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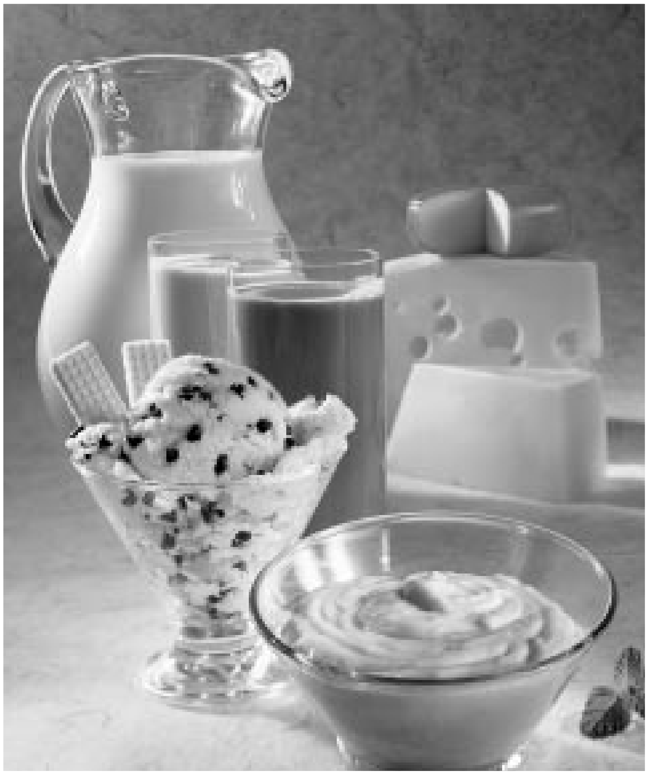
Identifying and Eliminating Off-flavor Sources in Milk and Dairy Products



Executive Summary

No food or beverage product is immune to off-flavor (OF) development. Milk is rather bland-tasting compared to many other foods and beverages. As a result, extremely low concentrations of contaminating organic chemicals can cause noticeable OFs, depending on the contaminant's chemical nature. Another problem with dairy products is that the lactose, protein and milkfat they contain are not only excellent nutrients for humans, they also are ideal nutrients for spoilage bacteria.

When OF problems occur, dairy processors must act quickly to determine their cause. Important steps are identifying the chemical(s) responsible for the OF and then determining the source. Thanks to new analytical techniques, this task can be accomplished more quickly and accurately than ever before. This report explains common sources of dairy OFs and how they can be identified and prevented—or at least minimized.

Introduction

The chemicals responsible for OFs and malodors in milk and dairy products can originate from incidental contamination from environmental (outside) sources—e.g, air, water, sanitizers or packaging materials—and from chemical reactions occurring within the dairy product itself—e.g., lipid oxidation, enzymatic degradation of proteins and triglycerides and microbial metabolic reactions. In addition, "imbalance" OFs can occur when certain ingredients normally present and often essential to the acceptability of the product are present in abnormally high or low concentrations. Also, OFs can occur in correctly formulated products subjected to inappropriate processing conditions—e.g., overheating.

When OF problems occur, several questions should be answered as quickly as possible. Answers to the following questions can avoid expensive product recalls and minimize damage to a dairy processor's reputation:

- What is the mechanism of OF formation?
- Is this an isolated problem caused by a single case of product abuse by one customer, or is this the result of processing, ingredient, packaging or microbiological problems involving an entire production run and requiring product recall?
- Does the OF signal a problem that is a potential public-health threat?
- Has the same OF type occurred in previous products?
- How can future episodes of this particular OF problem be prevented?

OFs' negative impact on milk sales should not be under-estimated. To appreciate the extent of OF problems, consider the number of milk samples suffering from light-induced oxidation OFs. Exposure of milk in blow-mold plastic containers to fluorescent lights in supermarket dairy cases prompts OF development in some 80% of store samples, according to one study conducted by retired Professor S.E. Barnard, Penn State University.

