precipitate the pectinaceous material and aggregate the cellular material containing the carotenoid pigments. Filter aid is added to facilitate filtration and removal of the water-soluble constituents. Extraction of the aqueous-methanol filtrate with hexane has shown that no lycopene or carotene are discarded with this filtrate. With adequate blending, three extractions of the pulp-filter aid mixture with a 50 to 50 acetone-hexane solution is sufficient to remove all of the carotene and lycopene. Removal of the acetone and drying the hexane is necessary before good adsorption of the pigments can be obtained upon chromatographing.

Saponification of the extract does not appear to have an effect on pigment values sufficiently great to justify the extra step. Carotene and lycopene are both relatively unstable pigments, and different absorbents and even different lots of the same absorbent may influence their measurement. Similarly, delay in analyzing the extracts for an hour or more may influence results. The experimental results reported in this paper emphasize the necessity of working through the analytical steps without undue delay.

Comparison of the values obtained for carotene and lycopene by the chromatographic-spectrophotometric method A, with those obtained by the spectrophotometric binary method B, show that the latter procedure gives values which are 10 to 15% higher. The reason is that the latter procedure, B, measures all the water-insoluble pigments present in terms of carotene and lycopene. Unpublished data obtained in the U. S. Fruit and Vegetable Products Laboratory confirm results reported by Khan (*4*) and Curl (*2*), that carotene and lycopene comprise approximately 80 to 90% of the total carotenoid pigments of Ruby Red grapefruit.

Method B offers a simple rapid procedure for estimating total pigment in terms of lycopene and carotene, for which most laboratories might be expected to have the skill and equipment required. A fairly close approximation of the analytical values for lycopene and carotene obtained with method A can be obtained with method B by subtracting correction factors from the results of the binary calculations, 10.3% for lycopene and 16.2% for carotene.

Method A is an analytical method. It requires the chromatographic separation of the pigments, the separate elution of the lycopene and carotene bands, and their quantitative spectrophotometric measurement. The absorption curves of these two pigments, separated in accordance with the recommended procedure, have been compared with absorption curves of pure β-carotene and lycopene. This comparison shows that the chromatographic procedure is successfully separating the two pigments. Precision and accuracy (recovery) tests have been made which show that method A is satisfactory.

Test data, as well as establishing the value and limitations of two methods for determining the pigment content of colored grapefruit, provide additional information confirming quantitative changes in the pigment content during the January–April period of the harvesting season (*5*, *6*). In this period the total pigment decreases as the season progresses; lycopene decreases regularly and carotene remains relatively constant until the latter part of the season. The trends are the same from year to year but the dates on which the pigment content changes significantly will vary. This fact undoubedly reflects changes in environmental conditions, sampling variables, and the like.

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MILK IRRADIATION

Irradiation Preservation of Milk and Milk Products

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A method was developed which increased the volatility of the off-flavor complex, or its precursors, of irradiated milk so that milk irradiated with 2 × 10^6 rep of gamma rays was obtained free from detectable off-flavors. The increase in the sensitivity of irradiated milk to browning, caused by the formation of reductones, was further studied. The production of chalky off-flavors from milk fat was caused partly by the formation of peroxides of the more saturated components, whereas oxidized off-flavors were caused by highly unsaturated fractions of the butterfat. A method was found by which milk or milk concentrates might be sterilized by cold sterilization—i.e., by the application of ionizing radiations.

Problems connected with the sterilization of milk by ionizing radiations have been delineated and described (*4*, *6*, *9–11*, *18*, *19*).

The study of the nature and origin of some of the chemical changes in milk and the possible development of means for preventing some of these changes is described herein.

Two reasons make it desirable to pay particular attention to the problems encountered in the radiation preservation of milk: The application of ionizing radiations results in stronger and more undesirable off-flavors than in any food as yet studied, and experience gained from research on these flavor changes in milk may prove applicable to other less difficult foods. Furthermore, milk is one of the few foods that can be separated into groups of components whose behavior can be studied independently, checked objectively, and then evaluated by recombining them into milk of the original composition.

Approximately 5 to 7.5 × 10^5 rep have been found to be sufficient for the

Table I. Effects of Irradiation of Various Butterfat Fractions with 2 × 10^6 Rep

Fraction No.	% of Total	Melting Range, ° C.	Iodine Value, Wijs		Peroxide Molar Equivalent of O_2		Flavor[a]	
			0	2 × 10^6 rep	0	2 × 10^6 rep	Chalky	Oxidized
1	4.4	52–54	9.7	9.6	0.85	7.38	****	*
2	6.0	45–47	19.0	17.6	1.06	4.24	***	*
3	1.3	41–43	29.2	29.0	0.75	6.6	***	*
4	18.2	30–32	18.7	18.2	0.94	3.19	*	**
5	41.3	16–18	31.7	32.1	1.57	2.10	None	***
6	10.6	−8–6	48.4	47.8	2.15	3.14	None	****
7	5.3	−14–12	48.8	49.0	3.67	8.83	None	****
8	12.9	−30–28	62.8	62.2	5.13	4.28	None	****

[a]Judgment of five members—the score * for trace and **** for very strong off-flavor. One gram of fat was emulsified into 100 ml. of unirradiated skim milk.

practical sterilization of raw milk (*15*). The assurance of complete sterilization under possible adverse conditions—such as an unusually high spore contamination—would, however, require a dose of 2 × 10^6 rep. The work on chemical changes was, therefore, carried out at this level.

