MILK FLAVORS

by

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Milk Flavors

INTRODUCTION

Milk is the normal secretion of the mammary gland and is defined by the United States Public Health Service (Pub. 229, 1965) as "the lacteal secretion, practically free from colostrum, obtained by the complete milking of one or more healthy cows, and that it meet the state minimum requirements for butter fat and milk solids not fat". The law does not say that it must be free from objectionable odors or flavors except with respect to adulterating materials such as water, medicines, and pesticides.

Good tasting milk, meets all requirements set forth in the law, and is characterized as having a pleasing, slightly sweet taste with no unpleasant aftertaste.

Off-flavors in milk are a major problem for the dairy industry. Innumerable experiments have been conducted, research reports published, and lectures and talks delivered on causes and corrections of off-flavored milks but problems with milk flavors continue to persist. An individual endeavoring to study off-flavors in milk is confronted with an apparently insurmountable maze of references.

The purpose of this report is to organize this large body of material in a simple and precise manner. It covers flavors of both raw and pasteurized milk, including the effects of feeds, processing and storage conditions and improper handling of milk as well as prevention and control of off-flavor development. Chapters are included covering transmitted, microbial, lipolyzed, heated, light-induced, oxidized, and miscellaneous flavors that include astringency,

foreign odors and flat. Finally, the flavor of ultra high temperature (UHT) milk is reviewed.

Thus, the objective of this report is to emphasize the significance of milk flavors. Numerous research papers and a few review articles point to the complexity of the subject. But with more emphasis being placed on the broader aspects of food science and less on commodity oriented programs at universities, dairy food processing centers of excellence are dwindling. Consequently, current organized material in such specialized topics of milk flavors is not available. This report in addition to its utility with dairy production and processing industry should serve as an important reference for the students and faculty in food science curriculums, FFA leaders, dairy products evaluation students, regulatory people and fieldman.

CHAPTER 1 TRANSMITTED FLAVORS

INTRODUCTION

Off-flavors in milk caused by the transfer of substances from the cow's feed or environment into the milk while it is in the udder are called transmitted flavors. They include flavors commonly described as feed, weed, cowy, and barny. This transfer of substances may be through the respiratory and/or digestive system and blood stream. Transmitted substances are responsible for most of the flavor changes that are encountered in fresh raw milk. As some of these flavors are mild, however, they may not be considered disagreeable by consumers.

The mechanism of flavor transmission was demonstrated at Cornell University in a series of experiments (13,43). Potential flavor producing substances were introduced directly into the lungs or rumen of two cows by means of tracheal and ruminal fistulae. The depth of penetration of eructated gases and the effect of these gases on the flavor of milk was determined by using a tracheal fistula. Air was pumped through a chamber containing odoriferous material into a tubing connected to the tracheal catheter to introduce the odors into the lungs. A T-tube was positioned at the top of the chamber to allow the introduction of odoriferous material into the oxygen or air stream to supplement the air passing into the lungs. Passage of air at the bottom and top of the chamber were adjusted in order to regulate the rate of flow of volatile materials into the lungs. Test substances also were introduced through the ruminal catheter to ascertain whether flavors could enter the milk, while lungs were being supplied with fresh air, through the tracheal catheter. The normal passage of air then was re-established by removing the tracheal catheter to determine, if the eructated gases from the rumen contributed to

off-flavors. Milk samples were taken before, and after the introduction of the test substances and later judged by an experienced panel. Results of one of these studies dealing with the transmission of onion flavors to milk is shown in Table 1 (13).

The above experiments proved that odors from a fresh onion slurry did not contain volatile materials that produce the onion flavor in milk. However, when the slurry was incubated with rumen material the characteristic onion flavor was produced and transmitted to the milk. Apparently the microflora in the rumen or the enzymes present liberated flavor substances from the onion. No odors were transmitted to the milk, inspite of the fact that the ingesta alone was odoriferous. Some flavor was still transmitted when the slurry was placed directly in the rumen while the lungs were supplied with fresh air. This suggested that part of the flavor was transferred through the rumen wall to the blood stream. When the cows were permitted to breath normally more flavor was transmitted. However, under these conditions the flavor substances passing through the rumen wall supplemented the eructated gases that entered the lungs. Nevertheless, when the source of the flavor materials was no longer accessible to the cow and the air she inhaled was devoid of odors, the volatile materials that are accumulated in the udder were returned to the blood stream and exhausted via the lungs.

1. FEED FLAVORS

Feed flavors of milk can be defined as undesirable flavors that are present in freshly drawn milk as a result of some feed taken into the body of the cow by eating or inhalation. They are different from flavors resulting from

Table I. Transmission of flavor from onions to milk (13).

Expt. no.	Sample[a]	Transmission route	Off-flavor intensity
1	Odor from fresh onion slurry	Lungs	None
2	Odor from onion slurry after incubation with rumen ingesta	Lungs	Strong
3	Odor from rumen ingesta	Lungs	None
4	Onion slurry (fresh air in tracheal cannula)	Rumen	Mild
5	Onion slurry (tracheal cannula removed)	Rumen & lungs	Strong

[a]Odors were introduced via the tracheal cannula; slurries were introduced into the ruminal cannula.

decomposition of the milk by microorganism or chemical changes occurring in milk during storage. They also differ from those resulting from handling prior to, or after processing (47). No geographic region is free from the problem of feed flavors but some areas are more affected than others. The type of feed flavors encountered are also different in different regions of the country.

Feed related off-flavors were first reported in 1757 by Bradley (45) who observed that feeding beet and turnip tops and roots produced a bitter flavor in the milk produced in an area near London, England. At the present time nearly two and half centuries, after the statements by Bradley, feed still continues to be one of the main contributing causes of off-flavored milk. They are important to the dairy industry because they curtail the consumption of dairy products (38). It has been reported that 75 % of customer complaints about off-flavors in milk are caused by feeds that are consumed by the cow (8,14). Trout (47) reported that if milk does not have a pleasant flavor or if the flavor is not uniform from day to day, the consumer fails to consume as much as he or she might otherwise. Adults and teenagers select food and drink that they like for this reason. Young children drink milk because it is fed to them. Unfortunately poor quality dairy products may create a dislike for these products in children which will adversely affect milk consumption in the years to come (5). Jensen in 1960 (20) reported that the most prevalent milk-flavor defect was of feed origin and was observed in 73 % of the milk samples analyzed. Although the widely used closed pipeline milking system avoids the incorporation of many feed and barn odors into milk, there is still a problem with flavor defects. In 1963 Shipe reported that 39 % of the samples tasted from 120 dairies in a New York milk flavor survey had feed flavor.

After the earliest recorded reference of feed flavor by Bradley other

observers stated that roughages have a more pronounced effect on the flavor of milk than grain concentrates. Reports from different sources regarding the off-flavors from feed imparted to milk began to appear in the 1890's and early 1900's.

a. Feeds of Economic Value that Impart Objectionable Flavor to Milk.

1. Silage. Silage in general, and corn silage in particular were the first feeds studied in relation to odors and flavors imparted to milk. King (22) in 1897 demonstrated that corn silage fed shortly before milking gave a sweetish odor to the milk. In 1905, Knisely (23) reported taste comparisons of milk from silage-fed cows and non-silage-fed cows. In that study of 372 comparisons, 60 % of those tasting favored milk from the silage-fed cows, 29 % preferred the milk from non-silage-fed cows, and 11 % indicated no preference. He concluded that silage of good quality, was one of the best feeds available for dairy when pasture was not accessible. Gambel and Kelly (17) in 1922 found that silage which was fed one hour before milking was absorbed so quickly that its flavor was distinguishable in the milk. Roadhouse and Henderson (39) in 1935, however, observed that objectionable feed flavors and odors were eliminated when a full ration of corn silage was fed and the feed was withheld during the 5 h interval before milking. In 1956 Owens et al. (32) found stronger feed flavors in the milk when cows were fed from crowded openings in a trench silo rather than from an open manger.

Two important factors relating to the strength of off-flavors in milk due to silage are the moisture content of the silage and the ventilation in the proximity of silage consumed (36). McCormick (27) stated "the greater the moisture content, the stronger the flavor". The logical explanation for this would seem to be that silage has greater possibility for mold growth and

spoilage in high moisture silage. In better quality silage proper acid concentrations inhibits spoilage. Feeding inside closed buildings allows odor to permeate and saturate the atmosphere. These odors are breathed continually, whereas feeding silage outside or in a well ventilated buildings lessen flavors from these surrounding odors (25).

The chemical compounds responsible for off-flavors in milk from cows fed silage have been reported by many researchers. For instance Morgan et al. (29) in a study of volatile constituents of grass and corn silage found mixtures of methyl sulfide, lower aldehydes, ketones, alcohols and simple methyl, ethyl, and propyl esters. They suspected methyl sulfide and esters as principal contributors to the off-flavors; those occurring from the inhalation of silage odor. The same researchers in 1963 found that fresh cut alfalfa hay contains high concentrations of trans-2-hexenal, and 3-hexenals and 3-hexenols which impart a green grassy flavor (30). Potts and Kessler (36) in 1957 reported that there was no apparent relationship between the concentrations of acetone bodies in the milk of cows fed grass silage and the flavor of the milk. More acetone and 2-butanone was found in milk from cows fed silage shortly before milking (24). Shipe et al. (43) reported that introduction of acetone, 2-butanone, methyl sulfide and cis-3-hexene-1-ol through rumen and lungs imparted flavors that closely resembled those that could be found in commercial milk. In 1956 Patton et al. (35) detected methyl sulfide in normal milk and suggested that this might contribute to characteristic flavor of raw milk. The threshold level of methyl sulfide was calculated to be 12 ppb in distilled water. At higher concentration in milk, the flavor was described as malty or cowy. Toan et al. (46) in 1965 reported the threshold level of methyl sulfide in homogenized milk was 115 ppb. This concentration was easily

detected by gas liquid chromatography. The flavor above this threshold was described as methyl sulfide-like, molasses-like or cowy.

Fresh milk also contains trace levels of ammonia, plus propyl-and hexyl amines. Feed flavored milk was found to contain higher concentrations of these amines (7). Also if these two compounds were added to good quality milk a feed like flavor would be produced. Honkanen et al. (19) in 1964 reported that when a series of aliphatic alcohols, aldehydes, ketones, and esters were introduced into the rumen of a cow with an odorless purified diet, only alcohols with odd number carbon atoms, lower ketones and esters were found to enter the milk to such an extent that they gave distinct flavor defects to milk. Aldehydes and some unsaturated alcohol and ketones were transferred to milk in trace amounts or none at all. This group also reported threshold values for some aliphatic alcohols, ketones and esters in milk. Forss (16) in 1979 reported that when protected sunflower seed oil in a basal diet of chopped lucerne hay and crushed oats were fed to cows, the milk had a sweet raspberry-like flavor due to a mixture of γ- dodec-cis-6-enolactone and γ--dodecanolactone. But when the basal diet was fed alone there was a sweet flavor due entirely to the saturated lactone.

Research by Shipe et al. (43) demonstrated the cow's ability to transmit a variety of substances commonly found in feed. A list of volatile materials that were studied and their effect on milk flavor are shown in Table 2. Fortunately, cows were able to prevent some substances such as butyric acid from being transmitted directly into the milk.

2. Rye. Contradictory opinions have been reported in connection with production of off-flavors in milk from cows on rye pasture. Babcock (2) as early as 1925, found that when dairy cows consumed 15 lbs of green rye one

Table II. Transmission of a variety of flavors to milk (43).

Flavor substances	Terms used to describe flavor
Ethanol[a]	sweet, vanilla-like, ester-like
Propanal[a]	Alcohol-like, vanilla-like, duco cement
2-Butanol	Sweet, ester-like, xylene
Acetone[a]	Feedy, cowy, sweet, silage
2-Butanone[a]	Hay-like, sweet, aromatic, cowy
Propanal	No detectable effect
n-Butanal[a]	Malty, chemical, butanal
n-Pentanal	No detectable effect
Butyric acid	"
Propionic acid	"
Methyl acetate	Sweet, ester, grassy
Ethyl acetate	Sweet, fruity, ester, odd
Propyl acetate	Sweet, vanilla-like, feed
Butyl acetate	Banana, ester, malty, odd
Dimethyl sulfide[a]	Weedy,cowy,unclean, onion
Cis-3-hexenol[a]	Grassy, weedy, musty, grass
Green pasture	Grassy, cowy, barny, feedy
Green corn silage	Barny, cowy, feedy, sweet
Grass silage distillate[b]	Fruity, sweet, ketone
Corn silage distillate[b]	Fruity, fermented, aromatic

[a] These substances were introduced by both the lung and rumen routes. The pasture and silage were consumed in the normal manner. The other substances were introduced by the lung route only.

[b] The silage distillates represent the neutral carbonyl-free fraction boiling between 36 and 100 C.

hour before milking, slight abnormal odors and flavors were detected in the milk. If the quantity of rye consumed were increased to 30 lbs, the off-flavors and odors increased. He also reported that feeding 30 lbs of green rye immediately after milking had no effect on the flavor of the milk at the next milking. In 1944, Trout and Harwood (48) observed that Balbo rye pasture did not have the adverse effect on the odors of milk as did common rye. The offensive odor characterstic of milk from cows pastured on common rye at certain growth stages, variously described as neutralizer, soapy or even fishy, was not observed in milk from cows pastured on Balbo rye. The off odor in milk caused by pasturing Balbo rye may be described as slightly grassy and resembling some what the off-flavors in the milk from cows milked soon after removal from blue green pasture. Herman and Garrison (18) at the Missouri Experiment Station, reported a method of grazing cows on Balbo rye and Missouri Early Beardless Barley which did not produce objectionable flavors in milk. Cows were grazed on barley or rye 3 to 4 h daily and the milk was sampled 1 to 2 h after the cows were removed from the pasture. The milk showed slightly different, but not objectionable flavor, when analyzed organoleptically on 18 successive days. The relative flavor scores of the milk were high and it was concluded that neither the Balbo rye nor the Beardless Winter Barley produced undesirable flavors in milk. Studies at Kansas State Agricultural Experiment Station (9,11,28) indicated a strong off-flavor was generally encountered in the milk when cows were pastured on Balbo rye until shortly before milking. This flavor was very objectionable and often described as "fishy". It was less objectionable when the cows were removed from the pasture 4 h before milking.

3. Bromegrass. The effect of grazing cows on alfalfa-bromegrass pasture was reported in 1940, by Trout et al. (49) as to the called "soda", "alkaline" off-flavor in milk. The intense off-flavor was found when cows were grazed heavily and milked 3 times daily. It was found that alfalfa contributed more to the off-flavor than did the bromegrass. When the cows were kept off the pastures 7 h prior to milking no feed flavors were noticeable in the milk. Colson and Bassette (8) studied the effect of sorghum silage, rye pasture and bromegrass pasture on the flavor of the milk. Milks from cows fed the above feeds were evaluated by a trained taste panel and by the percentage of milk returned in accordance with a prearranged evaluation by consumers. These results indicated that rye pasture produced a stronger off-flavor than bromegrass and sorghum silage, whereas sorghum silage produced the least taint in milk. Other researchers (15) found that cows on bromegrass pasture produced milk with an unclean flavor. About 10% of cows pastured on bromegrass developed this undesirable, unclean milk flavor to which customers objected. According to these workers the flavor does not always appear in the milk as it leaves the udder, but may develop after holding 1 or 2 d. After longer grazing the flavor becomes strong and persistent. The defect has been controlled by limited pasturing of bromegrass, supplemental hay feeding and removing cows from the pasture at least 4 h before milking.

2. WEED FLAVORS

Closely associated with feed flavors are weed flavors. Through weed infested pastures or weeds mixed with hay, silage or the concentrate portion of the ration, cows often ingest enough weeds to produce an off-flavor or taint in the milk. Certain weeds impart serious off-flavor to milk. One of the most common and readily recognized of these is wild garlic which was first reported

in 1757. The flavor components from some weeds are relatively non-volatile and are slowly eliminated from the cows body through the lungs. The milk flavor may be affected unless the flavor components are eliminated or otherwise metabolized by a process which may take as long as 12 h.

Babcock (3) reported that garlic flavor and odor were detected in milk, when samples were taken one minute after feeding garlic. The intensity of the garlic flavor and odor increased as the time interval between feeding the garlic and taking the milk samples increased. After 10 min an intense off-flavor was observed. When cows consumed half pound of garlic 4 h before milking, an objectionable flavor still was produced. As the time interval increased beyond this the off-flavor decreased until at 7 h it had practically disappeared from the milk.

MacDonald and Crawford (25) in 1927 reported that the substances responsible for the disagreeable onion flavor and odor in milk were carried almost entirely by the milk fat. They used mineral oil to extract the onion flavor and found that one washing with one part of mineral oil to 10 parts of milk removed most of the odor but that two washings were needed to remove the remainder of the onion odor and taste.

Park (33) reported that off-flavors produced when cows eat plants of Lepidium sps. were due to skatole and indole; with skatole being the major contributor. The primary cause of the flavor in milk produced by cows eating Coronopus or land cress is benzyl methyl sulfide (34). It was generally accepted that this compound is a metabolite of benzyl thiocyanate. Benzyl isothiocyanate, benzyl cyanide, indole and skatole also were found in traces in land cress tainted milk.

Weeds that produce off-flavors in milk and their suspected causative compounds are shown in Table 3 (41).

3. COWY FLAVOR

Josephson and Keeney (21) suggested that cows suffering from ketosis or acetonemia produce milk with "cowy-like" odor. The odor or the breath of the affected cow was found to be similar to that of their milk. In severe cases, the odor may be so strong that it would be transmitted to the milk of neighboring cows, in the presence of inadequate ventilation. The cowy odor was found to be due to ketone bodies released into the blood stream from incomplete metabolism of fat. However, Potts and Kessler (36) reported that acetone in milk also could be affected by acetone in feeds, such as silage etc. But these researchers were unable to find a relationship between the amount of acetone in the milk and intensity of off-flavor. Patton et al. (35) stated that above threshold levels of methyl sulfide also imparts a cowy flavor to milk. Later Toan et al. (45) reported that when commercial milk is contaminated with A aerogenes a cowy-like odor is produced due to the production of methyl sulfide.