Microbial metabolite OFs produced in temperature-abused milk also can negatively impact milk consumption. According to Professor Theodore Labuza, Department of Food Science and Nutrition, University of Minnesota, the major reason kids turn off to milk is the abuse of milk above refrigeration temperatures in distribution, at retail, in school foodservice and in the home. Kids learn to associate the OFs with milk, so consumption does not continue beyond the mandatory grades. School foodservice conditions have the most potential for loss of quality, according to Labuza. If we want to get kids to drink more milk, optimize its storage conditions.

A recent study, "The School Milk Pilot Test," supports Labuza's concerns. Results of a year-long pilot test sponsored by Dairy Management Inc.™ with funding from America's dairy farmers indicate school milk sales increased 22% among secondary school students and 15% among elementary school students after milk packaging, flavor, variety and storage temperature enhancements occurred. Dairy analysts estimate the pilot test sales increases would translate to an annual per capita fluid milk consumption increase of 1.4 gallons among kids ages 6 to 17—nearly 67 million gallons of milk per year. Equally important, evaluations of milk "plate waste" remaining after lunch indicated students drank more of the milk they took. The study, co-sponsored by the American School Food Service Association and the National Dairy Council®, was implemented during the 2001-02 school year and involved more than 100,000 students in 146 elementary, middle and high schools in 12 U.S. markets. Improved storage and refrigeration to ensure cold milk was one factor in this study contributing to increased milk consumption.

Another reason for milk's high susceptibility to OFs and malodors is its primary nutrients—lactose, casein, whey proteins and milkfat—are not only good substrates for microorganism growth which can generate OF metabolites, but also are subject to degradation by exogenous enzymes, oxygen, heat and/or light. As dairy processors try extending dairy-product shelf life through new processing techniques and packaging materials, the potential for increased OF incidence is high.

Types of OFs in Dairy Products

The first steps involved in resolving OF problems are identifying the chemicals involved and determining their mechanism of formation. The following describes OF types formed in dairy products, how they form and what adjustments can be made to reduce their occurrence.

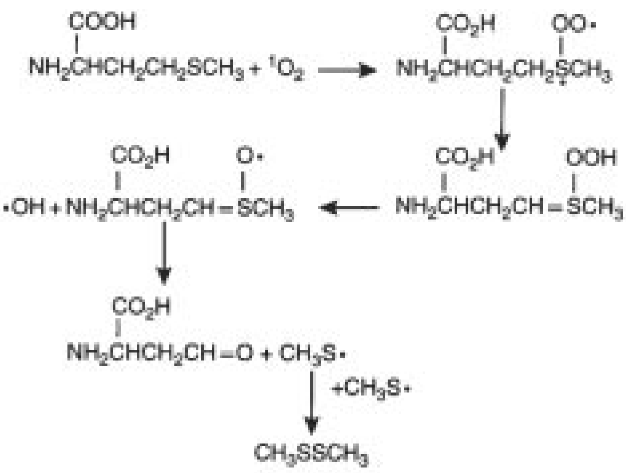
Light-Induced OFs

Light-induced OFs, the most common milk flavor defect, have two distinct components. Initially, a burnt, active sunlight flavor develops and predominates for approximately two to three days. Degradation of sulfur-containing amino acids of whey proteins is likely responsible for this OF, called light-activated flavor (LAF).

Specific chemicals responsible for LAF have not been definitively identified. For many years, scientists believed the mechanism generating LAF involved the light-induced reaction of methionine and riboflavin. This reaction produced methional, an unstable compound that degrades to several noxious, putrid compounds, including methanethiol, dimethyl disulfide and dimethyl sulfide—all contributors to LAF.

Recent studies, however, indicate methional might not be a LAF precursor. One postulated mechanism (Figure 1) indicates, dimethyl disulfide is formed by singlet oxygen oxidation of methionine.

