 20.3 seconds.

As a general rule of thumb, gelation is **inversely related to the holding time** at any given temperature treatment.

A distinction should perhaps be made between gelation and coagulation. When casein coagulates, whey forms and the process is irreversible. Gelation is a process in which a jelly-like structure is produced, no whey forms and, initially, the reaction is reversible.

* Increases the stability of evaporated milk. Preheating of milk prior to evaporation and sterilization does much to prevent gelation of the finished product during storage. The process is called **forewarming**. Both low temperature/long-hold and high temperature/short-time treatments are used. The former is a process of heating to about 203 F (95 C) for 10 minutes. The latter involves temperatures of about 302 F (150 C) for 25 seconds.

In addition to forewarming, the salt balance also plays an important role in stability of casein during storage. In order to achieve maximum stability, a certain balance must be maintained between calcium and magnesium content (components that tend to destabilize casein) and phosphates and citrates (components that tie up calcium and magnesium and stabilize casein).

Both **disodium orthophosphate** (DSP) and **monosodium orthophosphate** (MSP) may be used as stabilizing salts. Sometimes a 50:50 mixture of these compounds serves better than either one singly. Level of addition may range up to 0.2%. To determine the appropriate compound(s) and level of use, trial runs are usually made.

Although amounts as high as 0.2% of these stabilizing salts may be added, lower levels (0.1% maximum) ensure absence of salty flavors in the finished product.

To some extent, DSP and MSP enhance stability of evaporated milk by altering pH. In this regard, they produce opposite effects. Only pilot tests can determine which will serve best.

When the acidity of milk is slowly increased, casein tends to coagulate as a soft, watery curd. Both addition of more acid and/or application of heat hastens the expulsion of whey. Stirring--agitation--also causes whey to be expelled more rapidly. These are important considerations, of course, in cheese manufacture, both in cheeses processed with rennet and those, like cottage cheese, processed without it. It is in the body/texture of cheeses that these factors are often reflected.

The effect of acid, heat, and agitation are also important considerations in manufacture of casein as a product in and of itself. Both **hydrochloric** and **sulfuric acids** have been used for this purpose. These are strong mineral acids. As such, they must be diluted before use in order not to cause localized variations in texture of the curd. Sulfuric acid is not appropriate for manufacture of casein if the whey is intended for use as animal feed.

Lactic acid may also be used in production of casein, and the finished product(s) is similar to that of casein processed from mineral acids. The lactic acid for such use may be derived from bacterial fermentation of whey. Certain **lactobacilli** are able to produce as much as 2% lactic acid, a level which can serve in direct precipitation of casein. S. cremoris is also used for this purpose. When lactic acid per se is added to skimmilk, casein begins to coagulate at a pH of 5.3 or a titratable acidity of about 0.5%.

Casein curd processed at temperatures below 95 F (35 C) tends to be soft and to consist of very fine particles. Severe agitation causes the curd to be finer yet and to pose difficulties in efficient separation.

High quality casein can be produced by acidifying skimmilk to pH 4.6 at 35 F (2 C), holding 1 hour at that temperature, then warming to 86 - 95 F (30 - 35 C). The initial low temperature treatment causes formation of a very fine casein curd. The holding time of 1 hour provides time for colloidal calcium phosphate (associated with the casein) to dissolve--allowing ready removal. The curd thus prepared is recovered by **centrifugation** in commercial equipment.

The curd must be thoroughly **washed** to remove **lactose** and **milk salts**. Complete removal of lactose is essential to avoid the **browning** reaction in the event the casein is used in a food system that is exposed to high temperatures. Coffee creamers are an example. The browning phenomenon is covered in detail in the discussion of lactose.

Casein curd may be dried in **fluid bed** dryers or other dryers specifically designed for the purpose.

Casein processed at temperatures between 95 F and 100 F (35 C and 37.8 C) forms a coarse curd as long as agitation is not too intense. However, the curd remains fairly soft.

A firmer (also readily separable) curd can be produced in one of two ways: (1) by heating to a temperature between 100 F and 118 F (37.8 C and 47.8 C) or (2) by increasing the acidity to pH 4.1 without increasing the temperature. The precise temperature in method (1) depends upon the acidity level.

acid whey. It is also a product that may be manufactured either by direct acidification or through bacterial cultures.

Data in Table 6 indicate the average composition of sweet and acid whey, both in fluid and dry products. The major difference lies in ash/mineral content as caused mainly by the higher levels of calcium and phosphorus found in acid whey.

It should be pointed out that, unlike casein, the proteins of whey do not vary in composition whether originating under acid or rennet cheese manufacturing processes.

Important characteristics of casein

* Casein has a large number of hydrophobic (apolar, non water-attracting) amino acids which would tend to render it much less soluble in water were it not for the presence of rather large levels of phosphate groups. The latter, as well as presence of the sugar-containing (glycosidic) kappa-casein, promote solubility. As much as 20% solubility is possible at 176 -194 F (80 - 90 C). The high temperatures are necessary to counteract the otherwise high viscosity.

* Casein has a high water-binding capacity (2.5 grams per gram of protein). This characteristic makes it valuable as an ingredient in sausage, comminuted meat products, instant dessert products, and artificial/synthetic whipping cream.

* Casein is subject to proteolysis without prior denaturation by acid or heat.

* Kappa-casein interacts with beta-lactoglobulin, a whey protein, upon heating to temperatures at which the latter undergoes denaturation. In the process of denaturation, SH groups are exposed in the beta-lactoglobulin which, in turn, interact with K-casein. The process may start at a temperature as low as 149 F (65 C); it is more pronounced at 167 F (75 C) and higher.

This complex of K-casein and beta-lactoglobulin has significant impact on heat stability of milk, on rennet coagulation and strength of rennet curd.

* Casein is rich in lysine, an important essential amino acid.

* The kind of bond (covalent) that attaches phosphorus to casein is very strong and can only be broken by severe treatment, either in the form of high pH or by use of enzymes.

* Both acid and alkaline phosphatase enzymes readily attack the ester linkage that ties phosphate to casein. Casein treated with these enzymes takes on entirely new characteristics.

* Most casein (95%) in milk exists in small particles called micelles. These particles are distributed throughout milk as a colloid. They are large enough to reflect (scatter) light. In doing so, casein micelles become the cause of the white color of milk.

Proteose peptone can be isolated by adjusting the pH of skimmilk to 4.6 and heating to 203 F (95 C) to co-precipitate both casein and whey proteins. Proteose peptone remains in solution.

Beta-lactoglobulin exists as a dimer. That is, it is made up of two identical sub-units, each with a molecular weight of 18,400. Above 104 F (40 C) the dimers split into their component monomers (individual units). It is these units that become significant in denaturation processes. It is also noteworthy (and will be mentioned in other considerations) that this protein contains sulfur in the form of disulfide bridges (S-S) and also as free sulfhydryl (thiol) groups (chemically depicted as -SH). Under certain conditions, as already indicated, these sulfur compounds can be activated to serve as antioxidants.

For many years no explanation could be offered for the presence of beta-lactoglobulin in milk. Recently, though, it has been found that the protein can bind retinol (vitamin A). What this implies is that beta-lactoglobulin may be one of the processes by which the vitamin is carried through the stomach to the small intestine where it can be absorbed into the body. Beta-lactoglobulin would be well adapted to such function because of its resistance to proteolytic enzymes. That is, it would pass through the gut scarcely altered and could, indeed, carry retinol with it.

The isoelectric point of beta-lactoglobulin is pH 3.55. It is structured as a dimer from pH 5.0 to 7.5. The dimers split apart at pH 2.0

Alpha-lactalbumin is the most heat-resistant of the whey proteins. It is also the smallest in size. It, too, carries disulfide bridges which enter into a reversible denaturation process under given levels of heat treatment. There is evidence that residues of the amino acid lysine are to be found at the surface of the molecule.

Alpha-lactalbumin binds calcium, likely through the attraction of negatively charged carboxyl groups to the positively charged ion. Uptake of calcium corresponds with the potential to take up water.

Binding of calcium is of significance in the denaturation process that occurs at about pH 4.0. The reaction is to an extent reversible. However, as pH is lowered, the protein molecules tend to aggregate into more stable (less reversible) particles.

The isoelectric point of alpha-lactalbumin is pH 5.2. As pH is lowered below 5.0, calcium is lost and the protein aggregates and precipitates at fairly low temperatures. The protein has minimum solubility in 0.5 molar sodium chloride at pH 4.8.

Alpha-lactalbumin enters into the biochemical process in which cows produce lactose. This has significance in the composition of milk produced under treatment of cows with bovine growth hormone. Because of the need to maintain proper tissue pressure (osmotic balance), the cow responds to the hormone by attempting to maintain adequate levels of lactose. In the process, larger

magnesium sulfate. They can be further separated, by **dialysis**, into **euglobulin**, a true globulin, and **pseudoglobulin** (meaning false globulin). Together, these proteins make up the **immunoglobulin** fraction.

Table 6. Composition of sweet and acid whey, both fluid and dry (amount per 100 grams of product)

	SWEET WHEY		ACID WHEY	
Component	Fluid	Dry	Fluid	Dry
Gross Composition:				
Water (g)[1]	93.1	3.2	93.4	3.5
Protein (g)	0.85	12.9	0.76	11.7
Fat (g)	0.36	1.07	0.09	0.54
Carbohydrate/lactose (g)	5.14	74.5	5.12	73.5
Ash (g)	0.53	8.35	0.61	10.8
Minerals:				
Calcium (mg)[2]	47	796	103	2054
Phosphorus (mg)	46	932	78	1348
Magnesium (mg)	8.0	176	10	199
Potassium (mg)	161	2080	143	2288
Sodium (mg)	54	1079	48	968
Iron (mg)	0.06	0.88	0.08	1.24
Zinc (mg)	0.13	1.97	0.43	6.31
Vitamins:				
Vitamin C (mg)	0.10	1.49	0.06	0.90
Thiamine (mg)	0.036	0.52	0.042	0.62
Riboflavin (mg)	0.158	2.21	0.14	2.06
Niacin (mg)	0.074	1.26	0.08	1.16
Pantothenic acid (mg)	0.383	5.62	0.38	5.63
Vitamin B_6 (mg)	0.031	0.58	0.042	0.62
Folacin (mcg)[3]	1.0	12	2.0	33
Vitamin B_{12} (mcg)	0.277	2.37	0.179	2.5
Vitamin A (IU)[4]	4	10	1.0	9
(RE)[5]	16	44	7.0	58

Adapted from United States Department of Agriculture (1976)

1 Grams
2 Milligrams (1/1000 of a gram)
3 Micrograms (1/1,000,000 of a gram)
4 International Units
5 Retinol equivalents (1.0 retinol equivalent = 3.33 International Units of retinol or 10 International Units of beta-carotene)

most important consideration is the activation of thiol (sulfhydryl) groups, reducing compounds that inhibit the onset of oxidized flavors.

Cooked flavors and presence of free (activated) sulfhydryl groups are related phenomena. Both involve sulfur compounds present in whey proteins. Both are processes in which sulfur linkages are disrupted or altered. Beta-lactoglobulin is the primary source of sulfur compounds liberated by heat.

As already mentioned, sulfur in whey proteins exists to some small extent as free sulfhydryl (-SH) groups. It is also present as disulfide bridges (S-S) which help hold the protein in its native globular or coiled shape. It is present also as an integral element of the amino acids cystine and cysteine. As temperature is raised or prolonged at high enough levels, some of the S-S linkages are broken, the coil-like shape of the protein disrupted, volatile sulfur compounds (sulphides) formed along with larger numbers of free -SH groups. In addition, some amount of H_2S, the gas giving rise to the rotten egg odor, evolves.

Moderately intense cooked flavors are currently well accepted by the public. Minimal pasteurization causes only very slight flavor development. More pronounced flavors arise at temperature treatment in the range of 158 - 161 F (70 -71.6 C) for 30 minutes and 169 - 172 F (76.1 - 77.7 C) for a few seconds.

Cream shows evidence of alterations in sulfur compounds at temperature treatment of 160 F (71.1 C) after momentary holding. This is a lower temperature response than that observed for skimmilk.

Heating cream to very high temperatures in the buttermaking process has become common procedure. This is done purposely to produce high levels of free -SH groups which, in turn, markedly retard development of oxidized flavors. Temperature of treatment may vary from 192 F (88.9 C) for 20 seconds to temperatures of 194 - 200 F (90 - 93.3 C) or slightly higher for momentary holding or holding for up to several (five) minutes.

Although the cooked flavor may be relatively pronounced initially, it gradually disappears as sulfhydryl groups are lost during storage and subsequent oxidation.

Creaming of milk is also influenced by heat treatment sufficient to cause denaturation of whey proteins. Even minimal pasteurization results in loss of some globulins that would otherwise be active in bringing about clumping of fat globules. Clumping increases the effective size of the particles of cream and is important in hastening their rise and the formation of a cream layer. For cream products generally, minimal pasteurization is usually advised.

At temperatures high enough to cause major whey protein denaturation, the white color of milk is enhanced. This occurs just prior to development of caramel colors due to the browning reaction. At this point, whey proteins are denatured and aggregated into large size particles, their visible presence resulting in the whitening property.

Table 8. Classification of nonfat dry milks by a measure of undenatured whey protein

Classification	Whey Protein Nitrogen (mg/g)	Approximate % Denaturation
High Heat	not > 1.5	80
Medium Heat	1.51 - 5.99	21 - 79
Low Heat	not > 6.0	20

Adapted from American Dry Milk Institute (1971)

As data in Table 8 indicate, the lower the heat treatment the higher the level of undenatured whey protein in the product.

Functional properties of whey proteins

Whey proteins are of value mainly as ingredients in a variety of foods. They are only useful for such purposes if their presence does not detract from the flavor and textural properties characteristic of the given food. They are of more value if their presence adds to the quality of the product. Those chemical and/or physical characteristics of a protein(s) that govern its behavior in other food systems are known as **functional properties.** And these properties are important as they influence the food system during processing, storage and, finally, through consumption.

For example, egg white proteins can serve needed functional properties in whipped toppings. Gluten and glutenin, proteins of wheat, provide leavening in bread. Casein is the protein that makes up the structure of cheese.

Other proteins may be able to substitute for the examples given. A whey protein might well replace egg white in a whipped topping. But it will do so only if it functions similarly to egg white in the final product. That is the critical factor.

From the above discussion, it is apparent that processing of whey to yield appropriate products for use in foods requires attention to a number of details. Yet products are processed regularly and regularly they meet the demands of the marketplace. These demands vary, but can be identified in terms of a few important functional properties: These include solubility, thickening, whipping and foaming, binding of water, emulsifying capacity, gelation, adhesion and binding of flavors. Adhesion is the ability to hold particles together. This is an important property for proteins used in meat products such as hotdogs, sausages and the like.

Solubility: In some foods, the protein ingredient must dissolve or disperse in, usually, an aqueous liquid. For this application, undenatured proteins serve best. And they serve best whether the final pH is acid, alkaline, or neutral.

* pH—as it effects heat denaturation. pH of 5.0 is optimum for heat treatment at temperatures under 158 F (70 C) and pH 7.0 for treatment at temperatures above 176 F (80 C). These conditions result in the dissociation of dimers.

* Presence of calcium—generally decreases foamability by enhancing aggregation.

* Increased solids—enhances whipping properties. A protein concentration of 10% appears optimal.

* Ionic strength—enhances whipping and foaming characteristics at low levels, but is dependent upon the type of ions present.

* Presence of reducing agents—inhibits foaming. Free -SH groups are reducing compounds. Denaturation must be controlled in order to prevent formation of high levels of these units.

* Level of fat—should be low as possible prior to heat treatment of the whey protein product.

Emulsifying properties:

Emulsifying properties make it possible to hold oil/fat and water together in a food product. The best emulsifying compounds carry distinct hydrophilic (water-loving) and hydrophobic (water-hating) characteristics on the same molecule. In proteins, the emulsifying capability depends upon specific amino acids, some being more or less hydrophobic or hydrophilic than others. Because of the generally random positioning of amino acids in whey proteins, emulsification potential is lessened to some extent.

