is obviously essential. A comparison of this work with that reported by Deutsch and Samuelsson (*4*) and by Samuelsson (*12*) shows a difference in the characterization and/or identification of several of the peaks. The present work failed to detect methionine sulfoxide, citrulline, and asparagine, which were reported by those investigators. However, those investigators were unable to find phenylacetylglutamine and sarcosine, which were identified here.

In contrast to the possible shortcomings of the ion exchange method mentioned above, the superiority of ion exchange chromatography even as a qualitative tool over paper chromatography should be pointed out. Thus, paper chromatography of the ninhydrin-positive components in desalted milk serum has been applied by several investigators (*2, 8, 19*). That paper chromatography alone is quite limited in its scope is borne out by the fact that a maximum of only 14 spots were detected, compared to 31 peaks in the present study. This limitation is set by the amount of material which can be applied to a sheet of paper, and by the possible disproportionality of the constituents present in the sample.

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MILK PRESERVATION

The Use of Antioxidants in Frozen Whole Milk

A. J. GELPI, Jr., L. L. RUSOFF, and ENRIQUE PINEIRO[1]

Dairy Department, Louisiana Agricultural Experiment Station, Baton Rouge, La.

Various antioxidants—ethyl hydrocaffeate, gentisic acid, sodium gentisate, and Ionol—were tested with whole milk at −10° F. for a 6-month storage period with and without the addition of copper. All compounds, with the exception of Ionol, showed good antioxidant properties, the sodium gentisate being superior to the others. Ionol imparted an off-flavor to the milk and precipitated the milk proteins. By use of proper antioxidants and storage conditions, frozen whole milk may be kept in good condition for at least 6 months.

THERE are numerous occasions when it might be advisable to store frozen milk for future use; however, the development of oxidized flavors in stored frozen fluid dairy products usually presents a serious economic problem. This problem has attracted the attention of research workers in the field of dairy and food technology (*7–9*). Prevention of oxidized flavor development in stored frozen cream has been investigated by using various substances and procedures, including high temperature heat treatments, homogenization, the use of ascorbic acid (*1, 2*), and nordihydroguaiaretic acid (N.D.G.A.) (*7, 8*). Gelpi *et al.* (*5, 6*) have reported that butyl hydroxyanisole, Tenox II (butylated hydroxyanisole + citric acid + propyl gallate in propylene glycol), ethyl caffeate, and ethyl hydrocaffeate showed varying degrees of effectiveness in delaying or preventing the development of oxidized flavors in stored frozen cream. The results indicated that ethyl caffeate and the hydrogenated form (ethyl hydrocaffeate) possessed some outstanding properties as antioxidants. Frozen cream containing 0.004% of ethyl hydrocaffeate + 0.5 p.p.m. of copper failed to develop an oxidized flavor after 12 months of storage at −10° F.

The present study includes the use of ethyl hydrocaffeate, gentisic acid, sodium gentisate, and Ionol as antioxidants in frozen milk for a 6-month storage period.

The gentisates (2,5-dihydroxy benzoic acid), as represented by gentisic acid and sodium gentisate, possess good antioxidant properties in certain food fats (*4*). They are appreciably water-soluble, fairly heat stable, and do not show carry-through properties in the Schaal oven. Gentisic acid has been

[1] Present address, Superi 1771, Buenos Aires, Argentina.

Table I. The pH of Whole Frozen Milk at Different Periods of Storage with and without the Addition of Antioxidants

Anti-oxidant, %	Storage, Months 0	2	4	6
Control	6.65	6.61	6.62	6.56
Ethyl Hydrocaffeate				
0.01	6.62	6.62	6.67	6.6
0.005	6.54	6.61	6.67	6.65
Gentisic Acid				
0.15	6.15	6.09	6.15	6.17
0.1	6.41	6.3	6.4	6.3
0.05	6.52	6.38	6.49	6.4
0.01	6.67	6.52	6.68	6.4
0.005	6.64	6.6	6.62	6.67
Sodium Gentisate				
0.15	6.62	6.63	6.68	6.48
0.1	6.68	6.68	6.72	6.7
0.05	6.63	6.63	6.68	6.65
0.01	6.7	6.62	6.7	6.55
0.005	6.7	6.63	6.68	6.65

approved from a toxicity standpoint as an analgesic, and extensive toxicity tests show a level of toxicity well within the food approval range. Ionol (butylated hydroxy toluene) is an antioxidant which has been accepted by the U. S. Food and Drug Administration for use in the stabilization of foods and drugs. It is a white crystalline product, insoluble in water, but soluble in oils, a variety of alcohols, and other solvents.