Figure 1. Postulated Mechanism for Formation of Dimethyl Disulfide by Singlet Oxygen Oxidation of Methionine (from Jung et al., 1998)

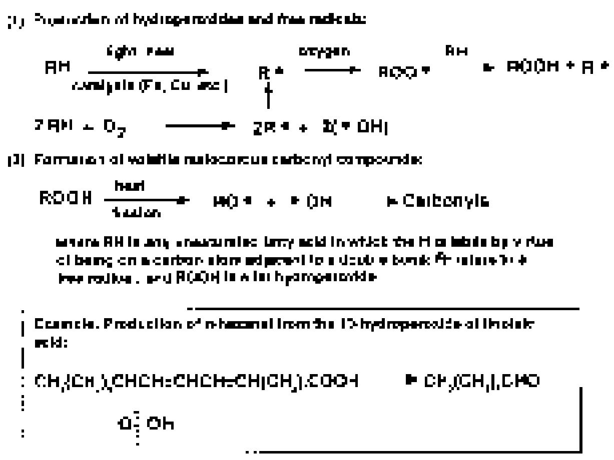


A second LAF component is attributed to lipid oxidation. This OF, often characterized as metallic or "cardboardy," usually develops after two days and does not dissipate. Aldehydes, especially pentanal and hexanal, and, to a lesser degree, ketones (e.g., 1-hexene-3-one and 1-nonen-3-one), alcohols and hydrocarbons form as a result of these lipid oxidation reactions.

Various malodorous carbonyl compounds are produced from light reacting with unsaturated fatty acids in milkfat triglycerides (triacylglycerols). Autooxidation of unsaturated fatty acids involves a free radical reaction, forming fat hydroperoxides that thermally degrade to various malodorous compounds, e.g., hexanal, the predominant lipid reaction byproduct produced from linoleic acid (Figure 2).

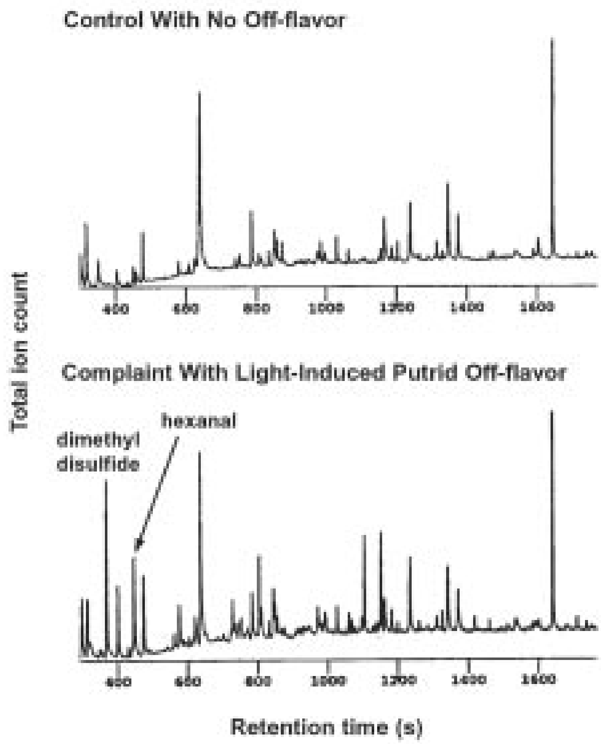
Milk isn't the only dairy product susceptible to LAF. A vanilla ice cream sample developed a strong putrid flavor during distribution. The GC-MS chromatogram of this sample compared to a control (normal-tasting vanilla ice cream) is shown in Figure 3. Elevated levels of hexanal and dimethyl disulfide clearly indicate the sample was light-abused.

