for amounts equivalent to concentrations expected in prepared feeds and premixes. Figure 1 shows absorption curves for furazolidone and nitrofurazone, made by a Beckman DK-2 ratio-recording spectrophotometer.

Discussion

The specificity of the color reaction with respect to solvent was investigated. No color was developed with the compounds being studied in the presence of potassium hydroxide and any of the following solvents: acetone, ethyl ether, nitromethane, dioxane, chloroform, carbon tetrachloride, and 1,1,1-trichloroethane.

Several other medicaments (vitamin A, Enheptin, Nitrophenide, diethyl stilbestrol, arsanilic acid, sulfaquinoxaline, phenothiazine, Nicarbazin, and Nitrosal) were dissolved in dimethylformamide and tested for any color development. None developed a color that would interfere with the method described. Nitrosal gave a yellow solution in dimethylformamide, but was not changed when the potassium hydroxide solution was added. Enheptin developed a light yellow and Nicarbazin a deep yellow color after alkali was added. Nitrophenide developed a brownish pink color when the alkali was added.

The use of dimethylformamide obviates the possibility of an incomplete extraction, as the compounds sought are soluble in this solvent, compared to alcohol and other mixtures used in other methods.

Several brands of chicken and turkey feeds, both mashes and pelleted, were mixed, ground together, and remixed. This composite sample was used throughout the experimentation. None of the feeds in this mixture contained any of the medicaments (except vitamin A) mentioned earlier in the paper.

Table II. Bifuran Recovery from Poultry Feed

Sample No.	Nitrofurazone, Mg.		Furazolidone, Mg.	
	Added	Recovered	Added	Recovered
61	0.556	0.556	0.0825	0.082
62	0.556	0.560	0.0825	0.082
63	0.556	0.554	0.0825	0.100
		Av. 0.556		0.088
71	1.120	1.12	0.165	0.175
72	1.120	1.13	0.165	0.180
73	1.120	1.17	0.165	0.180
		Av. 1.14		0.178
81	2.240	2.30	0.330	0.320
82	2.240	2.10	0.330	0.330
83	2.240	2.24	0.330	0.350
		Av. 2.21		0.333
91	3.360	3.50	0.495	0.480
92	3.360	3.36	0.495	0.490
93	3.360	3.20	0.495	0.525
		Av. 3.35		0.498

Nitrofurazone develops a color similar to furazolidone in pure dimethylformamide solution, when a few drops of potassium hydroxide solution are added. The color is characteristic for nitrofurazone, but was not used for this method because of poor recoveries after chromatography and increased manipulations to remove the alcohol. Nitrofurazone gives a red solution in acetonitrile on addition of an alkali. Nitrofurazone is only slightly soluble in acetonitrile, however.

Feeds that contain mixes of furazolidone, nitrofurazone, and 3-nitro-4-hydroxyphenylarsonic acid may also be analyzed by this procedure. The chromatography step holds the 3-nitro-4-hydroxyphenylarsonic acid on the column. Solvents such as alcohol, acetone, or diethyl ether do not remove this material.

The phenylhydrazine method of Buzard (*1*) may be applied to the solutions after the components of the mixture have been resolved.

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HEAT EFFECTS ON MILK

Review of Organic Chemical Effects of Heat on Milk

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Many heat-induced changes in milk and its products can be studied appropriately by an organic chemical approach. Research progress using such an approach is discussed in terms of lactose-protein interaction (browning) and flavor changes in the fat and nonfat phases of milk. The need for research concerning effects of heat on milk fat is emphasized. To make milk a more flexible and useful raw material and to overcome some of its tendencies toward chemical deterioration, custom manufacture of milk components, such as the fat and protein, should be more extensively developed by the dairy industry.

PRACTICALLY ALL MILK and all its products are heat-processed to some degree. In fact, many dairy products gain their identity through one or a combination of processing steps in which heat treatment is inherent: pasteurization, homogenization, preheating (forewarming), mixing, blending, condensing, superheating, sterilization, and drying. Such measures are not without effect on milk, a delicately balanced biochemical system. Because milk contains considerable organic matter, some of these effects can be investigated to advantage from the standpoint of organic chemistry.

