Dry Coconut The hard coconut shell is removed and the mature coconut is sun dried. The dry coconut can then be stored and used throughout the year. The coconuts are grated and used in various preparations. The keeping quality of dry coconuts is greater than that of the fresh coconuts, as a nut and as a part of the prepared product. However, dry coconuts lack the characteristic delicate flavour of fresh coconuts. The dry coconut is usually roasted lightly and used in preparations such as chutney powders as an ingredient in dry masalas, and in sweets (chikki, biscuits etc.).

Gingelly Seeds Two varieties are available—black and white. The white seeds are widely used. Use of the black seeds is restricted. Very few food preparations require black gingelly seeds.

Oil seeds and the oil extracted from them are used in food preparation. Gingelly seeds are used in curries and gravies as thickening agents: Chutneys and powders are made out of gingelly seeds. This serves as a base for some masalas.

Gingelly seeds give us 18 per cent proteins, 43 per cent fat. It is a very rich source of calcium. Gingelly seeds are a fair source of iron, thiamin and niacin.

Pumpkin Seeds, Cashew nut, Walnuts, Almonds, Pistachios

These nuts are usually used as garnishes. These nuts are quite expensive and hence they are used occasionally for their rich flavour and colour. The nuts are roasted, and used whole, as chopped nuts or used after grinding.

Gardencress Seeds These seeds contain 25 per cent protein, and are a rich source of iron and calcium. These seeds are used to prepare very nourishing preparations such as *ladus* and porridge for nursing mothers, as it is a galactogogue.

Poppy Seeds (***Khaskhas***) are small white, seeds. Their most common use is in gravies as thickening agents. These seeds contain 21 per cent protein and are a very rich source of calcium. Their use is restricted to certain preparations for nursing mothers such as poppy seed *ladus*, *porridge*, *vadis* etc.

Buying and Care of Nuts and Oil Seeds

Clean nuts and oilseeds should be selected, so that these are free from dirt, foreign matter and stones. Choose nuts, which are evenly coloured as unnatural spots on nuts is a sign of deterioration.

Nuts and oilseeds have a high oil content and can turn rancid if stored over long periods.

Nuts and oilseeds should be destoned and made free from foreign matter. Nuts and oil seeds should be consumed within reasonable time as they turn rancid easily. All the nuts and oil seeds, if sun dried before storage, keep well.

Milk and Milk Products

Milk has a very special place in the Indian dietary. It is an essential part of our morning's cup of tea or coffee. Curd and butter milk are made from milk. Milk is also used to make popular sweets such as *pedhas*, *barfi* and a variety of *halwas*. A number of desserts from milk made for special feasts

include *kheer*, *shreekhand*, *rasgullas*, *gulab jamuns* etc. In fact we in India, may boast of having the largest number of preparations made out of milk. Therefore, its important for us to understand the composition and nutritive value of milk.

Composition of Milk

Milk from different animals is used as food, but in India, buffalo and cow are the two species which are most important for the commercial production of milk and milk products.

Milk is a complex food, which contains more than 100 components. Most of these components are suspended in water and thus milk is a colloidal solution and is opaque. The composition of milk and milk products is given in Table 10.3 in which besides water, only eight nutrients are included.

The major components of milk are water, protein, fat, the sugar lactose and minerals. The composition of milk varies with species. It may be observed from Table 10.3 that buffalo's milk contain twice as much fat as cow's milk.

TABLE 10.3 Composition of Milk and Milk Products (per 100 g)

	Moisture (g)	Calorie	Protein (g)	Fat (g)	Lactose (g)	Ca[1] (mg)	Fe[2] (mg)	Vit. A (mcg)	Thiamin (mg)	Riboflavin (mg)
Cow's milk	88	67	3.2	4	5.0	120	0.2	57	0.05	0.19
Buffalo's milk	81	117	4.3	9	5.0	210	0.2	53	0.04	0.10
Human milk	88	65	1.1	3	7.4	28	—	137	0.02	0.02
Curd	89	60	3.1	4	3	150	0.2	34	0.05	0.16
Butter milk	98	30	0.8	1	4	30	0.8	0	—	—
Butter	19	730	—	81	—	—	—	317	—	—
Ghee, Buffalo's	0	900	—	100	—	—	—	89	—	—
Ghee, Cow's	0	900	—	100	—	—	—	198	—	—
Channa, paneer	54	265	18.3	21	—	208	—	121	0.07	0.02
Cheese	40	348	24.1	25	6.3	790	2.1	—	—	—
Khoa	31	421	14.6	31	—	650	5.8	—	—	—
SMP (Skimmed milk powder)	4	357	38	0.1	—	1370	1.4	0	0.45	1.64
WMP (Whole milk powder)	4	496	26	27	—	950	0.6	462	0.31	1.36

1. Ca – Calcium
2. Fe – Iron

The main proteins in milk are *casein* and *lactalbumin*. Casein, which accounts for 87 per cent of the total proteins present is a *phosphoprotein*. The reactions of these proteins are important in milk preparations.