Experimental

The milk used in the following trials was produced locally and was of average quality as determined by bacteriological and chemical quality tests which included standard plate counts, coliform counts, titratable acidity, pH, per cent fat, and per cent total solids. The milk was pasteurized and cooled under normal commercial conditions by U.S.P.H., HTST method (165° F. for 16 seconds), homogenized, and cooled to 40° F. The flavor was normal before and after processing and no indication of any off-flavors was present.

The experimental milk was divided into two equal portions. One portion received 0.5 p.p.m. of copper as copper sulfate and the other remained untreated. Samples of each served as controls. Samples of the remaining two portions were then treated with the antioxidants ethyl hydrocaffeate, gentisic acid, sodium gentisate, and Ionol, at the following levels: 0.15, 0.1, 0.05, 0.01, and 0.005%, respectively, on a volumetric basis with and without copper addition. The ethyl hydrocaffeate was dissolved in a small amount of ethyl alcohol before being added to the milk so as to facilitate complete dispersion. The water soluble gentisic acid and sodium gentisate were added to the milk in solution and thoroughly agitated with a mechanical agitator. The Ionol, being fat soluble, was added to the milk in corn oil and mixed by vigorous mechanical agitation.

The treated milk was distributed in pint, standard Sealright plastic-lined paper ice cream containers to eliminate a possible source of cappy or other similar flavors. Immediately after filling and prior to freezing, pH determinations were made on all samples. The containers were then stored in an ice cream hardening room at −10° F. Organoleptic examinations of the samples were made by three experienced milk flavor judges every 30 days for a 6-month period. The samples to be examined were held at 40° F. for 24 hours, then tempered to room temperature (76° to 78° F.) for 30 minutes before examination.

Table II. The Effect of Adding Ethyl Hydrocaffeate, Gentisic Acid, and Sodium Gentisate to Whole Milk Stored at −10° F.

Antioxidant, %	Cu Added, P.P.M.	Storage, Months 1	2	3	4	5	6
Ethyl Hydrocaffeate							
Control		−	−	−	−	+	+
Control	0.5	+++	+++	+++	+++	+++	+++
0.01		−	−	−	−	−	−
0.005		−	−	−	−	−	−
0.01	0.5	−	−	+	+	+	+
0.005	0.5	+	+	+	+	+	+
Gentisic Acid							
Control		−	−	−	−	+	+
Control	0.5	+++	+++	+++	+++	+++	+++
0.15		−	−	−	−	−	−
0.1		−	−	−	−	−	−
0.05		−	−	−	−	−	−
0.01		−	−	−	−	−	−
0.005		−	−	−	−	−	−
0.15	0.5	+	+	++	++	++	++
0.1	0.5	+	+	++	++	++	++
0.5	0.5	+	+	++	++	++	++
0.01	0.5	+	+	++	++	++	++
0.005	0.5	++	++	++	++	++	++
Sodium Gentisate							
Control		−	−	−	−	+	+
Control	0.5	+++	+++	+++	+++	+++	+++
0.15		−	−	−	−	−	−
0.1		−	−	−	−	−	−
0.05		−	−	−	−	−	−
0.01		−	−	−	−	−	−
0.005		−	−	−	−	+	+
0.15	0.5	−	−	−	+	+	+
0.1	0.5	−	−	−	+	+	+
0.05	0.5	−	−	−	+	+	+
0.01	0.5	−	−	−	+	+	+
0.005	0.5	+	+	+	+	+	++

− Not oxidized.
+ Slight oxidation.
++ Pronounced oxidation.
+++ Very pronounced oxidation.

Results and Discussion

The effect of frozen storage on the pH of milk is shown in Table I. Although gentisic acid initially lowered the pH of the milk slightly, no further change occurred during the entire storage period. This is in agreement with Doan and Warren (*3*) who reported no changes in titratable acidity or pH in concentrated skim milk held in frozen storage.