Reports in the literature on the relative strength of off-flavors formed in irradiated milk have differed widely. Recently, Bierman and Proctor (*4*) showed that the threshold of recognition for irradiated milk is at 2 × 10^4 rep for skim milk and at 7 × 10^3 rep for whole milk. A dose of more than 5 × 10^5 rep produced little additional off-flavor.

The off-flavors of irradiated skim milk were volatile, and, when irradiated skim milk was distilled under vacuum, the condensate contained the characteristic off-flavor. Carbonyls and organic sulfides in the oxidized form (*18*) were also found in the condensate.

Studies on Fat Compounds

Milk fat in whole milk and cream is responsible for the production of another, somewhat less volatile, group of off-flavors that are characterized by oxidative rancidity. A third type of off-flavor, which also originates with milk fat, is nonvolatile and has a strong, lasting, chalky, metallic, persisting taste. The formation of these lipoidal off-flavors occurs quite independently of any interaction with the aqueous phase of the milk, as they are obtained in equal strength and character when anhydrous butterfat is irradiated. This was confirmed by organoleptic evaluation with triangle tests which showed no detectable difference between irradiated skim milk that contained emulsified irradiated butterfat and samples that contained butterfat emulsified in skim milk before irradiation.

Solutions of anhydrous butterfat in acetone were fractionated according to their solubility at various temperatures; eight components of decreasing melting point range were thus obtained. After the removal of the solvent, the fractions were irradiated with 2 × 10^6 rep and emulsified skim milk. The data are presented in Table I.

These fractions which give generally increased iodine values with decreasing melting point range show distinctly different behavior on irradiation. Distinct differences were noted in the changes which took place in the fractions of lower and of higher unsaturation. The oxidized flavor component, which is present only in traces in the more saturated fat, increased sharply with increasing unsaturation. The chalky off-flavor, on the other hand, was strongest in the more saturated fractions. Iodine values did not change significantly on irradiation. Peroxide formation, however, increased significantly when those fractions which produced chalky off-flavor were irradiated. The more unsaturated fractions, which produced predominantly oxidized flavors, showed a lesser increase or even decrease in peroxides after irradiation.

Off-flavors of irradiated butterfat and of fractions thereof were much more pronounced when emulsified in milk than when the butterfat was tasted directly. There is no evidence of the chemical nature of the chalky off-flavors. There are strong indications that peroxides or hydroperoxides may be responsible, and that the fatty acid chain is not broken upon their formation.

Browning and Reductone Formation in Irradiated Milk

The increased sensitivity of irradiated milk to browning on heating and the discovery that the browning precursors originated with lactose (*19*) have been reported. Studies on model systems containing lactose, galactose, or glucose revealed that irradiated galactose was somewhat more and glucose somewhat less sensitive to browning than lactose. These precursors to browning react as carbonyls, and when they are removed with carbonyl-binding reagents, browning sensitivity is also removed. The formation of carbonyls was also confirmed by evaluation of the infrared spectra of irradiated, lyophylized samples. Their ability to reduce 2,6-dichlorophenol indophenol and acidic silver nitrate characterized them as reductones. Data on the formation of reductones from irradiated aqueous lactose solutions are presented in Figure 1. The determination of reductones was carried out as follows:

Essentially, the method of colorimetric determination of ascorbic acid with 2,6-dichlorophenol indophenol as outlined by the Association of Vitamin Chemists, Inc. (*2*) was followed to determine reductones in aqueous sugar solutions. Radiation-induced reductones in milk were determined by direct titration with Tillman reagent (*7*).

A sufficient amount of sample was used to give, after dilution to 10 ml., a reading of 55 to 80% transmittance. The sample (up to 7 ml.) was pipetted into a 10-ml. volumetric flask. Two milliliters of citrate buffer and 1 ml. of 5% oxalic acid were added, and the volume was adjusted to 10 ml. with distilled water. Five milliliters of this solution was taken and mixed with 5 ml. of dye solution in a colorimeter tube, and a reading was taken after 20 minutes, with a Lumetron photoelectric colorimeter and the 530-mμ filter. The time interval between mixing the dye and reading absorbance was modified for the determination of reductones because of the prolonged reaction time of these components. The standing time of 20 minutes was chosen for two reasons: After 20 minutes, the fading for the dye in the blank was parallel—i.e., at the same rate—to the fading of the sample, which indicated that reducing substances had completed their reaction by this time; and, with known concentrations of purified triose reductone (as standard), the ratios of absorbances corresponded to the ratios of concentrations after 20 minutes.