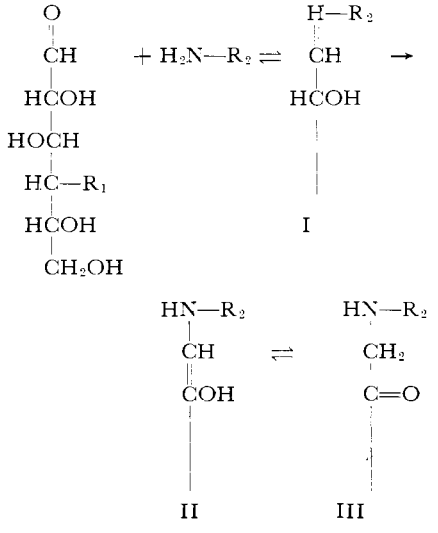
Appreciation of classical organic chemistry must be modified somewhat in applying it to a food system. It is not clear what may be reacting with what when a complex medium such as milk is heated. Thus understanding of re-

action mechanisms may be prevented by ignorance of reactants. Moreover, there are no high-yield reactions, which are the ideal in synthetic organic chemistry. Nonetheless, it is the trace quantities of end products which serve as the starting point in our understanding of organic chemical changes in food systems. If these products can be identified, the reactants can be determined through study of simplified systems, and with reactants and end products established, the reaction mechanisms can be clarified. Tremendous advances are possible in this field, as various spectrophotometric and chromatographic procedures make it unnecessary to recover substantial quantities of end products in pure form. For example, maltol (2-methyl-3-hydroxy-4-pyrone) in heated milk was initially identified from many liters of material rigorously heated for hours (*12*). More recently this compound was shown in less than 1 liter of commercial evaporated milk (maximum heat treatment 116° C., 15 minutes) by a combination of paper chromatographic methods (*20*). A further example concerns the demonstration of methyl sulfide, a flavor compound, in both heated (*24*) and unheated (*18*) milk. Without the aid of gas chromatography, this identification would have been unlikely.

Effects on Skim Milk Phase

Two principal areas of interest in the chemistry of heated milk are: changes associated with the milk serum proteins and changes related to the browning reaction. Jenness (*8*) has discussed changes in the serum proteins. The browning reaction and related considerations in milk have been the subject of a review (*15*) through 1954. There have been several findings of interest since that time. In considering research on browning and associated changes in milk it should be remembered that what transpires rather quickly at elevated temperatures may be accomplished in a parallel manner by months of storage at room temperature.

Patton and Flipse (*16*) have studied the interaction of lactose with proteins in heated (97° to 121° C., 0 to 60 minutes) milk using lactose-1-C.[14] They established that a sugar-protein complex is formed, which on further heating gives rise to limited but noticeable browning. The binding of sugar by milk protein, particularly casein, prior to heat-induced browning has been confirmed by Schober and Christ (*21*) using glucose-C.[14] Binding was greatest in the unheated state and heating produced a decline to approximately 70° C., above which temperature there was a steady rise in protein-bound sugar. The implication this holds for the binding of lactose by native milk protein is of sufficient practical and fundamental importance to warrant investigation. One wonders what groups of the protein serve as binding sites and whether the phenomenon is reversible. If irreversible, it is clearly one of the most heat-sensitive reactions of milk protein. On the basis of evidence from simplified systems, Hodge and Rist (*5, 6*) have postulated that browning in sugar-protein systems proceeds initially through the Amadori rearrangement. This may be represented schematically as follows for lactose (R_1, glucose) and casein (R_2, NH_2, where the NH_2 groups appear to be predominantly the epsilon-NH_2 of lysine):

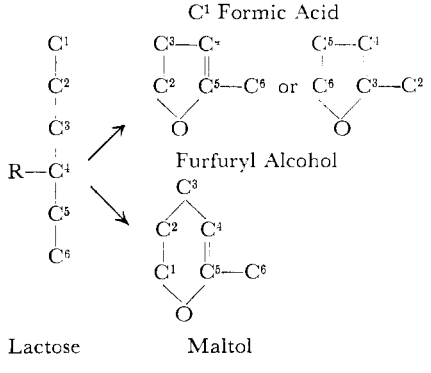


The first product in this series is a Schiff's base–type compound (I). The reaction by which it is formed is reversible, particularly under acid conditions. However, under the nearly neutral conditions that exist in milk, some rearrangement of I to the 1-desoxy-1-aminoglycoside, II (enol form) and III (keto form), would be expected.