The extent to which whey proteins attach themselves to fat globules is highly dependent upon pH. Individual whey proteins differ in this respect. Beta-lactoglobulin adsorbs best at neutral pH, with alpha-lactalbumin improving in this respect as pH is lowered.

Calcium inhibits emulsifying properties of whey proteins, and for the same reason that it is detrimental to solubility and whipping properties, i.e. it encourages aggregation of protein molecules.

Indeed, the factors that influence whipping and foaming properties likewise influence the emulsification potential. Please see the preceding section.

As a general rule, temperature treatment of 158 F (70 C) or less favor solubility, whipping and foaming, and emulsification properties. Temperatures above 158 F favor enhancement of viscosity (thickening) and uptake of water.

Gelation:

Gel formation may serve a useful functional property, as it does in custard products. Or it may be an undesirable attribute, as in the gelling of sterilized milk products (evaporated milk, UHT milk). Gels can also pose

Factors influencing functional properties

Whey protein composition and properties can be influenced at all stages of production and processing.

On the farm, primary factors that impact on functional properties of finished whey protein products are related to milk quality. Storage time and temperature and sanitary practices bear directly on bacteria numbers and kind. Psychrotropic bacteria readily attack proteins, splitting them into fragments and altering their native characteristics. High somatic cell count and presence of the enzyme plasmin likewise induce protein break down. Whether or not a whey protein product will function properly in a food system depends largely on the original quality of the milk.

If whey is derived, as most of it is, from cheese manufacture, then functional properties of the whey proteins are related to the kind of rennet/chymosin used (calf vs bacterial or other bioengineered product), the kind of cheese manufactured (sweet vs acid fermentation), addition of calcium, whey recovery technologies, agitation, and sanitary handling of the whey to time of further processing.

After cheesemaking, status of the whey proteins is influenced by the technologies used to concentrate them prior to further processing, and then drying of the concentrate to a finished dry product. Processes such as vacuum evaporation, reverse osmosis, ultrafiltration and/or drum or spray drying each reflect a different potential to cause denaturation. Membrane systems (reverse osmosis, ultrafiltration etc.) usually produce less denaturation than other technologies.

If the whey is dried and stored prior to use as such or prior to further processing, the state of denaturation of the whey proteins will depend upon the moisture content of the product and the conditions of storage. Chemical reactions involving proteins are enhanced by higher levels of moisture. High storage temperatures also hasten chemical changes.

Heat treatment may be used as a process by which whey proteins are concentrated or fractionated/isolated as individual components. The heat treatment itself is a factor in denaturation. In addition, though, heat treatment effects vary depending upon pH, amount of calcium ions, overall ionic strength of the whey, dielectric constant, presence of air and fat, and also solids content of the product undergoing the heat treatment.

If the protein component is concentrated prior to drying, or if the proteins are concentrated or isolated as individual components, the technologies for performing these operations must also be considered as factors in the physical/chemical state of the proteins. Here, a variety of different technologies may be used, either alone or in conjunction with each other. These technologies include, among others, reverse osmosis, ultrafiltration, electrodialysis, ion exchange and gel filtration. More will be said about these processes later.

9. To concentrate the beta-lactoglobulin (remaining yet in the liquid after alpha-lactalbumin is precipitated), adjust the pH to 7.0, concentrate by diafiltration, and spray-dry. Beta-lactoglobulin of over 98% purity is thereby obtained.

Products of whey processing

Composition of whey products varies greatly. Some few products, however, are regulated, with both the name of the product and its composition defined (see Table 9).

Table 9. Name and compositional parameters of various whey products

	Percent of Dry Product				
Product Name	Protein	Fat	Lactose	Ash	Water
Dried whey	10 - 15	0.2 -2.0	61 - 75	7 - 14	1 - 8
Reduced lactose whey	16 - 24	1 - 4	60 (max.)	11 - 27	1 - 6
Reduced minerals whey	10 - 24	1 - 4	85 (max.)	7 (max.)	1 - 6
Whey protein concentrate	25 (min.)	1 - 10	60 (max.)	2 - 15	1 - 6

Adapted from Federal Register (1981)

Technologies for processing whey proteins

The technologies used for processing whey proteins are, in essence, technologies of concentration and separation. To purify the proteins, it is necessary to remove fat and other fatty substances (namely lipoproteins that originate in the fat globule membrane), lactose, and minerals.

Fat, as such, is removed by mechanical separation. Lipoproteins can be removed by precipitation with calcium, as indicated in the previous section.

Further processing consists of steps designed to perform one or more of the following unit operations (1) concentrate the liquid product, (2) concentrate and separate protein components, (3) remove minerals, and (4) drying.

1. Concentration technologies:

Evaporation under vacuum:

Evaporation under vacuum is the oldest, most common technology used to concentrate milk and milk products, whey included. Evaporators are constructed as single- or multiple-effect. The former consists of single vacuum pan in which the product is concentrated, vapors removed. Multiple-effect evaporators range from double on up to five or six evaporation units joined together and

Reverse osmosis is one of several systems of concentration and/or fractionation categorized as membrane processes. In these systems, some portion of the treated product (whey, as example) is lost, some is retained within the membrane. Substances that are lost make up the permeate, i.e. that which permeates--passes through--the membrane. The permeate has significance both as a source of the compounds present in it--which may be further concentrated or fractionated--and, if no further use is made of it, as a product requiring disposal by some form of waste treatment. The permeate of reverse osmosis, though relatively low in milk components other than water, nonetheless may carry a Biological Oxidation Demand (BOD) of up to 300. Tighter membrane systems, non-cellulosic in nature, have been developed and will allow production of permeates with a BOD of less than 100.

With reverse osmosis technology, it is possible to concentrate whey to 25 to 30% solids. It is also important to note that, besides water, some amount of certain whey components of low molecular weight may also pass through the membrane. Lactic acid is one such compound. Some components of the nonprotein nitrogen fraction also cross the membrane along with small amounts of minerals. In fact, reverse osmosis allows passage of some amount of those molecules ranging up to a molecular weight of about 100. However, it is not as porous as membrane systems prepared specifically for the removal of ionic salts (such as sodium chloride). These "thin-skinned" membrane systems are given the name loose reverse osmosis (or ultra-osmosis), and are referred to in the section on demineralization.

The membrane system most frequently used for reverse osmosis is one of a family of membranes produced from cellulose acetate.

Freeze concentration:

In a product like milk or whey, pure water can be isolated from the mixture of other compounds by freezing it from solution. In freeze concentration, water is frozen into ice crystals and then removed in a piston-like device called a wash column.

Freeze concentration offers the following advantages: (1) low comparative energy requirements, (2) no loss of desirable flavor compounds, (3) no thermal breakdown of product ingredients, (4) no microbial growth during processing, and (5) continuous operation requiring no intermediate shut-downs for cleaning and sanitizing purposes.

2. Protein separation technologies:

Heat precipitation:

Proteins from acid whey can be precipitated by heating at 194 F (90 C) for 10 minutes. To separate proteins from sweet whey, it is necessary to adjust the pH to 4.6 in order to obtain a protein concentrate low in mineral content. Addition of calcium at that pH enhances recovery of proteins. These treatments result in products that are extensively denatured.

Diafiltration:

Diafiltration can be considered a secondary step to ultrafiltration. It is used to permit UF to be carried to higher levels of protein concentration without interference from lactose crystallization.

In this process, the retentate (carrying the proteins) is diluted back to its original volume with water, and UF treatment is repeated. UF treatment followed by dilution and further UF processing is called diafiltration.

If one-half the lactose is removed in the first UF step, then one-half of the remaining lactose is removed the first time diafiltration is applied. The next diafiltration treatment would then remove one-half of what is left of the lactose from the first diafiltration. In this way, UF followed by two diafiltration treatments would result in removal of 87.5% of the lactose (1/2 + 1/4 + 1/8 or 50% + 25% + 12.5% = 87.5%).

In commercial operations, UF is used to concentrate proteins of whey to about 4% by weight of the retentate. Diafiltration can then be used to further concentrate the proteins to 16%. Evaporation and drying of the latter result in whey protein concentrates of 50 to 75% protein. Because UF and diafiltration are low-heat processes, whey proteins thus produced are not denatured to a significant degree (assuming that the products are spray-dried under low-heat conditions); they yet carry 95 to 100% solubility potential.

Gel Filtration:

Once a commercial process, gel filtration was originally scaled up from a laboratory procedure used to separate proteins from contaminating substances.

The gel medium, sephadex G-25 in this case, is prepared in a cylinder-shaped column. Whey or other liquid mixture of proteins is allowed to trickle down the column. Small molecular weight compounds become trapped in the interior of the gel matrix while the proteins simply pass over or around the matrix. They are eluted (drained off) first.

A gel filtration technique using a basket centrifuge to hasten the filtration process has been found to be most practical in producing a whey protein concentrate of about 50% protein. The centrifuge system also overcomes to a significant extent problems of large volumes of dilute concentrations of protein produced by column processes.

The protein in products generated by this process are largely undenatured.

Ion Exchange (process 1):

The ion exchange process is similar in principle to the process used for softening water. Because proteins are amphoteric, however, carrying a net positive or negative charge depending upon pH, technologies have been developed in which the protein particles per se are directly adsorbed to the ion exchange material. This differs, of course, from conventional ion exchange, where

cation exchanger. Regeneration is accomplished with ammonium bicarbonate (NH_4HCO_3).

Milk contains both anions and cations. Major anions include citrates, phosphates, lactates, and chloride ions. Casein whey processed from sulfuric acid will also contain sulfate anions.

Major cations of whey include sodium, potassium, calcium, and magnesium.

Two general formulas describe the conventional procedure for demineralization of whey:

Cation exchange: $R\text{-}H + Na^+ + Cl^- \longrightarrow R\text{-}Na + H^+ + Cl^-$

Anion exchange: $R\text{-}OH + H^+ + Cl^- \longrightarrow R\text{-}Cl + H_2O$

In the cation exchange formula, sodium in sodium chloride serves as the example of cation removal. In the anion exchange formula, hydrochloric acid (HCl) serves as the source and example of removal of an anion (Cl-). In both cases, "R" is the symbol for the exchange resin. An ion exchanger may also utilize sodium ions, to be replaced with calcium ions. This is the principle of home water softeners and works in the same manner for the removal of calcium from milk and whey.

Most of the ion exchange technologies of the above types require that whey be treated first in one and then the other of the two ion exchange media (anion and cation). Ions are removed in that fashion, the proteins are eluted, then evaporated and dried.

Exchange resins must be regenerated after a period of usage. This is usually done by countercurrent flow, i.e. flow up through the resin column. Regeneration chemicals vary according to the kind of exchange system employed. Sodium hydroxide (NaOH) is an example of a chemical that can be used to regenerate an anion resin, hydrochloric acid (HCl) a chemical that serves certain cation exchange systems.

Protein products of these processes can be produced without serious denaturation. If lactose is not removed, dried products may contain over 10% protein. Such products, however, will carry about 82% lactose. Nonhygroscopic powders (powders that will not pick up moisture from the air and form rock-hard blocks) can be processed if the liquid demineralized whey is simply evaporated and dried directly.

Loose Reverse Osmosis:

Loose reverse osmosis (which may be referred to under the trade name "Ultra Osmosis") is a process essentially the same as reverse-osmosis. The major difference lies in the size of pores. Membrane pores in loose reverse osmosis are sized to fall somewhere between conventional reverse osmosis and ultrafiltration. The systems are designed to be highly porous to mineral salts, but impervious to lactose.

Electrodialysis may also be joined with ion exchange to produce a whey protein concentrate of 95% or higher protein content. The former is used to remove 50 to 70% of the mineral components, the latter to further reduce the level of minerals. This kind of dual demineralization system may be used in lieu of one or the other alone as a means of decreasing the overall cost of mineral removal from whey.

4. Drying:

Two drying technologies are used commercially. By far the most common drying method is spray drying. The other technology is drum or roller drying. The former is used for producing both low- and high-heat powders, the difference lying in the heat treatment rendered to the liquid product prior to drying. In drum/roller drying, the concentrated product is dried directly on the hot surface of a revolving drum. Only high-heat, denatured protein products are derived from this technology.

In general, therefore, processes that maintain low temperature treatments through the concentration of milk or whey proteins make it possible to produce spray-dried products with proteins possessing low-heat properties.

Although initial spray-drying temperatures are usually very high (302 - 608 F) (150 - 320 C), the product temperature throughout much of the drying process is low because of the continuing evaporation of moisture from the drying particles. Only when most of the moisture has been removed is the product susceptible to heat damage. For this reason, the temperature of incoming hot air is not nearly as critical as the temperature of the exit-air, the final heat treatment received by the product.

Drying milk and whey, especially whey of high lactose content and also acid whey (from cottage cheese) is complicated by the tendency of lactose, under certain conditions, to pick up moisture and form rock-hard lumps and cakes. The drying operation is further complicated by the tendency of the partially dried product to become very gummy and sticky and to deposit on hot metal surfaces. When this happens, drying conditions are altered, product cannot be efficiently transferred to the next step in processing, and desirable properties of the finished product are lost.

For the above reasons, drying of whey products must be carefully carried out under precise drying conditions. These conditions will vary depending upon the lactose content of the whey and on the type of drying equipment. In all cases, however, the first step in the process is to concentrate the product. Concentration methods have been described in preceding sections. Most whey products are generally concentrated to pre-drying concentration in a vacuum evaporator.

If lactose is not crystallized prior to drying, concentration of whey is limited to about 42 to 45% solids. The inlet air temperature should not exceed 356 F (180 C). The outlet (exit) air temperature during the critical final stage of drying must of necessity be fairly high in order to prevent sticking and caking and, ultimately, a dry product of poor dispersability, poor solubility, and of dusty, hygroscopic nature. The latter refers to the

Table 10. Summary of major commercial technologies used in whey processing

Technology:	Critical Operational and Product Characteristics
To Concentrate:	
Vacuum concentration	low-heat process; significant energy consumption unless combined as multi-effect operation; proteins do not undergo significant denaturation
Reverse osmosis	low-heat, membrane process; used primarily to separate water from whey; also results in loss of some lactic acid, minerals, and nonprotein nitrogen compounds; uses pressures of 400 to 800 psi; little or no denaturation; can concentrate whey to 25 to 30% solids
Freeze concentration	low energy requirements; no loss of desirable flavor compounds; little or no heat denaturation; no microbial growth during processing
To separate proteins:	
Heat	usually, though not always, results in a denatured protein product; often applied at some specific pH (acid) level; protein products vary in functional properties; generally insoluble protein; can prepare products of 80 to 90% protein
Ultrafiltration	low-heat, membrane process; uses 10 to 100 psi pressure; provides for separation of compounds with molecular weights of up to 10,000; also separates from protein a number of small organic molecules; used to prepare whey protein concentrates of 35% protein content; soluble proteins
Diafiltration	low-heat, membrane process; extension of ultrafiltration by diluting retentate back to original concentration; used to prepare whey protein concentrates of 50 to 75% protein; soluble proteins
Gel filtration	done on Sephadex G-25 gel; column process; small molecules trapped in gel matrix; proteins eluted off the column; soluble proteins
Ion exchange	a process in which the proteins per se are adsorbed to the ion exchange material; lactose and minerals eluted first, then protein; protein undenatured

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Chapter V

The Chemistry and Properties of Lactose

Lactose is the chemical name of **milk sugar.** Milk contains other "sugars," but only in very small quantities. Lactose, however, averages nearly 5.0% by weight of milk solids. That is higher than the level of all proteins and higher than the average level of fat in milk. Lactose is a major component of milk.

Chemically speaking, lactose is a member of a family of compounds called **carbohydrates.** As the name implies, carbo-hydrates consist of the elements **carbon, hydrogen,** and **oxygen.** Hydrate, as used here, refers to water. The general structure for carbohydrates can be described as $C_x(H_2O)_x$, where x indicates the possibility of one or more carbon atoms joined with an equal number of the elements that make up water.

