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Influence of Pasteurization and Homogenization Treatments on Photocatalyzed Oxidation of Cream

J. FOLEY, J. J. GLEESON, and J. J. KING

Department of Dairy and Food Technology
University College, Cork, Ireland

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ABSTRACT

Heat treatment within the temperature range of 60 to 94 C promoted photocatalyzed lipid oxidation of cream. The sulfhydryl compounds cysteine hydrochloride, glutathione, thioglycolic acid, and mercaptoethanol at concentrations of 0.1 to 0.001% acted as antioxidants. Copper migrated from the serum phase to the fat globule surfaces when cream was heated and acted as an oxidation catalyst. The latter influence predominates over the antioxidant effect of the free sulfhydryl groups in heat treated cream. Homogenization of pasteurized cream inhibits lipid oxidation depending on the pressure used.

Much of the literature on lipid oxidation in dairy products relates to liquid milk and to ripened cream butter. Many factors including light, ascorbic acid, riboflavin, temperature treatment, and traces of copper and iron are known to influence the susceptibility of milk to oxidation. Studies on cream oxidation are few and seem, in the main, to confine attention to the effect of heat treatment on the general keeping quality and in some instances oxidative tendencies of the resultant butter.

In general, the earlier work relating heat treatment of cream to butter quality indicates that relatively high temperature treatment improved the keeping quality of butter and retarded oxidation. It was considered that the sulfhydryl (-SH) groups freed from the serum proteins acted as antioxidants. The widely held view that heat treatment is effective in preventing oxidized flavor through liberation of active -SH reducing groups was challenged by Aurand et al. (*1*) who found that addition of cysteine hydrochloride to milk did not inhibit development of oxidized flavor.

Smith and Dunkley (*11*) found that pasteurization of milk increased its susceptibility to oxidized flavor, a result, which they observed was consistent with commercial experience.

At least two changes which may influence lipid oxidation take place in milk during heating: (*a*) production of free sulfhydryl groups, and (*b*) migration of copper from the serum to the fat globule surfaces.

Copper plays a dominant role in oxidative changes in dairy products. Almost all of the copper in milk is bound to the protein and there is little if any present in the ionic form. The percentage of copper in milk which is associated with fat globules is vitally important in lipid oxidation. King and Dunkley (*4*) attributed 10 to 35% of the copper of milk to the fat globule portion, while Samuelsson (*7*) found that 15 to 20% of the natural copper of milk is associated with the fat globules. Heat treatment causes migration of copper associated with milk serum to fat globule surfaces, and thus influences distribution of added copper among the different fractions of milk (*8, 10*).

Homogenization of milk is known to retard development of oxidized flavor. The influence of homogenization on the oxidative stability of cream has received little attention but it is generally assumed that the effect is the same as on milk.

The objects of this study were to determine the influence of different heat treatments on the photocatalyzed lipid oxidation of cream, to explain any trends observed and to establish the effect of homogenization and varying homogenizing pressures on oxidative changes in cream.

MATERIALS AND METHODS

Cream samples

Cream containing 35% fat was mechanically separated from well bulked milk which had been preheated to 40 C. Pasteurization was done either in a commercial HTST plant or in a laboratory pasteurizer.

The cream samples (100 ml) in 125-ml Pyrex containers were held in a cabinet at 5 C under a natural color fluorescent lighting strip which gave a light intensity, measured by an AVO light meter of 50 lumens/ft^2 on the cream samples. Peroxide value determinations were done in duplicate after predetermined holding times.

Separation of the fat of cream for peroxide determinations

The method of Stine et al. (*13*) was used. It employs a surface active agent (BDI reagent consisting of 30 g of Triton ×-100 and 70 g of sodium tetrapyrophosphate made up to 1 liter with distilled water) combined with heat treatment to de-emulsify the fat globules followed by separation of the fat layer by centrifugation.

Peroxide test

Peroxide analyses were made by the ferric thiocyanate method of Loftus Hills and Thiel (*5*) as modified by Holloway (*3*). Quantities of 0.5 ml or of 0.1 ml of fat were used depending on the magnitude of the expected perioxide values. Peroxide values were expressed as m equiv. of oxygen per kg of fat.

Determination of copper concentration

Copper determinations were made using a Perkin-Elmer Atomic Absorption Spectrophotometer Model 103 fitted with a copper hollow

cathode lamp. The following instrument settings were used: Wavelength, 3247 A°; Oxidant, air at 45 psi pressure and a flow rate of 26.5 l/min; fuel, acetylene gas at 8 psi pressure and a flow rate of 3.5 l/min; lamp current, 8 ma.

A standard graph of copper concentration against transmittance readings was prepared each time the instrument was used by dilution of a stock solution of cupric chloride to give a range of copper concentrations. The instrument scale was adjusted to obtain a 100% reading for 1 ppm of copper.