Evidence that these reactions occur in heated milk has been offered recently by Adachi (*1*). Through tryptic hydrolysis of the proteins from evaporated milk, amino acid glycosides corresponding in structure to formulas I and II (or III) have been demonstrated. The task of establishing the chemistry between these glycosides in milk, the brown discoloration, and the many fragments of lactose that are known to be formed is the substantial task which remains. Presumably it is in the form of such *N*-glycosides that sugars are rendered highly susceptible to the dehydration, fragmentation, and condensation reactions associated with browning. Rigorous evidence validating this proposal for food systems has not yet been offered.

Findings from the author's laboratory indicate that casein acts in the manner of a basic catalyst, through its free amino groups, so far as browning and lactose decomposition in heated (120° C., 20 minutes) milk are concerned. The intact protein is more effective in this regard than amino acid hydrolyzates of it (*14*). The sugar fragments are primarily those resulting from alkaline degradation of sugars. They include acetic, lactic, formic, and pyruvic acids, carbon dioxide, acetol, methyl glyoxal, acetaldehyde, furfural, furfuryl alcohol, hydroxymethylfurfural, and maltol (*15*). Hydroxymethylfurfural is formed in trace amounts when milk is heated at normal pH, 6.6. Larger amounts are recovered under acid conditions (*13, 20*).

Regarding sugar fragments, one approach which appears to hold promise concerns the use of compounds labeled with carbon-14. Recent observations (*17*) on formic acid, furfuryl alcohol, and maltol from heated milk are of interest. When these compounds are recovered from highly heated (121° C., 4 hours) milk, fortified with lactose-1-C^{14} prior to heating, the carbon from formic acid and maltol contain substantial amounts of isotope (lactose to product carbon-14 ratios of 1 to 0.5 and 1 to 0.8, respectively), whereas the furfuryl alcohol exhibits no activity. Such results establish carbon-1 of lactose as a primary source of the formic acid in heated milk and make attractive the hypothesis that the carbon chain of the glucose moiety serves as the origin for maltol, and without carbon-1, provides the skeleton for furfuryl alcohol. The validity of this scheme could be evaluated further with lactose containing uniformly labeled galactose. The relationship of the lactose and maltol carbons in this scheme is suggested by the observation that iodoform from maltol-C^{14} (from lactose-1-C^{14}) shows no activity.



Effects on Fat Phase

Current knowledge of the effects of heat on milk fat is inadequate. With the exception of a very few isolated observations, there is no guiding information. Some questions which may be raised are: Does the heat treatment normally employed in the processing of milk cause any fat hydrolysis, isomerization of double bonds within the unsaturated fatty acids, polymerization of unsaturated fatty acids, or interchange of the fatty acids within the triglycerides? In fact, what is known about the structure of triglycerides in milk fat? Granted that heating milk fat hastens oxidative deterioration, what

specifically happens from a chemical standpoint? Are hydroperoxides formed and decomposed? To what are they decomposed? As the lipides in milk are finely dispersed in an aqueous medium, does any hydration of double bonds occur during heating, or on the contrary, is the degree of unsaturation increased? Why has investigational work to date ignored the possible interaction of lipides with nonlipide constituents as a result of heating milk. Continued research on the chemistry of milk fat, one of our most important dietary fats, is needed.

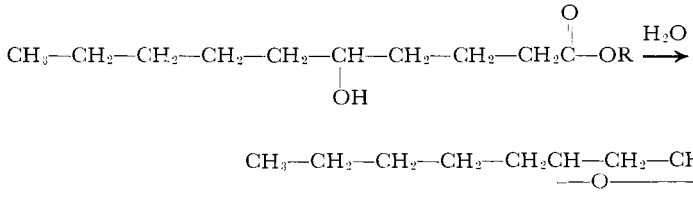
Effects on Flavor

Flavor is one of the most important practical considerations with any food commodity. There is no question that chemistry is bound to play an important role in the sciences of flavor and odor, as volatile organic compounds provide a basis for stimulation of the taste and odor receptors. Pasteurization of milk (143° F., 30 minutes, or 161° F., 15 seconds) is considered to have little if any effect on flavor. However, as heat treatment progresses beyond this point, changes in flavor become more numerous and more intense. At about 74° C. (165° F.) so-called "cooked" flavor begins to become evident (*4*, *9*). At this point in heating, flavor change is generally attributed to volatile sulfides and particularly to hydrogen sulfide (*23*). It is known that this hydrogen sulfide comes substantially from the heat denaturation of β-lactoglobulin (*7*). Theoretically its origin is the amino acid cysteine, but the mechanism of the reaction has not been established.