Milk fat contains some volatile fatty acids (e.g., caproic and butyric acid). These are released when butter turns rancid. Their presence is noticeable in rancid butter due to their bad smell.

Lactose is the sugar present in milk. It is present in the milk serum. Milk is an excellent source of the minerals calcium and phosphorus. The minerals in milk are present partly in solution, partly in suspension and some as components of proteins and fats. For example, most of the phosphorus is suspended in the form of calcium phosphate, but a little is combined with casein and another trace is found in combination with the fat.

Milk contains all the vitamins known to be beneficial to human nutrition.

Nutritive Value

The composition of milk from various species of mammals differs markedly (Table 10.3). The milk of each species is designed to fulfill the needs of the young of that species.

It may be noted that human milk has the highest lactose content and lower protein, fat and mineral contents than those in cow's and buffalo's milk. The high fat content of buffalo milk is reflected in the higher calorific value. These differences need to be taken into account when milk formulae are prepared for human babies using milk from other species.

In India, buffalo's and cow's milk accounts for 96 per cent of the total production of milk. Therefore, the discussion will be restricted to these only.

Milk contains proteins of high biological value, which can support life through the first critical six months. Milk proteins contain certain essential amino acids, which supplements those of proteins from cereals. Milk proteins are easily digested to the extent of 97–98 per cent.

Milk fat is in an emulsified form and hence is readily utilised in the body. It is liquid at body temperature, therefore, it is digested quickly.

Lactose, the only sugar in milk contains galactose, which is essential for brain development Lactose is the least fermentable sugar. Lactic acid is formed on fermentation of lactose. Lactic acid formed, provides acidity in the intestinal tract, which facilitates absorption of calcium and phosphorus. It provides a substrate for lactic acid bacteria, and tends to supress putrefactive bacteria in the intestine.

Milk is an excellent source of calcium and phosphorus. Thus, in presence of vitamin D in the milk, these minerals can be readily used for bone development.

Milk contains very little iron. Therefore, infants need supplements of iron rich foods by the fifth month, when the prenatal store of iron is used up.

All vitamins known to be essential for human nutrition are present in milk. Milk is an excellent source of riboflavin, a vitamin of the B-complex group. It is low in niacin, but a good source of tryptophan, an amino acid, which acts as a precursor of niacin.

Milk contains only about 2 mg ascorbic acid per 100 g. As milk is used only after boiling in India, part of the vitamin C is destroyed. Therefore, milk-fed babies need supplements of foods containing ascorbic acid.

Milk is a fair source of vitamin A, as it contains both the vitamin and its precursor, beta carotene. The vitamin content varies with the feed of the animal. All processed milk products from which fat is removed, contain very little vitamin A e.g., skimmed milk powder.

Processing of Milk Milk may be given various treatments like heating, concentrating, drying or altering its pH to obtain a number of different products. The objective of such treatments is to preserve milk and add variety to our meals. This is done by:

(i) exposing milk to high temperatures at which microorganisms are killed,
(ii) binding or reducing the water present in milk and thus making moisture unavailable to micro-organisms,
(iii) by increasing the acidity of milk to a level that does not permit growth of spoilage microorganisms, and yet imparts a desirable sour flavour to the product.

Some of the processing methods employed and the common milk products available are discussed below.

Pasteurization of milk is a process which consists of heating milk to a certain temperature, for a definite time to ensure destruction of pathogenic bacteria, which are likely to be present.

There are three methods used to pasteurize milk.

Holding Method Milk is heated to 62.7°C and held at that temperature for 30 minutes.

Higher Temperature Short Time Method (HTST)—Milk is heated to 71.6°C at least for 15 seconds.

Ultra High Temperature Process Milk is heated to a temperature of 89–90°C or more for 1 second or less.

In all three processes, milk is immediately cooled to 10°C or lower and held at that temperature. Since cold storage facilities are not commonly available in most homes, milk is routinely boiled prior to use in India, which results in improving the shelf-life of milk. Therefore, pasteurization does not have the same significance here from the health point of view as in the western countries.

The purpose of pasteurization in India is to increase the keeping quality so that the additional time required to deliver it from the plant to the various parts of large cities does not result in its spoilage. Even then, the consumer boils the milk when received to ensure its keeping quality for a day.