The term **sugar** is also commonly used in reference to carbohydrates. It is important to note, however, that this is a generic use of the word. Many chemically different sugars exist. Table sugar—**sucrose**—is but one, and this sugar is distinctly different from lactose, milk sugar.

Sucrose is also referred to as **cane** sugar or **beet** sugar, a reference to major sources of this particular carbohydrate. **Fructose** is the sugar associated with fruit products. **Galactose** has been called **brain** sugar or **cerebrose;** significant amounts of this carbohydrate are found in brain tissue. One of the most commonly occurring sugars, **glucose,** is variously referred to as **blood** sugar, **grape** sugar, and **corn** sugar.

It can be useful to recognize that chemical names of various sugars usually end in the suffix **"ose."** This can often be taken as evidence that a chemical compound, perhaps otherwise unfamiliar, is in fact a member of the carbohydrate family.

Other terms and distinguishing names

A variety of names and/or terms may be used to identify carbohydrates. A knowledge of these names can be helpful in interpreting information, particularly scientific information.

Carbohydrate is the most inclusive term used to identify this family of related chemical compounds. It is the umbrella under which the entire family exists. **Saccharide(s)** is a term that means **much the same** as carbohydrate. It is perhaps more narrowly defined as meaning **simple sugar** or a compound that, broken down into its basic units, produces only simple sugars. The term carbohydrate can and usually is taken to include more complex chemical compounds.

"Simple sugar" refers to any one of the basic molecular units that make up a carbohydrate. These sugars occur as single molecules of a given carbohydrate or combinations of several molecules. They are distinguished from longer, more complex carbohydrates which consist of many molecules of simple sugars.

50 as **high conversion.** Starch products of 42 to 50 DE are considered to be **medium conversion,** products of 28 to 32 DE, **low conversion.**

Starch is the end product of the process by which plants store energy. Animals, including humans, also store energy in the form of carbohydrates. This reserve of energy is called **glycogen.** It is found in large part in the liver, but also in muscle tissue. As occurs in starch, hydrolysis of glycogen results in the intermediate formation of dextrins, then, ultimately, glucose.

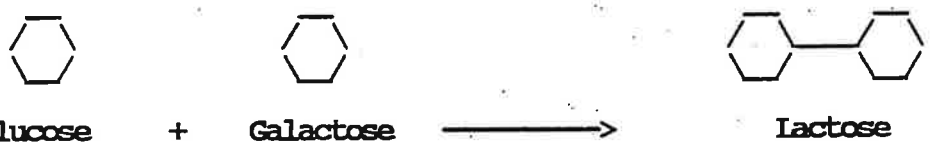
In summary, lactose is correctly referred to as any one of the following:

* carbohydrate
* simple sugar
* disaccharide
* oligosaccharide
* a pair of hexoses
* a disaccharide of glucose and galactose.

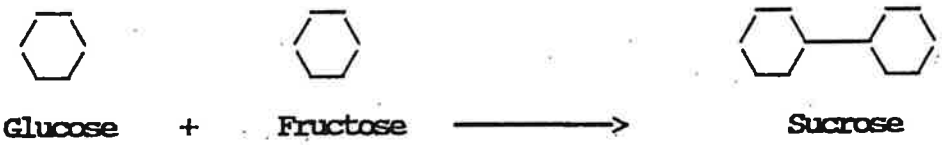
Carbohydrates also combine with proteins (**glycoproteins**) and with individual amino acids (**glycosamines, amino sugars**) and with fats (lipids) (**glycolipids**).

The chemical structure of lactose

Being hexoses—simple sugars containing six carbon atoms—and also because they exist as ring structures, glucose and galactose can be diagrammed as six-sided figures. They can then be joined to provide a visual model of a molecule of lactose:



If table sugar—sucrose—is similarly diagrammed, the equation looks like this:

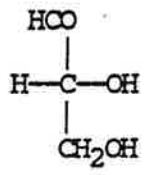


atoms surrounding the asymmetric carbon atom(s), a given sugar, dissolved in water, will rotate the plane of polarized light either to the right (the dextro effect) or the left (the levo effect).

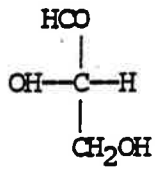
4. Glucose and galactose differ only in the apparent location of the OH and H on carbon number 4. This difference, which in two-dimensional format is used to reflect a mirror image of a given molecule, gives rise to the concept of isomers. An isomer is a molecule exactly alike another in chemical make-up—kind and number of elements—but different in some other respect(s). Glucose and galactose are isomers—stereoisomers—of each other.

5. Both glucose and galactose are designated as D forms of the molecule. Both D and L forms occur. They are distinguished solely by the placement of the H and OH atoms about the next-to-the-last carbon atom. On a hexose consisting of 6 carbon atoms, carbon atom number 5 is considered next-to-last. In a pentose, a sugar of 5 carbon atoms, carbon number 4 is the significant atom in this respect.

Please note that, in both configurations of glucose and galactose, the H and OH on carbon number 5 are on the left and right, respectively. This is the D form. In the L form, the positions of the H and OH would be reversed (H on the right, OH on the left). This method of distinguishing one form from the other is arbitrarily based on the configuration of D and L glycerose:



D-Glycerose



L-Glycerose

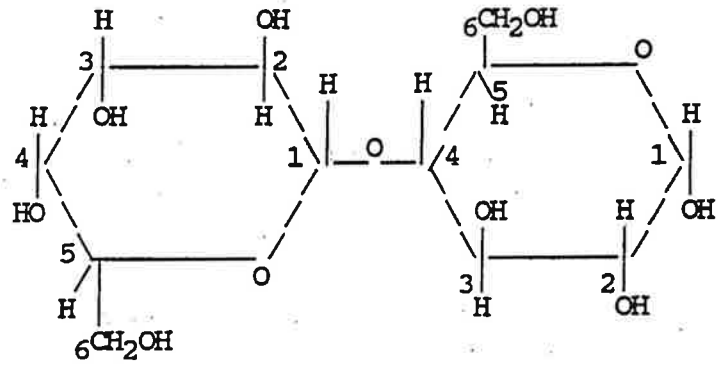
In the glycerose structures, the H and OH have been spread out from the middle carbon atom in order to more clearly indicate the difference between the two configurations. A three-dimensional figure would more accurately describe the true nature of the difference.

D and L forms of a molecule represent different families of compounds. As such, individual members of a given family often react quite differently in the human body than do members of the other family.

Among compounds found in D and L forms, some individual components may express dextro (right) rotation, others levo rotation. Often these differences are designated as (+) and (-) symbols, respectively. In the glycerose molecules shown above, it would be appropriate to add a + sign after the D: D (+) glycerose. The L form, in this case, could be expressed as L (-) glycerose.

The two diagrams shown are alpha and beta representations appropriate to any hexose. Specifically, they can reflect the two forms possible for either glucose or galactose.

The following diagram shows the ring structure combination of glucose and galactose as they join to form lactose. Remember, the two units join at carbon number 4 of glucose and carbon number one of galactose. To more clearly reflect this fact, the diagram shows galactose on the left, the number 1 carbon directed toward the number 4 carbon of glucose, on the right.



beta-D-galactose alpha-D-glucose

alpha-LACTOSE

The above diagram represents lactose in the way the two ring structures of galactose and glucose combine, showing the 1,4 linkage, respectively, of the two hexoses.

The beta form is shown and can be recognized by noting the manner in which the OH and H units are attached to the number 1 carbon of the glucose moiety. They are identical to the beta form shown on page 6. In the reverse position, the OH and H groups would reflect the alpha form.

Some physical properties of lactose

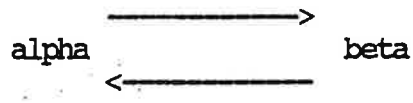
The preceding chemical and structural configurations of lactose give rise to a number of specific chemical and physical properties. Some of the more important of these follow:

Specific rotation:

Lactose, like other simple sugars, causes a specific rotation of plane-polarized light. This is an important characteristic, one which is used to determine the concentration of lactose in solution.

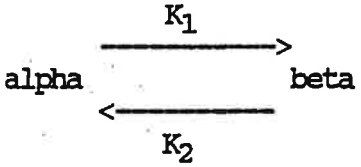
Specific rotation is defined as the rotation produced by one gram of the pure substance dissolved in one milliliter of water as observed in a polariscope tube one decimeter in length. Results are expressed in angular degrees.

to the other. This change--this dynamic state of flux--gives rise to the term mutarotation. It may be represented as follows:



The reaction above is either progressing in one direction or the other or is balanced at the point of equilibrium.

The rate of change can be described by ascribing a constant "K" to the reaction:



Then: K (constant) = $K_1 + K_2$

The rate of mutarotation (how fast it progresses in one direction or the other to the point of equilibrium) is influenced by four factors: (1) temperature, (2) pH, (3) milk salts, and (4) concentration of lactose.

To reach equilibrium at 68 F (20 C) requires hours. At 158 F (70 C), equilibrium is reached in a matter of minutes.

Mutarotation progresses more slowly in the pH range 2.0 - 6.0. The rate is significantly faster at pH less than 2.0 and above pH 6.0.

The rate of mutarotation is increased by a factor of two at higher concentrations of milk salts.

As the concentration of lactose increases, the rate of mutarotation decreases. The following chart describes this influence in practical terms:

Time to reach equilibrium at:	Very High Solids (2% moisture)	Less High Solids (6% moisture)
At 77 F (25 C)	months	days
At 122 F (50 C)	hours	less than 1 hour
At 176 F (80 C)	minutes	———

The above phenomenon is most significant in dairy processing because of the different characteristics of the two forms of lactose. They differ in (1) solubility, (2) crystal size and shape, (3) hygroscopicity--ability to pick up moisture and, thereby, cause textural changes, (4) specific rotation and (5) sweetness. Some of these changes relate directly to the ability to store nonfat dry milk and dry whey and whey products without serious defects occurring. They also bear directly on such problems as grittiness in

Beta-lactose anhydride is both more soluble and sweeter than alpha-lactose monohydrate, but only slightly so.

Lactose glass or amorphous lactose does not have crystalline form. However, it is of much significance in that it readily picks up moisture from the air, then crystallizes into the alpha-hydrate form, which causes caking (solidifying) of dry milk and dry whey products. With sufficient humidity, lactose glass in these products will pick up about 8% moisture. To prevent this problem from occurring, it is necessary to cause the lactose to crystallize under controlled conditions during processing. This aspect is discussed in following sections.

Solubility:

When either alpha- or beta-lactose are dissolved in water, they produce a mixture of both forms. The proportion and speed with which this happens depends upon the temperature of the solution. Nevertheless, lactose in solution moves toward and establishes an equilibrium mixture of the alpha and beta forms. At a temperature of 63 F (17.2 C), for example, the ratio of alpha to beta forms is 1:1.65.

Due to the equilibrium process, the addition of lactose crystals to water results in an initial solubility--the amount of a given crystalline form that dissolves at a given temperature--and then, after the alpha/beta equilibrium is ultimately established, a final solubility.

The final solubility, therefore, represents the solubility of the two forms of lactose at equilibrium.

Data in Table 1 indicate the initial solubility of both alpha- and beta-lactose and also the final, equilibrium solubility.

When a saturated solution--a solution at maximum concentration of dissolved substance--is cooled below the temperature of saturation, the solution is said to be supercooled. At this point, the dissolved compound is in a state of supersolubility or supersaturation. It may remain dissolved for a prolonged period of time if the cooling progresses slowly and is not taken significantly below the saturation temperature. This condition is referred to as the metastable state.

As cooling progresses below the saturation temperature, nuclei eventually form. Nuclei can be considered the core material around which crystals form. At this point, the solution is considered to be in the labile state, in effect the condition in which crystals will form.

Not only is lactose less soluble in presence of sucrose, it dissolves more slowly in such a mixture. At 95 F (35 C), rate of solution is increased by 30 to 60%.

Table 2. Solubility of lactose in presence of various concentrations of sucrose.

Temperature °C	Temperature °F	Lactose Alone	In presence of: 40%[1] Sucrose	50%[2] Sucrose	60%[3] Sucrose	70%[4] Sucrose
		(grams of anhydrous lactose per 100 grams of water)				
25	77	21.6	16.1	13.6	11.0	9.1
40	104	31.8	24.4	20.6	17.0	14.1
50	122.2	49.0	32.7	28.1	23.1	18.7
60	140	59.5	43.8	46.5	40.6	29.5
80	176	93.6	83.7	71.8	65.7	59.8

Adapted from Foremost-McKesson, Inc.
[1] 66.6g/100g water
[2] 100g/100g water
[3] 150g/100g water
[4] 233g/100g water

Relative sweetness:

Lactose is generally considered to be about 1/6 as sweet as table sugar (sucrose). Nevertheless, at the concentration found in milk, it does impart a slight sweet flavor to fluid milk.

Sugars tend to vary somewhat in sweetness, and data in Table 3 provide a basis of comparison. If sucrose is arbitrarily given a base sweetness value of 100, then lactose must be rated as 16.

Table 3. Relative sweetness of a number of simple sugars

Sugar	Relative Sweetness (Sucrose--table sugar = 100)
Fructose (levulose)	173
Invert sugar	130
Sucrose	100
Glucose (dextrose)	74
Corn syrup	60
Galactose	32 [1]
Maltose	30
Lactose	16

[1] Values in the scientific literature range from 32 to 58.

Some chemical reactions of lactose

Lactose enters into a number of chemical reactions. It does so as a direct result of its specific chemical composition. In recent times, much more attention has been given these attributes as a way of attempting to find new uses (and new markets) for this sugar. The most common reacting groups of lactose (and other similar carbohydrates) are (1) the reducing group (CHO) of glucose, (2) the 1-4 linkage between galactose and glucose, (3) the hydroxyl (OH) groups of both glucose and galactose, and (4) the bonds connecting the carbon atoms (C—C).

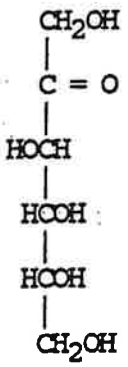
Some of the more important chemical reactions of lactose follow:

1. Lactose as a **reducing sugar:**

A reducing sugar is one that carries a free (exposed) or potentially free aldehyde group. If you will refer to the structural diagram of glucose and fructose on page 4, you will find the aldehyde group shown in position number 1. This chemical grouping is designated as CHO.

Such groups are chemically reactive. That is, they enter into a variety of chemical reactions. They are important in that regard, and this is an important aspect of lactose chemistry.

Not all sugars possess a free aldehyde group. Fructose, one of the monosaccharides that makes up sucrose, does not. The structural formula for fructose is as follows:



Fructose

Both ends of the fructose molecule carry CH_2OH groupings. An aldehydic group does not exist. There is a keto (C = O) group, however. Such groups are reactive, but not in the way an aldehyde group reacts. It is presence of the keto group that gives rise to a generic name for such sugars. They are called ketoses. Major difference exist in the way ketose sugars react compared to reducing sugars like lactose.

certain food systems. One of these influences is reflected in a color reaction as noted in item number 2 below.

2. The **Maillard** (or **browning**) reaction:

One of the major chemical reactions that occurs during processing of certain foods results in the production of a brown color. Where a reaction of this kind naturally takes place and consumers have grown accustomed to it, a brown color is considered desirable. Baked goods are an example. How would bread be perceived if the crust were white or toast was not brown? How would people react to beer that was lacking in color?

On the other hand, brown colors are not considered appropriate in many foods, (nonfat dry milk being one such example), and for these food systems browning reactions must be avoided.