Figure 2. Oxidation of Unsaturated Fatty Acids in Milkfat



When this OF problem occurred, the ice cream manufacturer reacted quickly. Ice cream sampled from roughly half the cartons (round 1.5 gallon paperboard with a clear plastic lid) developed the defect, which formed on the top surface of the ice cream but not in ice cream from the carton interior. Records showed the flavor defect became noticeable when samples were stored in a new freezer warehouse. Dairy technologists suspected the ice cream had absorbed some type of solvent stored in the warehouse. However, the flavor chemist, who was aware that hexanal and dimethyl disulfide are light-induced OF byproducts of dairy products, advised checking for abnormally high light-exposure conditions. Re-inspection of the warehouse showed samples were stored in close proximity to high-intensity lights. Lighting adjustments were made, remedying the problem.

Figure 3. GC-MS Chromatograms of Control and Complaint Vanilla Ice Creams

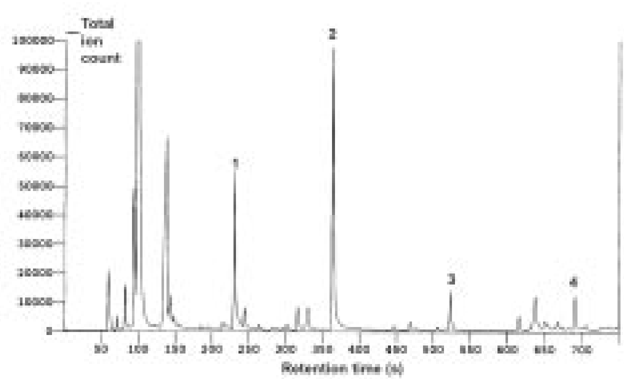


Adjusting packaging/carton materials can minimize LAF. Memphis-based Mayfield Dairy, for example, is one of the first U.S. dairies to use opaque cartons with light-blockers incorporated into the high-density polyethylene carton. Another useful tactic is to control lighting conditions in supermarket dairy cases.

Oxidation OFs Catalyzed by Prooxidant Metals

The presence of prooxidant metals (most commonly iron, copper and nickel) can significantly accelerate lipid oxidation reactions and generate strong oxidation OFs. Figure 4, for example, shows a chromatogram of a highly oxidized sample of sour cream. High concentrations of pentanal, hexanal, heptanal and octanal—all lipid oxidation byproducts—caused the severely cardboardy OF. The dairy technologist's numerous attempts to create sour cream with pilot plant processing equipment produced the oxidation OF. A processing equipment examine revealed a copper valve recently had been used to replace a worn stainless steel valve. Replacing the valve solved the problem.

Figure 4. Oxidation Off-flavor Chemicals Formed in Sour Cream



Chromatogram of sour cream oxidized by copper contamination from processing valve. Sample analyzed by GC-MS using solid-phase microextraction (SPME). Numbered peaks are malodorous aldehydes formed from the oxidation of unsaturated fatty acids in milkfat. Peak identities as follows: (1) pentanal; (2) hexanal; (3) heptanal; (4) octanal.

One interesting aspect of this problem is sensory analysts were unable to identify the OF as an oxidation problem. While the dairy technologists were experts at identifying oxidation OFs in milk by taste, the strong culture flavor background of the sample altered the perception of the oxidized flavor so dramatically that oxidation wasn't even considered a potential cause. This example illustrates the valuable of good analytical chemistry support in resolving OF issues.

TABLE 1. Examples of Dairy Food Off-flavor Studies Under Way at University Dairy Research Centers*

Researcher	Affiliation	Study Description
Valente Alvarez	The Ohio State University	• Flavor changes during extended shelf life of ultrapasteurized milk in PET bottles.
David Barbano	Cornell University	• Sensory threshold of off-flavors caused by proteolysis and lipolysis in fluid milk. • Effect of somatic cell count on proteolysis and lipolysis in pasteurized fluid milk during shelf life.
MaryAnn Drake & Keith Cadwallader	University of North Carolina University of Illinois	• Milk powder lexicon.
Joseph Irudayaraj & Donald McMahon	Pennsylvania State University Utah State University	• Process technology to improve the flavor of heated milk.
John McGregor	Clemson University	• Enhancing the shelf life of whole milk powder.
Donald Palmquist	The Ohio State University	• Contribution of fat source and micronutrients in the cow's diet to development of spontaneous oxidized flavor in milk.
Gary Reiniccius	University of Minnesota	• Flavor losses in spray-dried cheese powders. • Factors influencing flavor quality in concentrated and dry whey ingredients. • Improving the shelf life of flavored milk products.
Zata Vickers	University of Minnesota	• Milk aftertastes and their acceptability.

