

BORDEN AWARD ADDRESS

FLAVOR ORIGINS

Review of Chemical Mechanisms Affecting Flavor Acceptability of Dairy Products

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Milk is a complex biological fluid which spontaneously undergoes chemical change under a variety of conditions. Heat, light, air agitation, trace metal contamination, and microorganisms are a continuous threat. Frequently the only measurable manifestation of these chemical reactions is change in flavor or odor. Milk and dairy products made from it pose two main flavor problems: prevention of development of off-flavor and maintenance or development of characteristic flavors. Recent efforts in dairy research have been directed toward elucidation of chemical mechanisms involved in flavor change. Photolysis of methionine in milk, responsible for the "sunlight" flavor; heat denaturation of β -lactoglobulin, causing "cooked" flavor; decomposition of lactose and formation of furan compounds, associated with "caramelized" flavor; and secretion of acetone bodies causing "cowy" flavor.

FLAVOR CONTROL has evolved as one of the most exclusive problems in the dairy industry. By its very nature, milk is extremely susceptible to chemical change and under certain conditions even fresh milk may exhibit atypical flavors. Once the product enters the channels of commercial procurement, processing, and distribution, it is constantly subject to outside forces such as heat, light, air, metallic contamination, and bacteria capable of inducing chemical changes that give rise to flavor problems.

Milk is an extremely complex biological substance. This fact alone has undoubtedly discouraged many a biochemist from pursuing fundamental chemical studies with this product, and progress in this field has been rather slow. Much technological progress has been made in dairy engineering, bacteriology, nutrition, processing, and distribution, yet many of the problems with milk are the same as 25 years ago. This is due in large part to a lack of fundamental knowledge of the chemical nature of this biological fluid.

Flavor Acceptability

Milk and milk products are admittedly basic in the diets of most Americans. To be acceptable however, they must possess pleasing flavor qualities. The average human being is equipped with an extremely sensitive taste mechanism with which to evaluate flavor, as well as a taste memory to which he can refer

in establishing his acceptance of a food. The taste mechanism with its three distinct parts, gustatory (tongue), tactual (touch), and olfactory (smell), depends primarily upon chemical stimulation in establishing the composite sensory response we describe as flavor. In evaluating foods, the consumer does not voluntarily sort out the various flavor qualities made evident by the individual taste mechanisms, but involuntarily evaluates them in terms of the over-all taste response. Individuals vary widely in taste acuity, but the average consumer discriminates against atypical flavor qualities in any food for which he has an established taste memory.

A striking example of this fact was experienced during World War II with whole milk powder. Here was a concentrated food of unquestioned value in the field ratio of soldiers, which, because of its atypical flavor qualities, was generally rejected as a reconstituted beverage. To the average soldier it was not milk and he refused it solely because it did not possess the taste qualities of the fresh fluid product to which he was accustomed.

Milk is a very bland tasting food and to many people is actually lacking in a describable flavor. It is, however, very delicately balanced chemically and is subject to chemical and physical changes under a variety of conditions. Heat, light, air agitation, trace metal contamination, and a host of microorganisms are a constant threat to the degradation of many milk components. Frequently

the only measurable manifestation of the chemical reaction is change in flavor or odor. In other words, trace changes which escape microanalytical techniques are often sufficient to make a milk product unacceptable from the flavor standpoint.

Because flavor is the primary index of food acceptance and food product improvement, work in this field is particularly worth while. The dairy industry has had to rely rather heavily on trial and error methods in establishing processing procedures which minimize or control flavor changes. However, recent efforts have been directed toward elucidation of the intricate chemical mechanisms involved in flavor changes. It is from such fundamental work that processing procedures can be developed to control and protect the characteristic flavors of dairy foods.

Heated Flavors

Because of perishability and for public health reasons, all dairy products are heated at some stage of manufacture. Commercial temperature treatments vary from a minimum of 143° F. for 30 minutes to 240° F. for 15 or more minutes. It is not surprising, therefore, that "cooked" or heat-induced flavors in milk are a common problem in this industry. In 1938 the author was assigned to a project to investigate the nature of cooked flavor in milk and since that time has been vitally interested in the many manifestations of heat and

its effect on flavor and other changes in dairy products.