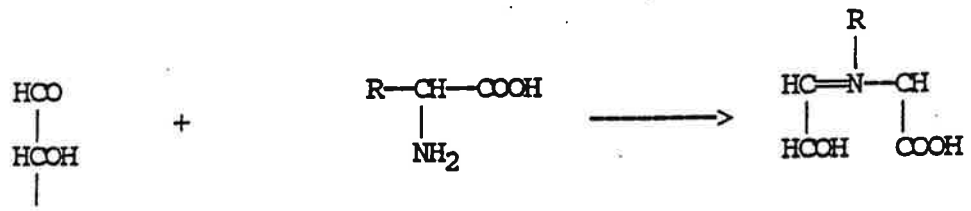
These are the kinds of issues that arise when certain sugars are used as ingredients in various foods. Other issues, of nutritional consequence, are also posed.

Because of the difference in heating method, microwave cookery does not produce a browning response, even when the appropriate compounds are present, and this has been a significant problem in developing food systems for heating in microwave ovens.

Two kinds of browning are recognized, **non-enzymatic** and **enzymatic**. The latter is of the kind often associated with discoloration of certain fruits and fruit products, apples for example. It is the former, however, that will be considered in this discussion because the reaction involves lactose and certain other sugars.

Non-enzymatic browning is often referred to as the **Maillard** reaction, after the person who first described it. It is a color change caused by a reaction between a **reducing sugar(s)** and the **amino group** of certain **amino acids** of proteins. Although the reaction can take place at relatively low temperatures, it is nonetheless associated with **high heat treatment** of milk and various milk products.

The reaction can be described as follows:



Aldehydic/reducing component of a sugar

Amino (NH_2) group of amino acid/protein

Brown-colored compound

The above data indicate why browning is less of a problem with fluid and dry cream products than certain other dairy products. Both evaporated milk and nonfat dry milk readily undergo browning. Dry whey, dry ice cream mix and sweetened condensed milk also exhibit the defect under the right conditions of processing or storage. Cream products, too, will undergo browning, but only under severe heat treatment or prolonged storage under adverse conditions.

* Browning reactions in most fluid dairy products will occur at temperatures ranging between 230 and 300 F (110 to 150 C) at holding times ranging from 1.0 to 30 minutes. For comparison purposes, it may be noted that browning of evaporated milk requires about 100 seconds at 248 F (120 C) and less than 30 seconds at 300 F (150 C).

Table 4 provides some benchmark temperatures and holding times for initiation of browning in various fluid dairy products.

Table 4. Approximate time required for initiation of browning at three different temperatures in various dairy products

Product	230 F	275 F	300 F
Cream, 30-40% fat	26 min.	8 min.	3.3 min.
Cream, 10% fat	22 min.	5 min.	1.6 min.
Milk, 3.5% fat	18 min.	2.5 min.	50 sec.
Milk, skim	8.3 min.	1.2 min.	25 sec.
Evap. milk, (25% total solids)	3.6 min.	40 sec.	17 sec.

The minimum treatment required to **sterilize** (kill all microbial life) canned, evaporated milk is 239 F (115 C) for 15.3 minutes (or 257 F—125 C—for 1.53 minutes). The former falls well within the temperature/time range for browning. The latter also falls within the range needed to initiate the reaction.

However, UHT—ultra high temperature—sterilization is a process in which the product is heated in the range of 275 to 300 F (135 to 150 C) for a few seconds (usually in the range of 2 to 4 seconds). As the above chart indicates, these temperature/time processes fall well outside those in which browning occurs in fluid milk products. UHT processing, therefore, not only sterilizes the product, but does so without development of objectionable color changes.

In manufacture of evaporated milk, it is necessary to pre-heat the product prior to sterilization in order to prevent gelation and/or separation of milk components. This treatment is called **forewarming**. It should be noted, therefore, that prior heat treatment in the range of 185 to 212 F (85 to 100 C) for an extended period produces changes that are additive to the browning

Lactose is not readily hydrolyzed by weak, organic acids such as citric and lactic acids. For this reason, hydrolysis is usually carried out using strong mineral acids. Both hydrochloric and sulfuric acids have been used. The rate of reaction can be greatly enhanced by heat.

Acid hydrolysis may be carried out at pH 1.2 at temperatures ranging from 194 to 300 F (90 to 150 C). At the latter temperature, a 10% solution of lactose will be completely hydrolyzed in about one hour. Using 2 - 3 normal acid and a reaction temperature of 140 F (60 C), about 80% hydrolysis will occur in 20 hours.

A major problem associated with acid hydrolysis of lactose is brown discoloration of the product due to the browning (Maillard) reaction (see item (2) above). Because most common sources of lactose (whey and whey permeate, for example) contain protein or fragments of protein, acid hydrolysis must either be accompanied by a color-removal procedures (with activated charcoal, for example) or the method must be limited to hydrolysis of pure lactose. Acid hydrolysis is relatively inexpensive and an easy process to control.

Cation exchange resins are used in two ways to hydrolyze lactose. They either serve as a reactor as such or as a process applied solely for the purpose of lowering pH. In the latter application, the cation is of the kind that produces H+ ions. After pH adjustment takes place, the reaction is continued by treatment similar to Ultra High Temperature (UHT) processing. Products intended for this method of processing should first be treated to remove minerals. Otherwise the exchanger quickly loses potency.

The following chart provides some examples of rate of hydrolysis under various conditions:

Processing Condition			Percentage Hydrolysis
Time	Temperature	pH	
70 minutes	203 F (95 C)	----	90
50 seconds	302 F (150 C)	0.8	85
11 minutes	284 F (140 C)	1.35	94
3 minutes	284 F (140 C)	1.35	50

Beta-galactosidase (lactase) enzymes are commonly used in hydrolysis of lactose. The enzymes are derived from certain bacteria, yeasts, and molds. Streptococcus thermophilus and Bacillus stearothermophilus are examples of two bacteria which are reasonably good sources of the enzyme. Saccharomyces lactis and Kluyveromyces fragilis are examples of lactase-containing yeasts. Two molds commonly used a sources of lactase in commercial operations are Aspergillus oryzae and Aspergillus niger.

Lactase enzymes are applied in one of three ways: (1) as single-use

* Ultrafiltration is applied in a continuous system. In this technology, the hydrolysis reaction takes place in a vat (or reactor vessel), but the reaction mixture is continuously moved through a UF membrane. The enzyme is retained by the UF process and returned to the reaction mixture; permeate (containing the products of hydrolysis) is removed and retained separately.

This above system has been applied to whey permeate as a way of hydrolyzing the lactose therein. A 10% concentrate of permeate was used, and the hydrolysis was carried out at pH 4.6 at 131 F (55 C). These conditions were selected as a way of minimizing microbial growth.

Immobilized enzyme technology operates similar to ion exchange. The lactase enzyme in this system is attached to porous, plastic or silica or other material constructed as beads of relatively small size. The beads are placed in a column similar to ion exchange. Because the enzyme is bound to the beads, it does not leach away during passage of whey or other lactose source through the column. Yet the enzyme is able to serve its usual function and remains available for re-use over an extended period of time (several months). The technology is referred to as a fixed bed reactor.

Other than the fact that the enzyme can be re-used, these systems have the advantage of operating continuously, the end-product quality is reasonably consistent, and the enzyme does not become a contaminant of the finished product. However, this technology is expensive and requires skilled technicians to operate properly. In addition, one must guard against microbial contamination i.e. cleaning requirements are exacting.

Common cleaning procedures involve backflushing with water, acetic acid, mild alkali and special detergents. Caution must be applied in use of chlorine as sanitizer; enzyme activity can be lost.

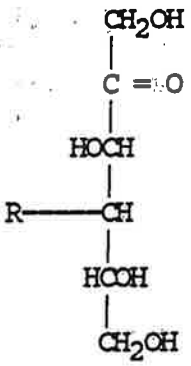
Even under the best of conditions, the enzymes of immobilized systems lose activity over time and must be replaced.

Characteristics of several lactase enzymes from different sources are given in Table 5.

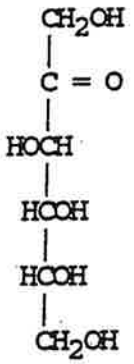
Table 5. Characteristics of lactase enzyme derived from various sources

Source	Optimum pH	Optimum Temperature (F)	Activating Ions	Inhibitors
Aspergillus niger	3.2-4.3	122-140	none	galactose
Escherichia coli	7.3-7.5	122	?	?
Saccharomyces lactis	7.0	98.6	Mn^{2+}, $K+$	Ca^{2+}
Aspergillus oryzae	4.5	122-131	none	galactose

Adapted from Sprossler and Plainer (1983) and Zadow (1984)



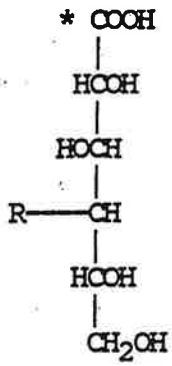
Lactulose



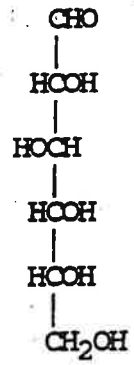
Fructose

5. Oxidation products:

A large number of products can be produced by oxidation (addition of oxygen) of lactose using a variety of oxidizing agents and various conditions of treatment. Lactobionic acid is one product that has received considerable attention, mainly because it is an end product in one of the quantitative tests for lactose. Although lactobionic acid might serve as an acid in various food systems, little work has been done to research and promote this function. Below, the structural formula for lactobionic acid, using "R" to represent the galactose (beta-D-galactopyranosyl) portion of lactose, is compared to glucose.



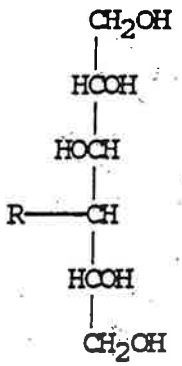
Lactobionic acid



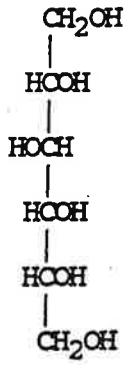
Glucose

* Please note that the aldehydic group (CHO) of glucose has been converted to a carboxylic acid (COOH) group.

Mucic acid is another oxidation product of lactose, formed in presence of nitric acid. It is a dicarboxylic acid (carries two COOH groups) originating in the galactose portion of the lactose molecule.



Lactitol



Sorbitol

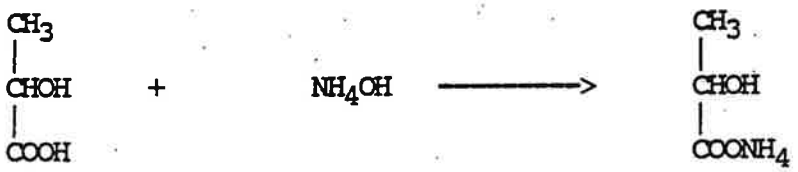
7. Lactose derivatives for animal feed (ammonium lactate and lactosyl urea)

Both ammonium lactate and lactosylurea, chemical derivatives of lactose, have been prepared for use as animal feed. Both have considerable value for this purpose and serve ruminant animals as substitutes for protein.

Ammonium lactate is prepared by fermenting lactose (in whey as such or an ultrafiltered whey) to lactic acid using Lactobacillus bulgaricus. This organism has a high tolerance for acid; it can survive the acid conditions that it produces during the fermentation process and completely convert lactose to lactic acid.

Following fermentation, the broth is treated with aqueous ammonia and then evaporated to 60% or higher solids content. The product has good physical stability. The high osmotic pressure created by the high level of ammonium lactate effectively prevents microbial growth.

The chemical reaction by which ammonium lactate is produced is shown below.



Lactic acid + Ammonium hydroxide → Ammonium lactate

Ammonium lactate provides the ruminant animal with both an energy source (the lactic acid portion of the molecule) and a nitrogen (protein) source (NH_4).

such byproducts is to ferment them using the mold Aspergillus oryzae. BOD can be reduced by as much as 95% in 12 hours. In addition, the mold biomass produced in the process has been found to serve as feed for dairy cattle.

Alcohol production can also be enhanced to some extent by first hydrolyzing (splitting) lactose into glucose and galactose. A larger number of organisms are capable of effectively fermenting the breakdown products than lactose per se. One yeast that has been found particularly useful in producing alcohol from hydrolyzed lactose is Saccharomyces cerevisiae.

Citric acid has been produced from acid whey permeate using the mold, Aspergillus niger. The process requires 8 to 12 days of fermentation at 86 F (30 C).

For coversion of lactose to methane, anaerobic (absent oxygen) conditions usually serve best. Lactose from permeate or other dairy byproducts can be used in such fermentations.

Lactic acid can be produced by a number of lactic organisms. However, many are killed by their own acid secretions long before the lactose has been totally converted. For this reason, acid-tolerant organisms serve best, and Lactobacillus bulgaricus is used extensively for production of this specific end product.

N-butanol, a useful industrial solvent, has been processed from whey permeate using a strain of the organism, Clostridium acetobutylicum.

In addition to the above, lactase enzyme, the enzyme that breaks down lactose, has also be produced by fermentation processes. One yeast used for this purpose is Candida pseudotropicalis.

A variety of gums are used to control texture of food products. Some, for example, serve as stabilizers in ice cream. Guar gum, locust bean gum, xanthan gum and carrageenan are examples of complex polysaccharides that have the ability to bind water and slow formation of ice crystals during repeated freezing and thawing.

Substances of this kind derive from plant sources, but may also originate as secretions of certain bacteria. Because of the commercial importance of these compounds, it is well to note that certain bacteria are capable of using lactose in whey or hydrolyzed whey and producing extracellular polysaccharides.

Both Alcaligenes viscosus and Zooglea ramigera have been found to produce gums through fermentation of whey. Organisms from the genera Xanthomonas and Arthrobacter are capable of producing gum, but only from the glucose of hydrolyzed whey or whey products.

Processing lactose

Processing of lactose is often an integral part of the processing of whey or whey permeate. Where whey is concerned, the foremost consideration may lie in the production of a concentrated whey protein product rather than lactose. For

processing and refinement of lactose. Data in Table 7 indicate the major mineral elements found in sweet and acid whey and whey permeates.

Table 7. Major minerals of sweet and acid whey and whey permeates

	Sweet Whey			Acid whey		
Mineral	Liquid	Liquid Permeate	Dry Permeate	Liquid	Liquid Permeate	Dry Permeate
Ash (%)	0.52	0.50	8.07	0.59	0.56	12.2
	(mg/100g)					
Calcium	22.5	21.0	480	86.0	82.0	1410
Phosphorus	42.5	49.6	638	63.0	62.7	857
Magnesium	5.8	5.3	113	8.9	8.2	155
Sodium	35.0	31.8	780	40.0	32.0	770
Potassium	109.0	108.0	2610	133.0	114.0	2535

Adapted from Hargrove et al.

Processing lactose from sweet whey (general process)

* Heat whey to near boiling to initiate precipitation of whey proteins.
* Add lime (Calcium oxide—CaO) to precipitate albumin and phosphates.
* Evaporate to 30% solids.
* Filter.
* Evaporate to 80% solids.
* Cool, centrifuge, and then refine with carbon black.

Low-temperature processing of lactose from sweet whey

More recent processes of lactose manufacture have been designed around the need to produce a high-grade lactose while maintaining the low temperatures necessary to minimize whey protein denaturation. The basic steps of manufacture are:

* Preheat the whey to 176 F (80 C), flash treatment, prior to evaporation.
* At temperatures not to exceed 158 F (70 C), concentrate the whey by evaporation to 50 to 62% solids. Higher solids levels must be avoided in order to prevent spontaneous crystallization and/or a product viscosity too high to

* Dry the lactose. Lactose may be dried in a fluid bed dryer, spray or roller dryer. The former two drying processes result in the production of alpha-lactose monohydrate. Because of the high drying temperatures, the latter will produce significant quantities of beta-lactose anhydride.

Lactose production from sweet whey permeate

The manufacture of lactose from permeate is to some extent complicated by the the fact that it is saturated with calcium. Concentration of the product results in precipitation of calcium phosphate and citrate, compounds which cause fouling and scaling of equipment. In addition, calcium salts become contaminants of lactose crystals and, as such, are difficult to remove because of their low solubility. For these reasons, permeate must be pre-treated prior to or during evaporation with processes for removal of calcium and other minerals.