4. BARNY FLAVOR

The primary cause of barny flavor is inadequate ventilation. Cows inhale odors of a damp, dirty barns and transmit the off-flavors to milk. However, the nature of barny flavor has not been characterized or distinguished clearly from cowy flavor (6).

CONTROL OF TRANSMITTED FLAVORS

Table III. Different weeds and their suspected causative chemical compounds (41).

Name	Botanical classification	Suspected chemical compounds
a) Land cress	Coronopus didyma Senebiera didyma	Benzyl mercaptan
b) Penny cress French weed or Stink weed	Thalaspe arvense	Allyl isothiocyanate
c) Penny royal	Mentha Pulegium	Pulegone
d) Peppar grass	Lepidium virginicum	Indole
e) onion and garlic	Allium cepa	Di-n-propyl sulfide Isopropyl mercaptan Propionaldehyde

Cows' milk has more or less prounced flavors and odors, varying from those which are pleasing, to others which are objectionable. Factors that are responsible for the intensity and persistence of feed and weed flavors in milk are: a) time of feeding, b) ventilation, c) fertility of soil, and d) health of the cow.

1. Time of Feeding. The most crucial factor, responsible for feed and weed flavors in milk is the time interval between feeding and milking. An aromatic flavor, which is characteristic of feed is detected in milk when cows eat and/or inhale strong odors from different feeds up to 2 to 4 h. before milking. A feed flavor may not be produced if these feeds are fed after milking or withheld 4 to 5 h before milking (2). As the flavor components are mobilized through the blood stream to the mammary glands, the time it takes to produce a detectable off-flavor is governed by the concentration of the flavor components in the blood. The concentration in the blood depends on the amount being supplied to the cows lung or rumen. But as the source is removed it is assumed that there is some reversal of this process with consequent reduction of flavors in milk.

2. Ventilation. Another important factor, contributing to feed-and weed-flavored milk is improper ventilation. When cows are fed in a barn with inadequate ventilation, the odors of feeds or weeds they eat are imparted to the milk. In order to reduce or eliminate these odors, cows must be placed in a well ventilated barns. It is obvious from the research done at Cornell University (13,43) that milk flavors may be transmitted by odor laden air

3. Fertility of Soil. Soil fertility may contribute to feed and weed flavors in milk. Al-Hasani (1) in 1962 reported that cows grazed on a plot which had low or no nitrogen fertilization produced significantly poorer flavored milk than milk from cows grazed on a highly fertilized plot. However, there was no definite relationship between nitrogen concentration of bromegrass and the flavor intensity of the milk.

4. Health of the Cow. The physical condition of the cow also may contribute to off-flavored milk. It is believed that accumulation of gases, as in bloat, results in off-flavor. Bloat is caused by excessive frothing of the rumen ingesta and accompanied by probable inhibition of eructation. It was stated that the amount of reduction in buoyancy of the rumen material is directly related to the quantity of the gas formed. As legumes quickly gravitate to the lower portions of the rumen, bubbles of gas formed on solid particles become entrapped in the rumen juice and the level of fluid in the rumen increases thereby preventing eructation. These entrapped gases over a long period are absorbed through the rumen wall into the blood stream and eventually into milk possibly giving milk an off-flavor (31). However, Reddy et al. (37) reported that when cows were administered with Poloxalene, a bloat preventive agent, there was no effect on milk flavor but reduced surface tension, pH of rumen fluid and effectively maintained reduced surface tension for 24 to 36 h.

In the production of palatable milk, preventive measures are always best. Dice (12) in 1944 listed three ways of avoiding or correcting off-flavors in milk. They are as follows: 1) careful herd management (feed an adequate ration and keep cows off weedy pastures for 3 to 8 h before milking); 2) good pasture management to control weeds; 3) vacuum pasteurization.

In late 50s and early 60s, processors were subjecting milk to different conditions of vacuum and vacuum-heat treatments for removing the objectionable feed flavors. Not only were some objectionable feed flavors removed, but there also was a standardization of flavor. It was a common belief that the objections from consumers came not because the feed flavor was so bad, but because of the change in flavor. This was the reason that flavor standardization was considered to be important.

Various degrees of effectiveness in removing objectionable flavors have been reported. Reports (4,9,10,40,41,42,44,50) stated that the amount of off-flavor removal is related to intensity of treatment as measured by temperature differentials and the amount of flash steam created. Although vacuum processing equipment eliminated some of the more volatile flavors in milk, the amount of improvement did not appear to be worth the added effort and expense. Hence most procesing plants have discontinued use of this type of equipment. Through better farm practices, the quality of raw milk has been improved significantly. Transmitted flavors still exist but the responsibility for controlling them has shifted from the processor to the producer.

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CHAPTER 2 MICROBIAL FLAVORS

INTRODUCTION

Accumulation of bacterial metabolism products result in a wide array of flavor defects in both raw and pasteurized milk. These include flavors commonly described as acid, malty, fruity, unclean, bitter and putrid. Such flavors are produced by the action of complex enzyme systems of the contaminating organism upon the constituents of milk. The type and number of these organisms are dependent upon sanitation of milk handling equipment on the farm and upon processing-, transporting-, storage-, and temperature-history of milk.

The organisms commonly responsible for milk spoilage are believed to originate from soil and associated plant life. They are well adapted to milk; an excellent culture medium for different types of bacteria. This is due to the fact that milk is high in moisture, rich in nutrients and nearly neutral in pH. Extensive sanitary procedures are essential to restrict the initial contamination of milk during its production. Rapid cooling and holding raw milk at 4.4°C or below to prevent the proliferation of possible contaminants is necessary if the flavor quality of the milk is to be maintained until pasteurization. All pathogenic and most non-pathogenic bacteria in milk are destroyed by pasteurization. However, off-flavors of bacterial origin which have developed prior to pasteurization are affected little by this process and most cannot be removed by commercial vacuum treatment processes.

Subsequent contact with unsanitary equipment may recontaminate properly pasteurized milk. Such contamination often includes psychrotrophic bacteria which are commonly responsible for flavor defects in pasteurized milk (6, 9, 37, 42, 43). These organisms multiply slowly at 4.4°C or less and

unless the contamination is appreciable flavor defects may not be perceived before 10 to 14 d storage. Nevertheless, psychrotrophic organisms may multiply rapidly and cause flavor defects in a few days (43), in milk which has not been cooled to at least 4.4°C immediately after pasteurization or which is stored at above 4.4°C.

Although bacteria may be responsible for a number of different flavor defects in both raw and pasteurized milk, only those defects described as acid, malty (28) and fruity (29) can be recognized as being of the microbial origin by sensory perception alone. The flavors described as stale, barny, unclean, bitter, foreign, rancid and feed can be caused by bacteria, but determination of the actual cause is often difficult without bacteriological analyses because of the similarities of these flavors to flavors due to other causes.

1. ACID FLAVOR

The principal acid producing bacteria in milk may be conveniently divided into 4 groups (40).

a. The Lactic Streptococcal Group. They are gram positive cocci and occur in pairs or chains. Their optimum growth temperature varies enormously but usually about 30°C. They are non- motile.

b. The Intestinal Bacteria. They are gram negative short rods found in the lower part of the intestine of most warm blooded animals and consequently contaminate milk either directly or indirectly via fecal material. The most prevalent of this group is Escherichia coli growing at room temperature or 37°C. They are short motile rods.

c. The Lactobacilli. This group are gram positive and usually exists as

long thin rods. The most predominant species among lactobacilli in milk is Lactobacillus bulgaricus. Their optimum growth temperature is 30-40°C.

d. The Lactic Micrococci. They are gram positive spherical occurring singly or in pairs. They grow between 22-37°C, most strains grow at 10°C but not usually at 45°C.

Due to the ubiquitous nature of Streptococcus lactis in the environment of milk production, most milk is unintentionally inoculated with this organism immediately after milking or during processing. If the milk is not cooled rapidly to 4.4°C or below, it eventually will develop an acid taste due to proliferation of S. lactis and their conversion of lactose to lactic acid. Pure lactic acid has a clean acid taste and because of its low vapor pressure has no odor. Sommer (46) reported that the "sour" odor was not due to lactic acid but to volatile substances such as acetic, propionic and formic acids, acetaldehyde, acetone, diacetyl and methyl acetylcarbinol and that the proportion of these to lactic acid governs the extent to which milk must sour before a change is detected by odor. Van Slyke and Baker (49) referred to the first perceptible sign of souring as a characteristic flavor discernible to the senses of both smell and taste, due to the presence of some volatile compounds formed in the souring process and not to lactic acid. The flavor appears before the milk begins to taste acidic. Developed titratable acidity in milk of 0.07 % to 0.10 % above the acidity normally present in milk, calculated as lactic acid, is commonly detected by most individuals. On the other hand the odor of the volatile acids may be detected by most individuals when the titratable acidity has increased by as little as 0.01 %.

Since S. lactis is destroyed by proper pasteurization of milk, acid development subsequent to pasteurization is unlikely. However, pasteurization

will not improve the flavor of the raw milk if acid already had developed.

2. MALTY FLAVOR

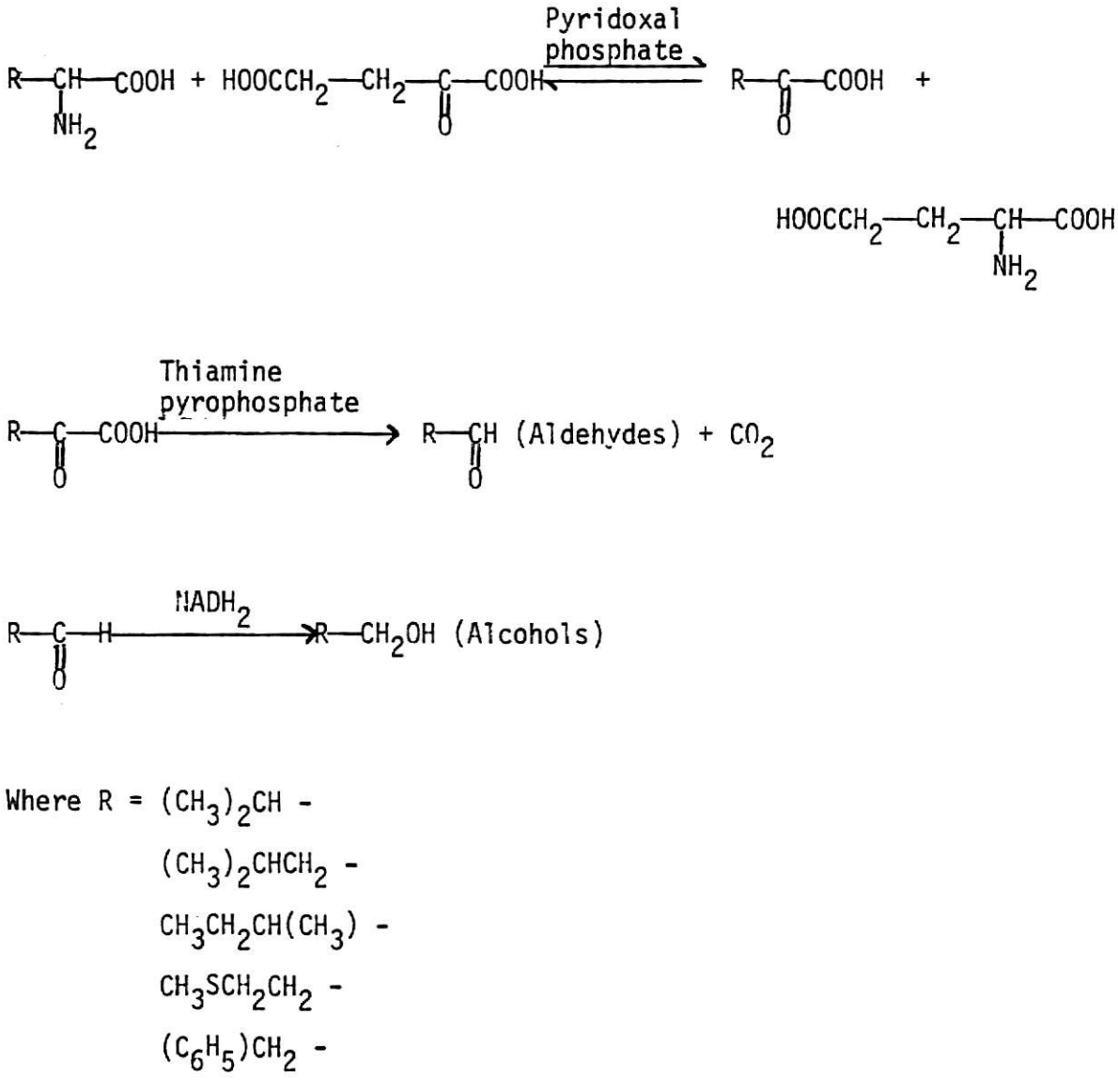
A flavor and aroma which has been described as cooked, burnt, caramel, grape nuts and malty, may develop in raw milk as a result of the metabolism of S. lactis subsp maltigenes (9). This organism is occasionally seen in milk in northeastern states but is rarely encountered in the Pacific Coastal states. Except for its ability to produce a detectable malty aroma in milk before any appreciable acid is produced the organism is identical to S. lactis (9). When grown in pure culture a malty aroma can be detected over the culture when the standard plate count reaches 10^7 to 10^8 per ml. The aroma becomes more intense as the acidity increases and the milk coagulates.

Morgan (30) reported that McDonnell who as early as 1899 described an organism he named Bacterium lactis acidi var maltigenum which produced a malt like flavor and aroma in milk. A burnt or caramel off-flavor in milk and cream produced in Iowa was reported by Hammer and Cordes (11). They found that it was caused by an organism similar to S. lactis except for its ability to produce off-flavor. Later Hammer and Baker (10) included S. lactis var maltigenes in descriptions of several variants of S. lactis. In 1947 Virtanen and Nikkala (50) stated that acetaldehyde produced by a "malt coccus" that is responsible for a malty flavor defect in Finnish butter and butter cultures. However, Zuraw and Morgan (51) in 1952 found that non-malt producing strains of S. lactis produced as much or more acetaldehyde in milk cultures as malty strains and that a typical malt flavor and aroma could not be simulated in milk by addition of acetaldehyde. Jackson and Morgan (15) conclusively established that the characteristic malty aroma of milk cultures of S. lactis

var maltigenes was due principally to the production of 3-methylbutanal. Although resting cells of S. lactis var maltigenes produced the corresponding methylbutanal in buffered solutions of leucine and isoleucine, the production of 2-methylbutanal seems to be negligible in milk cultures of the organism. A non-malt producing strain of S. lactis did not yield detectable amounts of 3-methylbutanal in milk or from buffered leucine substrates. The addition of as little as 0.5 ppm of 3-methylbutanal to milk simulated the characteristic malty flavor and aroma.

Several studies were designed to further elucidate the mechanism by which the organism produces aldehydes from amino acids is shown in Figure 1 (30). For instance Macleod and Morgan (22) in 1955 found that both S. lactis and the malt producing variant require leucine, isoleucine and valine for multiplication in a completely synthetic medium. They also found that the resting cells of these organisms are able to convert leucine and valine to α--ketoisocaproic acids and α -ketoisovaleric acid in the presence of glutamic acid. Only S. lactis var maltigenes cells concomitantly decarboxylates these two keto acids to the corresponding aldehydes. The same researchers in another study (23) in 1956 reported that the transamination reaction could be demonstrated using dialyzed acetone powders prepared for both malty and non-malt producing cells and that only the preparation from the malt producing cells contained a thiamine pyrophosphate mediated α -ketoacid decarboxylase. They also reported that the resting cells of S. lactis var maltigenes also were able to convert phenylalanine and methionine to phenylacetaldehyde and 3-(methylthio) propanal, respectively, presumably by the same mechanism (24). Tucker and Morgan (48) found that among the -ketoacids, the C_3-C_6 α -ketoacids branched at penultimate carbon were

Figure 1. Mechanism for formation of aldehydes and alcohols from amino acids by Streptococcus lactis var. maltigenes. (30)



Where R = $(CH_3)_2CH$ -

$(CH_3)_2CHCH_2$ -

$CH_3CH_2CH(CH_3)$ -

$CH_3SCH_2CH_2$ -

$(C_6H_5)CH_2$ -

converted most rapidly to the respective aldehydes by both resting cells and cell extract preparations from S. lactis var maltigenes. Pyruvate and α-ketobutyrate did not behave as α-carboxylase substrates in that oxygen was absorbed when they reacted with resting cells. Mixed substrate reactions indicated that the same enzyme was responsible for decarboxylation of both α-ketoisocaproate and α-ketoisovalerate. Morgan et al. (33) confirmed the identity of 2-methylpropanal and 3-methylbutanal by examining the volatile compounds in the head space over milk cultures of malty and non-malt producing strains of S. lactis by on column entrainment GLC-MS technique and found that appreciable amounts of the corresponding alcohols also were present. This also suggested the presence of the enzyme alcohol dehydrogenase in the cultures. Aldehyde reduction by a partially purified enzyme preparation from cell extracts was found to be stimulated by $NADH_2$.

Miller et al. (26) isolated several strains of an organism which they described as a new species and named Lactobacillus maltaromicus from milk samples having a malt like flavor defect. Like S. lactis var maltigenes, this organism also produces 2-methylpropanal, 3-methylbutanal and the corresponding alcohols. They suspect that the mechanism involved in their production is similar to those in S. lactis var maltigenes. Although the volatile compounds produced by S. lactis var maltigenes include a number of aldehydes and alcohols which are derived from amino acids, the characteristic aroma and flavor are due principally to the production of 3-methylbutanal from leucine.