Some of the earlier findings demonstrated that the appearance of cooked flavor in heated whole milks coincided with the appearance of mercapto ($-SH$, sulfhydryl) groups detectable by the nitroprusside test (3, 8). The critical temperatures at which this change took place in whole milk were $76.5^{\circ} C.$ instantaneous, $71^{\circ} C.$ for 15 minutes, and $68.5^{\circ} C.$ for 30 minutes. Cream liberated mercapto compounds at a lower critical range of temperatures, while skim milk required a slightly higher heat treatment to produce a similar effect. This work eventually led to the conclusion that mercapto compounds were liberated by heat from lactalbumin and the protein constituent of the fat globule membrane of milk and were wholly responsible for the cooked flavor. Later work in these laboratories and by others (5, 11, 12) demonstrated conclusively that the mercapto compounds so liberated came primarily from the serum protein β -lactoglobulin, the major component in the albumin fraction.

The presence of free mercapto groups in milk has many other interesting and practical implications. As the degree of heating is increased to approximately $90^{\circ} C.$, there is a progressive increase in the liberation of free mercapto groups and degree of cooked flavor, accompanied by a rather uniform decrease in the oxidation-reduction potential. As mercapto groups have definite reducing properties, it was not surprising to find that heated milks, showing evidence of free mercapto groups, were very resistant to the oxidative changes normally found in milk. Ascorbic acid, which normally oxidizes readily on storage, was rather stable as long as free mercapto groups remained. Likewise the lipide fractions of milk, which are highly susceptible to oxidation and are responsible for "oxidized flavor," remained stable and apparently unchanged, even in the presence of added copper. As the free mercapto groups were depleted, the cooked flavor disappeared and the normal oxidation of ascorbic acid and lipide took place. In other words, the mercapto groups are active antioxidants in protecting ascorbic acid against oxidation and preventing the development of the lipide-associated oxidized flavor in milk. It would appear that the mercapto groups oxidize preferentially and in doing so lose their flavor characteristics. The flavor of milk depleted of free mercapto groups becomes indistinguishable from similar unheated milk.

The antioxidant properties of heat-induced mercapto groups are used to advantage in the dairy industry today. When conventional pasteurization temperatures (145° to $160^{\circ} F.$) are employed prior to freezing, the storage life of

high-fat products is limited, because of the danger of spontaneous or copper-induced lipide oxidation. In the processing of cream for butter-making or cream to be placed in frozen storage, high temperature pasteurization is currently practiced. Such treatment imparts a strong cooked or even sulfide odor, as well as a high level of antioxidant activity in the product. As the storage period progresses, the mercapto compounds gradually become depleted and a good flavored product is available for the market.

One of the most challenging flavor problems is that with evaporated milk. Under commercial conditions this product is normally sterilized at $240^{\circ} F.$ for 15 minutes. Under such rigorous heat treatment, milk undergoes numerous changes chemically and flavorwise. This product has been used widely as an infant food and in cooking for many years, but has never been accepted as a beverage for older children or adults. Nutritionally, it has certain advantages over conventional fluid milk, but its characteristic "caramelized" and "astringent" or "rough" flavor makes it unacceptable for general beverage use.

For a number of years workers in these laboratories have been studying some of the complex chemical reactions and interactions which take place when milk is heated at or above $90^{\circ} C.$ (7, 10, 13, 14). These studies have yielded much fundamental and practical information. The chemical changes which occur during storage at room temperature over long periods of time have been found to resemble closely those taking place at elevated temperatures in the course of a few minutes. Some of the specific pathways of fat, protein, and carbohydrate decomposition as a result of high heat treatment have been established.