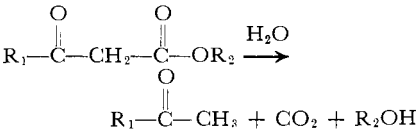
To state that cooked flavor under these conditions of heating is nothing more than a matter of hydrogen sulfide evolution is probably an oversimplification. Nonetheless, this is the extent of knowledge to date. As heat treatment of milk intensifies beyond the cooked flavor range, volatile sulfide evolution declines. Sulfhydryl groups in the medium disappear and at some indeterminate point caramelized flavor becomes evident. The appearance of this flavor is closely correlated with the onset of browning and undoubtedly it is one of the many ramifications of the browning reactions in milk (*15*, *19*, *23*). All that is known with reasonable certainty concerning caramelized flavor is that it results from the interaction of lactose with the milk proteins. The character of this flavor probably is determined also by certain heat-induced changes in the milk fat.

One compound which appears to be important in the flavor of heated milk, specifically evaporated milk, is methyl sulfide. Although this compound occurs naturally in unheated milk (*18*), it has been encountered at elevated levels in evaporated milk (*24*). Observations by the author and his associates suggest that methyl sulfide is of broad general importance in the flavor of many cooked foods. Its flavor threshold in water has been established as approximately 12 parts per billion. Its possible origin in the amino acid methionine is obvious; its mechanism of formation from such a source is not known.

The discussion of heat-generated flavor compounds thus far has concerned those from the skim milk phase. Evidence of two heat-induced changes associated with milk fat have come to light. When milk fat is heated or stored either as such or as a component of a dairy product, δ-decalactone is formed (*10*, *11*). This compound has a pronounced buttery, coconutlike odor and flavor. It accounts for much of the palatability derived from cooking foods in butter. On the other hand, δ-decalactone is distinctly out of place in beverage milk products and thus is one of the limiting factors in consumer acceptability of dry whole milk. The origin of this lactone in milk fat has not been established. One plausible source would be 5-hydroxydecanoic acid. Milk fat may contain traces of this acid in esterified form, which could rearrange to the lactone in accordance with the following scheme. The compound also might result in some unique manner from autoxidation.



A group of methyl ketones recently has been identified as trace constituents in heated milk (*24*). Specifically acetone, 2-pentanone, and 2-heptanone were revealed in the low-temperature, reduced-pressure distillate from evaporated milk. Additional members of this homologous series also may be present, as some limitation of volatility would be anticipated under the experimental conditions (40° C., 10 mm.). Although these findings are preliminary and further effort will be required to determine the specific origin and flavor significance of these ketones, some additional relevant information is available. Forss (*3*) has indicated that a common flavor defect of certain dairy products results from the presence of 2-pentanone and 2-heptanone. Extensive studies of heated skim milk at this laboratory have never revealed formation of such ketones. On the other hand, odor of steam distillates from milk fat frequently suggests the presence of 2-heptanone. Milk fat reacts with 2,4-dinitrophenylhydrazine and the resulting product shows an absorption maximum in chloroform at 364 mμ (*24*), in agreement with the absorption for ketone derivatives (*2*). This absorption cannot be removed from the fat by repeated washing with methanol. Over 40 years ago, Smedley (*22*) noted weak reactions to the nitroprusside test by milk fat, suggesting the presence therein of ketoacids. Thus one reasonable origin of the ketones noted may be from β-ketoacids, residual in the synthesis of milk fat, which on heating decompose:



where R_1 = *n*-alkyl
R_2 = balance of the triglyceride

Here again autoxidation of milk fat offers an alternative formative mechanism for the compounds in question. The number of saturated and unsaturated carbonyl compounds which are theoretically possible from milk fat by autoxidation is astounding.