The constituents of milk are not altered to any great extent, during pasteurization, since the temperatures used are not very high. If pasteurization is well controlled, the cream layer is not much affected.

Concentrated Milks These include *khoa*, evaporated milk, sweetened condensed milk and dried milk made from both whole and skimmed milk. Varying amounts of water are removed to make the concentrated milk products. These products have a longer shelf-life and some can be reconstituted to their original form.

When milk is heated to concentrate it by evaporating part of the moisture, colour of the milk changes to light brown. There is a change in aroma and flavour also. Milk is concentrated for preparing *basundi*, *rasamalai* etc.

Evaporated Milk is made by evaporating more than half the water from milk under vacuum. It is necessary to fore-warm milk 10 minutes to prevent coagulation of casein during sterilization after it is canned. Colour and flavour are best preserved if sterilization is carried out at high temperature for a short time.

Condensed Milk is concentrated to about 1/3 of its original volume and has about 15 per cent sugar added to the milk. Since the finished product contains at least 40 per cent of sugar, which acts as a preservative, it is not necessary to sterilize it before canning. Indian standards require 31 per cent total milk solids and 9 per cent fat in sweetened condensed milk.

Homogenization Homogenization is carried out by forcing milk through small openings under high pressure. Homogenization reduces the size of fat globules in milk and thus prevents their separation. This treatment increases the stability of the emulsion, as the cream does not separate on standing.

Dehydrated Milk Products include whole milk powder, skimmed milk powder, infant milk foods and malted milk.

Dried milk products are manufactured by two methods.

(i) Roller or drum drying in which the milk is sprayed on the surface of the heated metal cylinders in vacuum.
(ii) and spray drying in which the partially evaporated milk is sprayed into a chamber of heated air.

Indian Standards require that the moisture content of the dried milk be less than 4 per cent. Therefore, dehydrated milk is a concentrated source of protein, calcium and riboflavin and other nutrients.

Skimmed Milk Powder In production of skimmed milk powder, the milk fat is removed or skimmed from the milk, before the milk is dehydrated to a moisture content less than 4 per cent. It has therefore lower energy value, higher protein, calcium and riboflavin content as compared to dried whole milk and is devoid of vitamin A. The Indian Standards require that dried skimmed milk must contain less than 0.5 per cent fat and 96.5 per cent total solids.

Uses of Milk Powder When fresh milk or curd is unavailable, milk or curds can be prepared from milk powder. It is also used in various biscuit, chocolates, halwas, gulab jamuns. Milk powder is very handy and can be used in the place of milk during emergency.

Infant Milk Foods are fortified[1] with varying amount of certain nutrients, such as iron, vitamin C and vitamin D.

1. Fortification consists of addition of certain nutrients to the food to improve its nutritional value.

Malted Milk is made by combining the liquor of a mash, made up of ground barley and wheat flour with whole milk and drying the moisture; some of the malted milks are flavoured with chocolate. These are used to prepare beverages.

Khoa is made from milk by concentrating it in a broad, open pan till the desired consistency is reached. About 2/3 to 3/4 of the water is removed. It has a shelf-life of about *two to four days*; depending upon the atmospheric temperature. It can be preserved for a longer period if sugar is added, or if it is stored at low temperature. It is not produced on a commercial scale. It is mainly prepared to utilize unsold milk in the shops vending milk. These shops make milk candies, such as *pedhas*, *barfis* and *gulab jamun* from *khoa*. All the nutrients in milk are present in *khoa* in a concentrated form. Only a fraction of the thiamin which is heat labile may be lost due to the heating process.

Care of Milk in the Home

Fresh clean milk has a delicious rich taste. It is important to ensure that the taste of milk is retained during production and storage. It is very necessary to receive and store milk in a clean container in the home. Raw milk sours on storage due to the action of lactic acid producing bacteria, especially in summer months, when the atmospheric temperature is high. Milk should therefore be boiled as soon as it is received in the home to prevent spoilage. Further, it should be covered and stored in a cool place. If there is a refrigerator in the house, milk should be stored in the refrigerator after boiling and cooling. Never mix stale milk with a fresh lot of milk, as it hastens spoilage. In the refrigerator, milk must be stored covered and kept away from strong smelling foods, such as cut onion or other foods, as it absorbs their strong flavour.

Effect of Heat on Milk

In India, milk is boiled prior to use. A number of changes occur in the milk due to heat, the extent of changes depend on the temperature and period of heating. The changes affect the colour, flavour and viscosity of milk.