The yield of reductones is very small. This is in conformity with the results of Phillips (*14*), who found that uronic acids are the main chemical breakdown products formed from irradiated hexoses. The yield of reductones increases with

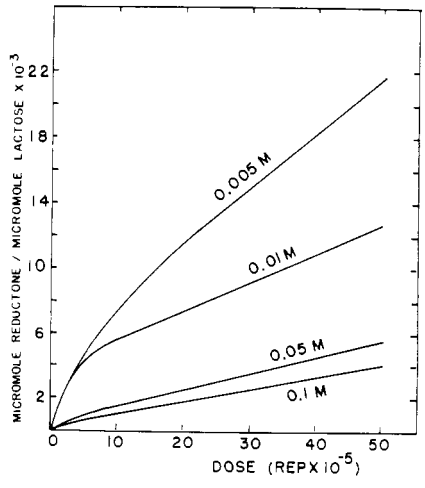

Figure 1. Formation of reductones from irradiated lactose in aqueous solution

Table II. Formation of Reductones on Irradiation with 2 × 10^6 Rep

	Yield, μmole Reductions/ μmole Substance × 10^{-2}	Ionic Yield, M/N × 10^{-3}
Lactose		
0.005*M*	1.18	9.86
0.01*M* (0.36%)	0.74	12.4
0.05*M*	0.24	20.1
0.1*M* (3.6%)	0.18	30.8
Amino Acids, 4% Aqueous Solution		
Glycine	0.012	1.12
Alanine	0.005	0.36
Serine	0.101	6.41
Histidine	0.40	15.0
Tyrosine	0.19	7.2
Tryptophan	0.43	13.9

increasing dose for each concentration level. The ionic yield (which represents the relation of functional reductones formed to the number of ion pairs produced by irradiation) of the functional reductones was of a low order and decreased with increasing dose but increased with increasing concentration. When reductone formation in various concentrations of lactose is plotted against dose in roentgen-equivalent-physical, a change in the slope of the curves occurs after approximately 1 × 10^6 rep, and, above this level, the curves have a constant slope of decreasing order with increasing concentration of lactose.

When lactose solutions are irradiated with a dose of 2 × 10^6 rep, the yield of reductone decreases with increase of concentration, and, in a concentration of lactose corresponding to that found in milk (0.1*M*), only 0.2% of the sugar was transformed into reductone (Table II). Aqueous solutions of certain amino acids also formed reductones on irradiation. α-Aminopropionic acids, substituted on the beta carbon, formed the largest amount of reductone; histidine and tryptophan produced about as much as lactose on irradiation. This suggests that certain end groups of proteins might be activated on irradiation in a similar manner. The radiation-induced reductones show an absorption maximum at 265 mμ, whereas triose reductone absorbs at 277.5 mμ.

Samples representing various dose rates and fractions of irradiated sugar solutions show the correlation of reducing value to absorbance, at 265 mμ (Figure 2). When irradiated galactose was heated at 95° C., browning was induced, the absorbance of the 265-mμ maximum also increased, and proportionate increase of reducing values was found. The position of these points on a straight line passing through the origin confirms the relationship of these components to browning.

Work on the identification of these components has been difficult not only because of their low yield but also because of their tendency to polymerize. They form yellow compounds, as does triose reductone, when caused to react with *p*-aminobenzoic acid (PABA) (*1*, *3*, *8*). In contrast to the triose reductone, however, they are water soluble and insoluble in glacial acetic acid.

Paper chromatography reveals that the R_f values of these reductones do not correspond with values obtained with triose reductone (Table III). There are indications, however, that these substances are related to reductones found by Weygand (*20*) to occur in alkali-treated sugars and which, like radiation-induced reductones, do not form insoluble lead salts.

Chromatography of the *p*-aminobenzoic acid compounds on magnesium silicate (Magnesol, industrial regular, Westvaco Chemical Division) columns allows the separation of one band which remains adsorbed on the top of the column. This brownish band, which can be separated from lower, more diffuse bands by washing with distilled water, was removed, by cutting, from the extruded column under ultraviolet light, where it was observed as a band of brilliant orange fluorescence. The component was eluted with a mixture of 50% acetone and 50% water.

Another *p*-aminobenzoic acid compound leaves the magnesium silicate column unadsorbed, whereas other diffuse bands and excess unreacted *p*-aminobenzoic acid remain adsorbed. The main portion of this unadsorbed fraction appeared before traces of *p*-aminobenzoic acid could be detected with *p* - dimethylaminobenzaldehyde. This fraction was further purified as follows:

Lead acetate was added and the mixture was filtered within the first 5 minutes after the addition (after approximately 20 minutes, the first traces of lead salt of galacturonic acid began to precipitate also). The precipitate was washed with acetone and with water, and was then suspended in acetone. Hydrogen sulfide was introduced for 30 minutes, and lead sulfide was filtered with suction. The precipitate was washed with acetone to which a few drops of water had been added (to increase the solubility of the *p*-aminobenzoic acid compound). The filtrate was evaporated to dryness and redissolved in a small amount of water and rechromatographed through magnesium silicate.