S.Lactis var maltigenes cells are inactivated by pasteurization but the volatile products of their metabolism are not affected by this process nor can they be removed effectively by any of the vacuum treatment processes currently used for improvement of milk flavor.

3. FRUITY FLAVOR

Fruity aroma which sometimes occurs in pasteurized milk and other processed dairy products is frequently produced from post pasteurization contamination by Pseudomonas fragi. The aroma has been described as strawberry-like, resembling a May apple, ester-like, and fruity. The term fruity, for this defect, seems to have priority by virtue of common usage.

Morgan (30) cited that both Eicholz and Gruber in 1902, described the organism which produced a strawberry-like aroma in milk and butter held at low temperatures. Morgan (30) also mentioned that Hussong in 1932, proposed the name P.fragi for organisms he isolated from defective Iowa dairy products. These organisms are found in soil and water and apparently widely distributed in the environment of milk during production and processing (14, 27). They are sensitive to heat and thus their presence in pasteurized products is most likely due to post pasteurization contamination. They multiply at 5°C to 7°C thereby outgrowing many other species during refrigerated storage (13). As they are aerobic, proliferation and flavor development is enhanced by aeration of milk after pasteurization and by storage in partially filled containers in home refrigerators. In agitated milk inoculated with P.fragi, unclean flavors have been noted when the plate counts reach 5.5×10^6/ml (37) and fruity aroma at 5.0×10^8/ml (38).

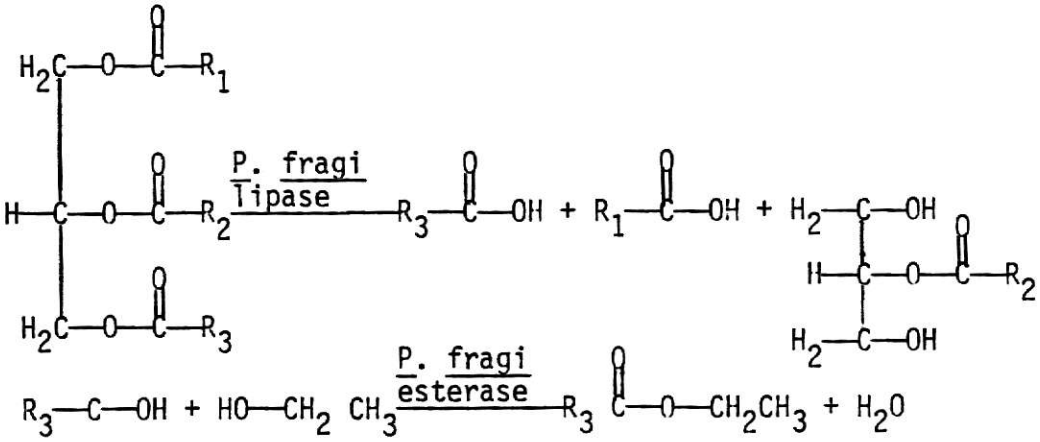
Pereira and Morgan (35) indicated that the fruity aroma of milk cultures of P.fragi was due to the production of ethyl esters, but mistakenly identified the principal compound as ethyl isovalerate. Reddy et al. (39) has confirmed that although organism produce some ethanol in milk cultures, production of a fruity aroma is enhanced by addition of 0.2% ethanol to milk medium. Such

cultures having developed a strong fruity aroma were found to contain ethyl butyrate and ethyl hexanoate in concentrations of 0.35 and 0.50 ppm respectively.

P.fragi is strongly lipolytic (12, 33) however, a 1, 3-position specificity for lipolysis of triglycerides was required with both crude and purified lipases obtained from this organism (25). Since most of the butyric and caproic acids of milk fat is esterified in the 3-position of constituent triglycerides (36) they are likely the major products of the lipolysis of fat in milk by this organism. Marked increases have been noted in the production of esters of these acids by the P.fragi in milk when the milk fat content was increased progressively from 0 to 4%, and esterification by the organism of added butyric and caproic acids with ethanol and propanol in a simple glucose-ammonium chloride salts medium. Reddy et al. (38) also noted that additions of milk fat or butyric acid to milk media containing ethanol were conducive to the production of a fruity aroma. From the foregoing it appears that P.fragi also contains an enzyme esterase responsible for the esterification of butyric and caproic acids with ethanol hence the origin of the fruity aroma in dairy products. It also seems likely that production of fruity-aroma esters by this organism would be enhanced in both milk and cottage cheese containing increased amounts of ethanol due to feeding silage (4) or growth of other organism which produce either acetaldehyde or ethanol (17).

Hosono et al (12, 13) elucidated the mechanism for the production of the fruity aroma. They demonstrated the presence of an esterase in crude cell extracts from Pseudomonas cells, capable of esterfying butyric and caproic acids with ethanol. A summary of the mechanism involved in the formation of ethyl ester by P.fragi is shown in Figure 2 (30).

Figure 2. Mechanism for formation of ethyl esters by Pseudomonas fragi (30).



Where $R_3 = -(CH_2)_2CH_3$

$-(CH_2)_4\ CH_3$

Strains of bacillus also have been isolated from milk which have characteristics similar to those of some species of Pseudomonas and are able to produce similar defects, e.g. fruity flavor. These spore forming organism may be the cause of flavor defects in aseptically packaged milk and fluid products.

4. UNCLEAN, BITTER, and PUTRID FLAVOR

Although unclean, bitter and putrid flavors may be due to other causes, often they are produced by psychrotrophic organism in pasteurized milk. These organism can multiply at or below 7°C irrespective of their optimum growth temperature. Even though most raw milk psychrotrophs are heat sensitive gram-negative rods, some heat resistant gram-positive species also have been isolated (5, 39). These latter organisms may cause spoilage of stored heat treated milk or milk products either during their growth or from the involvement of specific enzymes that they produce. Psychrotrophic gram-negative bacteria are not present in heat treated milk or milk products. However, the number of gram negative rods present due to post pasteurization contamination has a considerable influence on the shelf life of milk, and the range found in commercially pasteurized milk was reflected in a wide range of shelf lives (41). The two groups of these enzymes of major importance are the proteinases and lipases which, because they are produced extracellularly, can act directly on micellar casein or on the fat globules in milk thus producing unclean, bitter, and putrid flavors. They can act in raw milk while it is being stored and the psychrotrophs are multiplying, but they may also continue to act in heat treated liquid milk and other products.

Shipe et al. (44) revealed that 12 of 24 commercial milk samples developed fruity fermented flavors by the time they reached the "sell-by" date. The 12

fruity fermented samples had high bacterial counts. Apparently, these samples had been contaminated and consequently had a short shelf life. Later Bassette et al. (3) in a survey of milk flavor and quality at Kansas State University found that of the six brands of milk analyzed, all brands except two deteriorated in quality due to microbial growth after one week storage. Increase in n-pentanal, n-hexanal and acetaldehyde were shown to parallel increases in off-flavors in those milks when analyzed by GLC.

a. Psychrotrophic Proteinases.

Some organisms produce significant amount of proteinases during refrigerated handling of raw milk before heat treatment. For example, Law et al (19) in 1977 showed that strains of Pseudomonas and Acinetobacter species growing to approximately 10^7 cfu/ml and above produce sufficient proteinase to degrade casein to an extent detectable by polyacrylamide or starch gel eletrophoresis. They also found that when milk in which the Pseudomonas species had grown were subsequently UHT-sterilized and stored at 20°C those which had contained $5x10^7$ and $8x10^6$ cfu/ml before sterilization gelled after 10-14 d and 8-10 wk, respectively. Uninoculated low count milk or milk containing only $8x10^5$ cfu/ml of the pseudomonad remained liquid for at least 20 wk after sterilization, although a sediment gradually formed in the latter. The ß and k caseins were extensively degraded in gelled UHT milk and some loss of casein was seen. Whey proteins were not detectably degraded.

Other workers have reported that casein is degraded by proteolytic species of Pseudomonas. Adams et al. (1) in 1976 investigated 9 raw milk isolates and showed that in most strains, k-casein degradation was detectable even before the population had reached 10^4cfu/ml. ß-casein was degraded more than ∝-casein by most strains though it was detected at much higher

populations. Some strains also degraded the whey proteins, ß-lactoglobulin and α-lactalbumin. In 1977 Cousin and Marth (7) studied milk coagulation by heat (63-85°C) after growth of a natural psychrotrophic flora or of psychrotrophic species of Flavobacterium, Pseudomonas, Lactobacillus and Micrococcus. They reported that the natural flora, and also one of the Pseudomonas isolates, rendered the milk unstable when they reached $7x10^6$ and $4x10^7$cfu/ml respectively. The remaining isolates had similar effects on heat stability at higher populations. Refrigerated storage of the experimental milk lengthened rennet coagulation times regardless of the type of psychrotrophs present. The authors suggested that the changes in properties of the milk were due to breakdown of αs-and ß-caseins; they later confirmed this and showed that the native milk flora degraded ß-casein whereas the Flavobacterium and Pseudomonas isolates hydrolyzed both ß and α_s-casein. Skean and Overcast (45) in 1960 showed that proteolytic strains of P. fluorescens, P. fragi, P. putrefaceins degraded caseins and whey proteins when grown as pure cultures in pasteurized milk at low temperatures. Milk was spoiled by coagulation and bitter off-flavors were detected, however,the bacterial numbers were higher than would normally be expected in milk unless very heavy post pasteurization contamination occurred. Janzen et al. (16) in a recent study investigated the cause of a possible difference in shelf life of pasteurized skim milk and whole milk. They found that the relative protease activity was significantly higher in skim milk than it was in whole milk. This is due to the fact that skim milk in some way protects the enzyme activity of microorganism from heat denaturation. Therefore, a higher protease activity in skim milk may account partially for its decreased shelf life.

Ledford et al. (21) reported the influence of growth of coliforms on

flavor acceptability. Unsatisfactory flavor scores in 35 commercially processed samples found positive for coliforms by the preliminary incubation were 0, 11, 57, and 91% at 1, 7, 10, and 14 d. Samples positive for coliforms had higher standard plate counts and rapid psychrotrophic counts than coliform negative samples. Pyruvate content, lipolysis, and proteolysis were greater in samples positive for coliforms. Growth of coliforms may influence growth of psychrotrophic pseudomonads in commercial samples.

b. Psychrotrophic Lipases.

Deeth and Fitzgerald (8) found that, lipolysis in raw milk is often due to the action of native milk lipase which may act spontaneously. Its activity varies among other things according to the degree of agitation to which the milk has been subjected during transport and production. Muir et al. (32) in 1978 reported increasing free fatty acids concentrations in stored raw milk in which psychrotrophic bacteria have been multiplying. Lipolytic spoilage of heat treated milk due to enzymes from psychrotrophs is rarely reported. Nevertheless, Patel and Blankenagel (34) in 1972 reported that flavor defects were present in pasteurized milk which had contained 5×10^6 bacteria/ml before processing, but defects may already have been present in that raw milk. In 1975 Stewart et al. (47) suggested that off-flavor formation by the direct action of lipase on natural fats was unlikely since higher fatty acids have little flavor, but observations by Law et al. (20) in 1976 on lipolytic rancidity in cheese caused by P. fluorescens lipase have shown that when butyric acid and medium chain (C_6-C_{10}) 'soapy' fatty acids were released from milk fat, strong off-flavors can develop. The low incidence of bacterially induced rancidity in milk is probably due to the inaccessability of the triglyceride to the lipases unless the fat globule membrane is damaged by

excessive agitation; in these cases the natural lipases in raw milk is very active and would be expected to have a much greater effect than the relatively small amounts of lipase which would be produced by normally encountered numbers of psychrotrophs in stored raw milk. Anderson et al (2) in 1981 reported that, when a heat resistent microbial lipase was added to cows milk which was subsequently sterilized and stored at 8°C for 22 d, a tangible or perceptible change in Acid Degree Value (ADV) and flavor was monitored during storage. The samples containing lipase showed a rapid increase in ADV as compared to the reference and also produced a rancid flavor.

CONTROL OF MICROBIAL FLAVORS

Microbial contamination of milk can be prevented by utilizing extensive sanitary practices at all stages of production and processing. Milk should be cooled rapidly to 4.4°C or below and maintained at this temperature (18). Pasteurization of milk as soon as possible to reduce microbial growth and the production of heat resistent enzymes. Care should be taken in the packaging and storage of milk to minimize post pasteurization contamination and growth of organism.

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CHAPTER 3 LIPOLYZED FLAVOR

INTRODUCTION

Lipolyzed flavor results from hydrolytic cleavage of fatty acids from milk fat by the enzyme, lipase. The liberation of these acids in milk even in very small amounts imparts a bitter taste and a sharp unpleasant aroma which is characteristic of acids such as buytric, caproic, and caprylic. The term "rancid" although most commonly used by people in the dairy industry to describe this defect, may be confusing since other segments of the food industry associate the term with lipid oxidation. Descriptive names such as "goaty", "soapy", "butyric", and "bitter" are used to describe the lipolyzed flavor. The term "bitter" however may be ambiguous since bitter flavors can occur from protein degradation. Also, lipases secreted by microbial contaminants in milk can produce flavor defects which usually are accompanied by bitterness from proteolytic enzymes with subsequent protein degradation. The stage of lactation, temperature of handling, storing, and processing procedures are among the factors that affect the activity of the lipase and consequently the development of lipolyzed flavor.

1. LIPASES AND ESTERASES IN MILK

Fox and Morrissey reported in a book edited by Birch et al. (3) that milk contains at least two lipases. One has been designated as the naturally active lipase, or membrane lipase, which is irreversibly absorbed on the fat globule membrane when fresh milk is cooled. It is associated with spontaneous lipolysis. The other is plasma lipase, which remains in the plasma associated with casein until adsorbed on to the globule by an activation treatment. In

general its optimum pH range is from eight to nine. Recently Anderson (2) discussed the origin of milk lipases and free fatty acids, together with properties of the principle enzyme responsible for lipolysis in raw milk. Sundheim et al. (30) found that milk also contains a lipoprotein lipase similar to that demonstrated in post heparin plasma, adipose tissue and heart. It attacks only fat bound to protein and when compared to normal lipase, is present in relatively high concentration in milk, but does not hydrolyze the triglycerides of cream unless blood serum is present.

Milk contains three distinct types of esterases. 1) A-type carboxylic ester hydrolases, which hydrolyze aromatic esters e.g. phenyl acetate. With little activity on tributyrin, and are not inhibited by organophosphates. 2) B-type esterases, most active on aliphatic esters, although they show some activity on aromatic esters; inhibited by organophosphates. 3) C-type esterases, highest activity on choline esters but hydrolyze some aromatic and aliphatic esters slowly; inhibited by organophosphates (3).

2. LIPOLYSIS

The physical state of fat in milk must be taken into consideration in order to have an understanding of lipolysis. Milk fat occurs in the form of disjointed globules averaging about four microns in diameter, scattered as an emulsion in the milk. The fat globules are dispersed and in liquid form as the milk leaves the cow. At this stage the various lipases are inactive as they are not in contact with the globule. It was presumed, that the lipases are loosely bound to the casein micelle and are not released until either the milk is cooled or an activation treatment is applied (10).

Willie and Duthie (33) reported two types of lipolyzed flavor (a) "unclean", occurring from foaming or spontaneous lipolysis; and (b) "sickening" resulting from the mixing of raw and homogenized milks, churning, intense agitation via Waring blender or temperature fluctuation.

a. Spontaneous Lipolysis

Milks from individual cows are known to differ in their tendency to develop lipolysis. It is a relatively simple matter to distinguish naturally lipase-active milks from "normal milks" (11). With naturally-active milks, lipolysis develops in the absence of agitation and is initiated by prompt cooling of fresh milk. High concentrations of free fatty acids (FFA) accumulate following relatively short holding periods regardless of the milk storage-temperature. This phenomenon is known as spontaneous lipolysis (11). The rapidity and extent of cooling, formation of ice crystals on the bulk tank and to some extent the stage of lactation, type of feed and the physiological status of the cow influence the spontaneous lipolysis (30).

1. Characteristic of spontaneous lipolysis of milk

a. Susceptibility to Induced Lipolysis. Normal milk is less susceptible to the various forms of induced lipolysis than "spontaneous milk" (3). So milk drawn from cows in final stages of lactation and on dry feed is more susceptible to physical changes than normal milk. This probably is due to a weakness in the fat globule membrane in spontaneous milks. Warm spontaneous milk on leaving the udder is particularly susceptible to activation so that air leaks, long pipe lines, and risers of milking machine installations induce

lipolysis. But the hydrolytic rancidity in raw milk by a naturally active lipase occurring in high concentration can be prevented by mixing spontaneous milk with normal milk within an hour after milking. The amount to be mixed depends upon the concentration of the lipase. Rancidity (lipolysis) can be prevented by mixing lipase milk with normal milk in a proportion of 1:4. To ensure the effectiveness of this method, the mixing must be made within an hour after milking, before cooling or immediately after cooling.

b. Cooling. Stored milk is prone to spontaneous lipolysis when it is cooled to temperature below 15°C soon after milking. The lower the temperature, without freezing, the greater the extent of lipolysis. Delayed cooling decreases lipolysis, so most spontaneous lipolysis can be prevented by delaying cooling for one hour after milking. Delay in cooling for several hours will not prevent some individual milks which are susceptible to this defect to undergo lipolysis. Even though uncooled milk is not subject to this type of lipolysis, milk in which spontaneous lipolysis has been started by cooling will continue to undergo lipolysis when rewarmed and stored at a higher temperature. Under these conditions, the rate of lipolysis then increases as the temperature of storage is increased.

c. Biochemical Factors. Susceptibility of milk to spontaneous lipolysis appears to be influenced by various properties in milk. The most important is the amount of lipase. The characteristic of milk fat globules such as the nature of its membrane determines whether the milk lipase can attack the fat when milk is cooled (21).

It has been suggested that there is an inhibitory factor in normal milk that is not present in spontaneous milk which hinders the development of

spontaneous lipolysis. When normal and spontaneous milks are mixed this inhibitory factor prevents the lipase from making close contact with the fat (22).