In another respect, permeate offers certain advantages over whey as a source of lactose. That is, absence of protein makes it possible to produce lactose more efficiently.

Lactose may be processed from sweet whey permeate by the following procedure:

* Lower calcium levels by means of ion exchange (exchanging calcium for sodium).

* Remove other minerals by electrodialysis and/or ion exchange.

In lieu of the above pre-treatment processes, pH may be lowered to inhibit formation of insoluble salts. Use of calcium chelating compounds such as sodium hexametaphosphate have also been suggested. These latter form insoluble complexes with calcium, which may then be removed prior to further processing.

* Heat and process permeate at temperatures ranging up to 203 F (95 C). High temperatures of heating decrease the rate of precipitation of calcium compounds, thus providing for longer operating times (more efficient lactose recovery and longer periods between clean-up).

* Concentrate to 65 - 70% solids by evaporation. The higher the concentration, the higher the temperature that must be maintained to prevent premature crystallization and excessive viscosity.

* Cool, seed, and further process the lactose by procedures similar to those applied to whey.

Lactose production from acid whey or acid whey permeate

Pre-treatment of acid whey or whey permeate is necessary to efficiently process lactose from these sources. In general, the procedures involve partial removal of calcium compounds by treatment with alkali and heat. The product is then clarified by centrifugation.

$$\% Y = \frac{a - b}{(100 - b)\ a} \times 10{,}000$$

where Y = percentage yield of crystallization

a = % lactose prior to crystallization

b = % lactose remaining after crystallization

As example, assume that a whey permeate contained 74.1% lactose. After lactose has been removed by crystallization, the whey permeate is found to contain 36.4% lactose. Then:

$$\% Y = \frac{74.1 - 36.4}{(100 - 36.4)\ 74.1} \times 10{,}000$$

$$= \frac{37.4}{(\ 63.6)\ 74.1} \times 10{,}000$$

$$= \frac{37.4}{4712.76} \times 10{,}000$$

$$= 0.0080 \times 10{,}000$$

$$= 80\%$$

Grades and specifications for lactose

The Federal Food and Drug Administration (FDA) has established a Standard of Identity for food-grade lactose. Specifications are as follows:

* lactose content of 99% by weight on a dry basis
* sulfated ash content no greater than 0.3% by weight on a dry basis
* pH of a 10% solution (wt/wt) not less than 4.5 nor over 7.0
* no more than 6.0% loss in weight after drying for 16 hours at 248 F (120 C)

The following chart provides a comparison of chemical and physical specifications for crude, edible, and U.S. Pharmacopoeia grade lactose.

2. Freezing rapidly and with agitation. Lactose will not crystallize if frozen quickly to temperatures below -9.4 F (-23 C). The sugar becomes supersaturated—ready to crystallize—at a temperature of 28 F (-2 C).

3. Reducing the lactose concentration. Ultrafiltration can be used for this purpose if the loss of nutrients is acceptable. Otherwise, the lowering of lactose level is best accomplished by hydrolysis.

Lactose content and the freezing point of milk

Milk freezes at a temperature just below the freezing point of water. The freezing point depression is used, in fact, as a means of assessing adulteration of milk with water. The higher the level of adulteration, the more closely the freezing point approaches that of water.

The two components most responsible for lowering the freezing point of milk are lactose and minerals. The higher the content of either or both of these milk constituents, the lower the freezing point. Factors related to freezing point depression are treated in a later chapter. At this point, it is worthwhile noting only that lactose content of milk varies to some extent. Changes in lactose level are compensated by opposing changes in level of sodium and chloride in milk. That is, as lactose content increases, level of the latter minerals decreases and vice versa.

Mastitis is perhaps the most significant factor influencing the content of lactose and minerals in milk. As mastitic infections are cured, lactose content rises. Indeed, there is an excellent inverse relationship between lactose content and freezing point of milk. Milk of high solids content and milk of high quality—low somatic cell count—will have a somewhat lower freezing point than milk of low solids and poor quality (high somatic cell count).

"Sandiness" in ice cream

If the lactose level in the water portion of ice cream mix is too high, lactose crystals form and can become large enough to lend a gritty texture to ice cream. This defect is termed, appropriately enough, sandiness.

Conditions favoring sandiness include (1) too high a level of serum solids in the ice cream mix, (2) high freezing temperature (as often occurs in batch freezers), and (3) widely varying temperatures during storage of ice cream following freezing.

Because of the potential for sandiness to develop, the level of lactose-containing serum solids in ice cream mix is usually limited. Serum solids are skimmilk solids, and may be formulated into ice cream mix as nonfat dry milk, condensed skimmilk, fluid skimmilk, wholemilk, whey solids or other products that contain some level of skimmilk components.

Proper kinds and use of stabilizers can do much to prevent sandiness. However, the most foolproof method of controlling the defect is to limit the amount of serum solids. For practical purposes, and under severe conditions, sandiness

Because lactose is a source of galactose, the sugar poses a potentially serious problem to a very small segment of the population (about 1 in 55,000) of newborn infants, specifically those unable to properly metabolize galactose. The condition is termed **galactosemia**. Some hospitals regularly screen all newborns for this malady. Treatment involves avoidance of foods that contain galactose.

Among the more significant nutritional benefits provided by lactose are:

1. Lactose aids calcium absorption from the intestinal tract into the body. Calcium is required for bone production and repair and is the essential nutrient in prevention of **rickets** and **osteoporosis**.

2. Aside from calcium, lactose also aids uptake of other minerals (**cations**—positively charged ions of mineral elements).

3. Lactose promotes growth of a variety of intestinal bacteria which in turn are able to synthesize the vitamins **biotin, riboflavin, folic acid,** and **pyridoxine**. In this function, lactose performs a **sparing** role; if intestinal bacteria manufacture vitamins which otherwise must be provided by the diet, the level of intake of these vitamins can be reduced by a like amount.

4. Some research indicates that lactose may aid in digestion of proteins, and possibly the less digestible proteins of plant sources.

Lactose intolerance or malabsorption is the end result of inadequate secretion of the enzyme **lactase** in the small intestine. Lactose then moves in tact into the large intestine. As a result, water is drawn into the intestinal tract. Bacterial fermentation occurs and gas is formed; the symptoms of these kinds of responses are abdominal pain, bloating and diarrhea.

Lactose intolerance occurs in three different forms: (1) **congenital** or hereditary, (2) **primary**—the usual, more frequent form, and (3) **secondary**—a condition brought about by damage to the skin (**epithelial layer**) of the small intestine (as encountered in **coeliac disease**).

Researchers recognize two different states of reduced lactose digestability: (1) **reduced absorption of lactose** as a consequence of low lactase activity (diagnosed by suitable tests procedures) and (2) **lactose intolerance**.

Low lactase activity is expressly defined as less than 2 units of lactase activity per gram of **wet mucosa**. This latter is the tissue within the intestinal tract that produces lactase.

Lactose intolerance, by definition, differs from the above and is restricted in use to those persons showing "clinical signs" of digestive distress following the feeding of a standard dose of lactose in water. In other words, a test must be administered to determine whether or not an individual is truly adversely influenced by lactose intake.

Blood glucose levels are measured in a **lactose tolerance** test. The individual is first required to fast, then fed a standard amount of lactose. As lactose

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Chapter VI

Minerals/Salts of Milk

In relation to other major components, the minerals of milk are present in relatively small amounts. Their significance to dairy processing, however, far outweighs their quantitative aspects.

As ash, this group of constituents averages about 0.68% by weight of milk. In the form in which they are found in milk, minerals have a direct bearing on such processing considerations as **formation of curd** during cheesemaking, the **storage stability** of concentrated/evaporated milk and Ultra-High-Temperature (UHT) milk, and the **stability of milk lipids.**

In this regard, the important considerations are:

1. The role of **calcium** in coagulation and gelation of casein in cheesemaking.

2. The balance in concentration of **calcium** and **magnesium** vs citrate and **phosphate** in providing stability to heat of milk and concentrated milk products; the role of **calcium** in gelation of casein and whey proteins.

3. Presence of **phosphorus** as esterified phosphate of **phospholipids**, the latter serving as emulsifiers for the fat in milk.

4. The role of **copper** and **iron** in inducing oxidation of milkfat and development of **oxidized** flavors.

Ash vs mineral content of milk

Almost always the level of minerals in milk is expressed as the amount of ash remaining after the milk has been incinerated, i.e. all organic matter burned off.

The Association of Official Analytical Chemists (AOAC) method requires that a known quantity of milk (5.0 grams) be evaporated to dryness over a steam bath, then ignited in a **muffle furnace** at about 1022 F (550 C). When only ash remains, the amount is weighed and compared to the original weight of milk. Results are calculated as percent ash.

Ash, therefore, is correctly defined as the residue remaining after incineration. It does not accurately reflect either the kind or amount of true minerals of milk. The reasons are as follows:

* Both citrate and **carbon dioxide** (CO_2) are lost (volatilized) during incineration.

* Some alkali metals (**sodium** and **potassium**) are partially lost, again by vaporization.

cheesemaking as well as stability of milk following sterilization, either as evaporated or UHT milk.

Fluctuation in level of milk salts is a most significant factor in dairy processing, and an appreciation of the causes is essential. The most common of these follow:

* Infections of the udder:

  This is one of the most important causes of changes in mineral content of milk. Relatively major changes take place in milk of cows suffering severe mastitis infections. In general, sodium and chloride levels increase to compensate (osmotically) for a reduction in lactose content. Lowered lactose levels result from impairment of the process by which the sugar is synthesized. These changes have been used as an index of mastitis in a calculation of the Koestler number. The calculation is as follows:

$$\text{Koestler number} = \frac{100 \times \% \text{ chloride}}{\% \text{ lactose}}$$

  A value of 1.5 to 3.0 is considered normal. During mastitis infections the value increases.

  Calcium content may decrease as a result of infections, but only to a relatively slight extent. Magnesium levels drop perhaps to 60% of normal and iron, copper, and zinc levels increase slightly. Level of inorganic phosphorus and citric acid also drop to some extent.

  Implications of the above changes are: (1) a salty-flavored milk (due to the increase in sodium chloride content) and (2) lowered stability of milk to heat and/or other destabilizing factors as a result of the imbalance between calcium/magnesium salts and phosphate/citrate content.

* Stage of lactation:

  The following changes in mineral content appear to occur during lactation:

  Total calcium--generally high in both early and late lactation milk

  Colloidal inorganic calcium--low (minimal) in early lactation and high in late lactation

  Phosphorus--tends to decrease as lactation advances

  Sodium and chloride--high levels at the beginning of lactation followed by a quick decline, then a gradual increase through the remainder of lactation

Major ions of milk

To better understand the factors that relate to stability of milk proteins to heat and acid, it can be helpful to view the major cations (positively charged ions) of milk side by side with major anions (negatively charged ions). They are as follows:

Major Cations	Major Anions
Calcium (Ca^{++})	Citrate ($Citr^{-}$, $Citr^{2-}$, $Citr^{3-}$)
Magnesium (Mg^{++})	Phosphate ($H_2PO_4^{-}$, HPO_4^{2-}, PO_4^{3-})
Sodium (Na^{+})	Chloride (Cl^{-})
Potassium (K^{+})	Sulfate (SO_4^{2-})
	Carbonate (HCO_3^{-}, CO_3^{2-})

The significant relationships are those that occur between calcium/magnesium and phosphate/citrate to impart heat stability to fluid milk products and the changes in amount of calcium ions that occur either as a result of changes in pH or temperature.

Forms of salts in milk

Salts in milk exist in two major forms. Some are present as soluble (dissolved) salts, others in colloidal dispersion. The former include both ions as such and soluble complexes of calcium and other ions with phosphate and citrate.

Essentially all of the colloidal salts are found associated with casein and consist mainly of calcium and phosphorus integrated into the casein micelle as colloidal calcium phosphate. Some small amount of citrate is also associated with casein.

Salts in milk exist in a state of dynamic equilibrium. This is a most significant aspect because it implies that a shift in amount of one salt or one form of salt results in a counterbalancing shift in levels or forms of other salts of milk.

In simplified format then, salts occur in milk as follows:

Soluble		Colloidal
	equilibrium	
As ions	——————>	Colloidal calcium phosphate
Complexed	<——————	associated with casein

The soluble salts

Please note in the above list of anions that some occur in two or more different ionic forms. These are anions of relatively weak acids. The amount of each form that exists in milk can be calculated in reasonable approximation using the Henderson-Hasselbach equation:

and casein occurs between the amino acid serine and a phosphate group of the colloidal compound. Or the phosphate of serine phosphate is a connecting link between the protein and colloidal calcium phosphate.

Colloidal calcium phosphate represents about 6% of the dry weight of casein. That is a relatively small amount. Yet it is this level of mineral salt that accounts for many of the unique properties of milk. These include:

* the physical structure of the casein micelle
* the coagulation of casein in presence of rennin
* the remarkable stability of casein/milk to heat
* the stability of milk to calcium ions
* the curd tension of milk
* the color of milk

Level and distribution of soluble and colloidal salts in milk

Data in Table 1 summarize the average level and distribution of mineral salts in milk. It should be noted that the concentrations given are those found at the normal pH of milk, i.e. 6.6-6.7. As will be noted later, the level and distribution of these salts alters significantly as pH and a number of other factors are varied. Data in Table 1 represent the norm for milk as it comes from the cow.

Factors influencing the equilibrium between soluble and colloidal salts

As mentioned previously, milk salts exist in a state of dynamic equilibrium. Any alteration in one salt or the form in which it exists causes a shift in the balance and/or form of other salts. Particularly important in the processing of milk products is the equilibrium between soluble and colloidal salts. And in this regard, it is the change in form of calcium that impinges directly on processing.

As a general rule it should be kept in mind that the net result of factors which cause a reduction (loss) in colloidal calcium phosphate is an increase in calcium ions. Conversely, an increase in colloidal calcium phosphate implies, as equilibrium is established, a decrease in calcium ions.

Depending upon the severity of treatment, some changes in salt equilibria may or may not be reversible.

Among the more important factors influencing the equilibrium between soluble and colloidal salts are:

* addition of acid (lowering pH). As pH is lowered, progressively more colloidal calcium phosphate is solubilized. At pH 4.9, all colloidal calcium phosphate is lost from casein, i.e. is dissolved.

As the temperature of milk is raised, even nominally (i.e. between 68 and 86 F—20 to 30 C) level of colloidal calcium phosphate decreases, level of soluble phosphate increases. The change taking place between 68 and 86 F results in about 7% more soluble calcium and 4% more soluble phosphate. This change is completely reversible.

At a temperature treatment of about 200 F. (93C) for 11 minutes, some 50% of soluble calcium and 18% of soluble phosphate reverts to the colloidal (non-ultrafilterable) form. Upon cooling, the reaction is 75% reversible.

Changes in pH induced by temperature treatment

Two changes occur when milk is heated, one that would tend to lower pH, the other increase it. First, carbon dioxide, one of the gases dissolved in milk, is lost. Carbon dioxide forms carbonic acid in milk and loss of the acid results in decrease in acid reaction. Titratable acidity decreases.

However, the loss of acidity is more than compensated by an increase in colloidal calcium phosphate. The net effect, then, is a decrease in pH. Milk held at 68 F (20 C), for example, has a pH of about 6.64. Milk held at 104 F (40 C) has a pH of 6.45, milk at 140 F (60 C), a pH of 6.23.

The basis for this change is the acidic nature of the colloidal form of calcium phosphate:

$$4CaHPO_4 \xrightarrow{\text{heat}} Ca_3(PO_4)_2 + 2H^+ \text{ (an acid reaction)}$$

The reverse reaction occurs upon cooling, thus resulting in an increase in pH, i.e. a lowering of acidity level.

Changes in pH induced by dilution and concentration

Because milk is saturated with calcium and phosphate, dilution causes a lowering of the level of calcium ion (Ca^{++}) and HPO_4^{2-}. It also causes some amount of colloidal calcium phosphate to shift to the soluble form. The net result is a more alkaline reaction; pH increases.