These *p*-aminobenzoic acid compounds reduce 2,6-dichlorophenol indophenol. According to Euler and Angier (*1*, *8*), the endiol group remained intact and only 1 mole of *p*-aminobenzoic acid reacted with these reductones.

The spectrum of these *p*-aminobenzoic acid compounds indicates maximum absorption at 272.5 mμ (Figure 3) and is similar to pure *p*-aminobenzoic acid in this region, although the extinction coefficient of the reductone compound was considerably lower. This observation, as well as the result of elemental analysis, indicates that the chain length of these reductones is greater than six carbons.

Elemental analysis of the eluate fraction gave the following results: 43.24% carbon, 5.87% hydrogen, and 1.62% nitrogen. If four molecules of galactose-endiol had polymerized and then reacted with 1 mole of *p*-aminobenzoic acid, the empirical formula would be:

$C_{31}H_{45}O_{26}N$ or $(C_6H_{10}O_6)_4C_7H_5O_2N$

with a content of carbon, 44.7%;

Table III. Paper Chromatography of Reductones[a]

R_f	Triose Reductions	PABA Comp. of Triose Reductions	PABA Comp. of Alkaline Galactose	PABA Comp. of Irrad. Galactose	PABA Comp. of Irrad. Galactose, Eluote
0.96–0.95			Spot	Very faint spot	Faint spot
0.88		Spot			
0.79–0.75	Spot				
0.37–0.35			Spot	Spot	Spot

[a] Solvents: butanol, water, and acetic acid. Developer: 1% aqueous solution of silver nitrate.

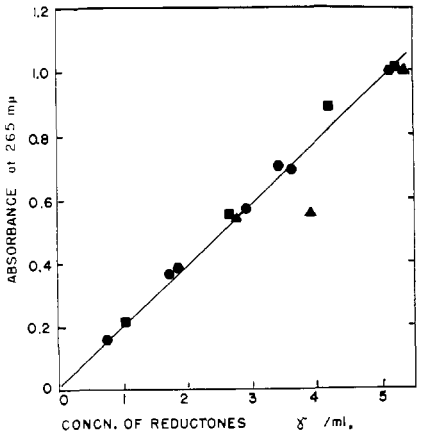

Figure 2. Browning and reducing value of irradiated, heated, aqueous galactose solutions

▲ Galactose solution irradiated and heated
■ Galactose solution irradiated
● Solute fraction from methanol
● Precipitated fraction from methanol

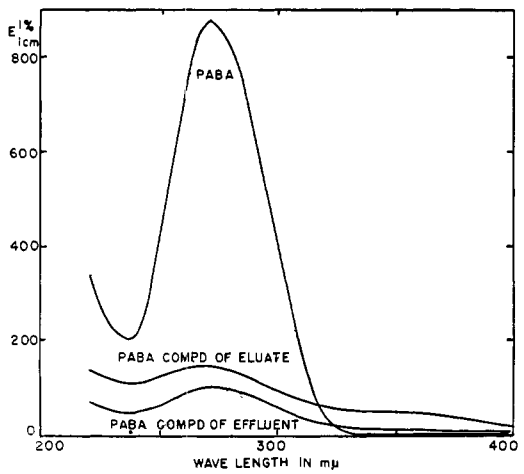

Figure 3. Transmittance of p-aminobenzoic acid and its compounds

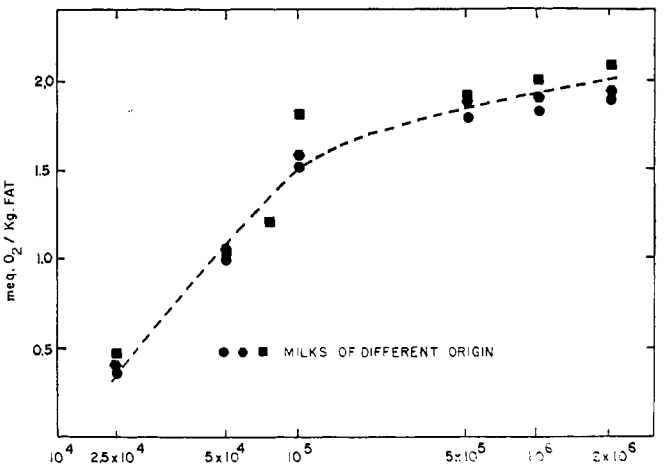

Figure 4. Homogenized milk irradiated at room temperature, peroxide value plotted against dose

hydrogen, 5.4%; and nitrogen, 1.68%. This is in good agreement with the values found by analysis. The extinction coefficient calculated from the extinction of *p*-aminobenzoic acid at 272.5 mμ in relation to the respective molecular weights would be 146, which is also in agreement with a value of 145 found in the eluate fraction.