Some researchers have claimed that spontaneous milk contains a special lipase activating substance which is not found in the normal milk. This substance is thought to originate from the blood stream and leaks into the milk before secretion due to some malfunction of the udder. The reason for suspecting this is that when blood serum is added to normal milk, it activates the milk lipase and breaks down the milk fat without further treatment (30).

2. Cow factors

Wide variation in the susceptibility to spontaneous lipolysis has been observed in milk from individual cows. Various physiological factors such as stage of lactation, feed and nutrition, season, breed and heritability, mastitis, as well as milk and fat yield have been found to influence this variability in lipolysis and their importance has been investigated.

a. Stage of Lactation. The most important factor in determining if a cow will produce spontaneous milk is the stage of lactation. Krienke (19) has reported that several cows produced lipolysis at the beginning of their lactation period while others produced it only at the end. He also stated that rancid milk was produced by some cows at the beginning and at the end of their lactation period with an intermediate interval during which the defect was not present. Also cows producing spontaneous milk in one lactation period may not produce it in the following lactation and vice versa. It has been reported that all cows will produce spontaneous milk in late lactation if they

are given feed of sufficiently poor quality.

b. Feed and Nutrition. The other important factor influencing the incidence of spontaneous lipolysis is related to the quality and quantity feed. Cows sufficiently low in nutrition especially in late lactation will produce spontaneous milk. It can be said that milk from well fed herds is seldom susceptible to spontaneous lipolysis. Jellema and Schipper (16) suggested that the high incidence of lipolysis in late lactation may be related to the absence of pasture feeding or to dietary changes. They reported that when cows were fed poor quality grass silage with low energy level, lipolysis in milk increased markedly a few weeks later. The increased susceptibility to lipolysis was preceded by a sharp decrease in milk production suggesting that the influence of feeding on lipolysis is related to an effect on milk production. Feeding trials indicate that a change from a low level of nutrition to a high level is effective in reducing spontaneous lipolysis (8).

c. Season. Seasonal variation was reported to influence the incidence of spontaneous lipolysis. Autumn and winter are the seasons in which most problems are occur (8). Weather conditions such as temperature and relative humidity have not been found to be significant. Parodi (23) in 1972 found that the lipolytic flavor is at a minimum during spring and summer when the cows are on pasture. They also stated that the season for the greatest incidence of spontaneous lipolysis during a particular season appears to relate to the proportion of cows in late lactation and the quality of feeds. Thus although considerable seasonal variation is observed, other factors such as the stage of lactation and availability of good quality feed are found to be more responsible for the spontaneous lipolysis than just the change of season.

d. Breed and Heritability. It has been suggested that the milk from Jersey cows contain larger fat globules and these upon agitation may rupture easily and liberates fat upon which the lipase may act. Similar tendencies in the production of spontaneous milk were observed in cows which are closely related by inbreeding. Lipolyzed and unclean flavors were the most heritable of the flavors found in milk. So it is suggested that careful breeding may reduce the number of cows producing "spontaneous milk".

e. Mastitis. The development of rancidity in individual milk has been related to mastitis. Cows with mastitis or a history of mastitis have shown a higher incidence of lipolysis. It has been noted that the initial FFA level was 75% higher in mastitic milk. Similarly Tallamy and Randolph (31) reported that FFA accumulation may be 1.5 to 2.0 times more rapid in milks with a positive Wisconsin Mastitis Test. Jellema (15) observed that lipolytic defects are more common on farms with leucocyte counts in the range 3.0-5.0 x 10^6 cells/ml. Furthermore, Downey (11) reported in a doctoral dissertation that Salih showed a striking relationship between the initial FFA levels and cell counts in milk from udders containing large numbers of bacteria, suggesting that the increased susceptibility of the high cell milk was due to some alteration in the stability of the fat globule membrane. He also reported that the rancidity in milk is due to the presence of lipase in the body cells which are present in milk in high numbers during mastitis and also due to the presence of blood serum components. Gudding (14) reported that the concentration of FFA in milk was positively correlated with California Mastitis Test scores and with somatic cell counts. Milk from cows with infectious mastitis had greater concentrations of FFA than milk from cows with non-specific mastitis.

Lipolysis was greatest in milk from quarters with Staphylococcus aureus mastitis. All these factors combine to cause lipolysis more frequently in mastitic milk than in non-mastitic milk.

f. Milk and Fat Yield. A significant inverse relationship was found by Jellema and Schipper (16) between the degree of spontaneous lipolysis and milk yield. They reported that this effect is related to the level of nutrition and stage of lactation but not due to the concentration of enzyme in small quantities of milk. Illness causes a decrease in milk production and increase in the level of lipolysis. Spontaneous lipolysis increases with an increase in fat. Nevertheless, it is difficult to separate the effect of fat content from the stage of lactation since both fat content and susceptibility to lipolysis increases with advancing lactation.

b. Induced Lipolysis

If raw milk or cream is subjected to excess agitation or turbulence, the fat globule membrane may be disrupted, resulting in the lipolytic enzyme having access to triglyceride which are accordingly hydrolyzed. This is known as induced lipolysis. Several activation treatments such as agitation and foaming, homogenization, freezing, and temperature fluctuation of milk activates the plasma lipase (9), which is responsible for induced lipolysis.

1. Activation treatments

a. Agitation and Foaming. Vigorous mixing of air and milk was found to be a major cause of induced lipolysis (3). Deeth and Fitzgerald (9) reported that the action which causes foaming or frothing, can occur during passage of

milk through pipelines or with any treatment involving vigorous agitation or stirring. Little or no activation has been observed when milk is agitated in the absence of air or without incorporation of air. The severity of agitation and the temperature of milk during activation governs the amount of activation and hence of subsequent lipolysis during storage. The more severe the treatment and higher the temperature the greater is the amount of lipolysis.

b. Activation by Temperature Changes. Deeth and Fitzgerald (8) reported that lipolysis may occur when fresh raw milk which has been precooled to 5° C or lower is warmed to 25-35° C and then recooled and stored. A greater degree of activation is seen when the milk is warmed to 30° C and recooled to 10° C. Milk from different cows have shown greater variation in their susceptibility to this kind of activation. They observed that the milk from individual cows or sometimes the whole herd is resistent to this kind of activation. The reasons for variability are not clear although it appears that spontaneous milks are more susceptible to lipolysis than normal milks. Like spontaneous lipolysis it is not prevented by mixing with non-susceptible milk.

c. Homogenization. Lipoloysis is enhanced greatly by breaking down the milk fat to smaller and more uniform size globules (16). This is due to the fact that the milk fat loses its natural protective membrane and becomes coated with a new membrane consisting largely of small casein particles. The fat is more sensitive as the new membrane covering is less structured and more premeable than the natural membrane. Homogenization of raw milk brings the lipase associated with the casein and the fat into close contact and

lipolysis begins immediately. In order to overcome this problem milk should be pasteurized to destroy lipase before being homogenized. Where this is not feasible pasteurization should immediately follow homogenization. Lipase from raw milk will attack the fat from homogenized milk when pasteurized, homogenized milk is mixed with raw non-homogenized milk. Hence it is essential that pasteurized milk not be contaminated with raw milk.

d. Freezing and Thawing. The fat globule membrane in milk can be ruptured by the surrounding ice crystals. On thawing some fat is set free from the damaged globules and become susceptible to attack by lipase. The amount of disruption is increased by repeated freezing and thawing. More damage is caused to the globules on freezing by slow cooling than by rapid cooling.

2. Effect of temperature

a. Temperature during Activation. Fitz-Gerald (12) reported that temperature has a strong influence over the amount of activation that occurs when milk is agitated and foamed. Cow's milk when drawn is 37°C and conducive for lipase activation. Cold milk is more resistant to activation than warm milk, however during cold storage it becomes susceptible due to disintegration in structure of fat globule membrane. The susceptibility of fresh milk to activation increases as the temperature increases from 5°C to 15°C. So it is important that the temperature of milk during handling be kept as low as possible to reduce induced lipolysis.

b. Temperature during Storage. The degree of lipolysis that occurs following activation is affected by the temperature of milk during storage. So when milk is activated by homogenization or by agitation and foaming, less lipolysis occurs at lower storage temperatures.

3. Farm problems

a. Milking Machines. Lipolysis in farm milk depends in part on the type of treatment that raw milk receives during and after milking. Sundheim et al. (30) have reported that lipase activation normally results from a faulty design and installation and inadequate maintenance of milking machines. Leaks in teat cups or claws, loose fitting joints in the system, small holes and cracks in rubber tubes and inflation allows enough air to cause an appreciable degree of activation. The absence of a shut-off valve at the claw or failure to manually cut off the vacuum when cups are transferred from one cow to next, also can result in considerable activation. Low milk flow rates are susceptible to activation of milk since a small amount of milk with large amounts of air results in back and forth movement through a system before it reaches the reservoir. Such a situation can occur where cows are slow milkers, where cups are left on cows after milking is finished, and where vacuum pumps of insuffient capacities are used.

b. Pipelines. Pipelines are also responsible for activation of milk. Pillay et al. (25) reported that the amount of activation increases with the increase in the length of the pipeline and number of joints and elbows. Fittings in the vacuum section such as in-line filters and tees with closed ends also cause activation by producing turbulence and foaming. Vertical risers also are responsible for the activation of milk. The degree of activation increases with increasing number and heights of risers. Therefore high line milking machines have greater influence on lipolysis than low line machines. Damage to milk also can be caused by small diameter pipelines which have tendency to get plugged with milk and results in high velocity turbulent flow. Milk should flow

along the bottom of the line with no agitation. Furthermore, positioning of the lines so that milk flows under gravity as much as possible reduces the risk of activation.

c. Pumping. Activation of milk is caused by the continuous operation of centrifugal pumps below full capacity or with admission of air through faulty seals. For pumping of milk from a small holding tank to the bulk vat, as in the case of low line milking system, automatic control should be used so that the pump operates only when there is enough milk to ensure full flow.

d. Bulk Tank. Foaming in bulk tank was found to be responsible for the activation of lipolysis. Crawford (7) observed that foaming can arise from splashing of milk into the tank from a high inlet pipe or from excessively vigorous action of the agitator; speed and duration of violent agitation control or influence activation. Small quantities of milk in large bulk tanks are most susceptible to activation especially if the agitator is not completely covered. Nevertheless, bulk tanks should have little effect on the activation of lipolysis. However, vacuum tanks have been found to lead to milk rancidity. Temperature changes as previously described result in activation when warm milk is added to a smaller amount of cold milk and the temperature of the mixture is allowed to increase substantially before being cooled. Activation also can occur if the refrigeration is off for a period during storage on the farm. Hence refrigeration on bulk vats should not be turned off between the first milking and collection of subsequent milking.

e. Storage. Lipolysis continues during storage after the milk lipase has been activated. The time and temperature of storage governs the amount of lipolysis. It decreases with time but does not stop until the lipase is

inactivated. The lipolysis will increase when further activation treatment is given to milk after the initial lipolysis has slowed down. Mishandling of milk in the plant leads to a second activation after it has been activated on the farm. There is a greater possibility that bacterial growth contributes significantly to the amount of lipolysis when milk is stored for a longer period.

4. Transport

Transportation of milk from the farm to the plant in cans or in bulk tankers does not usually have a significant influence on activation of lipolysis. Any problems involving tankers which have been encountered have been traced to faulty design or construction.

5. Milk processing plant problems

Pumping and separating are the main source of lipase activation in a plant. Continuous pumping induces damage to raw milk and cream when air is incorporated into the system through loose pipeline connections and faulty seals, and from centrifugal pumps run below capacity. When the milk or cream is pumped through long pipelines with vertical sections and excessive agitation in vats, the activation is increased. Temperature fluctuation and mechanical damage are the two ways by which activation occurs during plant separation of milk. Milk is usually warmed to 30°C to 50°C for separation after being held at 3°C. The cream then is cooled at 3°C after separation. These temperature changes may contribute to lipolysis. Mechanical damage to fat globule occurs in some non-air tight separators where cream leaves under high pressure causing partial homogenization. Mixing of homogenized milk with raw milk or incompletely pasteurized milk is an other effective methods of lipase

activation which may originate on the farm, and are increased by improper storage conditions in the plant. Inadequate refrigeration and increases in storage times of raw milk and cream are responsible for increased lipolysis in activated milks. The growth of lipolytic bacteria is favored by these storage conditions, and causes a further decrease in quality.

3. CHEMICAL ASPECTS OF LIPOLYZED FLAVOR

Chemically, among the various off-flavors in lipolyzed milk are well defined. Volatile short chain free fatty acids released by the action of lipase on milk glycerides are responsible for the rancid (lipolytic) flavor of milk. Nelson and Trout (21) stated that this flavor is due to free butyric acid, whereas Al-Shabibi et al. (1) reported that of 16 fatty acids evaporated in milk, only caproic, caprylic, and lauric acids produced rancidity. The C-10 and C-12 acids yield the most characteristic rancid flavor. Addition of buytric acid to milk is said to impart a flavor resembling butyric acid but lacking the sensation encountered in lipolyzed milk. However, Scanlan et al. (26) reported that the lipolyzed flavor is due primarily if not exclusively to the C-4 to C-12 volatile free fatty acids. They found that no single fatty acid in the butyric to lauric series exerted a predominating influence in its contribution to rancid flavor and long chain fatty acids C-14 to C-18 contributed little if any to lipolytic flavor. Kolar and Mickle (18) proved that very short chain fatty acids, formic, acetic and propionic are important in lipolyzed flavor. In a review Shipe et al. (28) mentioned that Paulet et al. demonstrated that sodium salts of capric or lauric acids imparted a soap like taste to water at 0.25 ppm or greater whereas the threshold level in a sucrose solution was 50

ppm and in a saline solution, 100 ppm. Tuckey and Stadhouders (32) reported that lipolyzed flavors can be detected more readily as the test medium pH is decreased. Pillay et al. (24) determined the free fatty acid and the thresold values for the detection of the lipolyzed flavor by using two methods namely "Bureau of Dairy Industries" detergent procedure and modified version of Frankel and Tarassuk method (13). They reported that the linear relationship and correlation which was found to be high for both the methods could be used for predicting ADV for one method from known value by the other. The threshold values for lipolyzed flavor detection in milk was within the range of 4.1 to 4.5 ADV as determined by the Frankel and Tarassuk method.

4. CHEMICALS RESPONSIBLE FOR INHIBITING LIPOLYSIS

A number of chemicals are responsible for affecting the lipase activity (5). Aureomycin, penicillin, streptomycin, and terramycin inhibit lipase activity by as much as 7.6 to 49.8%. Hammarsten casein, acid casein, α-casein, ß-casein, γ-casein, α-lactalbumin, and ß-lactoglobulin all inhibited lipase (27). Shahani et al.(27) postulated that the inhibitory effect of these proteins was due to the formation of a complex between them and the lipase. However, k-casein, lactalbumin, pseudoglobulin and euglobulin activated the enzyme to varying degrees. Lipase also can be inhibited by a number of salts but these have not had a significant impact on lipolysis in milk. Lipase is known to contain free as well as masked sulfhydryl groups that are associated with enzymic activity. For this reason, the enzyme can be inactivated by oxidation and is sensitive to metal ions such as copper and iron. Its activity can be reduced by hydrogen peroxide and exposure to light. Shipe et al. in 1982 (29)

reported that lambda carrageenan, a polysaccharide containing primarily 1, 3 D-galactose-2-sulfate and 1, 4 D-galactose-2, 6-disulfate inhibits lipolysis. This water soluble polymer reacts readily with milk protein and as little as 0.05% can inhibit lipolysis in milk that is subjected to either thermal or mechanical activation. It has been postulated that the inhibition is due primarily to an interaction between the carrageenan and the enzyme, although adsorption on the fat globule membrane may also be involved. Marshall and Charoen (20) reported that lecithin and casein protected a milk fat emulsion from attack by added bovine lipase. Lecithin was found to be more effective than casein. It appeared that the inhibitory effect of these compounds was due to a partial encapulation of the fat globule. Addition of trypsin of milk fat emulsion reduced the inhibitory effect of casein. Chrisope and Marshall (6) in a subsequent study found that lipolysis was increased in both milk fat emulsions and in raw milk by the action of phospholipase C. Since this enzyme cleaves phospholipids it is presumed that it enhances lipolysis by reducing the protective covering provided by the phospholipids. Shipe reported in a book edited by Charalambous (4) that a number of surfactants inhibit lipolysis. He found that Triton X-155 a polyoxyethylene octylphenol,was the most effective compound tried and although it did not inhibit the enzyme, it provided a protective coating for the substrate in a manner similar to phospholipids.

CONTROL OF LIPOLYSIS

A lipolytic flavor produces serious problemon that will immediately affect the marketing of milk. It will result in consumer complaints, returned milk, and possibly a permanent loss in customers. Immediate steps should be

taken on the part of the dairy plant management and the field staff to track down the source of the problem. In order to avoid lipolysis, the following steps should be taken: a) milk should be cooled to 5°C or below with minimum agitation immediately after milking and avoid freezing as ice crystals disrupts the fat globule membrane; b) maintain milk at 5°C; c) avoid long distance hauling of partially filled containers or tanks. Stirring and pumping of milk should be kept to a minimum; d) pasteurize milk as soon as possible at a temperature high enough to inactivate lipase. A thermal temperature equivalent to about 76.7°C for 16 s is recommended; e) avoid post pasteurization contamination with lipase producing organism; f) distribute milk as rapidly as possible and keep at 5°C below to avoid possible development of lipolysis; g) periodically sample milk to ensure that the consumer is getting milk free of lipolyzed flavor.

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CHAPTER 4 HEATED FLAVORS

INTRODUCTION

Off-flavors that are produced by the thermal processing of fluid milk are characterized as cooked or heated flavors. The pasteurization time and temperature of milk govern the nature and intensity of flavor. The heat induced flavors have been classified into the following four types: cooked or sulfurous, heated, caramelized and scorched (20).