Concentration of milk causes formation of higher levels of colloidal calcium phosphate, hence an overall acid reaction.

The two reactions may be described by the following chemical equations:

$$\text{Dilution:} \quad Ca_3(PO_4)_2 \xrightarrow{H_2O} 3Ca^{++} + 2\,HPO_4^{2-} + OH^-$$

$$\text{Concentration:} \quad 3Ca^{++} + 2\,HPO_4^{2-} \longrightarrow Ca_3(PO_4)_2 + 2H^+$$

Table 2. Summary of changes in salt balance of milk under various conditions

Condition/Factor	Level Increases	Level Decreases
pH lowered	Ca ions, sol. complex[1]	CCP[2]
pH raised	CCP	Ca^{++}, sol. complex
Add divalent cations i.e. Ca^{++}, Mg^{++} etc.	CCP	sol. phosphate, pH
Add secondary calcium phosphate ($CaHPO_4$)	CCP	serum Ca, Ca ions
Add citrate	soluble Ca, soluble phosphate, pH	Ca ions, CCP
Increase temp.	soluble phosphate	CCP, pH
Dilution	pH	Ca ions, HPO_4^{++}, CCP
Concentration	CCP	pH

[1] Soluble complexes of salts
[2] Colloidal calcium phosphate

Milk contains approximately 0.05 grams of sodium per 100 grams of milk (i.e. 0.05%) and about twice as much chloride (.103 grams per 100 grams of milk (0.103%). The sum of these two levels would suggest a sodium chloride content of 0.153% (0.05 + 0.103 = 0.153). This amount, however, is higher than the actual level of sodium chloride, which, in normal milk, is considered to range between 0.095 and 0.11% of total mineral salts (or 0.086 to 0.099% by weight of fluid milk). The latter values assume a total mineral content of 0.9% (hence 0.095 X 0.9 = 0.086 and 0.099 X 0.9 = 0.099).

The problem raised by the above facts concerns the appropriateness of using a chloride test as a measure of sodium content of milk or whey under the assumption that sodium and chloride are present in equimolar concentrations. In fact, they are not.

A molar solution is one which contains one gram-molecular-weight of a compound per liter. In the case of mineral elements, the atomic weight is the "molecular weight." Hence, a one molar solution of sodium would contain the atomic weight of sodium in grams per liter. The same is true of chloride.

ultra-osmosis. This technology retains lactose and whey proteins while allowing passage through the membrane of salt and lactic acid. "Salt" whey is first concentrated to 60% of the original volume, then diafiltration water is added at a rate equal to one-half the permeate flux-rate. The process is continued until the volume reaches 25% of the original volume. At this point, diafiltration is continued with water added at a rate equal to the permeate flux rate and maintaining constant volume. About 90% of the salt can be removed by this process.

Use of ion exchange for removing minerals from milk or whey

Ion exchange technologies consist of a resin (porous plastic material in bead form) or combination of resins which act as insoluble acids or bases that remain insoluble when converted to salts. These resins, then, are able to exchange certain mobile ions with ions in solution. Characteristically, the ions which are exchanged are of the same charge as the ion(s) removed from solution.

An acid ion exchange resin, therefore, picks up positively charge ions (cations), and releases H^+. A base ion exchange resin releases OH^- and picks up negatively charged ions (anions). If R is considered to be the immobile component of an ion exchange resin, then an equation for the reaction taking place in a cation exchanger in presence of sodium ions would be as follows:

$$R{-}H + Na^+ \rightleftharpoons R{-}Na + H^+$$

The arrows expressing an equilibrium reaction reflect the fact that the direction of the process depends upon the ion concentration on the resin and in solution.

In general, ions of higher valency are selected in preference to those of lower valency. Ca^{++} would be selected—removed from solution—in preference to Na^+. At the same time, ions of the same valence are preferentially selected on size (atomic weight). Calcium ions, therefore, are removed ahead of magnesium ions.

Selectivity of removal of major milk and whey cations proceeds as follows:

$$Ca^{++} > Mg^{++} > K^+ > Na^+$$

For anion removal, selectivity proceeds as follows:

$$Citrate^{3-} > HPO_4^{2-} > NO_3^- > Cl^-$$

Ion exchange resins may be both strongly and weakly acidic or both strongly and weakly alkaline in reaction. Examples are:

Mineral content of milk and milk products

The average mineral content of a number of major minerals of milk and several dairy products is shown in Table 3. It should be noted that the amount of sodium (Na) in both cheddar cheese and creamed cottage cheese reflects both the natural level and the sodium added as salt (NaCl).

It is also of interest to compare the calcium content of cheddar and cottage cheese and the sweet and acid whey, respectively, produced from them. Cheddar cheese contains considerably more, its whey considerably less calcium than cottage cheese and its whey. The reason for this difference lies in the fact that less acid is developed in the manufacture of cheddar than cottage cheese; more calcium is retained by the cheddar curd. The more acid conditions used in cottage cheese processing results in loss (solubilization) to the whey of calcium from the colloidal calcium phosphate of the casein.

Table 3. Average amount of some select minerals in milk and various dairy products.

	Mineral (mg/100 g)						
Product:	Ca	P	Mg	Na	K	Fe	Zn
Wholemilk, fluid	119	93	13	49	151	0.05	0.38
Skimmilk, fluid	123	101	11	52	166	0.04	0.04
Nonfat dry milk	1257	968	110	535	1794	0.32	4.08
Cheddar cheese	721	512	28	620 [1]	98	0.68	3.11
Cottage cheese, dry curd	32	104	4	13 [2]	32	0.23	0.47
Cottage cheese, creamed	60	132	5	405 [1]	84	0.14	0.37
Sweet whey, fluid	47	46	8	54	161	0.06	0.13
Sweet whey, dry	796	932	176	1079	2080	0.88	1.97
Acid whey, fluid	103	78	10	48	143	0.08	0.43
Acid whey, dry	2054	1348	199	968	2288	1.24	6.31

Adapted from USDA (1976)

Please note: Data in this table were derived either by atomic absorption or flame photometric methods.

[1] This value reflects both the naturally occurring sodium and that added as salt.

[2] This value reflects only the naturally occurring sodium; no salt/sodium was added.

CHAPTER VII

SPECIFIC GRAVITY AND VOLUME/WEIGHT CONVERSIONS

Legal temperature standards

Legal standards may vary, but 68 F (20 C) has often been the temperature specified for volume measurements. This is the standard temperature used in calibration of laboratory glassware; it is also "room temperature," a convenient temperature to use.

In addition, 68 F is an appropriate temperature for determining the proper fill-weight of a container of product, like heavy cream, that expands significantly as temperature increases. Proper fill at 40 F could result in container overflow at 68 F unless excessive head space were made available.

Federal regulations require that volume be expressed at 68 F (20 C). A U. S. gallon is given as 231 cubic inches. Quarts, pints and fluid ounces are, respectively, 1/4, 1/8 and 1/128 of a gallon, i.e. 57.75, 28.875 and 1.80 cubic inches, respectively.

Measuring specific gravity

Specific gravity can be measured in several ways:

* hydrometer (or, as applied to milk and cream, the lactometer)
* hydrostatic balance
* pycnometer or equivalent methods

Hydrometer/lactometer methods:

A hydrometer/lactometer operates on the principle that an object floating in liquid displaces an amount of liquid equal to that object's weight. A lactometer, then, is a device that can be floated in milk or other liquid dairy product and measure the amount of milk (dairy product) displaced.

A number of different lactometers have been devised and used over the years. The Quevenne lactometer saw much use in the past. It is graduated in scale from 24 to 37 units, subdivided by 0.1 of a unit. It is designed for use at 60 F. To express results as specific gravity, a value of 1.0 is place ahead of the reading. A reading of 31 becomes a specific gravity of 1.031.

At 60 F, some milkfat is liquid, some solid. As the fat changes from liquid to solid or solid to liquid, lactometer readings vary slightly. Milk held cold and warmed to 60 F yields slightly different lactometer readings than milk cooled down to 60 F from some higher temperature.

Due largely to this problem, a lactometer was devised for use at 102 F (39 C). It is named the Watson lactometer after its designer, and is considered the most accurate of all lactometer methods. The Watson lactometer scale is graduated from 26 to 37 units. For pooled herd milk, total solids calculated from a Watson lactometer reading may be expected to agree with the Mojonnier analysis to within -0.18 to +0.19%.

Pycnometer:

A pycnometer is literally any container that, when filled with liquid to some predetermined level at some given temperature (and pressure), is known to carry a precise volume. In application, the pycnometer is weighed while filled with the standard reference fluid (water) and volume calculated:

$$\text{Volume} = \frac{\text{weight of water at given temperature}}{\text{density of water at given temperature}}$$

Knowing the volume, density of milk is determined by filling to the appropriate level at a given temperature (t). Then:

$$\text{Density of milk} = \frac{\text{weight of milk at (t)}}{\text{volume of pycnometer at (t)}}$$

Knowing the density of milk, the specific gravity becomes the density of milk divided by the density of water at (t).

The pycnometer method, though tedious, can be a very precise method of determining density/specific gravity.

In a very comprehensive study, the United States Department of Agriculture used the 8% Babcock test bottle as a pycnometer in evaluating weight per gallon of milk, milk products, and cream. From this study were developed formulas that could be applied to milk and cream products of varying fat and solids-not-fat content to determine weight per gallon of product. This consideration is treated in a later section.

Specific gravity of milk and milk products

The average specific gravity of pooled herd milk in the United States is usually given as 1.032 (68F/68F). This number may be interpreted in the following way. If a given volume of water at 68 F weighs 1.000 pound, then the same volume of herd milk of average composition will weigh 1.032 pounds.

Various milk products and milk constituents vary significantly in specific gravity. Table 1 provides some data in this regard.

Milkfat has a lower specific gravity than other milk solids. Hence, the higher the fat test of a product, the lower the specific gravity. Data on cream products in Table 1 give evidence of this fact. Conversely, addition of nonfat solids, as in fortification of milk or skimmilk products, increases the specific gravity. Removal of water, of course, is another way of increasing solids, with a proportionate increase in specific gravity. Compare, for example, the specific gravity of evaporated milk with normal milk.

The Baume lactometer

It should be noted that a special lactometer has been devised for use in condensed and evaporated milk products. It is called the Baume lactometer. Different types are designed for use in products varying significantly in density. For example, a Baume hydrometer used in a heavy product like sweetened condensed milk will be graduated in scale ranging perhaps from 29 to 41 units with 0.1 subdivisions. For a less dense product, like evaporated milk, the scale will range from 0 to 15 or 5 to 20 units, again with 0.1 unit subdivisions.

Baume lactometers use arbitrary scale units, but units with a specific relationship to specific gravity.

Baume lactometers are often designed for use at 120 F (49 C), the approximate temperature at which a vacuum pan of a milk evaporator operates. In triple or quadruple-effect pans, the final temperature of evaporation may be nearer 100 F (38 C). In any event, a correction factor of 0.03 units is applied for every 1.0 F deviation from 120 F. The factor is added to the reading if the temperature of the sample is above 120 F; it is subtracted from the reading if the temperarture is below 120 F.

For example, assume that the Baume reading was made at 130 F and was found to be 32.1 for sweetened condensed milk. The Baume reading at 120 F would be:

$$\text{Baume at } 120 = 32.1 + (0.03) \times (130 - 120)$$

$$= 32.1 + (0.03) \times (10)$$

$$= 32.1 + 0.3$$

$$= 32.4$$

In practice, the specific gravity (at 60 F) of sweetened condensed or evaporated milk of given composition is calculated by formula. That specific gravity value is then converted to a Baume reading at 60 F and then to a Baume reading at 120 F.

An example of the calculations follow:

Assume that evaporated milk is being processed. The expected composition of the finished product is 7.5% fat, 25% total solids. At what Baume reading at 120 F should the evaporation process be stopped?

Step 1. Determine solids-not-fat content:

$$25\% - 7.5\% = 17.5\% \text{ SNF}$$

Step 5. Convert the Baume reading at 60 F to the Baume reading at 120 F. Use the following formula:

$$\text{Baume at 120 F} = \text{Baume at 60 F} - (0.031) \times (120 - 60)$$

The Baume reading at 60 F has been found to be 8.85. Then:

$$\begin{aligned}\text{Baume at 120 F} &= 8.85 - (0.031) \times (120 - 60) \\ &= 8.85 - (0.031) \times (60) \\ &= 8.85 - 1.86 \\ &= 6.99\end{aligned}$$

You will perhaps note that the temperature conversion factor is 0.031 as distinguished from 0.03, the factor suggested for use in sweetened condensed milk. All factors are peculiar to the particular lactometer being used for any given product, and manufacturer's recommendations should be used.

Calculating specific gravity by equation/formula

A consideration of the preceding discussion indicates that specific gravity of a fluid is directly related to the specific gravity of the individual components of which it consists. The specific gravity of milk bears a direct relationship to the specific gravity of the fat and SNF that make it up.

In addition, the specific gravity at any given temperature is directly related both to the specific gravity of the individual components and also to the specific volume that these components occupy at any given temperature.

From the above information, it is possible to create a universal equation for calculating specific gravity. That equation is given as follows:

$$\text{Specific gravity} = \frac{100}{\frac{A}{\text{Sp. Gr. of fat}} + \frac{B}{\text{Sp. Gr. of SNF}} + \frac{C}{\text{Sp. Gr. of water}}}$$

where A = % by weight of fat

B = % by weight of SNF

C = % by weight of water

An example of the use of the above equation may provide insight into the development of equations for determining specific gravity at any given temperature.

$$\text{Expansion factor for fat at 50 F} = \frac{1}{0.9541} - 1.0$$

$$= 1.04811 - 1.0$$

$$= 0.04811$$

$$\text{Expansion factor for SNF at 50 F} = \frac{1.0}{1.6275} - 1.0$$

$$= 0.61444 - 1.0$$

$$= 0.38556$$

The factor for fat indicates an increase of 0.04811 units in specific volume for every 1.0% increase in percentage of fat.

The factor for SNF indicates a decrease of 0.38556 units in specific volume for every 1.0 % increase in SNF.

The above factors make it possible to simplify the equation for calculating specific gravity:

$$\text{Specific Gravity} = \frac{100}{100 + \%\ \text{fat (Fat factor)} - \%\ \text{SNF (SNF factor)}}$$

For milk of 3.5% fat and 8.5% SNF, the calculation becomes:

$$\text{Specific Gravity} = \frac{100}{100 + 3.5(0.04811) - 8.5(0.38556)}$$

$$= \frac{100}{100 + 0.168385 - 3.27726}$$

$$= \frac{100}{100.16838 - 3.27726}$$

$$= \frac{100}{96.8911}$$

$$= 1.0321$$

Assume that a milk product contains 8.4% SNF and 3.6% fat. The specific gravity of the mixture is 1.0291 at 102 F. The specific gravity of fat at that same temperature is considered to be 0.913. Give water a specific gravity of 1.0. Then:

$$\% \text{ water} = 100 - (3.6 + 8.4)$$

$$= 100 - 12.0$$

$$= 88$$

$$\text{Sp. Gr. of SNF} = \frac{8.4}{\frac{100}{1.029} - \left(\frac{3.6}{0.913} + \frac{88}{1.0}\right)}$$

$$= \frac{8.4}{97.182 - (3.943 + 88)}$$

$$= \frac{8.4}{97.182 - (91.943)}$$

$$= \frac{8.4}{5.239}$$

$$= 1.603$$

By calculations similar to this, the specific gravity of SNF at different temperatures can be determined. The following chart provides this data at four common temperatures.

Temperature	Calculated Specific Gravity of SNF
40 F (4.4 C)	1.6453
50 F (10 C)	1.6275
68 F (20 C)	1.6167
102 F (38.9C)	1.5952

Calculating SNF when percent fat and specific gravity are known

By rearranging the universal equation, a related equation can be formulated that allows calculation of SNF when the percentage of fat and the specific

prevent excessive overage—and the added cost of such overage. Hence, both legal aspects and profitability are directly tied to specific gravity and its application to dairy processing.