*DMI funded on behalf of America's dairy farmers.

Microbial OFs

Acid, malty, fruity, bitter, stale, putrid and unclean OFs in dairy products have been linked to bacteria contamination. Various OF chemicals are produced as metabolites during the bacteria's growth phase.

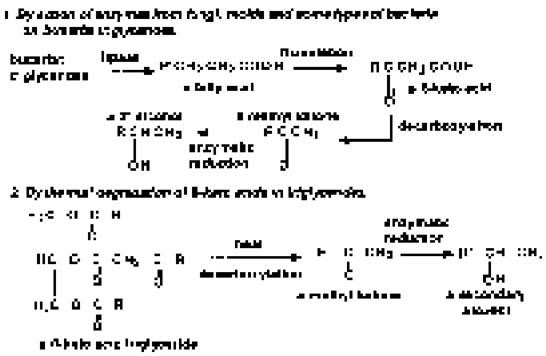
Post-pasteurization contamination of milk with psychrotrophic bacteria can cause OFs. Some characteristic OF notes in milk are clearly associated with specific psychrotrophs. For example, contamination by *Pseudomonas fragi*, a psychrotrophic Gram-negative organism, often causes "fruity" off-notes in milk. *Pseudomonas fragi's* lipase and esterase enzymes hydrolyze short-chain fatty acids in milkfat, converting the acids to ethyl esters by reaction with ethanol. Strains of *Bacillus* spp. also have been observed to produce fruity off-notes in milk.

Another common psychrotroph associated with a particular flavor defect is *Lc. Lactis* var. *maltigenes*. Production of 3-methylbutanal causes the malty aroma of milk cultures of *Lc. Lactis* var. *maltigenes*. 3-Methylbutanal concentrations as low as 0.5 ppm in milk generate the characteristic malty flavor defect. 3-Methylbutanal is produced from the action of bacterial enzymes on leucine. *Lb. Maloaromicus* also can produce 3-methylbutanal.

Other common metabolites include ethyl acetate, dimethyl sulfide, dimethyl disulfide, ethanol and other alcohols, methyl ketones, C4-C10 fatty acids, lactic acid, pyruvic acid and bitter peptides.

One problem complicating the determination of OF causes is that two or more mechanisms can produce many of these chemicals. For example, production of methyl ketones and secondary alcohols (Figure 5) and lactones in milk can result from microbial reaction pathways, as well as from high heat treatment. Dimethyl sulfide is generated (but dissipates after a few hours) by pasteurization heat treatment and by microbial spoilage. Dimethyl disulfide is produced when milk is light-abused, as well as by enzyme-induced reactions on milk proteins.

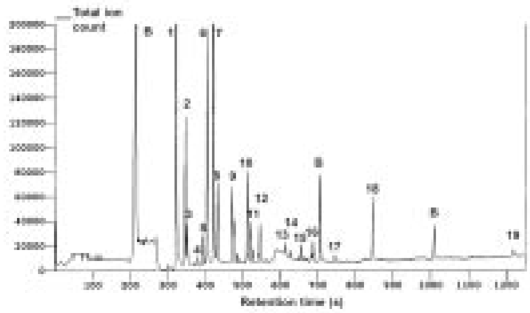
Figure 5. Production of Methyl Ketones in Dairy Products



Lactones, which can impart a burnt, fruity, stale or coconut-like OF to dairy products, can be produced by various microbial reactions. Yeasts, such as *Candida* spp. and *Saccharomyces cerevisiae*, and molds, such as *Penicillium notatum* and *Cladosporium butyri*, are known sources.