Colour changes occur when milk is heated. A light brown tinge develops when milk is concentrated by heat. The brown colour is due to the reaction of milk protein with reducing sugars such as lactose, glucose and fructose. A change of aroma and flavour accompanies the colour change. This change is very much liked by Indians.

The dispersion of calcium phosphate in milk decreases, when milk is heated and a part of it is precipitated at the bottom along with the coagulated lactalbumin. Volatile elements such as iodine, tend to be lost when milk is heated.

Scum Formation

Scum forms when milk is heated. As the temperature of heating increases, a scum forms at the top, which can be removed. But as soon as it is removed, another layer of scum forms. The milk boils over due to the scum formed. The scum is a tough, leathery, insoluble layer. The scum is forced

upwards due to the pressure built up under it and the milk flows out of the pan under this pressure. The tendency to boil over is increased when the pan is covered during heating of milk. Scum consists of a mesh of coagulated albumin, mineral salts such as calcium phosphate and fat globules.

It is necessary to prevent scum formation in preparation of milk recipes. This is achieved by agitating or stirring with a rotary motion during heating of milk. Use of a milk cooker to boil milk, prevents loss of milk due to boiling over.

When milk is heated, it scorches due to the film of coagulated albumin, which collects at the bottom of the pan. This is prevented by heating milk in a double boiler or a milk cooker.

The film of protein, which surrounds the fat globules, breaks on heating and the fat rises to the top. This is cream or *malai*, which can be removed if we wish to reduce the calorie content of milk.

Coagulation of Milk It was mentioned in chapter 2 that coagulation of proteins occurs between 65–90°C. Lactalbumin, a milk protein, begins to coagulate at about 66°C. This is evident from the coagulum that coats the utensil, when milk is heated. The coagulum is flocculent and hence results in the thickening of milk, which is heated for long time. Thus, there is an increase in viscosity of milk. Casein, the major protein of milk is not coagulated by heat *alone*. When the concentration of casein increases due to evaporation of water, the time required to precipitate it decreases. It takes a combination of factors such as heat and acidity to coagulate casein.

Influence of Acids and Salts Milk, which appears normal, and is not acid enough to taste at room temperature may curdle when heated. It is known that increase in acidity hastens the coagulation of milk protein by heat. A very good example of this phenomenon is the preparation of *paneer*, where acid and heat are used to coagulate milk. As you are aware *paneer* is prepared by adding lemon juice to boiling milk.

Addition of acid foods, like tomatoes to milk in cooking, may curdle it. The coagulation of milk proteins by heat is affected by the kind and amount of salts present.

In certain preparations, such as cream soups, it is necessary to prevent the curdling of milk, to get an acceptable product.

Curds (Yoghurt) Curds is a very popular milk product in India. It ranks second to milk in the extent of consumption.

Milk is boiled and cooled to about 50°C and a teaspoon of curd from an earlier batch of curd is added and mixed thoroughly. The lactic acid bacteria present in this sample of curd curdle the milk. They utilize the lactose in milk and break it down to lactic acid. The formation of lactic acid increases the acidity of milk. When the pH reaches 4.6, the milk protein, casein, coagulates as curd. Since the process is gradual, the milk serum is bound in the mass of coagulated proteins.

The optimum temperature for the formation of curd is 35–40°C. The time needed for curd formation varies from 8–12 hours depending on atmospheric temperature. Once made, curd keeps well at refrigerator temperature of 2–3 days. It is used as dressing on salads made from fresh vegetables. It combines well with plain cooked rice (Fig. 10.1).

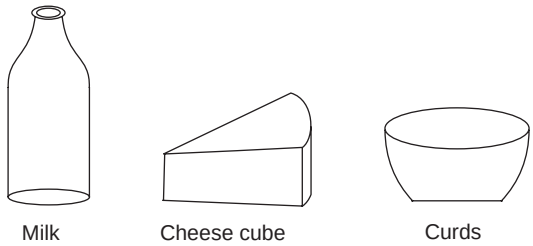


Fig. 10.1 Milk and Milk Products

Preparation of curd is one method of prolonging the shelf-life of milk by several days. Soured milk does not readily undergo proteolysis and other undesirable changes. Curds can be held at refrigerator temperature for several days without loss of acceptability. It is reported that the riboflavin and thiamin content increases during curd formation. It is also reported that fermented milk inhibits the growth of *Bacillus typhosus, Bacillus dysenteriae*, and *Vibrio cholerae* to a certain extent.

Paneer is prepared by addition of lemon juice or citric acid to hot milk and precipitating the casein. The liquid released in this process is known as *whey*, which contains most of the soluble nutrients from milk. Paneer contains about 18 per cent protein and is a good source of it.

