The data of Nilsson and Willart (1960) indicate that heating at 80°C for 20 sec is sufficient to destroy all lipases in normal milk. Their studies included assays after 48 hr of incubation following heat treatment. At lower temperatures for 20 sec, some lipolysis was detected after the 48-hr incubation period after heating. Thus, 10% residual activity remained at 73°C. Below the temperature of 68°C the amount of residual activity was enough to render the milk rancid in 3 hr; temperatures below 60°C had no appreciable effect on lipolysis. With holding times of 30 min, 40°C produced only slight inactivation, and at 55°C 80% inactivation was reported.

The data of Harper and Gould (1959) are essentially in agreement with those of Nilsson and Willart. These authors also detected no inactivation until a temperature of 60°C for 17.6 sec was reached. At 87.7°C (17.6 sec) some lipase still survived.

Shipe and Senyk (1981) reinvestigated the effects of various pasteurization times and temperatures on lipolysis (Table 5.2). They concluded that processing at 76.7°C for 16 sec should be sufficient to protect most milks from lipolysis problems for 7 days after pasteurization.

Fat apparently protects the lipases to some extent from heat inactivation, 1° to 2°C higher temperatures being necessary for whole milk than for skim milk (Frankel and Tarassuk 1959; Harper and Gould 1959; Nilsson and Willart, 1960; Saito *et al.* 1970).

Harper and Gould (1959) indicate that besides the protective effect of fat on lipase inactivation, the solids-not-fat content is also a factor. A higher solids-not-fat concentration, within limits, affords some protection.

***Inhibition by Light and Ionizing Irradiation.*** The milk lipoprotein

**Table 5.2. Effect of Pasteurization Time and Temperature on Lipolysis[a, b]**

| Temperature[c] (°C) | Holding Time[d] | | |
|---|---|---|---|
| | 16 s | 20 s | 24 s |
| 72.2 | 2.1 | 1.7 | 1.3 |
| 74.4 | 1.0 | 1.0 | 0.9 |
| 76.7 | 0.9 | 0.9 | 0.9 |
| 78.9 | 0.9 | 0.8 | 0.8 |
| 81.1 | 0.8 | 0.8 | 0.8 |

[a] Shipe and Senyk (1981).
[b] Average ADVs for six pasteurized-homogenized milk samples after storage for 7 days at 5°C. Average raw milk ADV was 0.7.
[c] ADV means for 72.2°C significantly different from all others ($P<0.01$); values for 74.4°C differed from 81.1°C ($P < 0.05$); no significant differences between others.
[d] ADVs for different holding times were different ($P < 0.05$) at temperatures below 74.4°C.

certain set of conditions and may or may not hold when these conditions are changed.

The apparent temperature optimum for the milk lipase system is reported to be around 37°C both on milk fat and on tributyrin (Frankel and Tarassuk 1956A; Roahen and Sommer 1940). This temperature has been recorded both at pH 8.9 and pH 6.6 for milk fat (Frankel and Tarassuk 1956A) and at pH 8.0 and pH 6.6 at tributyrin (Frankel and Tarassuk 1956A). Although the enzyme appears to be most active at 37°C, activity is rapidly lost at this temperature (Egelrud and Olivecrona 1973). Studying the effect of temperature on the activity of lipases of psychrotrophs, Driessen and Stadhouders (1974B) observed two optimum temperatures: a relative optimum at 37°C and an absolute optimum at 50°C; Kishonti (1975) found two temperature optima at 30 and 55°C.

***Stability.*** Some discussion regarding stability of milk lipases was presented in the preceding section. Egelrud and Olivecrona (1973) found that the enzyme fractions from heparin-Sepharose can be stored frozen at -20°C with less than 10% loss of activity in 2 weeks. The purified enzyme had only moderate stability at 4°C; high concentrations of salt or a pH below 6.5 or above 8.5 increases the rate of inactivation.

**Some Effects of Lipolysis.** The most serious effect of lipolysis is the appearance of the so-called rancid flavor which becomes detectable in milk when the ADV exceeds 1.2–1.5 mEq/liter (Brathen 1980). The fatty acids and their soaps, which are thought to be implicated in the rancid flavor, have been studied in an effort to assess the role of the individual acids in the overall rancid flavor picture. Scanlan *et al.* (1965) reported that only the even-numbered fatty acids from C4 to C12 account for the contribution of fatty acids to the flavor, but that no single acid exerts a predominating influence. Another study has implicated the sodium and/or calcium salts of capric and lauric acids as major contributors to the rancid flavor (Al-Shabibi, *et al.* 1964). Butyric acid, assumed to be the compound most intimately associated with the flavor, was not singled out in either study as being especially involved.

Besides changing the natural flavor of milk, lipolysis may produce a variety of other effects. One of the most noticeable of these is the lowering of surface tension as lipolysis proceeds (Schwartz 1974). Fatty acids, especially their salts, and mono- and diglycerides, being good surface-active agents, depress the surface tension of milk (see the discussion "Methods for Determining Lipase Activity"). Milk fat ob-

Dunkley 1959A) and neocuproine (Smith and Dunkley 1962B), among others (Samuelsson 1967), have also proven their effectiveness as inhibitors of autoxidation.