Note that microorganisms do not have to be living to generate OFs. Some types of exogenous enzymes lysed from dead microbes can survive pasteurization heat treatment and react with lactose, dairy proteins or milkfat to produce OFs. These OF metabolites can be monitored by GC-MS. Figure 6 is a GC-MS chromatogram of an actual milk sample spoiled by this mechanism. This OF problem, which developed a few days after production, prompted a multi-state product recall costing a dairy more than $1 million. Microbial plate count testing (SPC) showed that no processed milk samples from that day's production had elevated counts.

Figure 6. Off-flavor Chemicals Formed in Processed Milk by Exogenous Enzymes



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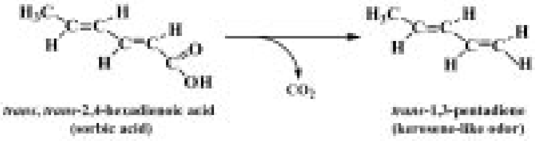
To minimize spoilage by exogenous microbial enzymes, raw milk should never be held longer than 48 hours in refrigerated silos before pasteurization. According to David Barbano, Director, Northeast Dairy Foods Research Center, carbon dioxide can be added to milk that is being held in silos (or during transit) to prevent bacteria growth. This is one way, says Barbano, to extend stored raw milk's life.

Microorganisms can generate OFs in unexpected ways. Potassium sorbate often is used as a fungistat in cultured dairy products and cheese. It has been shown that a large number of molds in the genus *Penicillium* can grow in the presence of up to 7100 ppm potassium sorbate (Marth, 1966). The occurrence of a plastic, paint, garlic-like or kerosene OF in feta cheese, cottage cheese and other dairy products has been attributed to production of *trans*-1,3-pentadiene, a strong odorant produced from enzymatic decarboxylation of sorbic acid (*trans, trans*-1,4-hexadienoic acid). (See Figure 7 for details.)

Figure 7. Production of trans-1,3-Pentadiene From Sorbic Acid



Packaging Material OFs

Ironically, packaging materials, which are designed to preserve food flavor and freshness, can directly cause OF defects in dairy products. Although plastic packaging materials consist primarily of nonvolatile, high molecular weight polymers, volatile low molecular weight compounds often are added to improve functional properties of these materials: plasticizers to improve flexibility; antioxidants to prevent oxidation of the plastic polymers or the food material inside the packaging; and UV blockers to prevent "yellowing" of the polymer material when exposed to light. Additional additives include polymerization accelerators, crosslinking agents and antistatic chemicals and lubricants.

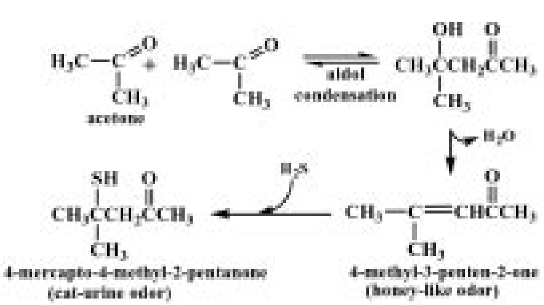
Occasionally, packaging materials are not cured sufficiently before use. As a result, a small amount of solvent associated with the manufacturing of the packaging material might contaminate the material. Malodorous solvent in packaging materials can migrate into the dairy product, resulting in OFs. Residual styrene monomer from polystyrene packaging is a common example.

A dairy product that developed a severe OF because of a packaging solvent occurred with half-and-half packaged in 14-ml polystrene cups. Normal-tasting control samples and complaint samples with a "chemical, solvent-like" OF were analyzed by purge-and-trap GC-MS. The most significant differences in the chromatograms of control and tainted samples were an increase in the acetone levels and the presence of a significant propylacetate peak in the tainted samples. The odor of the propylacetate was characterized by GC sniff port experiments (also referred to as GC olfactometry or GC-O experiments) and judged to be similar to the odor defect problem.