The formation of reducing substances can also be measured as their ability to reduce ferric to ferrous ion. This reducing power increases with the radiation dose applied to the milk. This system seems to coexist with an oxidizing medium in the fat phase. The peroxides, formed in the milk fats during irradiation, were determined by their ability to oxidize ferrous to ferric ion (*11*, *17*).

Under the experimental conditions of irradiation between 10° and 20° C., peroxide formation as determined by the method of Stine and coworkers is shown in Figure 4. A sharp increase in the rate of production is noted up to 1×10^5 rep. At higher dose rates, the curve flattens considerably. The formation of peroxides was only partly dependent on external oxygen. At 2×10^6 rep, a value of 2 meq. of oxygen for irradiation in air corresponded to approximately 1.5 meq. for deaerated milk irradiated under vacuum. No difference in off-flavor, between these samples in air and vacuum, could be detected. The formation of molecular oxygen in water by irradiation can be explained by reactions of free radicals. Hydroxyl radicals can combine to form hydrogen peroxide, the latter reacts with atomic hydrogen formed to produce molecular hydrogen and hydroperoxide. The latter then combines with another hydroperoxide radical to form hydrogen peroxide and molecular oxygen.

When oxygen is passed through the milk during irradiation, a value of 8 meq. is obtained and the intensity of off-flavor is considerably increased. The decreasing rate of peroxide formation above 10×10^4 rep (Figure 4) therefore does not seem to indicate limits of off-flavor production but rather some state of equilibrium which is reached under the conditions of irradiation.

Reduction of Off-Flavor Development

The application of free radical acceptors (*5*) has been reported as a means of reducing radiation-induced off-flavors for various foods, and the application of sodium ascorbate and of ascorbyl palmitate was effective to some degree in reducing off-flavor development in milk (*16*). Irradiation of milk and of other foods in the frozen state as described by Proctor and associates (*16*) effectively reduces the reactivity of free radicals and substantially diminishes off-flavor development. However, the threshold dose for milk off-flavor is so low (*4*) that additional means are needed to reduce the radiation-induced flavor changes to acceptable levels.

Observations made in these laboratories indicate that during the first phase of radiation changes, some unstable components may be formed (primary events), and secondary reactions of these components may be then responsible for the formation of the so-called radiation-induced off-flavors. The formation of off-flavors would therefore be aftereffects rather than primary reactions of the radiation-produced free radicals with molecules of the solute. Such mechanisms are suggested by observations such as the failure to detect the characteristic off-flavor of irradiated whole milk when tasted within 30 seconds

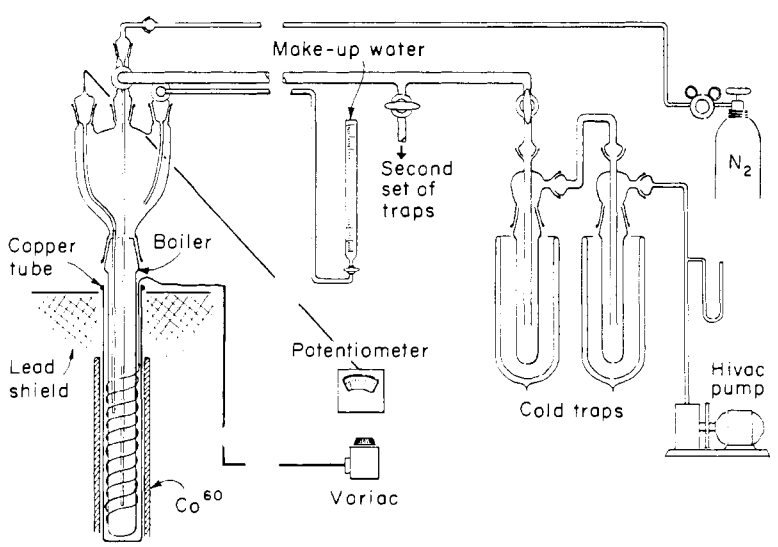

Figure 5. Radiation-distillation apparatus

after irradiation by cathode rays of high dose level.

A strong off-odor, which is noticed in the milk immediately after irradiation, disappears very rapidly to give place to the irradiation odor noted later. This first odor also disappears when milk is irradiated in closed cans; the change in odor may therefore be due to a chemical reaction or condensation.

Although the irradiation flavor of skim milk becomes diminished somewhat on storage, as reported by other workers, it becomes less volatile with increased storage time at 40° F. Almost all the off-flavor can be removed by vacuum distillation of the milk soon after irradiation. If this irradiated milk is stored for 1 or 2 days, however, increasing amounts of off-flavor remain in the distilling flask after distillation. Therefore, the formation of off-flavors probably proceeds in some steps or chains of reactions, and the time of formation of these radiation-induced off-flavors may exceed the half life of free radicals by several orders of magnitude. There were also indications that some of these intermediaries might be volatile and this suggests that if the irradiation process can be carried out under conditions that would allow the continuous removal of volatiles, such chains of reactions might be interrupted.

To test these hypotheses, a vacuum evaporator was constructed which would fit into the Massachusetts Institute of Technology Department of Food Technology's cylindrical cobalt-60 source, approximately 1.5 inches in diameter. This system is shown diagrammatically in Figure 5.