## Oxidative Deterioration in Fluid Milk

Fluid milks have been classified by Thurston (1937) into three categories based on their ability to undergo oxidative deterioration: (1) spontaneous, for those milks that spontaneously develop off-flavor within 48 hr after milking; (2) susceptible, for those milks that develop off-flavor within 48 hr after contamination with cupric ion; and (3) resistant, for those milks that exhibit no flavor defect, even after contamination with copper and storage for 48 hr. A similar classification has been employed by Dunkley and Franke (1967).

With the advent of noncorrodible dairy equipment, oxidative deterioration in fluid milk as a result of copper contamination has decreased significantly, although it has not been completely eliminated (Rogers and Pont 1965). However, the incidence of spontaneous oxidation remains a major problem of the dairy industry. For example, Bruhn and Franke (1971) have shown that 38% of samples produced in the Los Angeles milkshed are susceptible to spontaneous oxidation; Potter and Hankinson (1960) have reported that 23.1% of almost 3000 samples tasted were criticized for oxidized flavor after 24 to 48 hr of storage. Significantly, certain animals consistently produce milk which develops oxidized flavor spontaneously, others occasionally, and still others not at all (Parks *et al.* 1963). Differences have been observed in milk from the different quarters of the same animal (Lea *et al.* 1943).

The resistance of certain milks to oxidation, even in the presence of added copper, may be attributed to its poising action, i.e., the resistance of milk to a change in the oxidation-reduction potential (Parks 1974). That a correlation exists between the appearance of an oxidized flavor and conditions favoring milk oxidation, as measured by the oxidation-reduction potential, was shown by several researchers (Parks 1974). This apparent correlation, as well as other factors, tend to discredit theories on the role of enzymes as catalytic agents in the development of oxidized flavor. Xanthine oxidase has been proposed as the catalytic agent in the development of spontaneously oxidized milk (Astrup 1963; Aurand and Woods 1959; Aurand *et al.* 1967, 1977). The studies of Smith and Dunkley (1960), among others (Rajan *et al.* 1962), do not corroborate these findings, and the authors conclude that xanthine oxidase is itself not a limiting factor in the off-flavor. However, reports persist on the involvement of enzymes in the generation of various types of oxygen that may be involved in the autoxidation of milk

Other studies suggest that the preponderance of certain carbonyls or group of carbonyls is involved in the off-flavors of various dairy products. Forss *et al.* (1955A,B) reported that the $C_6$ to $C_{11}$ 2-enals and the $C_6$ to $C_{11}$ 2,4-dienals—and, more specifically, 2-octenal, 2-nonenal, 2,4-heptadienal, and 2,4-nonadienal—constitute a basic and characteristic factor in the copper-induced cardboard flavor in skim milk. The same workers concluded that "while these compounds in milk closely simulate the cardboard flavor, the resemblance is not complete" and that "the defect contains further subsidiary flavor elements."

Bassette and Keeney (1960) ascribed the cereal-type flavor in dry skim milk to a homologous series of saturated aldehydes resulting from lipid oxidation in conjunction with products of the browning reaction. The results of Parks and Patton (1961) suggest that saturated and unsaturated aldehydes at levels near threshold may impart an off-flavor suggestive of staleness in dry whole milk. Wishner and Keeney (1963) concluded from studies on milk exposed to sunlight that $C_6$ to $C_{11}$ alk-2-enals are important contributors to the oxidized flavor in this product. Parks *et al.* (1963) concluded, as a result of quantitative carbonyl analysis and flavor studies, that alk-2-4-dienals, especially 2,4-decadienal, constitute a major portion of the off-flavor associated with spontaneously oxidized fluid milk. Forss *et al.* (1960A,B) reported that the fishy flavor in butterfat and washed cream is in reality a mixture of an oily fraction and 1-octene-3-one, the compound responsible for the metallic flavor. *n*-Heptanal, *n*-hexanal, and 2-hexanal were found to be constituents of the oily fraction in washed cream, and these three carbonyls plus heptanone-2 were constituents of the oily fraction isolated from fishy butterfat. Badings (1970) identified 40 volatile compounds in cold storage cultured butter which had a trainy (fishy) off-flavor. Included among the 14 compounds which were present at above-threshold levels were 4-*cis*-heptenal; 2-*trans*, 4-*cis*-decadienal; 2-*trans*, 6-*cis*-nonadienal; 2,2,7-decatrienal; 3-*trans*,5-*cis*-octadien-2-one; 1-octene-3-one; and 1-octen-3-ol. Keen *et al.* (1976) reported on the carbonyls in ultra-high-temperature milk. They found acetaldehyde, hexanal, heptanal, octanal, and decanal in this milk but not in the control and ascribed the presence of these aldehydes and the higher concentration of nonanal in the ultra-high-temperature milk to the heat treatment.

Comparative studies by Forss and co-workers (1960A,C) on the fishy, tallowy, and painty flavors of butterfat tended to emphasize the importance of the relative and total carbonyl contents in dairy products with different off-flavors. These researchers showed that three factors distinguished painty and tallowy butterfat from fishy butterfat. First, there was a relative increase in the *n*-heptanal, *n*-octanal, *n*-non-