The packaging lidstock consisted of three layers: an outer paper layer impregnated with water-based inks, a middle metal foil layer and an inner plastic film used for heat sealing. The inner heat-seal film, actuated by heat and pressure to seal the lid to the cup, is applied to the foil as a slurry and dried. The packaging supplier used propylacetate to form the slurry. By allowing a longer cure time for the solvent to evaporate from the film in the drying ovens, the residual propylacetate solvent was more thoroughly evaporated from the film, and the OF problem ceased. This example illustrates another benefit of having good analytical support: When packaging suppliers know sensitive analytical techniques are being used to screen for OF chemicals that might be originating from their packaging, suppliers' Q.C. specifications are more likely to be followed and packaging quality will be higher.

Acetone, another common packaging solvent, can cause a cat-urine (ribes) odor in dairy products. This occurs when two acetone molecules react via aldol condensation reactions to form 4-methyl-3-penten-2-one, which then reacts with hydrogen sulfide produced from microbial enzymatic reactions with sulfur-containing amino acids in the dairy proteins. The resulting compound that forms, 4-mercapto-4-methyl-2-pentanone (also called "cat ketone"), has a strong cat-urine odor. (See Figure 8.)

Figure 8. Production of 4-Mercapto-4-methyl-2-pentanone From Acetone and hydrogen Sulfide



When it comes to OFs, flavor concentration and food context are very important. For example, at high concentrations, 4-mercapto-4-methyl-2-pentanone has an off-odor associated with cat urine, but in lower concentrations and in the context of a cabernet sauvignon wine, it provides the typical, desirable flavor impression of the sauvignon grape.

Another packaging-related problem that can lead to OFs is "scalping"—the absorption/adsorption of beneficial flavor compounds from the food or beverage into or through the packaging material. This problem has been observed by dairies producing orange juice. Weak, watered-down orange juice flavor can result when acetaldehyde, ethyl butyrate, decanal and other flavor-important chemicals in orange oil are stripped from the juice by paperboard cartons.

With sales of flavored milks accounting for a growing portion of total fluid milk sales, it's important to monitor this OF development source. Professor Gary Reiniccius, University of Minnesota, is studying OF causes in flavored milks and ways to extend shelf life. Packaging- related scalping problems will be one OF source studied.

Preventing flavor scalping is a matter of judiciously selecting packaging materials compatible with the flavor chemicals in the food or beverage product. Packaging offering better barrier protection might be needed to replace less expensive materials. In general, the more effective the packaging material's barrier properties, the higher the cost. Dairy processors can use analytical studies in combination with sensory taste paneling to objectively determine product deterioration or scalping level, how much can be tolerated, and how much barrier protection will be required. Investing in insufficient barrier protection can cause OFs, but spending more than needed for barrier protection wastes money.

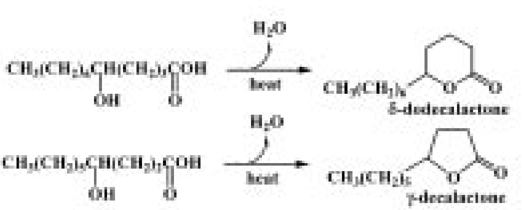
Feed-Related OFs

Cow diets can profoundly impact milk flavor. Feeds known to cause OFs include fermented/musty silage (maize, legumes and grass); alfalfa (green or hay); clover; hay; brewers' grain; and green barley.

In some cases, the chemicals responsible for the OF have been identified. Freshly cut alfalfa hay, for example, contains high levels of *trans*-2-hexenal, *trans*-3-hexenal and *trans*-3-hexenol, which impart a green, grassy flavor to milk. When ingested by cows, wild onion, garlic and related plants can impart severe malodors and objectionable tastes to milk.