1. COOKED OR SULFUROUS FLAVOR

With the advent of pasteurization of milk "cooked" was added as a possible flavor defect. This defect gave rise to some of the early discrimination against pasteurization, because the finished product did not have what many consumers called the "good raw milk flavor". At the present time, with flow diversion valves, recording thermometers and other refinements that make it possible to provide adequate pasteurization without overheating, consumer complaints of "cooked flavor" have practically disappeared.

However, pasteurization still imparts a slight cooked or sulfurous taint to milk and pronounced cooked flavors are observed when high temperatures are used (2,16). Gould and Sommer (7) reported that Butterworth and Sommer observed a flavor when milk was heated momentarily to 76°C-78°C. In contrast Marquardt and Dahlberg (15) found that no cooked flavor was detected even after heating milk for one minute at 76°C. Marquardt and Dahlberg also studied the influence of the temperature of the heating medium on the development of a cooked flavor in milk pasteurized at 62°C. When steam or boiling water was used to heat milk in a steel lined vat a cooked

flavor was produced, whereas the flavor was not produced when lower temperature hot water was used for heating. No correlation was found between the fat content of milk and cooked flavor, nor between the cooked flavor and the presence of a milk film in the vat. In other work (15) they found a tendency for the cooked flavor to increase in intensity with an increase in the fat content of the cream, particularly with samples containing 15 or more % fat. Webb (24) however, preheated 20% cream to 80°C before homogenization and noted that the cooked flavor obtained was not as pronounced as in evaporated milk; "due probably to the much smaller percentage of solids-not-fat"

Several studies have been conducted to ascertain the source of the cooked flavor. Davies (4) found that pasteurized milk possesses a different odor than raw milk, probably from the formation of volatile substances such as ammonia, hydrogen sulfide, mercaptans, and volatile phosphorous compounds that occur from protein break down. This odor can be removed by aeration. Blankenagel and Humbert (1) reported that when skim milk was heated in an UHT plate heat exchanger to temperatures ranging from 82°C- 140°C for a holding time of 3.5 s, the serum proteins were denatured. This resulted in sulfhydryl formation and finally in the appearance of the cooked flavor. Hutton and Patton (11) revealed that ß-lactoglobulin can account for practically all of the SH groups present. A study of the contribution of various major serum protein fractions to heat induced cooked flavor in skim milk demonstrated that ß-lactoglobulin was responsible for the flavor. Josephson and Doan (14) also reported that the sulfhydryl compounds seem to be wholly responsible for the cooked flavor and they also are responsible for

a decrease in oxidation-reduction potential. They act as active antioxidants and appear to be responsible for the inhibition of the tallowy or oxidized flavors in milk heated to temperatures over 76°C. As the sulfhydryls become oxidized they apparently lose their characteristic flavor and the milk or milk products which previously exhibited a cooked flavor become indistinguishable from similar unheated milk. The sulfhydryl substances in heated milk not only protect the milk against the development of tallowy flavor but actually acts as an antioxidant and protect ascorbic acid. The cooked or sulfurous note dissipates upon storage and may not be noticeable after 2 or 3 d of refrigerated storage.

2. HEATED FLAVOR

This flavor has been observed after the dissipation of strong sulfurous or cooked flavor note upon several days of refrigerated storage (21). The chemical nature of a rich or heated note has not been identified but recent evidences indicates that diacetyl is involved. Scanlan et al.(21) found in milk preheated at 82°C for 30 min and then to 146°C for 4 s the following compounds: $C_{3,4,5,7,8,9,10,11,13}$ n-methyl ketones, the $C_{8,10,12}$ delta-lactones, benzaldehyde, furfural, phenylacetaldehyde, vanillin, oct-1-en-3-ol, n-heptanol, 2-butoxy ethanol, maltol, acetophenone, benzonitrile, benzothiozole, and diacetyl. They also observed the following compounds in both raw and heated milk: a dichlorobenzene, a trichlorobenzene, methyl iodide and diacetyl. The concentration of diacetyl in heated raw milk was 5ppb while the amount in the heated milk was 38ppb. This is above the average flavor threshold level for diacetyl in milk. It is suggested that diacetyl contributes to the rich or heated flavor of heated

milk.

The role of dissolved oxygen in the development of oxidized flavors and the destruction of certain vitamins in milk is well known. Hand et al. (8) demonstrated that the removal of oxygen from pasteurized milk by controlled vacuum treatment, and its subsequent exclusion, prevented flavor defects due to oxidation and preserved the reduced ascorbic acid naturally present or added to milk. Later Ford et al. (6) demonstrated that UHT treated milk, containing less than 0.1ppm oxygen, and processed by direct heating retained both ascorbic acid and folic acid during storage. High oxygen concentrations resulted in complete destruction of both vitamins within a few days. There has been much concern expressed about the effect of oxygen on the acceptability of UHT milk with respect to cooked flavor intensity. Coulter and Jenness reported in a book edited by Van Arsdale et al. (23) that in heated fluid milk products the active SH compounds gradually disappear largely by oxidation. The cooked flavor is lost and the product become susceptible to oxidation. Zadow and Birtwistle (25) reported differing views regarding the effects of oxygen on flavor acceptability of UHT milk. It was suggested that sufficient oxygen must be present to react with the SH groups formed during processing, but that an excess should be avoided since oxidized flavors may result. Thomas et al.(21) reported a "cabbagey" flavor in milk immediately after UHT treatment. They reported that this flavor decreased within few days and the rate of decrease was more rapid when the initial oxygen level was high. However, high oxygen levels caused a rapid decrease in ascorbic acid and folic acid content. The beneficial effect of oxygen on flavor appears to be so slight and confined to such a short period, as to be completely

outweighed by the adverse nutritional effects. Jaddou et al.(12) reported that the length of heating time at 140°C also affects the intensity and rate of disappearance of the cabbagey flavor. They found that this flavor disappeared more rapidly in milk that was heated for 90 s than in milk that was heated for 3 s. They suggested that the cabbagey flavor might result from an interaction between S-bearing compounds, especially H_2S and CH_3SH, and one or more carbonyls. They speculated that there might be competing reactions for the S-compounds in the presence of Maillard reaction intermediate compounds. In the 3 s treatment compared to 90 s, there might be less Maillard reaction intermediates and therefore more S-compounds would be available for the formation of the cabbagey complex. A good correlation was found between the total volatile sulfur content and the cabbagey flavor. The volatile sulfur compounds includes H_2S, CH_3SH, CS_2, and $(CH_3)_2S$. A decline in these compounds parallel the decrease in cabbagey flavor.

3. CARAMELIZED FLAVOR

A sweet caramel flavor was observed in milk that had been processed at 135 or 143°C for 10 s, stored for 9 and 16 d, and suggested non-enzymatic browning as the cause (9). It was demonstrated that SH groups slowly disappear when milk is held for prolonged periods at high temperatures and that cooked flavor in the milk seems to give rise to a caramelized flavor (22,18). The caramelized flavor does not develop in heated fluid whey (18). It also was observed that the addition of ascorbic acid to raw milk will induce this flavor at a time and temperature lower than normally required(17).

There appears to be a natural inhibiting mechanism to heat induced browning in milk. Townly and Gould (22) noted that substantial browning

occurred at the time of a marked decrease in labile sulfide liberation. According to Patton and Josephson (18) the onset of browning in heated milk coincides with the disappearance of sulfhydryl groups. The mode of action of SH compounds in this connection is not known, but an interesting speculation concerns the addition of SH compounds at the double bond of the Amadori rearrangement product. Hodge (10) suggested that a reaction of this type might be effective against browning in certain systems. Several other agents are known to inhibit browning in milk systems. Patton (17) referred to a doctoral dissertation by Kosikowsky in which formaldehyde, sodium bisulfite, sulfur dioxide, and hydrogen peroxide were found to inhibit browning. However, the most important protection from browning in milk and milk products is to keep heat treatment, storage time and temperature at acceptable minima.

4. SCORCHED FLAVOR

Localized overheating such as that created by excessive "burn on" in a heat exchanger will produce a scorched flavor. Fortunately this is rarely encountered in milk.

CONTROL OF HEATED FLAVORS

Although cooked flavor is probably the most common defect in commercially pasteurized milk, it does not appear to have a significant impact on consumer acceptance. Cooked flavors were found to mask some undesirable flavors. Furthermore, the sulfhydryl groups that are activated by heating may contribute to the stability of milk oxidation. On the other hand, milk consumption is adversely effected by a pronounced heated flavor. Although high temperatures reduce microbial contamination and/or enzymatic

degradation, this advantage may be marred by heated flavors. Therefore, several methods have been used to minimize the development of this flavor.

Ferretti (5) found that certain compounds react with sulfhydryl groups and inhibit development of the cooked flavor. Specifically he observed inhibition by following 7 compounds: 2-aminoethyl 2-aminoethanethiosulfonate dihydrochloride, 5-aminopentyl 5-aminopentanethiosulfonate dihydrochloride, 2-acetamidoethyl 2-acetoamidoethane thiosulfonate, cystine sulfurdioxide, 2-aminoethane thiosulfuric acid, S-sulfocysteine, and S-sulfoglutathione. Although these compounds were effective at concentrations from 0.003 to 0.05%, they are not legally approved additives. Shipe reported in a book edited by Charalambous (3)that the addition of 30-70 mg L-cystine decreased the H_2S and cooked flavor. As cystine is a natural constituent of milk it would be permissible to use in some countries and perhaps might be approved even in the United States.

Cooked flavor can be removed by the use of sulfhydryl oxidase. This enzyme catalyzes the following reaction (13).

$$2RSH+O_2 \longrightarrow RSSR+H_2O_2.$$

Sensory evaluation provides the most practical method for monitoring the type and intensity of heated flavors. Different procedures (20) have been developed for producing these flavors to provide a means of training taste panel personnel. There is no simple objective test for measuring heated flavors; however, the nitroprusside test (18) provides an indication of the sulfhydryl content of milk and cooked flavor intensity. It does not measure the other heated flavors. Chromatographic techniques can be used to monitor

the volatile compounds associated with heated flavors. However, these methods are not suitable for routine quality control work.

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CHAPTER 5 LIGHT ACTIVATED FLAVORS

INTRODUCTION

In the past the quality of fluid milk usually was more predictable because it was produced, processed and delivered to consumers within 24 h. However today, milk is collected from commercial farms on an every other day basis, processed in plants throughout the week and is frequently 72 h old or more when in the hands of the consumer. In addition to the change in the length of time involved in collecting and processing milk, the marketing technique also has changed. New problems have evolved due to a shift from home deliveries to store purchases. Most of today's milk is sold wholesale, primarily to supermarkets and other stores, which bring the managers of the dairy departments of these stores into the picture. These managers must have knowledge about the potential problem areas in handling milk, including storage temperature, rotation of stock and particularly exposure of milk to light.

The effect of light on the flavor of milk and its products was reported as early as 1890 in Europe by Hanus and later, in 1907 by Burr both cited in a review of the subject by Stull (51). These investigators did not elucidate the exact nature of the observed flavor defect. In the U.S., references to the subject were made by Hammer and Cordes (29) in 1920 and by Frazier (26) in 1928.

Light-induced off-flavor development in milk was brought into the limelight during the late 1920's and early 1930's, with the advent of vitamin D fortification via ultra-violet radiation. Stull (51) reported that Drummond sounded an ominous note in 1927 when he conjectured that the benefits of

irradiating milk might be outweighed by the possibilities of off-flavor development and destruction of photosensitive vitamins.

In all of the early reports, the character of the flavor that developed in milk on exposure to light was apparently assumed to be singular in nature. In 1931 Davies (14) discovered two distinct flavors in milk due to light exposure. First of these, due to photo-induced lipid oxidation, was the familiar oxidized or tallowy defect reported by Brown and Thurston (11) in 1940 and by Greenbank (27) in 1948; Second and a distinctly different defect was described by others (14) as "sunlight" or burnt. Later other terms such as "burnt feather", "burnt protein", "scorched", "cabbage", and "mushroom" have been used to characterize the flavor defect thought to be due to degradation of proteins. Weckel and Jackson as cited by Stull (51), suggested that the flavor be referred to as the "activated" flavor.

1. FLAVOR ALTERATION

Flavor and shelf life are considered as the most important factors for the acceptance of milk as food. Barnard (3) found that the occurrence of oxidized flavors increased rapidly during a 4-year period from 1967 through 1970. Over 1600 milk samples collected from 400 retail stores in Pennsylvania and representing 250 brands were analyzed by a trained taste panel. In 1967, only 6.7% of 210 samples were criticized for oxidized flavor. But in 1970 this percentage increased to 23.7% of 443 samples. To further elucidate the magnitude of this problem, the same researcher (3) made compared the four major type of containers with respect to flavor and reported the following percentage distribution of oxidized flavor: blow-molded plastic containers,

86.1%; plastic bags, 50.0%; glass bottles, 46.4%; and the least was plastic coated paper, 12.7%. In 1974 the same researcher (3) reported that 84.2% of milk packaged in blow-molded plastic containers was oxidized. Bray et al. (10) reported that when 2000 consumers were surveyed at three county fairs in Vermont to determine their taste preference for samples of "good" milk and milk with light-induced flavor defect, more than 73% of the people surveyed perferred the "good" milk. More females than males could taste a difference between the two samples. Later Coleman et al. (13) asked a consumer taste panel of 781 people to rate three samples of milk, two of which had been exposed to fluorescent light (one exposure period of 1076 lux at 7°C for 12 h and another for 24 h), and the third was the control (non-light exposed). The panel using 5-point hedonic ranking scale, scored the control best, followed by milks exposed 12 and 24 h. In a recent survey conducted by White and Bulthaus (54), samples taken from plastic jugs were evaluted weekly for the frequency and severity of light activated flavors. Out of 90 samples examined, 53% were rated as having a moderate to strong light activated flavor. In addition, large consumer panels were run at two shopping centers to determine whether panelists could distinguish between "good" milk and milk with light activated flavor and which of these they preferred. Only responses from those consumers correctly identifying a difference were used to measure preferences. The age group of 25 yrs and younger was most successful in correctly detecting a difference; therefore, a survey also was conducted at a local college where 69 of 132 respondents correctly identified a difference. Preference testing over all portions of the study indicated 63% preferred the "good" milk, 27% preferred milk with light-activated flavor, and 10% stated no

preference.

2. CHEMICAL ASPECTS

a. Activated Flavor. Several attempts have been made to reveal characteristic flavor compounds in milk responsible for a sunlight or activated flavor. Weinstein et al. (53) stated that a protein in heat treated whey would produce the characteristic flavor. Later, Patton and Josephson (40) reported that the amino acid methionine was the precursor of activated flavor in milk. A pure solution of methionine after exposure to sunlight emitted a flavor similar to the activated flavor in skim milk.

The role of Strecker degradation in converting methionine into methional, ammonia and carbon dioxide as illustrated below was demonstrated by Samuelsson and Harper (42).

$$CH_3\text{-}S\text{-}CH_2\text{-}CH_2\text{-}CH\ NH_2\text{-}COOH \longrightarrow CH_3\text{-}S\text{-}CH_2\text{-}CH_2\text{-}CHO + NH_3 + CO_2.$$

They reported that riboflavin and oxygen are needed for this reaction. Moreover,they suggested that free amino acid also was necessary.

Forss (25) reported that free radicals derived from methionine, methional and cysteine combine to form the following compounds.

$$CH_3 - S + H \longrightarrow CH_3 - SH \longrightarrow \text{methanethiol}$$

$$2\ CH_3 - S \longrightarrow CH_3\text{-}S\text{-}S\text{-}CH_3 \longrightarrow \text{dimethyldisulfide}$$

$$CH_3\text{-}S + CH_3 \longrightarrow CH_3\text{-}S\text{-}CH_3 \longrightarrow \text{dimethylsulfide}$$

Samuelsson and Harper (42) also demonstrated the possible formation of methional, hydrogen sulfide, formaldehyde, acetaldehyde, propanal and various combinations of hydrocarbon substituents on sulfhydryl, sulfide and disulfide bases. Later Allen and Parks (1) showed that methionine was oxidized to

methional in presence of direct sunlight.

Dimick (19) identified the high molecular weight protein immunoglobulin as the primary source of the photosensitive amino acids. Finely and Shipe (22) believed that a low density lipid protein fraction is a principle source of light-induced flavors in milk. This would give support to the increased susceptibility of homogenized milk to light-activated flavor development since this fraction appears to be incorporated into the fat globule membrane during homogenization. Singleton et al.(48) reported that a non-dialyzable component in milk was responsible for sunlight flavor. Control skim milk developed the sunlight flavor, whereas the dialyzed skim milk did not. Likewise, when the dialyzate was added back to the dialyzed skim milk and later exposed to sunlight, the off-flavor was detected. In another trial, Singleton et al.(48) added riboflavin to dialyzed skim milk and then exposed it to sunlight. It then developed the sunlight flavor. They believed that a dialyzable component, presumably riboflavin, and a non-dialyzable component both were involved in the development of sunlight flavor in exposed milk. Also, since the flavor component once formed was not dialyzable researchers believed that the flavor component was a molecule of relatively large molecular weight such as protein. In a model system containing tryptophan and riboflavin, a complex formed upon exposure to light which was suspected as the flavor component.

Research on the photochemical changes in milk involving the protein fraction has led many investigators to believe that sulfur containing compounds are involved. Flake et al.(24) demonstrated that cystine, methionine, tryptophan and histidine upon irradiation possessed especially strong burnt flavors and may contribute to the development of this off-flavor

in milk. Upon the analysis of the protein fraction of low density lipid protein of exposed milk, believed by Finely and Shipe (22) to be the principle source of the light activated flavor, a loss was observed in the amino acids methionine, tryptophan, tyrosine, cysteine, and lysine.

b. Oxidized Flavor. The oxidized flavor produced by photoxidation of lipids has been thoroughly investigated. Tallowy or oxidized flavors develops more slowly than the activated flavors. Factors affecting development of the activated flavor influence development of the oxidized flavor except for homogenization and ascorbic acid. Homogenization retards the development of the oxidized flavor through the formation of a protective casein membrane around the newly formed fat globule following processing, but this same protective protein membrane is believed to be a possible source of the compounds responsible for the development of activated flavor. Ascorbic acid content has been shown to be related to the development of the oxidized flavor in milk (32, 33). The oxidation of fats occurs concurrently with the oxidation of ascorbic acid (46).