Cheese The milk is subjected to several process steps to produce cheese. The milk it held at about 27°C in vats and a lactic acid culture is added. When the milk gets acidic, rennet is added to it and the milk is allowed to coagulate. The curd formed is cut into strips and heated to about 37°C with stirring to separate the whey. The whey is drained. Salt is mixed with the curd and it is pressed to remove further amounts of whey. The cheese formed is coated with paraffin to prevent loss of moisture and allowed to ripen at low temperature.

Cheese contain about 24 per cent protein and is thus a concentrated source of protein.

Buttermilk Buttermilk is made by adding water to curd and churning to remove fat in the form of butter. The energy value is thus reduced due to fat removal. The concentration of the other nutrients is reduced in proportion to the dilution.

It is known to re-establish intestinal flora after an attack of diarrhoea.

Chakka Curd is tied up in a muslin cloth. All the water is allowed to drain out by hanging it from a hook for about 4–6 hours. The solid mass that remains in the muslin cloth is known as *chakka*. It is used for making *shreekhand*.

Eggs

Eggs are a good and an important source of protein in the human diet. Eggs of all birds may be eaten, but in India, eggs of hen and duck are mainly utilised for human consumption.

14.3.5.3 Applications

The cold test is a measure of success of the winterizing process. It ensures that oils remain clear even when stored at refrigerated temperatures.

14.3.6 Cloud Point

14.3.6.1 Principle

The **cloud point** is the temperature at which a cloud is formed in a liquid fat due to the beginning of crystallization.

14.3.6.2 Procedure

The sample is heated to 130°C and then cooled with agitation. The temperature of first crystallization is taken to be the point at which a thermometer in the fat is no longer visible.

14.3.7 Color

Two methods for measuring the color of fats and oils are the **Lovibond** method and the **spectrophotometric** method.

14.3.7.1 Procedure

In the Lovibond method, oil is placed in a standard sized glass cell and visually compared with red, yellow, blue, and neutral color standards. Results are expressed in terms of the numbers associated with the color standards. Automated colorimeters are available.

For the spectrophotometric method, the sample is brought to 25–30°C, placed in a cuvette, and absorbance read at the following wavelengths: 460, 550, 620, and 670 nm. The photometric color index is calculated as shown in Equation [1].

$$\text{Photometric color index} = 1.29(A_{460}) + 69.7(A_{550}) + 41.2(A_{620}) \quad 56.4(A_{670}) \qquad [1]$$

14.3.7.2 Applications

The color of fats and oils is most commonly evaluated using the Lovibond method. The spectrophotometric method (AOCS Method Cc 13c-50) is undergoing revision. Oils and fats from different sources vary in color. But if refined oil is darker than expected, it is indicative of improper refinement or abuse (13).

Though specifically developed for testing the color of cottonseed, soybean, and peanut oils, the spectrophotometric method is probably applicable to other fats and oils as well.

14.3.8 Iodine Value

14.3.8.1 Principle

The iodine value (or iodine number) is a measure of degree of unsaturation, that is, the number of carbon–carbon double bonds in relation to the amount of fat or oil. **Iodine value** is defined as the grams of iodine absorbed per 100-g sample. The higher the amount of unsaturation, the more iodine is absorbed; therefore, the higher the iodine value, the greater the degree of unsaturation.

A common practice is to determine **calculated iodine value** from the fatty acid composition (see section 14.6.1) using AOCS Recommended Practice Cd 1c-85. The calculated iodine value is not meant to be a rapid method, but instead gives two results (iodine value of triacylglycerols and FFAs) from one analysis (fatty acid composition).

14.3.8.2 Procedure

A quantity of fat or oil dissolved in solvent is reacted with a measured amount of iodine or some other halogen. Halogen addition to double bonds takes place (Equation [2]). A solution of potassium iodide is added to reduce excess ICl to free iodine (Equation [3]). The liberated iodine is then titrated with a standardized solution of sodium thiosulfate using a starch indicator (Equation [4]), and the iodine value is calculated (Equation [5]).

$$\underset{\text{(excess)}}{\text{ICl}} + \text{R—CH} = \text{CH—R} \rightarrow \text{R—CHI—CHCl—R} + \underset{\text{(remaining)}}{\text{ICl}} \qquad [2]$$

$$\text{ICl} + 2\text{KI} \rightarrow \text{KCl} + \text{KI} + \text{I}_2 \qquad [3]$$

$$\text{I}_2 + \underset{\text{(blue)}}{\text{starch}} + 2\text{Na}_2\text{S}_2\text{O}_3 \longrightarrow 2\text{NaI} + \underset{\text{(colorless)}}{\text{starch}} + \text{Na}_2\text{S}_4\text{O}_6 \qquad [4]$$

$$\text{Iodine value} = \frac{(B - S) \times N \times 126.9}{W} \times 100 \qquad [5]$$

where:

Iodine value = g iodine absorbed per 100 g of sample
B = volume of titrant (ml) for blank
S = volume of titrant (ml) for sample
N = normality of $Na_2S_2O_3$ (mol/1000 ml)
126.9 = MW of iodine (g/mol)
W = sample mass (g)

Calculated iodine value is obtained from fatty acid composition using Equation [6] for triacylglycerols.