Elevated levels of hydroxyacids might be incorporated into milkfat triglycerides (as hydroxyacid esters) when cows are fed stored forages rather than pasture. When incorporated into triglyceride molecules, hydroxyacids do not impart OFs to milk. However, the hydroxyacid esters, which are readily hydrolyzed from the triglycerides in the presence of water and heat, cyclize to form lactones, which can cause a stale flavor. (See Figure 9.) As previously pointed out, lactones can also be created in milk via microbial mechanisms.

Figure 9. Formation of Lactones by Heating Hydroxyacids



Cow diet can cause OF problems in unexpected, subtle ways. In one case, a fluid milk processor experienced dozens of oxidized-milk complaints from one U.S. region. The OF problem occurred during the late winter months for the previous four years, getting progressively worse each successive year. Because the oxidation OFs were so intense, dairy researchers suspected the milk was contaminated with high levels of copper or some other prooxidant metal. Another suspected contributing factor was lack of naturally occurring antioxidants (e.g., α-tocopherol) due to changes in winter feed regimens. Additional testing ruled out prooxidant metal contamination and lack of α-tocopherol as possible causes.

Further analytical investigations provided more details. When fatty acid profiles of complaint and normal-tasting control samples were compared, samples with intense oxidation OFs consistently contained 200%- 300% more linoleic acid than control samples. Linoleic acid is highly susceptible to photoxidation. The high linoleic acid content of the milk was traced to the cows' diet. Dairy farmers in this soybean-growing region were feeding cows increasingly high levels of soybeans each year. Feeding cows high levels of soybeans, whole cotton-seed and vegetable oils has been shown to increase milkfat's linoleic acid content and make milk more susceptible to oxidation. This problem was corrected by regulating levels of unsaturated lipids in feed and by supplementing feed with additional antioxidants (e.g., α-tocopherol).

Miscellaneous OF Sources

Besides the previously discussed OF sources, other less common culprits exist. Three examples include overheating, contamination by sanitizers and absorbed barn odors:

- ***Overheating.*** When milk is overheated, it produces cooked OFs. These OFs are caused by methyl ketones produced from thermal degradation of β-ketoacids in milkfat triglycerides, as well as by various sulfur compounds (especially dimethyl sulfide) liberated from the sulfhydryl bonds of the whey proteins. Joseph Irudayaraj, Pennsylvania State University, and Donald McMahon, Utah State University, are investigating processing technology to improve heated milk's flavor.
- ***Sanitizer contamination.*** When dairy processors neglect to flush processing lines previously cleaned with sanitizers, which are powerful oxidizing agents, it is not uncommon for a small amount of sanitizer to contaminate the first few bottles of milk filled on the line. This can lead to OFs in milk, especially with the newer peroxyacetic acid-based sanitizers. Peroxyacetic acid-based sanitizers, which contain a blend of acetic acid, hydrogen peroxide and an organic acid (e.g., octanoic or decanoic acid), are potent and robust sanitizers but can generate oxidation OFs if they contaminate milk.
- ***Absorbed odors.*** "Barny" flavor in milk can result when cows inhale odors in unclean, poorly ventilated barns. The major chemicals responsible for this OF appear to be methyl sulfide and dimethyl sulfide. A clean, well-ventilated barn is important to preventing this odor defect. In addition, milking and milk storage areas also should be kept free of these odors.

Conclusion

Numerous mechanisms exist for OF formation in dairy products. Furthermore, the potential for OF problems will likely increase in the future with dairy processors' heightened interest in extending dairy-product shelf life by using new processing techniques and packaging materials. Dairy processors must be vigilant, have monitoring techniques in place and respond quickly to problems when they occur to prevent product recalls and unhappy customers.

In some ways, discovering the chemical responsible for a particular OF can be like looking for a needle in a haystack. However, recent advances in sensory analysis, chemical instrumentation, olfactometry techniques, sample preparation methods for extracting odor-causing chemicals from foods prior to chemical analysis and the application of new statistical methods are helping increase the likelihood of finding that needle. New analytical techniques for identifying the chemicals responsible for dairy OFs will be discussed in detail in the next technical bulletin.

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