To determine weight per gallon of product at some given temperature, it is helpful to start with the volume, in milliliters, of 1.0 gram of water at such temperature. Information of this kind is found in the **International Critical Tables**. The milliliters of water in a gram of water at four commonly used temperatures are as follows:

Temperature	Milliliters of Water per Gram
40 F (4.4)	1.00106
50 F (10)	1.00133
68 F (20)	1.00283
102 F (38.9)	1.00846

With the above information, it is possible to determine the weight per gallon of water at those temperatures. One gallon of water is 3,785.33 milliliters. There are 453.592 grams per pound. At 40 F, therefore, one gallon of water weighs:

$$\frac{3{,}785.33}{453.592 \times 1.00106} = 8.3364 \text{ pounds}$$

By similar calculations, using milliliter values appropriate to the temperature under consideration, it can be determined that a gallon of water will weigh 8.3341, 8.3217 and 8.2752 pounds at 50 F, 68 F, and 102 F, respectively. These values can then be used to compute pounds of milk product per gallon:

Pounds of product per gal. = sp. gr. of product x pounds of water per gal.

Given 8.3364 pounds of water per gallon at 40 F and a specific gravity of 1.029 for a milk product at that same temperature, then:

Pounds of product per gal. = 1.029 x 8.3364

= 8.578

If the specific gravity of the product is not known, it can be calculated, knowing the percentage fat and SNF of the product. The equations for doing this have already been covered in an earlier section. However, it may be helpful to integrate those equations with known weights of water at various temperatures. Doing so provides a single equation for calculating pounds per gallon of product. Four equations are given below for determining pounds per gallon at four different temperatures:

products high in fat content. For example, the difference in weight of skimmilk at 40 F vs 102 F is 0.078 pound. The difference in weight of heavy cream at those two temperatures is just over one quarter pound (0.252 pounds).

These facts are most important in setting up a program of weight control of products sold by volume. They can be summarized as follows:

* Composition of fluid milk products has the greatest influence on weight per unit volume.

* The influence of temperature on weight of fluid milk products is sufficiently important that it should be taken into account when determining weight of a given volume of product.

* Differences in weight per unit volume of fluid milk products due to geographic location and season of the year are relatively unimportant.

Reasonable overfill and underfill weights of various common volumes

In setting up weight control programs for establishing fill of containers, some reasonable figures for over- and underfill can serve as guides. At the same time, it is desirable to use sensitive weighing devices for determining weight. Scales measuring weight in grams are appropriate.

The following conversion factors should be kept in mind:

One pound = 453.592 grams

One ounce (weight) = 1/16 pound = 28.35 grams

In controlling fill of containers, the preceding procedures can be used to determine the pounds per gallon of product. The next step in a control program should be the conversion of pounds to grams and establishing the grams per gallon, quart, pint etc. Suitably sensitive balances, providing weight in grams, can then be used for regular checking of fill-weights.

The following chart indicates some reasonable levels of over- and underfill of various commonly used volumes of fluid milk products. They can be used as guides to determine whether a given filling operation is performing within reasonable limits of weight control.

Simplified statistical methods have been worked out to handle these problems. A brief discussion of their use follows. However, it should be kept in mind that essentially the same statistical techniques can be used for controlling not just fill-weight, but also composition (fat and/or other components or ingredients in dairy products), and various processing functions. Any process that can be followed using some specific measure (inches, pounds, freezing point, specific gravity etc.) may be appropriately controlled using one or more of the following techniques.

Determine sensitivity of filler:

Before setting up control procedures, it is well to know the variability of the filler equipment, either the unit as a whole or individual filling heads, if desired. This information will tell you whether or not the equipment is operating within specifications of the manufacturer or whether adjustments are necessary to make delivery quantities less variable (more precise). The ability to control fill depends on the precision with which given quantities can be delivered.

A frequency chart can be used for this purpose. To produce such a chart, determine the weight of overfill and underfill in a given number of containers of some given volume(s). The following chart consists of one hundred weighings both before and after adjustment of filling equipment. Zero weight in the chart is the declared (legal) weight of fill. If container weight does not vary greatly, an average weight may be used and then subtracted from the total weight of container and product as a way of determining net weight.

Frequency Chart of Fill Weights

Weight (grams)	Frequency Measure Before Adjustment	Frequency Measure After Adjustment
+ 36	I	
+ 33		
+ 30	II	
+ 27		
+ 24	III	
+ 21	I	
+ 18	III	
+ 15	IIII	
+ 12	IIIII	
+ 9	IIIII IIII	IIIII IIIII
+ 6	IIIII IIIII IIIII II	IIIII IIIII IIIII IIIII IIIII IIIII
+ 3	IIIII IIIII IIIII IIIII	IIIII IIIII IIIII IIIII IIIII IIIII
0.0	IIIII IIIII	IIIII IIIII IIIII IIIII IIIII
- 3	IIIII IIIII	IIIII
- 6	IIIII III	
- 9	IIII	
- 12		
- 15	II	
- 18		
- 21	I	

Assume, as example, that the average weight of a 1/2 pint fiberboard container is 15.0 grams and that the standard deviation is 2.0 grams. That figure--2.0 grams--represents one standard deviation. Two standard deviations would be twice that, or 4.0 grams. In fact, 1.96 rather than 2.0 is the precise value to use for expressing results at 95% confidence. If 1.96 x 2.0, or 3.92, is added to 15.0 grams, the sum is 18.92 grams. If that amount is subtracted from 15.0 grams, the result is 11.08 grams. Ninety-five times out of 100, the weight of any given container will fall between 11.08 and 18.92 grams.

In setting a target weight of fill, a single standard deviation must be obtained, one that takes into account both variability of container weight--if that is a significant factor--and variability of delivery weight of product from the filler. That value, then, can be used to establish a target weight of container plus product that will provide some given statistical probability of underfilling or excessively overfilling.

The procedure requires a determination of (1) standard deviation of container weights. (2) standard deviation of fill weights, and (3) the integration of these two standard deviations into a single standard deviation value. The following steps set forth the procedures.

Step I. Determine the standard deviation of container weight:

1. Take a random sampling of containers in groups of five, i.e. five from each bulk carton or every three bulk cartons (or other appropriate number) of several lots of cartons. Groups involving numbers other than five can be used, but not with the same statistical factors that will be presented in this discussion. If groups other than five are used, the reader should refer to appropriate statistical tables for the respective factors required.

2. Weigh each container.

3. Determine the average weight of the containers in groups of five.

4. Determine the range in weight of containers in groups of five.

5. Determine the overall average weight of containers.

6. Determine the overall range in weight of containers.

7. Divide the overall average range in weight of containers by 2.326. This is a number that is used only when weighings or other measurements are made in groups of five. The value derived is a standard deviation of container weights.

Step II. Determine the average fill-weight and standard deviation of fill-weight using the same procedure indicated in Step I above.

Step III. Calculate the gross average weight of the filled containers.

Step IV. Calculate a single standard deviation that integrates the standard deviation of container weight and the standard deviation of fill-weight.

group number 1 totaled 75.6 grams, the average (total divided by 5) is 15.12, the range (15.4 -14.9) is 0.5 and so on across the chart.

The grand average container weight is the total of the average weight of each group divided by 10 groups. In this example, the average is 150.52 divided by 10 = 15.052 grams.

The grand average range of container weights is the average of the bottom row (going across) of range values divided by 10. The grand average range, therefore, is 3.4 divided by 10 = 0.34.

Calculate standard deviation as follows:

$$\text{Standard deviation} = \frac{\text{average range in container weight}}{2.326}$$

$$= \frac{0.34}{2.326}$$

$$= 0.146$$

At this point, then, it becomes necessary to determine the standard deviation of the range in fill-weight of product. This can be done using a chart identical to the one above and going through the process of determining standard deviation in exactly the same way.

Assume, now, that this process has been completed and the average range of fill-weight is 5.0 grams. The standard deviation is 5.0 divided by 2.326 = 2.15. We now have the two values required to establish a single standard deviation that accounts for both variations in container weight and variations in fill-weight.

Given 0.146 as the standard deviation of container weights and 2.15 as the standard deviation of fill-weights, determine the integrated standard deviation by the following equation:

Integrated standard deviation = square root of the sum of the square of each of the two standard deviations involved, i.e. container weight and fill-weight.

$$\text{Integrated standard deviation} = \sqrt{(0.146)^2 + (2.15)^2}$$

$$= \sqrt{0.0213 + 4.623}$$

$$= \sqrt{4.644}$$

$$= 2.15$$

Lower Control Limit (LCL), chart of average values:

LCL_X = target weight - (0.577 x average range of fill)

= 261.9 - (0.577 x 5.0) = 261.9 - 2.90 = 259.0 grams

Upper Control Limit, chart of range values:

UCL_R = average range x 2.114

= 5.0 x 2.114 = 10.57 grams

Lower Control Limit, chart of range values:

For groups of five, the factor is 0.0. Hence:

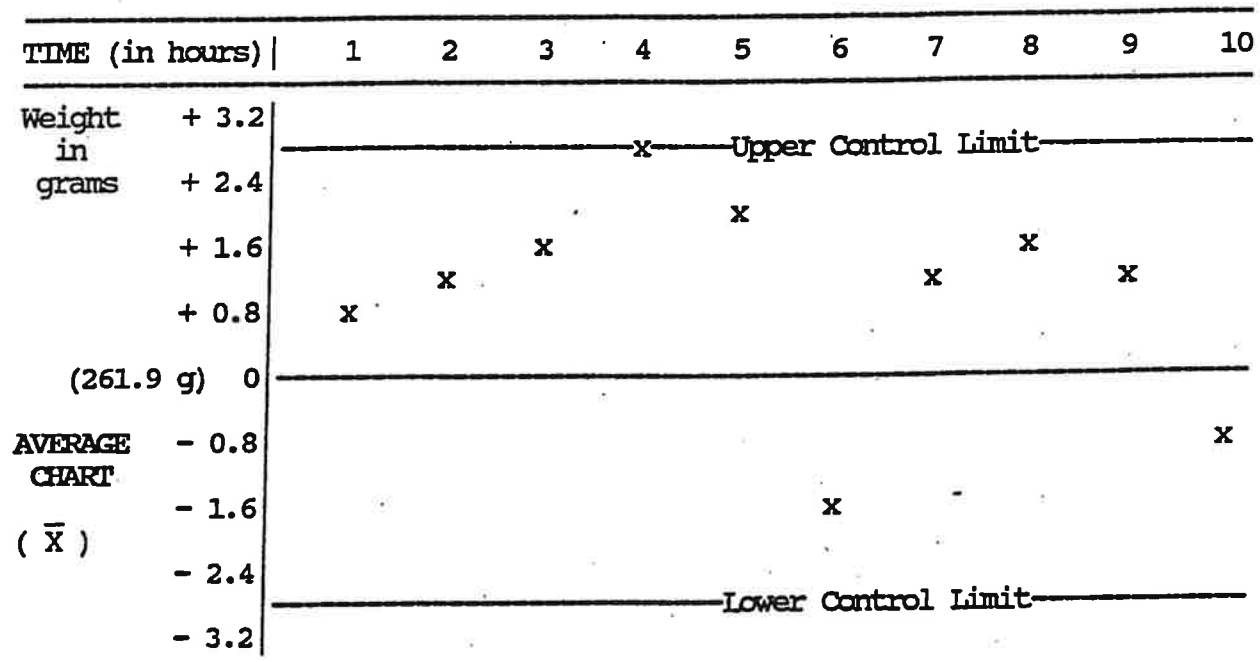
LCL_R = 5.0 x 0.0 = 0.0

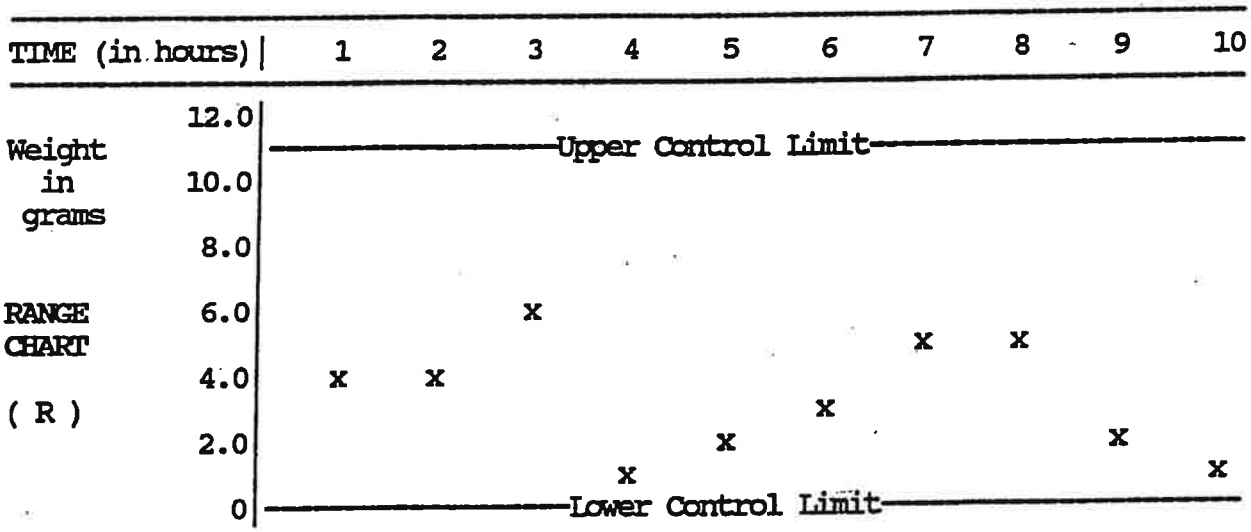
Setting up and using control charts

Both average and range charts are used in monitoring the stability of any process. The procedure is as follows:

1. Determine the time interval at which check-measurements will be made. In a filling line, a suitable time might range from every half hour to hour.
2. Take samples (filled containers of milk) off the filling line in groups of five at the pre-determined interval.
3. Weight each container, and record results.
4. Determine average weight and range in weight of each group of five filled containers.
5. Record results on both average and range charts.
6. In the event that upper or lower control limits are breached, re-sample immediately and check again. If control limits continue to be breached, make suitable adjustments in filling equipment.

An example of control chart data collection and use follows:





Selected References:

1. Davis, J., and F. MacDonald. 1953. Richmond's Dairy Chemistry. Charles Griffin and Co., Limited, London, England.

2. Hunziker, O. 1949. Condensed Milk and Milk Powder, 7th edition. Published by the author, LaGrange, IL.

CHAPTER VIII

PHYSICAL PROPERTIES OF MILK AND DAIRY PRODUCTS

* One BTU = 252 calories (gram calories).
* One calorie (gram calorie) = 4.186 joules.
* One BTU = 1054.8 joules

Factors related to specific heat of products

The specific heat of any given substance depends upon several factors:

* Product composition
* Temperature
* Moisture content
* Pressure
* Physical state (whether liquid, solid or gaseous)

The latter factor, physical state, is of significance in that given specific heat values assume no change in physical state of the product. Putting it somewhat differently, specific heat of any given substance varies depending upon the physical state of the substance. That is, specific heat of water differs from the specific heat of ice; specific heats of water and ice differ from the specific heat of steam. And specific heat varies, though slightly, with temperature irrespective of the physical state.

Additionally, given amounts of heat must be added or removed simply to take a product from one physical state to the other. This fact leads to the distinction between "sensible" heat and "latent" heat.

Sensible heat and latent heat

The term **sensible heat** refers to the heat taken in or given off as the temperature of a substance rises or falls without change in physical state.

The term **latent heat** refers to heat taken in or given off when a substance changes in physical state, i.e. milkfat crystals melt, water in ice cream mix freezes etc.