A similar equation allows calculation of the iodine value of FFAs.

$$\begin{aligned}\text{Iodine value (triglycerides)} &= (\%\ \text{hexadecenoic acid} \times 0.950) \\ &+ (\%\ \text{octadecenoic acid} \times 0.860) \\ &+ (\%\ \text{octadecadienoic acid} \times 1.732) \\ &+ (\%\ \text{octadecatrienoic acid} \times 2.616) \\ &+ (\%\ \text{eicosenoic acid} \times 0.785) \\ &+ (\%\ \text{docosenoic acid} \times 0.723) \qquad [6]\end{aligned}$$

14.3.8.3 Applications

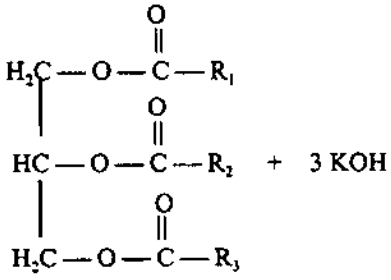
Iodine value is used to characterize oils, to follow the hydrogenation process in refining, and as an indication of lipid oxidation, since there is a decline in unsaturation during oxidation.

The calculated value tends to be low for materials with a low iodine value and for oils with greater than 0.5% unsaponifiable material (e.g., fish oils).

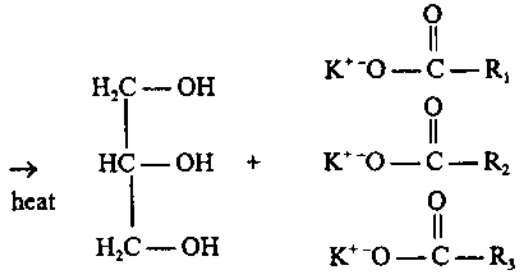
14.3.9 Saponification Value

14.3.9.1 Principle

Saponification is the process of breaking down or degrading a neutral fat into glycerol and fatty acids by treatment of the fat with alkali (Equation [7]).



triacylglycerol



glycerol

potassium salt of fatty acids

[7]

The **saponification value** (or saponification number) is defined as the amount of alkali necessary to saponify a given quantity of fat or oil. It is expressed as the milligrams of KOH required to saponify 1 g of the sample. The saponification value is an index of the mean molecular weight of the triacylglycerols in the sample. The mean molecular weight of the triacylglycerols may be divided by 3 to give an approximate mean molecular weight for the fatty acids present; the smaller the saponification value, the longer the average fatty acid chain length.

In common practice, the **calculated saponification value** is determined from the fatty acid composition (see section 14.6.2) using AOCS Recommended Practice CD 3a-94.

14.3.9.2 Procedure

Excess alcoholic potassium hydroxide is added to the sample and the solution is heated to saponify the fat (Equation [7]). The unreacted potassium hydroxide is back-titrated with standardized HCl using phenolphthalein as the indicator and the saponification value is calculated (Equation [8]).

$$\text{Saponification value} = \frac{(B - S) \times N \times 56.1}{W} \qquad [8]$$

where:

Saponification value = mg KOH per g of sample
B = volume of titrant (ml) for blank
S = volume of titrant (ml) for sample
N = normality of HCl (mmol/ ml)
56.1 = MW of KOH (mg/mmol)
W = sample mass (g)

The **calculated saponification value** is obtained from fatty acid composition using Equation [9]. The **fractional molecular weight** of each fatty acid in the sample must be determined first by multiplying the fatty acid percentage (divided by 100) by its molecular weight. The **mean molecular weight** is the sum of the fractional weights of all the fatty acids in the sample.

$$\text{Calculated saponification value} = \frac{3 \times 56.1 \times 1000}{(\text{mean molecular weight} \times 3) + 92.09 - (3 \times 18)} \qquad [9]$$

where:

Calculated saponification value = mg KOH per g of sample
3 = number of fatty acids per triacylglycerol
56.1 = MW of KOH (g/mol)
1000 = conversion of units (mg/g)
92.09 = MW of glycerol (g/mol)
18 = MW of water (g/mol)

assay is of value because the addition of synthetic D/L malic acid can be used to illegally increase the acid content of apple juice and apple juice products.