These results have placed the problem of manufacturing a more acceptably flavored evaporated milk clearly in focus. Many people look to this product, or an aseptically packed variation of it, to help solve storage, handling, and consumption problems of the milk industry. With the basic information that is now available, it can be predicted that an acceptable product, utilizing heat treatment for sterilization and room temperature for storage, is most unlikely. In addition to numerous lactose decomposition products which serve as the origin of caramelized flavor, heated milks exhibit protein hydrolysis with the formation of a "stewed-meat" type off-flavor and fat hydrolysis with the liberation of fatty acids and lactones which are responsible for a butter-scotch flavor. These flavors are not necessarily bad; they are merely out of place. So long as the American consumer has fresh fluid milk of good quality as a comparative standard, heat-

treated, aseptically packed milks will stand little chance of success in domestic markets. Minimizing initial heat treatment will not necessarily offset the chemical changes that normally occur when milk is stored at room temperature. Many who are considering application of the various "cold" sterilization process to fluid milks may need to consider these latter changes.

Dry Milk Flavors

Dry milk represents the most utilitarian form in which to handle and store milk, particularly during periods of surplus milk production. In addition, it is a very practical and necessary ingredient in rations for our defense forces. It supplies high-level nutrition with a minimum of weight. The Department of Defense, recognizing the importance of milk in the soldier's diet, has given whole-hearted support to research efforts for improving dry milks. Here again, a critical factor is flavor. Dry milk can be clean, free-flowing, readily dispersible in cold water, economical, and convenient to use, but if it makes an unpalatable beverage on reconstitution with water, all other advantages are to no avail.

Dry whole milk poses two primary flavor problems; one is concerned with flavor in the olfactory sense and the other involves the feeling of the reliquified milk in the mouth (a tactual defect). This latter defect, known as chalkiness or roughness, has been subjected to considerable study in these laboratories (4, 15). Results from this work indicate that so-called roughness or chalkiness in dry milk is due primarily to the formation of insoluble calcium-protein aggregates as a result of heat treatment and drying. The most useful procedures thus far developed for overcoming the defect are sequestering of calcium ion activity, removal of calcium ions from the milk, and/or low temperature processing of the dry milk.

The second flavor defect of dry whole milk is associated with the lipides and has proved particularly vexing. In recent years, autoxidation has been heavily stressed as the source of off-flavors in edible fats. To a certain extent autoxidation may be a factor in the deterioration of dry milk. However, the use of antioxidants and vacuum or inert gas packing of dry whole milk have been singularly unsuccessful in preventing a certain critical type of flavor deterioration of the milk fat. Rather substantial evidence has demonstrated that this process is nonoxidative and involves rearrangement of milk fat acids to lactones, which are detectable in milk at concentrations of 1 to 2 p.p.m. The only preventives developed thus far for this undesirable flavor change are refrigerated storage of the dry milk

from the day of its manufacture or hydrogenation of the milk fat used in manufacture of the dry milk.

Sunlight Flavor

All foods and beverages packaged and distributed in glass containers are susceptible to photochemical changes. In most foods the end products of moderate photochemical change do not seriously affect the flavor or consumer acceptance of the product. In milk, however, exposure to light results in the photolysis of certain vitamins and the formation of very undesirable flavor compounds. The losses of ascorbic acid and riboflavin are significant on prolonged light exposure, but the development of the so-called "sunlight" or "activated" flavor constitutes a far greater problem to the market milk industry. With the conventional method of distribution in flint glass bottles, this problem has definite economic and nutritional implications.

Little has been known concerning the mechanisms of photolytic decomposition of the components of milk. In earlier work (6, 7) it was demonstrated that in order to protect milk from the photochemical changes resulting in sunlight flavor, the container must prevent the transmittance of all wave lengths of light under 620 m μ . It was observed that milks vary considerably in their susceptibility to this defect and that the homogenized product is much more vulnerable than the unhomogenized product.