Table II shows the results of adding ethyl hydrocaffeate, gentisic acid, and sodium gentisate as antioxidants to whole milk in frozen storage. Samples of milk with added ethyl hydrocaffeate showed no evidence of oxidized flavor after 6 months of storage. With copper added, the sample containing 0.01% level of the antioxidant did not develop an oxidized flavor until the third month of storage. Gelpi *et al.* (*6*) reported better results when ethyl hydrocaffeate was used in frozen cream, indicating that this compound was a more effective antioxidant in cream than in whole milk. The results with gentisic acid show that all samples containing the antioxidant remained in good condition through 6 months of storage. With copper added, however, all samples developed an oxidized flavor during the first month. The samples to which

sodium gentisate was added also showed no oxidized flavor development during the entire 6-month period. With copper added, an oxidized flavor became apparent at the fourth month of storage at the 0.15, 0.1, 0.05, and 0.01% levels of sodium gentisate. A comparison of these results with those obtained by the use of ethyl hydrocaffeate indicates that sodium gentisate is slightly more effective as an antioxidant in frozen whole milk even in the presence of added copper.

The trials with Ionol were discontinued at the end of the first month, since, under the conditions of the present experiment, the compound imparted a peculiar flavor to the milk and also had a strong destabilizing effect on the milk proteins.

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MEAT IRRADIATION

The Role of Glutathione and Methionine in the Production of Hydrogen Sulfide and Methyl Mercaptan during Irradiation of Meat

SALVADOR MARTIN,[1] O. F. BATZER, W. A. LANDMANN, and B. S. SCHWEIGERT[2]

American Meat Institute Foundation, Chicago 37, Ill.

Experiments designed to determine the origin of the major volatile sulfur compounds formed during the irradiation of meat were carried out by adding to ground meat S^{35}-DL-methionine and S^{35}-glutathione, and irradiating with gamma rays at dosages from 2 to 10 megarads. Most of the methyl mercaptan appeared to be formed directly from free methionine. Some mercaptan was produced during the irradiation of glutathione, possibly indirectly or as a secondary product. Although H_2S was found in both cases as a product of the irradiation of methionine and glutathione, the observed isotope dilutions indicated that most of the H_2S apparently originated from other sulfur-containing precursors in meat.

THE PRINCIPAL COMPOUNDS in the volatile fraction of the off-odor material produced by irradiation of beef have been shown to be methyl mercaptan and hydrogen sulfide (*1, 11*). Studies (*4*) on the irradiation of amino acids with gamma rays showed that, with increased dosage, increased amounts of mercaptans are produced from methionine, while H_2S is apparently the result of secondary reactions, since the amount produced is not correlated with dosage. The radiation degradation of S^{35}-labeled methionine in solution has been followed (*6*), and the products containing S^{35} have been identified as methyl mercaptan, inorganic sulfate, methionine sulfoxide, methionine sulfoximine, methionine sulfone, homocysteine, and homocysteic acid. At a total dosage of about 5.6×10^6 rads, about 3 to 4 times as much sulfate as methyl mercaptan was produced. A similar production of sulfate was observed in in vivo studies on S^{35}-methionine in irradiated rats (*8*).

Production of H_2S by the irradiation of cysteine and glutathione has also been studied (*3, 12*). However, the interrelationship between the formation of mercaptan, H_2S, and the degradation of glutathione and cysteine, was not developed in these investigations.

The present investigation was undertaken to ascertain whether methyl mercaptan or hydrogen sulfide, found in the off-odor material in meat during irradiation, originated from free methionine or from the sulfur-containing amino acids in peptide linkage. In principle, an isotope dilution method has been employed. Since the radiation reaction results in a number of different products, the amount of isotope recovered as labeled methyl mercaptan or H_2S gives a measure of the extent of each particular reaction. It may be assumed that the specific activity per gram-atom of S^{35} will not change, if the product is formed exclusively from the original radioactive component. Therefore, any decrease in molar specific activity should indicate the degree of formation of mercaptan or H_2S from sources other than the labeled material.

Experimental

Experiment 1, Single Dosage. Samples consisted of 20.0 grams of uncooked, lean ground beef muscle to which 100 to 250 μg. of S^{35}-DL-methionine, in a solution containing about 50 μg. of methionine per ml., was added. Samples were refrigerated and irradiated at 4° C. with Co^{60} (ca. 234,000 rads per hour) to a total dosage of 5×10^6 rads. Unirradiated controls were held in a refrigerator at 4° C. during the time the samples were irradiated.

After irradiation, both the control and the sample were rapidly transferred with 60 ml. of H_2O to ebullition tubes for determination of CH_3SH and H_2S. The procedure for mercaptan was that described by Sliwinski and Doty (*10*), in which a trapping solution of mercuric

[1] United Nations Fellow, University of Mexico, Mexico City, Mexico, 1959.
[2] Present address, Department of Food Science, Michigan State University, East Lansing, Mich.