The boiler consists of an electrically heated borosilicate glass tube. Heat power is supplied from a variable transformer. A flash chamber made from a 1000-ml. three-necked flask remains outside the lead shield. Inlets for thermocouple, make-up water, vaporline, and glass capillary are provided. The apparatus is at first inserted empty into the source, the various connections are made to the equipment at a safe distance, and, after evacuation of the system, milk is drawn into the apparatus under such conditions that the boiling starts at the same time. Cold traps, pumps, and manometer are arranged in duplicate and, can be exchanged at hourly intervals without interruption of the distillation. Gas is inserted through a fine capillary in order to avoid boiling delays. As the level of the boiling milk cannot be observed during the operation, the apparatus had to be standardized in control runs made outside the irradiation source to coordinate evaporation rates with given settings of power input. These evaporation rates are checked in operation by measurement of the condensate and flow of make-up water is corrected accordingly. The total capacity of the boiler is 180 ml. and the evaporation rate can be increased up to 250 ml. per hour. To maintain constant level, distilled water is drawn into the apparatus at appropriate rates from a measuring pipet.

The first series of experiments with this device was carried out on a cobalt-60 source which emitted 1400 rep per minute. This required 12 hours of radiation time for the application of 1×10^6 rep. In a later series of experiments, a new and more powerful source was installed which has a dose rate of 5600 rep per minute and permitted a dose of 2×10^6 rep to be applied in 6 hours.

It is difficult to compare the intensity of off-flavor of samples irradiated under various conditions, as prolonged distillation alone may remove all of the proteinaceous and most of the oxidative lipoidal off-flavor. Quantitative comparison by organoleptic tests proves to be uncertain under such conditions except at very low off-flavor levels. It was necessary to apply additional means of objective evaluation.

The use of thiobarbituric acid (TBA) and browning reactions gave good indications of the general nature of changes occurring during irradiation, although there appears to be no direct correlation between the intensity of these reactions and off-flavor development. Figure 6 compares the intensity of the browning and thiobarbituric acid reactions under various conditions. Irradiation in the absence of air, followed by vacuum distillation for 12 hours, does not materially change these values. Although taste comparison is impossible, a strong chalky-metallic taste remains in the distilled sample. The addition of 1% sodium ascorbate appears to reduce the off-flavor development considerably but shows little change in thiobarbituric acid and browning. Milk, irradiated in the frozen state, shows the least amount of observed side reactions, although the flavor level is somewhat similar to that produced when milk, to which sodium ascorbate has been added, is irradiated. When milk is irradiated under concurrent evaporation, a further significant decrease in browning and thiobarbituric acid reactions is noted (Figure 6).

Some residual chalkiness is observed, although all other radiation-induced off-flavor is removed. The addition of sodium ascorbate to the milk with the retention of otherwise identical conditions gave the lowest residual values with the disappearance of all off-flavor except for some saltiness. Milk of double concentration showed reduction of browning and thiobarbituric acid to 70%. This did not change when this milk was distilled after irradiation. When milk is irradiated under concurrent radiation-distillation, further significant decreases are noted. When these operating conditions were maintained, the amount of side reactions and flavor development could be influenced by other variables, such as the speed of distillation, the temperature, and the atmosphere.

The thiobarbituric acid values for concurrent irradiation distillation for a total dose of 1×10^6 rep at different distillation rates are represented in Figure 7. With an increase of power input, the distillation rate increased and the thiobarbituric acid reaction

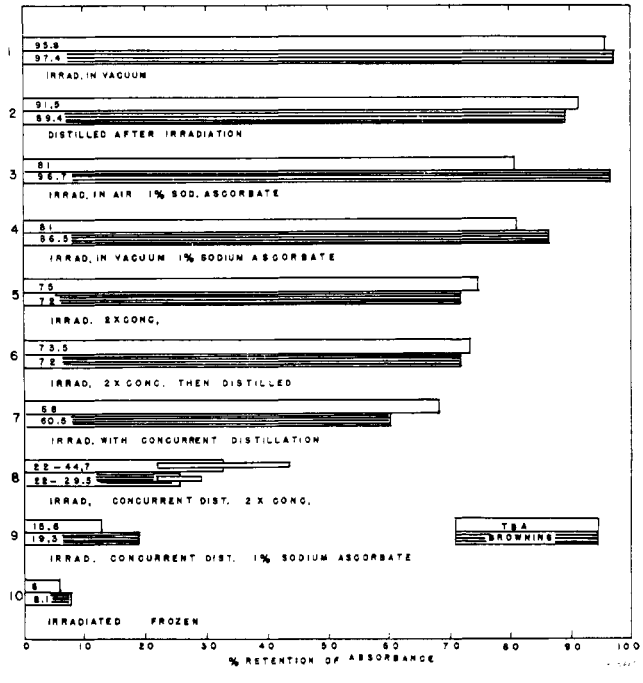

Figure 6. Thiobarbituric acid (blank areas) and browning reactions (shaded areas) of homogenized milk irradiated under various conditions