Latent heat of crystallization of fat of cream, for example, causes the temperature of cream to rise slightly above the initial temperature to which it is cooled. This fact is important, as discussed previously, in achieving the appropriate temperature of cream prior to churning in "continuous" churns.

General applications of specific heat

To calculate BTU's of heat required to raise the temperature of a given amount of milk by some given level, proceed as in the following example:

It is also noteworthy that whey has about the highest specific heat of any dairy product. It is close to 1.0 (0.98), the specific heat of water. This is a reflection of its low total solids and fat content.

Table 1. Specific heat of milk components and various dairy products

Product	Specific Heat (approx. average) (BTU per pound)
Water	1.0
Ice	0.48
Milkfat	0.50
Proteins	0.38
Carbohydrates	0.30
Minerals (ash)	0.20
Skimmilk	0.94
Wholemilk (3.5% fat)	0.92
Whey	0.98
Ice cream mix	0.80
Ice cream	0.45
Cream (20% fat)	0.86
Cream (30% fat)	0.84
Cream (40% fat)	0.79
Cream (50% fat)	0.77
Cream (60% fat)	0.75
Butter	0.48
Concentrated Milk (1:2.1)	0.85
Cheddar cheese	0.65
Cottage cheese	0.78

Adapted from Hammer and Johnson and other sources

Viscosity

In simplest expression, **viscosity** is resistance to flow (or agitation or shear). You might think of it as a kind of internal friction. A heavy syrup flows much less readily than water. A heavy syrup is much more viscous than water.

For engineering purposes, viscosity determines the amount of force that must be applied to move a given weight of product through various processing systems at some given rate of speed.

The physical state of both casein and whey proteins is important. The physical state of casein varies with:

a) pH
b) salt balance
c) heat treatment
d) influence of proteolytic enzymes
e) bacteria (as they influence pH or enzyme content of milk)
f) stabilizers

Whey proteins are also altered to more or less extent by factors (a) - (e), but the influence of whey proteins on viscosity is generally of less magnitude than that of casein.

* Physical state of the fat

This factor is somewhat less significant than physical state of casein, but is nonetheless an important consideration. Aspects influencing viscosity include:

a) size of fat globules
b) clumping or clustering of fat globules
c) homogenization
d) presence of added emulsifiers

Of the above, clumping or clustering of fat globules usually has the greatest impact on viscosity. Increase in viscosity of milk and cream from homogenization is due mostly to clustering of fat globules following treatment.

* Temperature

Viscosity of liquids decreases with increase in temperature.

* Age (length of storage)

In general, aging or storage of liquid dairy products results in increase in viscosity.

* Pasteurization or other heat treatment

Heat treatment of fluid dairy products causes varying influence on viscosity. In fluid milk, pasteurization at high temperatures destroys some of the protein components associated with the fat globule membrane, inhibits clumping of fat and, thereby, reduces viscosity.

High heat treatment of skimmilk increases the hydration—the amount of water associated with—casein, thus significantly increasing viscosity.

temperature increases to 248 F (120 C), temperatures well above those required for complete denaturation of whey proteins.

Viscosity of wholemilk follows the same general pattern as that for skimmilk, with a gradual increase all the way to the point of heat-coagulation.

* Homogenization:

Homogenization increases the viscosity of milk, though only slightly at fat levels under 5%. If milk of 6% fat is homogenized after cooling to 80 F., a marked increase in viscosity occurs.

As pressure of homogenization goes up, viscosity rises linearly.

Increase in viscosity by homogenization is due to increases in:

a) surface area of fat globules
b) amount and kind of proteins associated with fat globules
c) extent of clustering and clumping

The second stage of two-stage homogenization breaks up the clusters formed during the first stage of homogenization, thereby reducing viscosity.

* Temperature of separation or standardization of milk:

During cold separation of milk, the cryoglobulins that cause fat to come together in clumps stay mainly with the cream. At high temperatures of separation, these proteins are lost to the skimmilk. The potential for clumping of fat globules varies accordingly.

Cream products:

Viscosity of cream varies as follows:

* decreases with increase in temperature of pasteurization
* decreases under prolonged or unnecessary agitation, especially following pasteurization
* increases with aging (due to clumping of fat)
* increases with development or addition of acid
* increases under influence of fast cooling following pasteurization, i.e. cryoglobulins are more quickly adsorbed onto the fat globules, thereby enhancing clumping
* increases if, for whatever reason, pasteurized milk is re-warmed to temperatures approaching 86 F (30 C), held for 3 - 4 minutes, and then re-cooled

Yogurt:

A number of factors influence viscosity of yogurt. These include:

* increase in fat and solids-not-fat (SNF) content (increases viscosity); a fat content of 1% or more and addition of 3 - 3.5% SNF to normal-strength milk generally improves viscosity/body.

* heating the product to 194 F (90 C) for 15 minutes; this process maximizes viscosity and minimizes wheying off (syneresis) of stirred yogurt; denaturation of whey proteins enhances body/viscosity.

* heating to either 243.5 F (117.5 C) for 15.8 seconds or 284 F (140 C) for 36.6 seconds, both by direct steam injection--provides the same viscosity as heating to 158 - 176 F (70 to 80 C) for 30 minutes.

* heating to 243.5 F (117.5 C) for 13.9 seconds or 284 F (140 C) for 33.5 seconds by indirect heating methods--provides the same viscosity as heating to 158 - 176 F (70 - 80 C).

* homogenization; best viscosity is achieved at homogenization pressures of 2500 - 2800 pounds per square inch (psi) pressure at 140 F (60 C) or higher.

* presence of mucus from growth of certain bacteria--increases viscosity.

* minimal agitation during cooling, with little or no agitation until the temperature is 50 F (10 C) or lower, generally enhances viscosity.

* use of stabilizers--increases viscosity.

Rennet whey:

Rennet whey--the whey from hard cheese processing--varies in viscosity as a result mainly of changes in pH and temperature. Important aspects of these processing functions include:

* pH--the lowest viscosity is observed at pH 4.5 in whey which has received nominal heat treatment during cheese processing; on the acid side of pH 4.5, viscosity rises rapidly; on the alkaline side of pH 4.5 viscosity rises rapidly, shifts downward slightly, then levels off; in boiled whey, the lowest viscosity occurs at pH 5.5.

* Temperature/heat treatment; the relative viscosity of whey decreases slightly as temperature rises from 32 to 86 F (0 to 30 C); rennet whey shows a slight though uniform decline in viscosity as temperature increases between 86 and 149 F (30 and 65 C); above 149 F (65 C), viscosity of rennet whey increases.

Product	Viscosity (Centipoise, 68 F)
Water	1.005
Rennet whey	1.2
Skimmilk	1.5
Wholemilk	2.0
Half and Half	3.4
Whipping cream	27.0

Freezing point of milk and milk products

Water freezes at 32 F (0 C). Milk freezes at a slightly lower temperature—30.97 to 31.11 F (-.573 to -.494 C)—mainly because of the presence of (1) dissolved mineral salts and (2) lactose.

Only components of milk that are dissolved—found in true solution—influence the freezing point. Of course, components other than mineral elements and lactose are found in true solution. Whey proteins fall in this category. However, large molecules have far less influence on freezing point than small molecules. Hence, proteins in solution make only very minor contributions to freezing point of milk.

A rule of thumb: The more molecules of a given solute (dissolvable compound) per unit weight, the greater its influence on freezing point depression.

Sour milk will have a slightly lower freezing point than fresh, sweet milk. In the souring process, lactose is broken down into smaller molecules which then contribute that much more to lowering of the freezing point.

Whole milk, skimmilk and cream all freeze at the same temperature.

Processing operations other than those that involve concentration or dilution do not cause changes in freezing point. Heat treatments normally used in processing do not alter the freezing point.

Freezing point range and stability

Please note in the above figures that the range in freezing point of milk is very narrow. From the highest to the lowest values known to occur naturally, the difference is only about 0.14 F (0.079 C).

In a natural process, the cow maintains a balance between the osmotic pressure of the milk within the udder and the blood supply that nourishes the udder. This is necessary—essential—to life processes which require the transfer of nutrients and other chemicals between tissues of the animal's body.

$$^{o}C = [0.1915 \times (-^{o}H - 0.0004785)]/0.199$$

$$^{o}H = [0.199 \times (-^{o}C + 0.0004785)]/0.1915$$

An example of the application of each may help ensure proper calculations. For the first formula above, assume that you wish to convert a reading of -0.422 ^{o}H to centigrade. Then:

$$^{o}C = [0.1915 \times (-0.422 - 0.0004785)]/0.199$$

$$= [0.1915 \times (-0.4224785)]/0.199$$

$$= [-.0809046]/0.199$$

$$= -0.4065$$

As an example of conversion of centigrade to Hortvet, assume a centigrade reading of -0.598. Then:

$$^{o}H = [0.199 \times (-0.598 + 0.0004785)]/0.1915$$

$$= [0.199 \times (-0.5975215)]/0.1915$$

$$= [-.1189068]/0.1915$$

$$= -0.6209$$

Legal freezing point base as applied to water adulteration of milk

It has already been stated that milk, as it occurs naturally among the cow population of the United States, varies in freezing point from a low of about-0.573 to a high of -0.494 C (-0.595 to -.513 ^{o}H, respectively). The average value is -0.5205 C (-0.5404 ^{o}H).

However, the average value is not used as a legal base because it does not take into account natural variations among milk supplies, some of which are higher than the average.

Instead, the legal base is established as that freezing point that includes 99% of natural milk supplies at 95% confidence. Using such base, one is assured that a freezing point of a given milk supply falling above that level will contain added water 99 times out of 100 at least 95% of the time.

This base value has been established using a statistic called the standard deviation. In a study by Henningson, the standard deviation was found to be-.00676 ^{o}H. The upper base then becomes the average value plus 2.326 (a statistical factor) times the standard deviation:

A Land O Lakes, Inc. statistician, Dr. Joseph Dewbre, has suggested a statistically valid way of establishing a working base based upon an average of 30 or more freezing point determinations per month. The base may vary slightly from month to month, but will accurately pinpoint supplies of milk with added water.

The approach, in this method, makes the assumption that an average freezing point value of a sufficiently large number of tested milk supplies--just as they come from the farm--will closely approach and, in fact, be statistically indistinguishable from the true average value of the population of supplies as a whole.

The formula for deriving the working freezing point base then becomes:

$$\text{Working base} = [\text{avg. F.P.} + 2.326\ (0.00676/\sqrt{30}\ )]$$

where avg. F.P. is the average of 30 or more freezing point determinations of producer supplies

2.326 is a statistical constant encompassing 99% of the freezing point values at 95% confidence

0.00676 is the standard deviation of the freezing point of milk as determined by the Henningson study

$\sqrt{30}$ is the square root of 30 (or any other number over 30 that reflects the actual number of samples tested during a given month)

As an example of the calculation, assume that you have made 30 freezing point determinations of producer milk supplies and that the average of those 30 values is -0.521 C. Then:

$$\text{Working base} = [-0.521 + 2.326 \times (0.00676/\sqrt{30}\ )]$$

$$= [-0.521 + 2.326 \times (0.00676/\ 5.477)]$$

$$= [-0.521 + 2.326 \times (0.00123425)]$$

$$= [-0.521 + 0.00287]$$

$$= -0.518\ C$$

Any milk supply with a freezing point falling above the value thus derived (above -.0.518 C) becomes subject to appropriate action. Field visits to farms would be in order for those supplies consistently falling above this level, particularly if the observed freezing point is significantly higher than the calculated value.

Causes of variations in freezing point of milk

Keep in mind that the most serious cause of change in freezing point of milk is addition of water either at the farm or plant. A number of other factors, however, may influence freezing point, though generally only to a relatively minor degree. Rarely, if ever, will the naturally occurring highest freezing point level of milk be breached by any factor other than added water. Some of the causes of variation in freezing point of milk are as follows:

* **Cow differences:** are greater than those of pooled, herd milk.

* **Breed differences:** small differences exist, with Jersey milk generally lower in freezing point than either Holstein or Guernsey milk.

* **Developed acidity:** lowers freezing point by about 0.003 C.

* **Colostrum:** has a generally lower freezing point than normal milk.

* **Mastitis:** severe mastitis may lower the freezing point of milk.

* **Stage of lactation:** milk in late lactation may show slightly lower freezing point than milk of mid-lactation.

* **Nutrition:**
  1. pasture vs winter feeding may raise freezing point.
  2. low forage combined with higher than normal levels of grain raises freezing point about 0.003 C.
  3. high moisture silage (corn and sorghum) may cause somewhat higher freezing point than low-moisture (alfalfa) silage.
  4. sudden, drastic changes in cows' ration may produce somewhat higher or lower freezing points depending upon the kind of change that occurs.

* **Season:** higher freezing points are generally noted during summer months.

* **Amount of water consumed:** high levels of water consumption just prior to milking may cause an increase in freezing point; withholding water and/or drought may lower freezing point.

* **Regional differences:** regional/geographical differences in freezing point of milk are known to exist (possibly due to differences in feeding and management practices).

* **Freezing of milk:** freezing of raw and pasteurized milk increases the freezing point.

* **Vacuum treatment:** vacuum pasteurization or vacuum treatment of milk increases freezing point by 0.005 C.

curve for sucrose from mixes varying in level of lactose and corn syrup solids.

Boiling point of milk and dairy products

The average boiling point of milk at sea level (760 mm mercury, pressure) is 212.3 F (100.17 C). The boiling point is slightly higher than that of water as a result mainly of dissolved lactose and mineral salts. By comparison, the boiling point of sweetened condensed milk, a product to which sugar has been added, is 217.8 F (103 C). On the other hand, evaporated milk, which has no added sugar, has a boiling point of 212.79 F.

Boiling point of milk can be significantly reduced by lowering the pressure, i.e. treatment under vacuum. This is the principle behind use of vacuum processing of evaporated and condensed milk products. Under a 25-inch vacuum, milk boils at about 135 F (57 C). It is important to note that no less heat must be applied in processing under vacuum than at atmospheric pressure. However, rate of evaporation is greatly enhanced.

Color of milk and dairy products

The white color of milk is caused by reflection of light by casein and fat globules. The yellow color of milkfat is due to the presence of carotene, a precursor of vitamin A.

Whey has a greenish yellow color due mainly to presence of riboflavin, i.e. vitamin B_2.

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APPENDIX 1

Percentage of Nutrients in the American Diet Derived from Dairy Products (excluding butter)

Nutrient	Percentage
Macro nutrients and cholesterol:	
Total Energy	10
Protein	21
Carbohydrate	6
Fat	11
Cholesterol	14
Minerals:	
Calcium	76
Phosphorus	36
Magnesium	20
Iron	2
Zinc	20
Vitamins:	
Vitamin A	10
Thiamine (vitamin B_1)	9
Riboflavin (vitamin B_2)	35
Niacin	2
Vitamin B_6	11
Vitamin B_{12}	20

APPENDIX 3

Conversion Factors (Weights, Measures, Temperature)

Weight (mass):

1.0 ton = 1016.047 kilograms (kg)

1.0 pound = 16 ounces; 256 drams; 7,000 grains

1.0 pound = 453.59 grams (g)

1.0 ounce = 28.35 grams

1000 grams = 1.0 kilogram (kg)

1.0 gram = 0.0353 ounce

1.0 kilogram = 2.2046 pounds

1.0 milligram (mg) = 1/1,000 gram

1.0 microgram = 1/1,000,000 (one millionth) gram

Capacity:

1.0 gallon = 3.7854 liters; 128 fluid ounces

1.0 gallon = 231 cubic inches

1.0 gallon of water at 15 C. = 8.337 pounds

1.0 quart = 0.946 liters

1.0 liters (l)= 1.057 quarts

1.0 quart = 32 fluid ounces

1.0 pint = 16 fluid ounces

1.0 pint = 0.5682 liters

1.0 microliter = 1/1,000,000 liter

1.0 fluid ounce = 28.412 milliliters (ml)

1.0 milliliter (ml) = 1.0 cubic centimeters (cc)