The flavor compounds responsible for photoxidation of lipids are different from autoxidation or metal induced oxidation. Bassette (6) reported that the effect of light on production of some volatile compounds in milk exposed to sunlight. He found increases in some of the compounds which normally comprise the flavor in milk, such as acetaldehyde, propanal, n-pentanal and n-hexanal. Wishner (55) reported the presence of 2-enals in light exposed samples. This analysis did not reveal any 2, 4, dienals which are present in autoxidized milk. It has been postulated (55) that photoxidation involves the

monoene fatty acids of the triglycerides while autoxidation involves the polyenes of the phospholipids.

3. FACTORS EFFECTING LIGHT-ACTIVATED FLAVOR IN MILK

a. Source and Amount of Light. Amount and source of light are the most important factors responsible for activated flavor changes. When the effect of light on milk was first observed, sunlight was the only energy source considered to induced this oxidized or activated flavor. Stull (51) noted that the accurate measurement of sunlight intensity was difficult since it varied with season, geographic location, cloud cover and time of day. Holmes and Jones (34) used a pyrheliometer to record and control its intensity. The relationship among objectionable oxidized flavor, exposure time,and the light source was reported by Dunkley et al. (21). They found that light flavor was not detected immediately after short exposures to fluorescent light, but increased in intensity rapidly during the first 4 h of storage. Subsequent changes in flavor intensity depended on storage temperature at 32°F, remaining relatively constant; at 42°F decreasing after about 48 h; and at higher temperature decreasing after about 4 h. In milk given longer exposure the dominant flavor was tallowy or oxidized.

Stull (51) in a review reported that Francis observed that white, daylight, and Soft white fluorescent lamps increased the intensity of light activated flavor respectively, in exposed milk. However, Dunkley (21) concluded that Homeline, Cool-white, and day- light produced no significant difference in their ability to induce light activated flavor. Through the use of yellow, pink, or other colored fluorescent tubes, Hansen et al. (30) and Dunkley et al. (21)

reported the problem of light-induced off-flavors could be decreased. Comparing the effect of incandescent and fluorescent light, there was very little difference in the developed oxidized flavor intensities of milk bottled in clear glass containers exposed to the two light sources according to Smith and McLeod (49).

Variations in light intensities from 215-5380 lux in display cases in Vermont were reported by Bradfield and Duthie (9). Whereas de Man (15) stated that fluorescent light intensities in supermarket display cases in Guelph and Toronto, Canada varied from 550-5500 lux with many in the range of 1000-3000 lux. Hedrick and Glass (31) attempted to imitate average conditions in a grocery store display case, using fluorescent light adjusted in energy to 1614 lux and placed at 5.1 cm from the top of milk container. Flavors and composition of the milk are changed by these conditions when exposed for 5 h in both paperboard and blow-molded plastic gallon containers. No significant change in vitamin A, thiamin, and niacin in milk stored in paperboard or plastic containers. The same was true for 17 amino acids and 7 minerals. A significant decrease in vitamin C and riboflavin was observed when milk stored in both containers was exposed to fluorescent light. Dimick (18) conducted a similar test using a retail milk display case illuminated by Cool-white fluorescent lamps with 1076 lux, mounted parallel to the shelves and 45.7 cm from the containers. He used paperboard, blow-molded polyethylene and clear flint glass half gallon containers and the average light transmission was found to be 2.8% for paper board, 69.2% for plastic and 90.7% for glass container. Finally, flavor comparisons were made using a trained panel of 12 women. After 12 h of exposure all containers of milk were

rated lower than unexposed control milk. Flavor of milk stored in plastic and glass containers was similar and decreased remarkedly in acceptance after 12 h of exposure, while milk in paperboard containers required exposure to the light source for 48 h to reach similar flavor ratings.

Hoskin and Dimick (35) conducted an experiment to evaluate the ability of returnable polycarbonate, tinted polycarbonate, high density polyethylene, flint glass containers, and non-returnable unprinted paperboard cartons to protect homogenized milk from activated flavor development. Significant decrease in flavor scores were apparent when milk was exposed for 12 h under Cool-white fluorescent lamps producing 1076 lux. Milk held in paperboard containers had hedonic flavor ratings showing no statistical difference to non-illuminated control milk. Milk in glass, high density polyethylene and non-tinted polycarbonate could give little protection, while milk held in polycarbonate containers tinted with blocking material was effective against radiant energy in the 380-480 nm region and had hedonic flavor ratings in between those given milk in paperboard and for milk in glass, etc. Holmes and Jones (34) reported that as little exposure as 10 min will produce an activated flavor that can easily be distinguished. Nevertheless, the intensity of off-flavor decreases with prolonged exposure. Presumably, this is due to decomposition of methional developed during subsequent and prolonged exposure to light (34).

Barnard (4) reported that this flavor problem can be minimized by reducing the intensity of lighting in retail cabinets to 538 lux. Dunkley et al. (21) stated that an alternative was to use gold or "bug light" fluorescent lights in display cabinets.

b. Wave Length of Light. Sunlight flavor was recognized as an important defect, when milk was irradiated to increase its vitamin D content. Flake et al.(23) studied the effects of using different wavelengths of light and showed omission of wavelengths below 460 nm decreased the rate at which the activated flavor developed. Later, Herreid et al.(33) reported that ruby glass gave almost total protection against sunlight flavor development since no radiant energy below 600nm was transmitted through that glass. In addition, the rate of activated flavor development is reduced in the retail cabinets equipped with yellow fluorescent lights or fluorescent lights with yellow shields. Radiation spectra of these lighting systems show minimal energy emitted below a wavelength of 540 nm.

c. Temperature of Milk. Dunkley et al.(21) found that higher milk storage temperatures increases the intensity of light activated flavor. When temperatures of 0° and 11°C and later 1° and 16°C were employed, it was found that the flavor problem was due to both a fast reaction rate and an intense exposure.

Bassette et al. (7) observed less oxidized flavor in milk exposed to higher pasteurization treatments prior to irradiation. Milk heated at 90°C and then irradiated scored considerably higher in flavor with less light-activated flavor than 73°C irradiated milk. This means that the volatile materials that are characteristically related in light induced flavor tended to be higher in the milk pasteurized at the lowest temperature.

d. Time of Storage. The development of activated flavors was mainly due to the length of storage in illuminated cabinets or in sunlight. Bradfield and Duthie (9) reported that there are some locations in vertical display cases from which milk containers are never removed, mainly toward the back and center part. Also, in the low, reach-in variety of retail cabinet, the front row of cartons may not be sold for long periods, while containers in rows 2 and 3 were generally removed at faster rate.

In a study published by Market Facts of New York (5), 105 retail milk outlets were examined for disappearance of milk from cabinets as a means of assessing the length of exposure to fluorescent lighting in these display cabinets. The average light intensity in these stores was 2001 lux. In 15 outlets, in each of 6 cities, using time-marked milk containers, they found 71% of these containers unsold after 5 h, 58% unsold after 8 hr and 37% unsold after 24 h regardless of container size and type.

Dunkley et al. (21) found that 2 days of storage in the dark followed by exposure to a known amount of radiant energy resulted in less activated flavor than did a similar exposure immediately following processing.

e. Milk Containers. The type of container used to hold milk between processing and consumption plays an important role in minimizing flavor and nutrient alteration. Container materials generally used are: glass, blow-molded single service polyethylene container, paperboard container, plastic bags, and returanable high density polyethylene and polycarbonate container.

1. Glass. Hammer and Cordes (29) in 1920 reported that brown-colored glass milk bottles were effective in preventing the action of light on milk. Later, Herreid et al. (33) showed that amber glass offered protection from light for intervals up to 30 min, whereas ruby glass containers offered the best protection of all containers, including paper. Light of wavelengths less than 600nm did not pass through ruby colored glass. These researchers observed that processed milk taken from pasture-grazed cows and bottled in ruby glass containers showed no activated flavor after 2 h in direct sunlight, whereas milk in amber bottles showed only slight off flavor after 30 min in sunlight. In an experiment, to compare the protective effect of various colors of glass to clear glass, several researchers (18, 19) reported that the clear glass offered negligible or no protection from the sun's rays or fluorescent light. It was shown (35) that in 380 to 480 nm critical region, glass containers permitted 95% energy transmission.

2. Blow-molded single service polyethylene container. Single service plastic containers provide some protection from sunlight because of the opacity of the plastic (18). In a survey of market milk conducted in 1970, 86.1% or 31 of 36 samples of milk in blow-molded plastic were oxidized. A similar result, 84.2% was found in sampling in 1973 (3). Between 25-50% of the incident light is transmitted by blow-molded plastic containers. It was observed that plastic 3-quart milk containers allow 80% light transmission at 400 nm and over 80% at 700 nm. Another solution was offered to minimize this transmission by using such materials as titanium oxide etc to block visible radiation (3). However, de Man (15) reported a negligible effect in controlling

light-induced flavors by using titanium oxide in the bottle matrix.

3. Paperboard containers. Of all materials used for single service milk containers, paperboard best protects milk's flavor and nutritional qualities. The effects of sunlight on milk in different types of paperboard containers were studied first by Henderson et al.(32). They used a) white bleached paper, the thinnest of the three types, b) cream colored paper of intermediate thickness, and c) multiple layers of bleached white outer plies and unbleached and light brown innerplies, the thickest of the three paper boards. The paperboards were parafined, which should be similar to the current polyethylene coated paperboards. Milk stored in cartons made from material a) yielded a slight sunlight flavor in 1 h in noon sun; milk in carton b) was scored intermediate for off-flavor, while milk in carton c) showed no off-flavor at all.

The amount of light transmitted differs markedly in the unprinted portions of commercial milk cartons (15). It is known that the ink used absorbs light energy dependent upon its color. Therefore, cartons should be designed with large areas of red, brown, black, yellow or orange ink to absorb the shorter wave lengths of energy. Bradfield and Duthie (8) reported values for light energy transmitted through paperboard colored with red, blue, black and green inks. Using a 4304 lux fluorescent light, 100% transmission was observed in uncolored paperboard carton, then red gave 54% transmission, blue 27%, black 27% and green 18%. The same researchers reported that milk in uncolored cartons exposed to 2376-2690 lux of fluorescent light changed flavor in 54 h. At 4304 lux, milk in the uncolored cartons developed sunlight flavor in 12 to

18 h. Later Sattar and de Man (43) showed that light transmission values for paperboard containers at 400 nm was 0% and about 13% at 800 nm. Energy transmission values were reduced to 0 to 10% by printing.

It is obvious that any paper board laminate that contains an aluminum foil layer, such as used for long-life dairy products, would essentially eliminate transmission of light and increase in flavor and shelf life of the product contained.

4. Plastic bags. The main reason for not using this type of containers is the uncertainty or lack of presence of an outer protective cardboard box. Barnard (3) in a study found that 50% of the samples in plastic bags were oxidized. This is comparable to that for oxidized off-flavor in milk in glass, 46.4% or 26 of 56 samples.

Reif et al. (41) reported on flavor defects of 304 liquid milk products sampled at retail outlets in California. Among the principle flavor defects in 172 milks packaged in paper cartons were feed (9.9%), fruitness (8.1%), and lacks freshness (10.5%). The main defects in 132 milks in plastic containers were feed (12%), and light-induced flavor (45%). So it is suggested that steps be taken to reduce the incidence of light-induced flavors bottled in plastic containers.

5. Returnable high-density polyethylene and polycarbonate containers. These milk containers have shown limited consumer acceptance. Hoskin and Dimick (35) reported that gallon-sized high density polyethylene milk containers transmit 58% of fluorescent light. Gallon-sized polycarbonate containers on the other hand transmit 90% while these containers structured

with energy-blocking material transmit 75%.

4. INFLUENCE OF LIGHT ON THE COMPOSITION OF OTHER MILK CONSTITUENTS

Dimick (20) in a recent review reported that light effects several milk constituents. Among those affected are: 1) riboflavin, 2) ascorbic acid, 3) amino acids and proteins, and 4) vitamin A and ß-carotene, and the effects are related to and proportional to wavelength, intensity and duration of exposure.

a. Riboflavin. Loss of riboflavin is rapid when milk is exposed to sunlight or fluorescent light (2). Herreid et al. (33) indicated that in 30 min as much as 30% of riboflavin in milk was destroyed with this going to 80% in 2 h of exposure to sunlight. In a similar study, Singleton et al. (48) observed, 64% loss of riboflavin in 30 min of exposure and 89% loss in 2 h of exposure to sunlight. Sattar et al. (44) reported that degradation followed first order kinetics and the rate of destruction increased with temperature.

Stamburg et al. (50) stated that greater losses occurred in summer months, when products temperatures are high. Dunkley et al. (21) observed that the rate of destruction of riboflavin in milk is also directly related to the wavelength of light. Sattar et al. (43) reported the maximum riboflavin destruction when the milk was exposed to radiant energy between 415 and 455 nm while at wavelengths above 550 nm destruction was markedly reduced. The use of a gold lamp or "bug light" color is one possible solution to control both off-flavor and vitamin destruction in milk, stored in retail cabinets (21).

Maniere and Dimick (37) showed that the rate of riboflavin destruction increased when it was in free form and unassociated with proteins or fats in milk. A study (38) chemically defined lumichrome as the major degradation product of riboflavin.

2. Ascorbic Acid. Milk nutritionally is not a significant source of vitamin C. Stull (51) reported that light accelerates oxidation of ascorbic acid to dehydroascorbic acid and the rate is proportional to the amount of light transmitted through the container, the wavelength (33) and the presence of riboflavin (45).

Henderson et al. (32) indicated that brown or amber glass and paperboard containers offer the greatest protection from the actinic rays of radiant energy. Using paperboard containers and different light opacities, they showed that vitamin C destruction was proportional to light transmission and was indirectly related to flavor development.

Woessner et al. (56) showed that ascorbic acid is stable during normal thermal processing operations but disappeared rapidly during light exposure. Dunkley et al.(21) analyzed the active wavelengths that would destroy the ascorbic acid and reported that the energy between 400 and 550 nm was responsible for reducing flavor scores. Later de Man (16) reported that the decrease in vitamin C and flavor scores is proportional to light transmission. As with riboflavin, the greatest protective effect in illuminated retail cabinets would be from gold or "bug light" type lamps.

3. Amino Acids and Proteins. Dimick (18, 19) reported that even though

the sunlight flavor was attributed to oxidation of the essential amino acid, methionine, no significant change occurred in its concentration or the concentration of 16 other amino acids over a period of 144 h when milk in paperboard, blow-molded plastic and glass containers were exposed to fluorescent light. The amount of tryptophan lost on exposure of milk to sunlight was directly related to the loss of riboflavin and indirectly related to activated flavor intensity (28, 48). After 2 h of exposure to sunlight 15% of the tryptophan disappeared. Color changes in milk upon exposure to sunlight for 1 to 2 h and to fluorescent light for 4 to 5 h are caused principally by degradation of tryptophan and tyrosine in milk proteins (52). It is possible that photodegradation of isolated milk protein fractions may occur and involve both low and high molecular weight serum proteins.

4. Vitamin A and ß-carotene. The loss of vitamin A and its principal precursor could be markedly reduced by limiting exposure of milk to energy below 465 nm. A protective effect on vitamin A was observed when ß-carotene concentration was greater than 2.5 mg/ml. Senyk and Shipe (47) reported that added vitamin A was found to be particularly susceptible to destruction by fluorescent light. This destruction related inversely to fat content with 37, 44, 49,and 57% losses in whole, 2% fat, 1% fat and skim milk, respectively, when exposed to 2000 lumen/m^2 for 4 h in polyetheylene containers. The type of container was found to affect degradation of vitamin A with fiber and gold tinted containers providing the best protection. Later de Man (17) determined the vitamin A content of whole, 2% and skim milk packed in plastic pouches before and after exposure to 2200 lux intensity for

48 h at refrigeration temperature. He found that the vitamin A content of whole milk dropped to 67.7% of its original contents after 30 min and remained constant for 18 h whereas 2% milk dropped to 23.6% and skim milk to 4.2% of the original content.

CONTROL OF LIGHT ACTIVATED FLAVORS

The light activated flavors can be effectively controlled by 1) using light barrier for milk containers (pigmented bottles or cartons, opaque cartons) 2) controlling the type and intensity of irradiation. 3) Proper rotation of inventory of milk in display cabinets in retail stores.

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CHAPTER 6 OXIDIZED FLAVOR

INTRODUCTION

Terms most commonly used to describe the oxidized flavor in milk are "cappy", "cardboard", "metallic", "oily", "oxidized" and "tallowy". This off-flavor develops upon storage and is more prevalent in pasteurized cream line milk, skim milk and fluid cream products than in homogenized milk. Although it may be found immediately after milk is secreted by the cow, it generally requires time to develop.

Thurston (49) in 1937 classified milk into the following 3 categories with respect to differences in oxidation stability.