Practical Implications

Milk is currently viewed primarily as a finished product, which can be converted without substantial alteration into a number of usable forms. Unfortunately, some milk constituents are unstable or incompatible during processing and storage. Evaporated milk and dry whole milk are particularly problematical in this connection. Perhaps in the future it will be well to view milk, at least in part, as a raw material from which the important components can be recovered and custom manufactured to meet the needs of various dairy products as well as the processed foods in which they may be used. It seems probable that the presence of a reducing sugar (lactose) will always place some concrete limit to room temperature storage time for dried and sterile fluid milks. This is a natural consequence of protein-reducing sugar interaction, and modification of one or the other of the reactants or variables, such as pH, seems indicated for product improvement.

A second example concerns milk fat. Observations regarding flavor changes in certain dairy products suggest that the dairy industry may be well advised to consider tailor-making milk fat for various purposes in the same way that it makes nonfat dry milks for baking, cottage cheese making, and beverage use. In cooking, where flavors of the type provided by δ-decalactone are desired, the potential for such agents could be retained or perhaps developed to a maximum. On the other hand, where a flavor-stable milk fat is desired, such as in

dry whole milk, dried cream, dried ice cream mix, and heated sterilized fluid milks, refining measures as employed by the fats and oils industry may be desirable. In any event, it seems certain that flavor defects in a number of dairy products will be inherent so long as the same form of milk fat is considered equally appropriate for all product usage.

Acknowledgment

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BROWNING REACTIONS

Mechanism of Browning of Ascorbic Acid–Citric Acid–Glycine Systems

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To determine whether Strecker degradation of amino acids occurred in the browning of orange juice, the browning of ascorbic acid in citrate-buffered solutions containing radioactive glycine was studied. Neither Schiff bases nor volatile aldehydes were detected. Less than 3% of the carbon dioxide evolved was derived from glycine-C_1; less than 0.1% from glycine-C_2. The data indicated that the Strecker degradation does not occur, in detectable degree, under the test conditions. The browning of this system, therefore, does not follow the usual pathways reported for the glucose-glycine system.

IN THE "BROWNING REACTION" in foods and, particularly, in model systems, a major position has been assigned to the Amadori rearrangement and the Strecker degradation (*8*, *13*, *14*, *20*). The particular amino acid or polypeptides and the particular carbohydrate constituents involved in these reactions have been established for a few products only recently (*3*). In model systems, glucose and glycine have been used most commonly (*6*, *14*, *21*). In 1935, Joslyn and Marsh (*16*) reported that amino acids play a minor role in the oxidative nonenzymatic browning of orange juice; this has been confirmed recently for orange juice and model systems composed of ascorbic acid and glycine and other amino acids (*9*, *15*). Occurrence of a Strecker degradation in solutions containing oxidized ascorbic acid and glycine, however, has been reported by Abderhalden (*1*, *2*) and Schönberg (*17*, *18*).

To investigate this point in more detail and to determine the possible mechanism of browning of products such as orange juice, the browning of solutions containing radioactive glycine labeled in both C-1 and C-2 positions in the presence of ascorbic acid under both oxidative and reducing conditions was investigated. The results of these studies indicate that the initial reaction is not the formation of a Schiff base between glycine and dehydroascorbic acid, as would be expected on the basis of studies of glucose-glycine systems, and that the Strecker degradation resulting in the decarboxylation and deamination of glycine occurs very slightly, if at all, under the conditions used. Formaldehyde was not accumulated in detectable amounts and only part of the carbon dioxide liberated originated from the carbonyl group of glycine.

Experimental

The browning of ascorbic acid and glycine in citric acid–potassium citrate buffers at pH 3.7 and 7, under nitrogen and oxygen, was followed at 37° and 50° C. The citric acid buffer was prepared by dissolving 10.5 grams of C.P. citric acid hydrate in distilled water, adjusting to pH 3.7 or 7 by addition of C.P. potassium hydroxide pellets, and making up to 500 ml. A stock solution of ascorbic acid was prepared by dissolving 1.1 grams of Merck L-ascorbic acid in 100 ml. of buffer just prior to use. The stock glycine solution was prepared by dissolving 0.563 gram of glycine in 100 ml. of buffer prior to use. Glycine-