16.3.2 Enzyme Activity Assays

16.3.2.1 Peroxidase Activity

Peroxidase is found in most plant materials and is reasonably stable to heat. A heat treatment that will destroy all peroxidase activity in a plant material is usually considered to be more than adequate to destroy other enzymes and most microbes present. In vegetable processing, therefore, the adequacy of the blanching process can be monitored by following the disappearance of peroxidase activity (9). Peroxidase catalyzes the oxidation of guaiacol (colorless) in the presence of hydrogen peroxide to form tetraguaiacol (yellow brown) and water (Equation [37]). Tetraguaiacol has an absorbance maximum around 450 nm. Increase in absorbance at 450 nm can be used to determine the activity of peroxidase in the reaction mixture.

$$H_2O_2 + \text{guaiacol} \xrightarrow{\text{peroxidase}} \underset{\text{(colored)}}{\text{tetraguaiacol}} + H_2O \qquad [37]$$

16.3.2.2 Lipoxygenase

Recently it has been pointed out that lipoxygenase may be a more appropriate enzyme to measure the adequacy of blanching of vegetables than peroxidase (10). Lipoxygenase refers to a group of enzymes that catalyzes the oxidation by molecular oxygen of fatty acids containing a *cis, cis,* 1,4-pentadiene system producing conjugated hydroperoxide derivatives:

$$(\text{—CH=CH—CH}_2\text{—CH=CH—}) + O_2 \xrightarrow{\text{lipoxygenase}} (\text{—C(OOH)—CH=CH—CH=CH—}) \quad \text{(conjugated)} \qquad [38]$$

A variety of methods can be used to measure lipoxygenase activity in plant extracts. The reaction can be followed by measuring loss of fatty acid substrate, oxygen uptake, occurrence of the conjugated diene at 234 nm, or the oxidation of a cosubstrate such as carotene (11). All these methods have been used, and each has its advantages. The oxygen electrode method is widely used and replaces the more cumbersome manometric method. The electrode method is rapid and sensitive and gives continuous recording. It is normally the method of choice for crude extracts, but secondary reactions involving oxidation must be corrected for or eliminated. Zhang et al. (12) have reported the adaptation of the O_2 electrode method to the assay of lipoxygenase in green bean homogenates without extraction. Due to the rapidity of the method (<3 min including the homogenization), on-line process control using lipoxygenase activity as a control parameter for optimization of blanching of green beans is a real possibility. The formation of conjugated diene fatty acids with a chromophore at 234 nm can be followed continuously. However, optically clear mixtures are necessary. Bleaching of carotenoids has also been used as a measure of lipoxygenase activity. However, the stoichiometry of this method is uncertain, and all lipoxygenases do not have equal carotenoid bleaching activity. Williams et al. (10) have developed a semiquantitative spot test assay for lipoxygenase in which I^- is oxidized to I_2 in the presence of the linoleic acid hydroperoxide product and the I_2 detected as an iodine starch complex.

16.3.2.3 Phosphatase Assay

Alkaline phosphatase is a relatively heat stable enzyme found in raw milk. The thermal stability of alkaline phosphatase in milk is greater than the non-spore forming microbial pathogens present in milk. The phosphatase assay has been applied to dairy products to determine whether pasteurization has been done properly and to detect the addition of raw milk to pasteurized milk. A common phosphatase test is based on the phosphatase-catalyzed hydrolysis of disodium phenyl phosphate liberating phenol (13). The phenol product is measured colorimetrically after reaction with CQC (2,6-dichloroquinonechloroimide) to form a blue indophenol. The indophenol is extracted into n-butanol and measured at 650 nm. This is an example of a physical separation of product to allow the ready measurement of an enzyme reaction. More recently, a rapid fluorometric assay was developed and commercialized for measurement of alkaline phosphatase in which the rate of fluorophore production can be monitored directly without butanol extraction used to measure indophenol when phenylphosphate is used as substrate (14). The fluorometric assay was shown to give greater repeatability compared to the standard assay in which phenylphosphate is used as substrate and was capable of detecting 0.05% raw milk in a pasteurized milk sample. Similar chemistry has been applied to the measurement of acid phosphatase activity in meats as a means of ensuring adequate cooking via correlation of enzyme activity to endpoint temperature (21).