1. Spontaneous milk - develops oxidized flavor without exposure to iron or copper.

2. Susceptible milk - develops oxidized flavor in the presence of copper or iron.

3. Non susceptible milk - does not become oxidized even in the presence of iron or copper.

1. FACTORS AFFECTING THE DEVELOPMENT OF OXIDIZED FLAVOR

a. Heredity. Several studies have indicated that breed differences influence susceptibility of milk to oxidation. For instance, Corbett and Tracy (9) reported that milk from Ayrshire cows was slightly less susceptible to this flavor defect than milk from other breeds. On the other hand, Krukovsky (35) reported, that frozen cream and butter from Ayrshire milk had lower flavor ratings than these products made from Jersey, Brown Swiss or Holstein milk. Studies (40) conducted to determine the importance of heredity, indicate that

it is not a major contributor to the cow's susceptibility of milk to oxidized flavor.

b. Feed. It is generally agreed that feeds can influence the stability of milk against oxidation by affecting its composition. This effect was shown to be dependent on both type and quality of the feed. Anderson (2) reported that when cows were fed good-quality alfalfa hay they produced a milk more resistant to oxidation than when fed poor-quality alfalfa. Several workers observed that when cows were fed with green feeds or pasture, milk subsequently was more resistent to oxidation than milk from cows on dry feeds. Nevertheless, Greenbank (24) pointed out that the oxidized flavor in milk was not always inhibited by green feed. Krukovsky (35) found that, in general, pasture, silage, or hay made up predominantly of blade grasses or good-quality corn silage produced stable milk, whereas, red clover, ladino clover, and soybean silage rendered milk unstable. Dunkley et al. (16) reported that milk produced on alfalfa hay was much more susceptible to oxidation than milk produced from oat hay. Also the copper content a catalyst for oxidation was much higher in both alfalfa hay and the milk produced from its feeding than from oat hay.

It was shown (41) that, legumes have greater copper and lower manganese content than grasses. This may partially explain the differences between the susceptibilities of milk produced on these two types of roughages. The copper and manganese content of forage also varies with maturity (37). Consequently, the stage of maturity of a forage may influence the effect it has on oxidative stability of milk.

Even though the effect of feed on the copper content of milk has

received only limited attention, several studies were carried out to determine their effect on other constituents of milk which might be involved in oxidation. Brown et al.(7) conducted a series of investigations to determine the effect of feeds on ascorbic acid and carotene content of milk. In their initial study they found that feeding pure ascorbic acid, or tomato juice, or lemon juice reduced the tendency of milk to oxidation. Nevertheless, they did not observe an increase in the milk ascorbic acid content. However, they did noted a slight increase in carotene content. Feeding carotenes increased the carotene content of the milk and its resistance to oxidation. Krukovsky et al. (36) observed an important correlation between tocopherol (vitamin E) content of milk and its ability to resist oxidized flavor development. It is known that, some of the antioxidant effect of certain green feeds is due to their tocopherol content. Feeds supplemented with tocopherols (50, 12) ethoxyquin (15) N, N^1 diphenyl paraphenylenediamine (48) and cacao shells (39) which contain antioxidants have been used to increase antioxidant content of rations. Although these supplements help to protect lipids, direct addition of antioxidants to milk would be more reliable if it were more legal. Small amounts of antioxidants added to feeds are passed into the milk, for example only 2% of added α -tocopherol was secreted in the milk (50). However, both intravenous and intramuscular administration were found to be more effective but expensive (13).

A considerable amount of attention has been focused on the effect of feeds on the degree of unsaturation in the lipid fraction of milk. Crobett and Tracy (8) fed coconut and corn oils and found that the iodine values in milk subsequently produced were increased markedly. There was, however, only a slight increase in susceptibility of milk to oxidation with the more highly

unsaturated milk. Brown et al.(6) fed coconut oil to produce milk with a slight increase in the iodine number and a greater susceptibility to copper-induced oxidation. Krukovsky (34) reported that the type of roughages effects the iodine value of the milk fat. However, the stability of the fat against oxidation could not always be directly correlated with iodine number. He found that variations in the oleic acid were more closely associated with changes in oxidative stability than were variations in the linoleic acid content. Dunkley et al.(16) in comparing the effect of alfalfa hay with oat hay on fatty acids in milk fat produced from these rations observed some differences in unsaturated fatty acid. Even though the iodine values were lower in the milk produced on alfalfa hay, the milk was more susceptible to oxidation. However, the linoleic acid content was higher. These researchers speculated that, since polyunsaturated acids are more labile than oleic acid, small changes in them might be more significant than greater changes in oleic acid content. In another study (45) the same researchers found that infusing cottonseed oil into the bloodstream of cows increased the linoleic acid content of both the fats and phospholipids of milk. This was associated with an increase in the susceptibility to copper-induced oxidation. It is possible to increase the unsaturated fatty acid content of milk by feeding protected lipids, for example unsaturated lipids encapsulated to protect against hydrogenation by rumen microflora (21).

c. Stage of Lactation. Several studies were conducted to determine the effect of the stage of lactation on the oxidative stability of milk. Guthrie and Brueckner (25) were unable to observe any effect. Corbett and Tracy (9) reported that, milk from the first part of the lactation period is most

susceptible to oxidation, especially for heifers. King and Dunkley (29) reported that the copper content of early lactation milk was much higher than that in late lactation milk. A change in the percentage of copper associated with the fat phase as the stage of lactation progressed was reported by King and William (31). During the first 2-4 wk 15% of the copper was associated with fat globule, whereas at 10 wk 35% was associated with the globule. They also found high percentage of the copper associated with the fat in the case of spontaneous milk.

d. Milk Handling. Prior to automation of the dairy industry, oxidized flavor probably did not present a serious problem in the fluid milk. Milk was either consumed fresh or it became sour before it became oxidized. Cooling milk is one of the best means of increasing its shelf life, yet the cooling process was indirectly responsible for one of the first cases of oxidation to be reported. Shipe (42) reported that Golding and Feilman observed a mealy off flavor in milk in 1905 which was cooled on a poorly tinned surface cooler, which had exposed copper. They were able to duplicate this off-flavor by immersing a clean copper foil in fresh milk. Both copper and iron were found to be strong catalyst, with copper being more effective than iron,and ferrous iron more effective than ferric iron. King et al. (30) observed that added iron does not become associated with the fat globule, whereas some of the copper does. Some of the natural iron present in the fat globule membrane in the form of heme proteins may contribute to lipid peroxidation.

Henderson and Roadhouse (26) in 1940 reported that nickel, lead and zinc did not cause the oxidation of either ascorbic acid or lipids and Garrett (20) found that aluminum had no effect, but divalent manganese inhibited or

retarded the development of the oxidized flavor.

Exposure of milk to copper or iron in dairies and processing plants has been eliminated by the use of stainless steel and glass equipment. It was observed that white metals contributed copper contamination and Lusas et al. (38) reported that a stainless steel-white metal milk processing systems contributed an appreciable amount of copper to milk. Oxidation of milk was very rapid when it was processed in this equipment. This defect was attributed to the copper contamination from the white metal. Dunkley et al.(14) reported that copper from white metal fitting can become absorbed on stainless steel. This was facilitated by a circulation cleaning operations. The absorbed copper was not removed by water, but it was by milk or solutions containing Ethylene diamine tetra acetic acid (EDTA). It should be noted that 3-A standards no longer permit the use of white metal for equipment used in processing milk (27).

The incidence of oxidized flavor appears to be higher in milk stored at lower temperature (e.g. 5°C vs 10°C). This may be due to decrease in bacterial growth at the lower temperature. Lower bacterial growth could reduce the competition for oxygen and/or the production of reducing substances. The control of bacterial growth has led to prolonged storage of milk which provides enough time for chemical and enzymatic reactions to occur and may contribute to flavor problems.

e. Milk Processing. Some of the current processing procedures such as high-heat treatment and homogenization reduce the susceptibility of milk to oxidation. The inhibitory effect of homogenization increases as the homogenization pressure increase. Babcock (5) reported that it took

approximately ten times as much copper to produce an oxidized flavor in homogenized milk as in the corresponding unhomogenized milk. It was believed that homogenization resulted in enveloping the fat globule in a protective casein membrane. Krukovsky (34) believed that the unstable lipid components of the fat globule membrane were dispersed and drawn into the interior of the fat globule, where they were protected by the tocopherol associated antioxidant activity of the fat. Tarassuk and Koops (47) observed that the increase in protective effect from increasing homogenization pressure was related to the change in fat globule surface area. The concentration of phospholipids and the copper-protein complex per unit of fat globule surface area is reduced with the increase in homogenization pressure. It was postulated that homogenization causes a change in copper protein binding to produce a chelate with an antioxidant effect. Shipe (42) referred to Smith and Dunkley who observed that both homogenization and heat treatment altered the activation energy of the oxidation of ascorbic acid by copper. They attributed the change in activation energies to changes in the binding of copper.

Heating to higher temperatures has been found to inhibit oxidation. Kende (28) in 1931 believed that the protective effect of heat was due to destruction of an enzyme which he called oleinase. whereas Gould and Sommer (23), however, attributed the protective effect to the production of sulfides. In 1959, Aurand et al. (3, 4) revived the enzyme theory, partly on the basis that heat inhibited spontaneous oxidation. They believed that spontaneous oxidation involved an enzyme, whereas oxidation of susceptible milk did not and postulated that xanthine oxidase was the enzyme involved. They reported good correlation between xanthine oxidase activity of milk and spontaneous

development of oxidized flavor. Increase in Thiobarbituric acid (TBA) values when this enzyme was added to milk corroborated their theory. Smith and Dunkley (44) were able to show a correlation between xanthine oxidase activity and susceptibility of milk to spontaneous oxidation but suggested that the effect of the xanthine oxidase preparations might have been due to impurities with the enzyme preparation rather than xanthine oxidase itself.

Yee and Shipe (51) reported the effect of cysteine and glutathione on copper-and heme-catalyzed oxidation. In the copper-catalyzed system, cysteine exerted a strong prooxidative effect, and this effect increased with increases in concentration. Furthermore, oxidized glutathione did not have a prooxidant effect whereas reduced glutathione showed prooxidant activity. In heme-catalyzed system cysteine and reduced glutathione showed an antioxidative effect which increased with the increase in the concentration of the cysteine. Both anti and prooxidative effects of cysteine are eliminated by blocking the sulfhydryl groups by treatment of cysteine with iodoacetic acid. In the presence of sulfhydryl groups, copper promoted generation of the superoxide anion but heme did not.

CHEMICAL CHARACTERIZATION OF OXIDATION

In recent years a considerable amount of progress has been made in the chemical characterization of oxidized flavors. It is generally assumed that the flavor occurs initially in the phospholipid fraction rather than from the triglyceride. Furthermore, most if not all of the flavor components are believed to emanate from unsaturated fatty acids such as arachidonic, linolenic, linoleic, and oleic of phospholipids.

The mechanism for lipid oxidation (33) is believed to involve the classical

peroxidation pattern in which free radicals and hydroperoxides are formed. Although these compounds have no flavor, their scission products formed from the hydroperoxides or from the free radicals involved in the reaction yield a variety of flavorful aldehydes and ketones.

A number of carbonyl componds have been isolated from oxidized milk fat or phospholipids, Forss et al. (19), found acetone, ethanal, n-hexanal, C_4 to C_{11} mono unsaturated aldehydes from copper induced oxidized skim milk, which was described as "cardboardy". In several studies, Forss et al. (17, 18) and Stark and Forss (46) attributed specific flavors to specific compounds. They attributed the oily flavor to n-hexanal, n-heptanal, hex-2-enal, and heptan-2-one and the tallowy flavor to relatively large amounts of heptanal, octanal, nonanal, heptan-2-one, hept-2-enal, and non-2-enal. They found relatively high concentrations of n-pentanal and the C_{5-10} alk-2-enals, in samples of butter with painty flavors, reported that non-2-enal has a distinct cucumber flavor, a metallic flavor to oct-1-en-3-one, and a mushroom odor to oct-1-en-3-ol. Day et al.(10, 11) suggested that a complete spectrum of compounds is responsible for oxidized flavor rather than specific compounds and that combinations of subtreshold levels of carbonyl compounds are additive and thereby gives rise to detectable flavors.

Recently Schroder (43) reported that a high oxygen uptake in milk was not directly correlated with oxidized flavor development. Although nearly complete deoxygenation of whole pasteurized milk contaminated with copper prevented the formation of the formation of flavor, moderate deoxygenation resulted in even greater flavor intensity than non-deoxygenation.Allen and Joseph (1) in a recent study reported the consumption of oxygen during storage

of lab pasteurized milk at 7°C in dark for 14 d. The factors responsible for its consumption were quantitatively estimated. The principle ways in whiich oxygen was lost were: loss through septum, 7.1%, ascorbic acid oxidation, 34%, bacterial growth, 36.1%, sulfhydryl oxidation, 3.4%. Thus some 80.6% of the oxygen consumed was accounted for. Lipid peroxidation used only 0.07%. Nevertheless, preliminary sensory findings indicated that oxidized flavor was the predominant element in stale milk.

CONTROL OF OXIDIZED FLAVOR

One or more of the following procedures are recommended by researchers to control the oxidized flavor in milk.

Provide cows with feeds that impart antioxidant properties to the milk.

Implement suitable breeding programs that increase non-susceptible milk.

Avoid exposing milk to catalytic metals such as copper and iron.

Increase the heat treatment. A combination of heat and vacuum treatment has a marked protective effect because it releases sulfhydryl groups and removes oxygen (32). However, recently it was reported that suflhydryl groups have both anti and prooxidant effect (51).

Homgenization increases the resistance to non-light induced oxidation. Neither heat nor homogenization gives complete protection, especially if milk is exposed to copper or iron.

A variety of substances can be added to inhibit oxidation, however, these substances are not legally approved additives.

The concentration of ascorbic acid in milk affects oxidative stability. Low levels normally found in fresh raw milk (i.e. 10-20 mg/L) has a prooxidant effect whereas rapid and complete destruction of ascorbic acid inhibits

oxidized flavor development.

A mild trypsin treatment of milk can inhibit oxidation presumably by either exposing antioxidants such as sulfhydryl groups or reducing the activity of prooxidants (52).

Oxidation can be inhibited by the action of phospholipase C which breaks off the polar end of phospholipids to yield a diglyceride and a phosphoryl fraction. It is speculated that this cleavage reduces the exposure of the unsaturated lipid. The addition of phenolic type antioxidants prevents free radical formation, which binds metallic catalysts and inhibits oxidation. Nevertheless, it is illegal to use any of these antioxygenic treatments in processing milk (22).

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CHAPTER 7 MISCELLANEOUS FLAVORS

INTRODUCTION

Several miscellaneous off-flavors are encountered occasionally in milk. They may arise from poor practices, either on the farm or in the plant. Such flavor or tastes as "absorbed", "astringent", "bitter", "chalky", "chemical", "flat", "foreign", "lacks freshness" and "salty" fall in to this category.

1. ABSORBED FLAVORS

The term "absorbed" is applicable to flavors that are absorbed from the environment (1). In olden days it was thought that most feed and environmental odors were absorbed by milk directly from the air. But it is now understood that many such odors are transmitted through the cow. Nevertheless, some volatile substances may be absorbed directly from the air. For example fat soluble substances such as turpentine and other volatile solvents are absorbed readily. Hence, absorbed flavors can be a problem (3) when milk is stored near materials with strong aromatic flavors. Therefore, care should be taken to avoid this practice during milking and storage of milk.

2. ASTRINGENT FLAVORS

Astringent has been used to describe a dry,puckery oral sensation which involves the sense of touch or feel rather than taste. The terms rough, chalky or powdery also have been used to describe this sensation. Several researchers (5, 7) have associated astringency with milk products that have been processed at high temperature. In these cases the astringency has been attributed to large protein salt and salt particles. Protein particles with low mineral contents are responsible for astringency in acidified milk products. Astringent flavors also may be produced when milk is fortified with iron

salts, especially ferrous salts (4).

3. BITTER TASTES

Proteolysis usually is responsible for bitterness in milk, some peptides and amino acids produce bitter tastes (9). In fluid milk, the proteolysis usually is caused by microbial proteases since the activity of the natural milk protease is slight. Bitter tastes also may be caused by lipolysis or certain weeds e.g. bitterweed. An alkaloid may be responsible for the bitter weed taste since a number of alkaloids found in plants are bitter.

4. CHALKY TASTE

Chalky has been described as a tactual defect similar to astringent, giving a sensation that suggests finely-divided insoluble particles. Immediately, after the commercial introduction of homogenized milk, several reports indicated that homogenized milk had a chalky chararacteristic. This may have been caused by improper homogenization, however, there is no proof that proper homogenization does not causes chalkiness (12).

5. CHEMICAL FLAVORS

Flavors that are caused by the contamination of milk with a variety of chemicals associated with cleaners, sanitizers, and disinfectants (6, 11, 13) are included in this group. The most frequent contaminants are found to be chlorine and iodine. Phenolic compounds from disinfectant and some weed killers also are found occasionally in milk and cause off-flavors. A chlorophenol flavor was observed in milk (10), and attributed to products of a reaction of chlorine sterilizing reagents with phenol which were in a water supply.

6. FLAT

A flat taste is characteristic of low solids milk or milk that has been watered (2). Addition of as little as 3-5% water can produce this defect. Such milk lacks the normal, pleasing sweetness one expects in high-quality milk, but this defect generally does not produce consumer complaints unless the solids are excessively low when it is grossly watered. Some people feel that vacuum treatment of milk produces a flat tasting product. Flat tastes in milk can be minimized by the addition of solids-not-fat (10).

7. FOREIGN FLAVORS

Foreign and medicinal flavors are caused by the introduction of medications, disinfecting materials, sanitizers, fly sprays, gasoline or a countless number of other compounds commonly used on the farm or in the plant (8). These materials may enter the milk supply directly as in the case of medications used on the cow's udders, sanitizing compounds not properly rinsed from the utensils or disinfectant sprays drifting into open milk containers.

8. LACKS FRESHNESS

The term "lacks freshness" is used to describe an "old" taste or a minor off-flavor defect which cannot be positively identified. Even fresh milk may lack the full flavor of high quality milk. Thus it is hard to say with great confidence that this defect is necessarily due to milk age unless the sample had been judged when it was fresh.

9. SALTY FLAVOR

Saltiness in milk is identified easily by tasting. It is most commonly found in milk from cows in late lactation and occasionally from cows with mastitis (12).