In attempting to find the source of this flavor defect in milk, it was observed that sulfur-containing compounds found in whey were associated with the flavor. Others (2, 17) have related it with a derived protein from heated whey, which will produce the flavor on exposure to sunlight. More recent work in these laboratories (16) has indicated that the flavor substance has its source in methionine and that flavor production is dependent in a large measure on the presence of riboflavin. The importance of methionine was first revealed when dilute solutions of this amino acid were exposed to sunlight and observed to develop a flavor apparently identical to that observed in exposed milk. Additions of as little as 4 mg. of methionine per quart to milk before exposure enhanced the degree of the off-flavor, while increased concentrations up to 50 mg. per quart resulted in greatly intensified flavor production. The data indicated further that the flavor production mechanism could function independent of temperature. The more rapid rate of flavor production in milk as compared with pure solutions of methionine suggested that some other constituents of milk were probably involved in the mechanism. Riboflavin was considered to be

a logical possibility, as it was the only milk serum component showing appreciable light absorption in the visible range. This was confirmed in trials where distilled water solutions of methionine (20 mg. per quart) and riboflavin (1.5 mg. per quart) were exposed to light. Whereas solutions of methionine alone developed a slight but noticeable degree of typical sunlight flavor, those which contain both methionine and riboflavin developed an intense flavor.

Similar trials with the sulfur-containing amino acids, cystine and cysteine, revealed that they were incapable of producing the flavor. It, therefore, appears that the photolysis of methionine is primarily responsible for the presence of the sunlight flavor defect in milk.

The specific nature of this photochemical change in an essential amino acid is as yet unknown. The fact that it is activated by solar energy and intensified by the presence of a naturally occurring pigment poses an interesting lead regarding a number of biological processes. This laboratory is pursuing the matter, with the hope that it will lead to a better understanding of a flavor problem which has such practical implications.

Cowry Flavor

A flavor defect described as "cowry" or "barny" is prevalent in milk, particularly during the winter months. It was always assumed that the flavor was absorbed from foul stable air or from unsanitary practices on the farm. While this may be correct in some cases, investigations in these laboratories (9) suggested that this flavor can be attributed to chemical substances secreted in milk by cows suffering from ketosis or acetonemia.

The author's interest in this problem developed quite by accident when a graduate student, working on a wholly unrelated flavor study, noted a peculiar but familiar flavor in some of his samples. It developed that these samples contained traces of acetone and that the flavor produced was very similar to that of certain raw milks being delivered from farms to the University Dairy. Analysis by the methods of Van Slyke revealed that milks exhibiting the cowry flavor always contained free acetone bodies. It was established that β -hydroxybutyric acid was absent from all samples analyzed and that acetone plus acetoacetic acid accounted for all the acetone bodies present. Normal flavored raw milks were essentially free of these compounds. To determine the role of acetone in the development of cowry flavor, milks of good quality were treated with varying concentrations of redistilled c.p. acetone and compared in flavor with samples of naturally

cowry milk. In most cases where acetone equivalents were similar by analysis, the type and degree of off-flavors were indistinguishable. Acetone concentrations of 25 to 35 p.p.m. are sufficient to give milk an objectionable cowry flavor, but it is not uncommon to find milks containing 50 to 100 p.p.m. in herds afflicted with ketosis.

The problem of ketosis in dairy cattle is still critical in the dairy industry. It has been reported that the disease is similar to Addison's disease in humans and involves the endogenous energy metabolism of the animal body. Analyses of the blood and urine of cows suffering from the disease show relatively high concentrations of acetone bodies, so it is not surprising that some of these substances are secreted in the milk. This problem is further complicated by the fact that many animals may develop mild subclinical cases of the disease and continue to produce milk at normal levels. Under such conditions there are no outward symptoms except the presence of acetone bodies in the milk and other body fluids. This nutritional disease in dairy cattle has been a subject of investigation by animal scientists for many years. Until it is solved the cowry flavor will continue to be a problem to the dairy technologist.