16.3.2.4 α-Amylase Activity

Amylase activity in malt is a critical quality parameter. The amylase activity in malt is often referred to as

diastatic power and refers to the production of reducing substances by the action of α- and β-amylases on starch. The measurement of diastatic power involves digestion of soluble starch with a malt infusion (extract) and following increase in reducing substances by measuring reduction of Fehling's solution or ferricyanide. Specifically measuring α-amylase activity (often referred to as **dextrinizing activity**) in malt is more complicated and is based on using a limit dextrin as substrate. **Limit dextrin** is prepared by action of β-amylase (free of α-amylase activity) on soluble starch. The β-amylase clips maltose units off the nonreducing end of the starch molecule until an α-1,6- branch point is encountered. The resulting product is a β-limit dextrin that serves as the substrate for the endo cleaving α-amylase. A malt infusion is added to the previously prepared limit dextrin substrate and aliquots removed periodically to a solution of dilute iodine. The α-amylase activity is measured by changed color of the starch iodine complex in the presence of excess β-amylase used to prepare the limit dextrin. The color is compared to a colored disc on a comparator. This is continued until the color is matched to a color on a comparator. The time to reach that color is **dextrinizing time** and is a measure of α-amylase activity, a shorter time representing a more active preparation.

Because α-amylase is an endoenzyme, when it acts on a starch paste the viscosity of the paste is dramatically reduced, greatly influencing flour quality. Consequently, α-amylase activity is of great importance in whole wheat. Wheat normally has small amounts of α-amylase activity, but when wetted in the field, preharvest sprouting (pregermination) can occur in wheat, with a dramatic increase in α-amylase activity. Preharvest sprouting cannot be easily detected visually, so measurement of α-amylase activity can be used as a sensitive estimate of preharvest sprouting. The **falling number method** is a procedure in which ground wheat is heated with water to form a paste, and the time it takes for a plunger to fall through the paste is recorded (15). Accordingly, the time in seconds (the falling number) is inversely related to the α-amylase activity and the degree of preharvest sprouting. This method of measuring enzyme activity is a good example of using change in physical property of a substrate as a means of estimation of enzyme activity.

16.3.2.5 Rennet Activity

Rennet, an extract of bovine stomach, is used as a coagulating agent in cheese manufacture. Most rennet activity tests are based on noting the ability of a preparation to coagulate milk. For example, 12% nonfat dry milk is dispersed in a 10 m*M* calcium chloride solution and warmed to 35°C. An aliquot of the rennet preparation is added and the time of milk clotting observed visually. The activity of the preparation is calculated in relationship to a standard rennet. As opposed to coagulation ability, rennet preparations can also be evaluated for proteolytic activity by measuring the release of a dye from azocasein (casein to which a dye has been covalently attached). In this assay, the rennet preparation is incubated with 1% azocasein. After the reaction period, the reaction is stopped by addition of trichloroacetic acid. The trichloroacetic acid precipitates the protein that is not hydrolyzed. The small fragments of colored azocasein produced by the hydrolysis of the rennet are left in solution and absorbance read at 345 nm (16, 17). This assay is based on the increase in solubility of a substrate upon cleavage by an enzyme.

16.3.3 Biosensors/Immobilized Enzymes

The use of immobilized enzymes as analytical tools is currently receiving increased attention. An immobilized enzyme in concert with a sensing device is an example of a biosensor. A biosensor is a device comprised of a biological sensing element (e.g., enzyme, antibody, etc.) coupled to a suitable transducer (e.g., optical, electrochemical, etc.). Immobilized enzymes, because of their stability and ease of removal from the reaction, can be used repeatedly, thus eliminating a major cost in enzyme assays. The most widely used enzyme electrode is the glucose electrode in which glucose oxidase is combined with an oxygen electrode to determine glucose concentration (18). When the electrode is put into a glucose solution, the glucose diffuses into the membrane where it is converted to gluconolactone by glucose oxidase with the uptake of oxygen. The oxygen uptake is a measure of the glucose concentration. Glucose can also be measured by the action of glucose oxidase with the detection of hydrogen peroxide, in which the hydrogen peroxide is detected amperometrically at a polarized electrode (19). A large number of enzyme electrodes (biosensors) have been reported in the literature recently. For example, a glycerol sensor, in which glycerol dehydrogenase was immobilized, has been developed for the determination of glycerol in wine (20). NADH produced by the enzyme was monitored with a platinum electrode.

16.4 SUMMARY

Enzymes, due to their specificity and sensitivity, are valuable analytical devices for quantitating compounds that are enzyme substrates, activators, or inhibitors. In enzyme-catalyzed reactions, the enzyme and substrate are mixed under specific conditions (pH, temperature, ionic strength, substrate concentration, and enzyme concentrations). Changes in these conditions can affect the reaction rate of the enzyme and thereby the outcome of the assay. The enzymatic