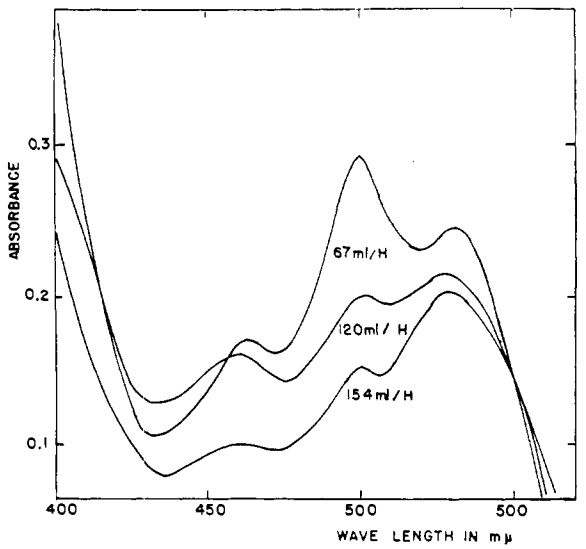

Figure 7. Change of thiobarbituric acid reaction with rate of distillation of homogenized milk during irradiation with 1 × 10^6 rep

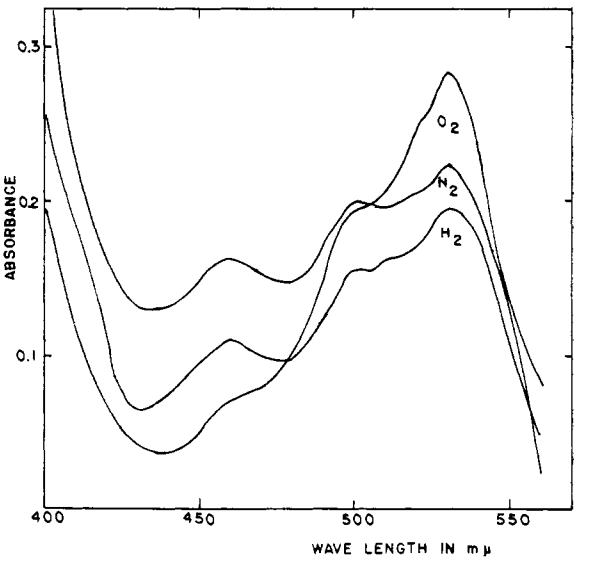

Figure 8. Influence of atmosphere on thiobarbituric acid reaction of homogenized milk during irradiation with 1 × 10^6 rep

diminished. The intensity of residual chalkiness also diminished until at a distillation rate of 150 ml. per hour freedom from radiation-induced off-flavors was achieved. This was confirmed by organoleptic panel tests. When oxygen was introduced during irradiation, a considerable increase in the formation of thiobarbituric acid pigments in the region of 535 mμ was noted (Figure 8), indicating increased fat oxidation. A decrease was achieved when hydrogen was introduced. Later experiments showed that this improvement was not due to chemical reaction of hydrogen. Traces of oxygen contained in prepurified technical nitrogen were eliminated with alkaline pyrogallol and the results obtained with oxygen-free nitrogen were identical to those obtained with the addition of hydrogen.

The installation of a new cobalt-60 source permitted a total dose of 2 × 10^6 rep to be given in a reasonable period of time (Table IV); when the first series of experiments was repeated, the distillation rates applied at the lower energy level were not sufficient to prevent off-flavor formation (Table IV, 1). By an increase in the rate of distillation to 250 ml. per hour, practical freedom from off-flavor was achieved at a dose rate of 336,000 rep per hour (*2*).

The method used to arrive at relative flavor scores consisted of the assignment of a value of 10 to the intensity of residual chalkiness remaining in the milk when it was distilled after irradiation. Noticeable trace of chalkiness was given a score of 1, 2 indicated the limit of acceptability, and a score of 3 signified that the sample was unacceptable. The improvement of flavor when hydrogen was introduced was duplicated when oxygen was removed from nitrogen (*3, 4*).

The high evaporation rates necessary in this operation at 336,000 rep per hour have made it desirable to investigate other environmental factors which would allow a reduction of these rates. When the distillation rate was again reduced, residual chalkiness again resulted, although oxygen had been carefully eliminated from the system (*5*).

The formation of free SH groups by heat treatment might act as an antioxidant as it does in other phases of milk processing (*13*). Warming of the milk before irradiation for 5 minutes at 200° F. showed a slight improvement (*6*). This was slightly increased when the time of heating was increased to 15 minutes (*7*). However, at this point, cooked flavors had already begun to be noticeable. Forewarming did not materially protect the milk fats during irradiation.