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CHAPTER 8 UHT-MILK

INTRODUCTION

Heating milk to ultra high temperature (UHT) for 3 s to extend the shelf life of pasteurized milk or when followed by aseptic packaging to make a sterile product has been practiced for over twenty years. The history of these developments is covered well by Westhoff (33). Although these processes are effective in destroying microorganisms in milk, changes that occur in the high temperature-treated milk during extended storage affect palatabiltiy of this milk. A thorough review of the properties of UHT milk by Mehta (23) describes the current status of this product

It is important in discussing ultra high temperature (UHT) processes for milk to distinguish between UHT-pasteurized and UHT-sterilized milk. UHT pasteurization was introduced into the United States in 1948 as an alternative to high temperature short time (HTST) pasteurization but was not officially accepted until 1965 (29). According to the Grade A Pasteurized Milk Ordinance (33) ultra pasteurized milk must be heated to 138°C or above for at least 2 s. Burton (8) explained that although UHT-pasteurized milk is not sterile, microbiologically it usually keeps better than conventionally pasteurized milk. It is heated to higher temperatures than HTST milk for long enough to destroy pathogens.

UHT-sterilization on the other hand involves heating the product to a high enough temperature for a long enough time to produce a commercially sterile product. Burton (7) and Hsu (15) reported that even in U.S.A. and Canada there is an increasing tendency to associate the terms "UHT milk" with "UHT-sterilized milk". Westohoff (33) and Staal (30) reported pasteurization

standards for the U.S. and sterilization standards for other countries, respectively.

Although UHT sterile milk has captured a major share of the fluid milk market in countries like Germany, Italy, and France (17), it has not been well accepted in the U.S. to date. There appears to be several reasons for the slow growth of UHT milk in the U.S. It was not until January 7, 1981 that non-hermetically sealed cartons legally could be used to aseptically package milk (1), and only in May 1982 did the first major processing plant begin marketing UHT sterile milk in flexible cartons (13). Initial market survey indicates that there is a reluctance of consumers to accept this sterile milk when it is in direct competition with fresh pasteurized milk (2). One of the main reasons for consumer lack of enthusiasm for the new product is the difference in flavor of the sterile milk

FLAVOR

Hostettler (14) reported that the flavor of UHT-milk is only slightly different from pasteurized milk. The aromatic substances of the milk which causes feedy, weedy or barny odors can be removed more efficiently at higher processing temperatures. Hence, consumers find the UHT-milk to be flat or "purer". On the other hand, some criticize it for off-flavors, such as cooked or stale. Hostettler explained that when steam is injected into milk or vice versa as in the case of direct heating UHT process, the condensed steam has to be removed. This is achieved when the milk is vacuum-cooled by flashing it into an expansion vessel under reduced pressure. This method of cooling also helps to eliminate a major portion of the aromatic components, sulfhydryl

compounds and oxygen. The deaeration helps in reducing oxidative changes in the milk.

According to Ashton (4) flavor changes that occur in UHT-milk packaged in waxed paper and polyethylene laminate cartons stored between 4 and 22°C could be separated into 5 stages or periods: (a) period 1, immediately after processing; the milk has an unpleasant taste and smell, hydrogen sulfide-, carbon disulfide-and boiled cabbage-flavors (b) period 2, at 2-3 d; there is a weaker hydrogen sulfide-, cabbage-and less unpleasant-flavor, slight residual "cooked" (c) period 3, at 5-12 d; UHT milk has its best flavor at this stage, with traces of initial unpleasant flavor, creamy taste similar to pasteurized milk (d) period 4, at 12-18 d; there is the appearance of flat-, chalky-or slight residual cooked flavor (e) period 5, at 19 d; UHT milk develops a slight incipient oxidative rancidity or "card boardy" flavor, becoming progressively more obnoxious with age.

According to Ashton (4), all the above stages progress faster if higher storage temperatures are used. The time necessary to pass through each stage is independent of the thickness of the carton's internal polyethylene layer. A black lining also does not affect the rate at which off-flavors develop; however, an aluminum foil lining increases the duration of each phase.

Hansen et al. (12) reported that milk processed at several temperatures between 107-143°C using direct steam injection and stored the milks at 1.7 and 7.2° C in polyethylene bags or clear glass containers, was judged from acceptable to good by a 25-member panel at 2, 9, 16, 23 and 30 d.

McKellar (26) reported the relationship between proteolysis and off-flavors pasteurized milk. In a research study milks were subjected to

proteolysis after incubation with different concentration of enzymes isolated from three different strains of Pseudomonas fluorescens and incubated for 20 h at 35°C. Proteolysis measured as the increase in trichloroacetic acid-soluble free amino acid groups. Increase in free amino groups were significant when pasteurized milk was incubated at both 4°C and 35°C for 20 h. UHT milk was approximately twice as sensitive as pasteurized milk to the action of crude proteolytic enzymes, but unlike pasteurized milk it did not coagulate when exposed to high concentrations of the enzyme.

a. Cooked Flavor and Sulfhydryls. Cooked flavor is one of two primary flavor criticisms of UHT milk. Hutton and Patton (16) reported that a cooked flavor is noticed first when raw milk is heated momentarily to about 75°C or when it is exposed to lower temperatures for a prolonged time. Sulfides and mercaptans result from the heat denaturation of only the serum protein (16). ß-lactoglobulin the major component of the albumin fraction in serum proteins, accounts for almost all the volatile sulfur-bearing components. Sulfur containing amino acids such as methionine, cysteine and cystine are probably responsible for the formation of volatile sulfur-bearing compounds. Patton (28) stated that the probable mechanism of the cooked flavor involves conversion of those amino acids to hydrogen sulfide and methyl sulfide. Jaddou et al. (18) correlated the cabbagy defect in UHT-milks with hydrogen sulfide, carbonyl sulfide, methanethiol, dimethyl sulfide and carbon disulfide.

Blankenagel and Humbert (6) observed that in the 82-140°C range, the primary effect of increasing the temperature of skim milk was denaturation of serum proteins, with ß-lactoglobulin completely denatured at 130°C. They also observed that denaturation produces sulfhydryl groups which cause the cooked

flavor. After a week of storage at room temperature, the volatile sulfur compounds dissipate; but at 4.5°C, the rate of their disappearance is much slower. Burton (7), Hostettler (14) and Ashton (4) found that UHT-milk has a hydrogen sulfide odor and a cooked flavor immediately after processing, that disappeares within 24 h after processing.

Patrick and Swaisgood (27) observed that the reactive sulfhydryl (SH) groups in UHT-skim milk were oxidized more rapidly at room temperature than at refrigeration temperature. Concentrations of reactive SH groups in UHT-milk correlated well with the undesirable "cooked" flavor, and it was speculated that they contribute to the instability of milk protein through disulfide interchange reactions. Lyster (22) suggested that the difference in SH stability at different heating temperatures, holding times, and storage times are due to the enzyme sulfhydryl oxidase which oxidizes SH groups linked to protein compounds.

Jordan (20) confirmed that the cooked flavor of milk disappeared within a few days because of oxidation. In the direct UHT process where direct injection af steam into milk or milk into steam is responsible for heating the milk, oxidation occurs more slowly as added steam and oxygen are removed by vacuum cooling. On the other hand when oxygen is passed through the product, oxidation of the reducing substances is accelerated. Such a process has been patented by AB Tetra Pak in Sweden (14) to reduce the cooked flavor.

b. Stale. Besides cooked, the other common flavor criticism of UHT milk is "stale". A stale flavor becomes noticeable after the cooked flavor starts to diminish (10, 32).

According to Thomas et al. (32), some investigations coupled the stale flavor with products of the Maillard reaction. The compound, 1-Amino-1-deoxy- 2-ketohexose was identified as the stale principle in sterile milk concentrate (32). This product of the Maillard reaction may be related to or parallel the development of browning and staling. Arnold et al. (3) identified the following various volatile chemical compounds in different kinds of stored milk products: C_1 - C_3, C_5 -C_{10}, C_{12}, C_{14}) n-aldehydes, (C_3-C_5, C_7, C_9, C_{11}, C_{13}, C_{15}) n-methyl- ketones, (C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}) n-fatty acids, 2-pentanone, 2-hexanone, 2-heptanone, δ -decalactone, δ--dodecalactone, furfuraldehyde, benzaldehyde, 2-methylheptanal o-aminoacetophenone acetone, phenylacetate and dimethyl sulfide. In experiements with stale sterile concentrated milk, Arnold et al., identified 2-heptanone, 2-nonanone, 2-tridecanone, benzaldehyde, acetophenone, naphthalene, a dichlorobenzene, δ -decalactone, benzothiozole, and o-aminoacetophenone. Of these compounds, only the dichlorobenzene and 2-heptanone were identified in the control milk. Control milk was stored at 1°C and experimental milk at 22°C. Jeon et al. (19) observed increases of acetone, methylketones, n-aldehydes and 1-butanol in UHT (145°C for 3 s) - milks stored at 3, 22 and 35°C for 5 months. Although the methylketones were most abundant, aldehydes appeared to be the most important contributors to the off-flavor of stored UHT-milk. Oxygen in milk affected the concentration of only the aldehydes, whereas storage temperature affected concentrations of aldehydes and the methyl ketones. Findings of Jeon et al. were similar to those of Mehta and Bassette (24) who found increases in stale off-flavor intensity for UHT-milk stored at 22°C occurred concurrently with increases in

propanal, n-pentanal, and n-hexanal and decreases in cooked flavor and methyl sulfide. Earley and Hansen (9) reported changes in milk processed at 138°C for 20.4 s, or 149°C for 3.4 s, packaged aseptically and stored at 24 and 40°C for 24 wk. They found that the fat soluble alkanals, methanal, propanal, butanal, and nonanal were identified in the two UHT samples in the beginning and later decreased during storage at both 24 and 40°C. Dissolved oxygen content increased linearly in samples stored at 24°C but decreased and remained at a minimum in samples stored at 40°C. As previously mentioned Bassette and Jeon (5) observed that increases in volatile aldehydes such as acetaldehyde, n-pentanal, and n-hexanal were closely related to the rapid decrease in product acceptability that was due mainly to increase in the intensity of stale flavor. Relatively little changes occurred in the concentration of these aldehydes in milk stored at refrigeration temperature. Mehta and Bassette (25) suggested that development of staling as cooked flavor disappeared suggested that the mechanism for staling depends on the oxidation-reduction potential. They also reported that refrigeration at 4°C helped decrease but not eliminate the rate of increase of the aldehydes and stale off-flavor. Hostettler (14) confirmed that UHT-milk resisted formation of oxidized flavors better than did pasteurized milk because of reducing substances. Keeney and Patton (21) associated some lactones with off-flavors in stored milk products. The flavor they described as non-oxidative was probably what other researchers referred to as "stale".

OXYGEN, PACKAGING AND MISCELLANEOUS

Zadow (34) investigated the effect of oxygen in sterile milk. After storage for short periods, milk with a high oxygen content was given

preference. After storage for prolonged periods, the flavor of milk with low oxygen was found to be better. The deterioration in flavor resulted in oxidized or slightly rancid off-flavors. These observations were confirmed by Zadow and Birtwistle in 1973 (35). Their work led them to conclude that the two main factors affecting the development of off-flavors in sterile milk are level of oxygen and temperature of storage. Tarassuk (31) studied the effect of oxygen content in the headspace of cans sterilized by autoclaving at 85-120°C. A correlation was found to exist among carbon dioxide produced, oxygen depletion, the intensity of cooked flavor and the degree of browning discoloration. There is a possibility of improving the flavor and color of sterilized milk by lowering the available oxygen in a can before sterilization. This also suggest that if the oxygen in the milk during UHT processing is limited, milk with a better flavor could be produced.

Thomas et al. (32) studied the effect of oxygen content on the flavor of indirectly heated UHT milk during a 150d storage period at room temperature. The UHT milk was prepared with initial oxygen contents of 8.9, 3.6 and 1.0 ppm which represent almost the entire range of concentration of oxygen expected under normal processing conditions. Flavor acceptability was maximum at 6 d, after which it slowly declined. The increase in acceptability from 0 to 6 d was associated with the decrease in the off-flavor described as cabbagy. After 6 d, the milks started acquiring a stale characteristic. A higher intial oxygen content, up to a period of 8-13 d, yielded a more acceptable flavor in the milk, however, after this period, the acceptability was independent of initial oxygen content. Decrease in sulfhydryl compounds, ascorbic acid and folic acid were less with lower initial oxygen and vice

versa. But the beneficial effect of high oxygen content on flavor is slight and is out weighed by the adverse nutritional effects.

The ideal packaging material for UHT milk should be odor-free, non-reacting, sterile, and impermeable to air and light (11). Hostettler (14) has reviewed the effect of packaging on the flavor of milk. Plastic foils used in the dairy industry have an odor of their own. For example, polyester foils may have terephthalic acid which has a paper-like odor; polyethylene foil, an odor of oxidized oil and wax; polypropylene foil, a burnt and phenolic odor. Hansen et al. (12) reported the noticeable flavor absorption in UHT milk packaged in a polyethylene bag and cardboard box. It is possible that the sizing glues and components in the cardboard were absorbed through the polyethylene film. However, the flavor absorption problem was eliminated when sterile amber and clear glass containers were used.

Liter polyethylene-coated cartons with aluminum foil (AC) and without aluminum foil (PC) were compared by Fluckiger (11). The AC cartons did not lose any weight during six-week storage period. During the same period, the PC cartons lost about 0.2% of the original weight during storage at 20°C and about 1% at 38°C. The oxygen in the milk in AC remained almost unchanged (1 ppm); however, in PC, the milk was saturated with oxygen (8 to 9 ppm) after a few days. Most of the oxidative changes in the PC took place in the first 2 or 3 d after processing. In other words, the reducing substances in the milk that offer protection against oxidation decreased rapidly in PC cartons. This milk was organoleptically acceptable up to 3 wk when stored at 15°C. The AC milk was organoleptically acceptable up to 2 months even at a storage temperature of 38°C. Later Mehta and Bassette (25) confirmed that

UHT-milk in AC cartons retained a desirable flavor longer than that in PC cartons, partly due to PC cartons were more permeable to gases. Cartons wrapped either with Saran or aluminum foil were harmful to flavor, as they not only prevent the outside air from entering milk but also prevent the escape of volatile compounds coming from either growth of microorganisms on the surface of the cartons or from the wrapping material itself.

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MILK FLAVORS

by

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This report is a review of off-flavors encountered in fluid milk. It includes chapters on transmitted-, microbial-, lipolyzed-, heated-, light-activated-, oxidized- and miscellaneous flavors. Finally, the flavor of UHT milk is reviewed.

Most of the literature cited for different off-flavors, covers the period since a comprehensive review of milk flavors by Strobel et al. 1953. In addition to causative agents and volatile materials associated with off-flavors, methods for control of each of the off-flavors are presented. This report is designed to serve the dairy production and processing industry, students and faculty of food science curriculums, dairy product evaluation students, regulatory people and fieldman.

Off-flavors usually considered as transmitted include feed, weed, cowy and barny. These flavors are transferred to the milk within the udder via the respiratory and/or digestive system. Different feeds responsible for this flavor are silage, bromegrass, wheat and rye. Transmitted flavors can be minimized by careful herd management and good pasture management.

Microbial flavors in raw and pasteurized milk are formed by the accumulation of the products of bacterial metabolism. These include flavors commonly described as acid, malty, fruity, unclean, bitter and putrid. They are encountered in raw and pasteurized milk through neglection or failure to refrigerate the milk at the farm or in the plant. They also may be due to improper processing, resulting in post-pasteurization contamination. Microbial flavors are controlled by utilizing extensive sanitary practices at all stages of production and processing.

Lipolyzed flavors results from hydrolytic cleavage of milk fat by the

enzyme lipase yielding free fatty acids. Terms such as "goaty", "soapy", "butyric", and "bitter" have been used to describe lipolyzed flavor. There are several factors responsible for this off-flavor, but the most important ones are agitation and foaming, and changes in raw milk temperature. Lipolyzed flavor can be prevented by cooling the milk immediately after milking to 5°C or below with minimum agitation and avoid freezing as it can cause disruption of the fat globule membrane. Pasteurization of milk inactivates lipase, thus preventing lipolysis.

Heated flavor produced by the thermal processing of fluid milk has been characterized as cooked. The flavor itself is from volatile sulfides originating from denaturable whey proteins of milk. These flavors can be minimized by precise control of time and temperature. Also certain chemical compounds react with sulfhydryl groups and inhibit development of the cooked flavor.

Light-activated flavors are caused by exposure of milk to various forms of radiant energy, namely direct sunlight and fluorescent light. This off-flavor is particularly prevalent in milk that is stored in plastic jugs under fluorescent lights in display cases. Light activated flavors can be controlled effectively by using light barriers for milk containers, controlling the intensity of irradiation and proper rotation of inventory of milk in display cabinets in retail stores.

An undesirable flavor namely oxidized, tallowy, oily, and fishy is often encountered in fluid milk and results from a reaction between molecular oxygen and lipids. It can be prevented by providing cows with feeds that impart antioxidant properties to the milk, and also by avoiding exposure of milk to catalytic metals such as copper and iron.

Several miscellaneous off-flavors are found occasionally in milk. They generally arise from poor practices on the farm or in the plant. Such flavors or tastes as "absorbed", "astringent", "bitter", "chalky", "chemical", "flat", "foreign", "lacks freshness" and "salty" make up this group.

UHT milk is somewhat different in flavor than pasteurized milk. The aromatic substances of the milk that causes feedy, weedy, or barny odors are removed more efficiently at higher processing temperatures. Hence, consumers find the UHT milk to be flat or "purer". On the other hand, some criticize it for off-flavors, such as cooked or stale. Cooked flavor is produced by the thermal processing of fluid milk and is from sulfide and mercaptans resulting from heat denaturation of proteins. Stale flavor becomes noticeable after cooked flavor starts to diminish. The stale flavor is complex and not yet well understood.