Conclusions

Applied fundamental studies in chemistry have had direct application to the every-day problems of the industry, but there are tremendous horizons in the foods field awaiting the work of chemists. We are a nation of nearly 162,000,000 people, fed on a plane of nutrition unequaled anywhere in the world. The food technologist has played a very important role in this accomplishment. However, with the population growing at a rate of 2,000,000 a year and a tillable acreage that has remained constant for 35 years, the need for continued technological progress is obvious. The quantity and quality of foods will undoubtedly be increasingly important factors in the future prosperity of this nation and to ensure an ample supply of high quality nutritious foods will require much progress in fundamental and applied chemistry.

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Received for review August 11, 1954. Accepted October 1, 1954. Presented before the Division of Agricultural and Food Chemistry at the 125th Meeting of the AMERICAN CHEMICAL SOCIETY, Kansas City, Mo.

CEREAL STORAGE EFFECTS

Deteriorative Changes in the Oil Fraction Of Stored Parboiled Rice

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Changes in the oil fraction of stored parboiled rice have been followed as criteria of loss in quality. Storage of parboiled rice in the dark at several temperatures permitted a study of rancidification and of nonenzymatic changes in the oil as measured by peroxides, monocarbonyl compounds, and free acids. In open-container storage, values for peroxides, monocarbonyls, and free acids remain low during an induction period, then rise markedly at or just before the time rancid odors appear. Peroxides and monocarbonyls again decrease to low residual values and rancidity disappears. Free acid values remain at the higher level. Original moisture contents of 11.4 to 12.5% fall to about 10% at 77° F., 6% at 100° F., and 3% at 140° F. Storage lives of two parboiled rices show the same relative order at 77°, 100°, and 140° F. Monocarbonyl maxima occurring at about 1 year at 77° F. are found after about 1 month at 140° F. and 1 week at 180° F. This observation may serve as a basis for accelerated testing of storage life. In closed storage at 77° and 100° F. peroxide and monocarbonyl changes are similar to those for open storage, though induction periods are longer. At 140° F. no rise is shown. Free acidity increases linearly for considerable periods at all three temperatures. The rancidifying effect of light on fat-containing foods was confirmed for parboiled rice during storage at 77° F.

PARBOILED RICE offers some unique advantages in studying the complex processes of deterioration in cereal grains, especially those occurring in the oil fraction. This fraction may be present as less than 1% of the grain, yet have marked effects on acceptability because of rancidity development. Both hydrolytic and oxidative rancidity may occur (7); hydrolytic action is favored by high moisture content and oxidative action by low moisture. The importance of biological factors in deterioration of damp grain has been summarized by Milner (17). The role of native cereal enzymes and of mold lipases has been discussed by Geddes (17), who points out that more research is necessary to evaluate their respective parts in rancidification processes.

Enzymes have been inactivated in parboiled rice. It is prepared by soak-

ing, steaming under pressure, drying while still in the husk, and then milling in the usual manner for white rice. Besides inactivating enzymes, this wet-heat processing enriches the endosperm in solubles such as sugars, minerals, and vitamins, at least partially gelatinizes the starch, and probably destroys some of the native antioxidants. Behavior of the oil fraction of parboiled rice in storage must reflect the absence of native enzymes, as well as of fungal enzymes under conditions that do not support fungal growth.

This report presents the changes in several characteristics of the oil fraction of parboiled rice over a range of storage conditions, and provides information useful for comparison with changes in the oil of rice containing active enzymes. The trends shown offer a basis for a

possible accelerated test for storage life of moist heat-processed rices such as parboiled and quick-cooking rice.

Materials

The main rice supply comprised two 100-pound lots from the 1952 harvest which had been commercially parboiled and milled. The 100 pounds of Pearl (short-grain, Pearl 1) had been prepared by a continuous process which involves the usual steeping, steaming, and drying. The Century Patna (long-grain) had been prepared by a batch process which involves subjecting the rice to a vacuum prior to addition of the steeping water. The rice was received directly after milling and was held at room temperature about 2 weeks before storage tests were started.

Another 50-pound supply of Pearl was