When off-flavors had been diminished to acceptable levels, some slight residual chalkiness remained which increased with storage. Peroxide values of 0.3 meq. were observed after irradiation with 2 × 10^6 rep and distillation. Hannan indicated (*12*) that these peroxides in milk, formed on irradiation, are unstable at elevated temperatures. When the pressure was increased to produce a boiling temperature of the irradiated milk of 34° C. for 1 hour

Table IV. Whole Milk Irradiated with 2 × 10^6 Rep

(Dose rate 336,000 rep/hour, concurrent radiation distillation)

No.	Dist. Rate, Ml./Hour	Retention, % Browning	Retention, % TBA	Taste	Score[a]	Experimental Condition
1	150	39.4	31.4	Chalky	4	Nitrogen
2	250	27	22.5	Chalky	1–2	Nitrogen
3	250	37	28	Chalky	0–1	Hydrogen
4	250	36	27.2	Chalky	0–1	Purified N_2 deaerated milk
5	150	38.2	31	Chalky	3–4	Purified N_2 deaerated milk
6	150	44	39	Chalky	3	Forewarming 5 min. 200° F.
7	150	32	38.3	Chalky	2–3 Cooked	Forewarming 15 min. 200° F.
8	150	31	28.4	0		1 hour after heating at 34° C.
9	150	23	15.8	0		1 hour after heating at 34° C., threefold concn.

[a] Empirical score indicating 10 for residual chalkiness at 2 × 10^6, 1, traces; 2, limit of acceptability; and over 2, unacceptable.

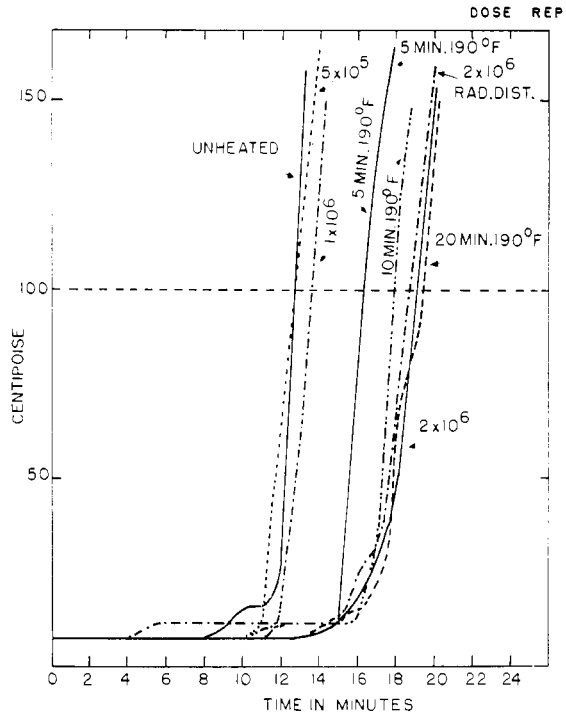

Figure 9. Rennet coagulation time of irradiated and heated milk

after irradiation, the peroxide value decreased to values between zero and 0.1 (*8*). This aftertreatment also caused the disappearance of residual chalkiness, even at the lower distillation rate. No difference could be detected between samples of such milk and samples of unirradiated, control milk by either a trained taste panel at the Department of Food Technology, Massachusetts Institute of Technology, or by panels at the Quartermaster Food and Container Institute, Chicago, Ill.

Table IV also shows that at this phase values for thiobarbituric acid and browning do not show a good correlation with off-flavor formation. It was not possible to eliminate either of these reactions entirely. A further reduction of these values was achieved when milk of threefold concentration was irradiated (*9*).

Descriptions of chemical changes within the protein phase of the milk indicate that ionizing radiations may cause some denaturation which can be demonstrated by changes in coagulation time. Reports on the increase of rennet coagulation time have been confirmed (Figure 9) and it has been verified that coagulation time increases with an increase in dose. Also, concurrent radiation-distillation does not change these values. The degree of change observed, however, does not seem to be greater than that caused by heat treatment. These changes caused by ionizing radiations may, therefore, eliminate the necessity of forewarming where this process is used to increase the stability of milk concentrates.

Progress has been made in the elimination of off-flavor under certain conditions of irradiation. Milk which normally shows off-flavor development at a dose of 7×10^3 to 20×10^3 rep can now be irradiated with a dose of 2×10^6 rep due to a concurrent radiation-distillation device that has been developed in the Department of Food Technology, Massachusetts Institute of Technology.

The absorption of 1300 rad per minute (0.77 joules per gram per hour or 8.3×10^4 gram per rep per hour) required a distillation rate of 0.83 ml. per ml. per hour to prevent off-flavor retention in milk. An increase of the dose rate with a new source to 5200 rad per minute (3.1 joules per gram per hour or 3.3×10^5 grams per rep per hour) required an increase in distillation to 1.4 ml. per ml. per hour to achieve freedom from off-flavor, and this rate may again be decreased to 0.83 ml. per ml. per hour when certain processing conditions are changed. Investigations are in progress to determine the effect of higher energy input in the range of 5000 to 50,000 rad per minute (3.0 to 30.0 joules per gram per hour or 3.3×10^5 to 30×10^5 gram per rep per hour).

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