The copper concentrations of cream sera were determined as follows: approximately 200 ml of each cream was centrifuged for 20 min at 3000 rpm. A 50-ml portion of the serum was collected using a syringe and 50 ml of 24%(wt/vol) trichloro-acetic acid added and the mixture shaken at 5-min intervals for 30 min to ensure complete precipitation of the protein. The precipitate was removed using a Whatman No. 42 filter paper.

Lanthanum oxide, 0.5 ml of 5% (wt/vol) solution was added as a suppressing agent and the filtrate concentrated about threefold by evaporation to obtain readings in the sensitive range of the atomic absorption spectrophotometer.

Homogenization of cream

Cream was heated at 72 C in a plate preheater and homogenized at different pressures in a Rannie single stage homogenizer. The samples were cooled to 5 C and placed in the display cabinet to catalyze oxidation.

Glassware

To avoid any possibility of copper contamination, all glassware, after washing with hot detergent solution, was steeped for 24 h in dilute nitric acid and then thoroughly rinsed with de-ionised water and dried.

RESULTS

Figure 1 shows the effect of heat treatment within the temperature range of 60 to 94 C on the peroxide values of cream held at 5 C under fluorescent light at an intensity of 50 lumens/ft^2.

The initial peroxide values of all samples after heat treatment and before exposure to light were practically identical. It is clear that as the treatment was raised from 60 to 94 C, the peroxide values, after exposure of the samples to light, increased. Differences in peroxide values are detectable after some hours and pronounced after 1, 2, and 4 days.

Double pasteurization of cream for buttermaking is a common practice in Ireland. Figure 2 shows that re-pasteurization of cream within the temperature range 60 to 94 C resulted in considerably increased peroxide values.

The control cream used in this experiment was pasteurized at 72 C for 15 sec and reached a higher peroxide value than the unpasteurized control used in the previous experiment.

When cream was heated, the copper concentration of the serum phase dropped. Figure 3 shows the percentage decrease in copper content in the cream serum.

Apparently copper migrates from the serum to the fat globule surfaces. At 66 C there was a reduction of about 5% of the serum copper while at 94 C for 15 sec the serum copper had diminished by 25%. Temperatures used for cream pasteurization usually fall within the range of 72 to 95 C and therefore within that where copper migration occurred.

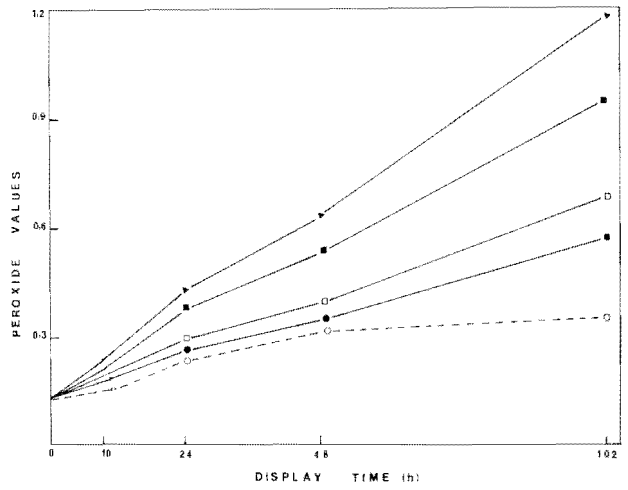

Figure 1. *The effect of the temperature of pasteurization on the peroxide values of cream. Pasteurization temperatures:* O — O *Control (unpasteurized),* ● — ● *60 C for 15 sec,* □ — □ *70 C for 15 sec.* ■ — ■ *82 C for 15 sec, and* ▲ — ▲ *94 C for 15 sec.*

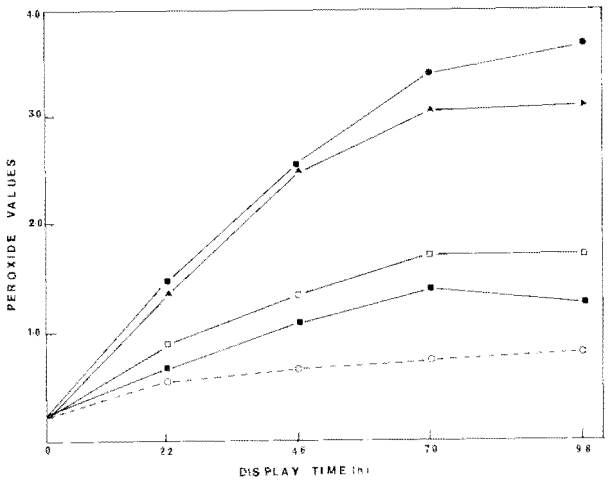

Figure 2. *The influence of the temperature of repasteurization on the peroxide values of cream pasteurized at 72 C for 15 sec.* O — O *control pasteurized at 72 C for 15 sec,* ■ — ■ *repasteurized at 60 C for 15 sec,* □ — □ *repasteurized at 70 C for 15 sec,* ▲ — ▲ *repasteurized at 82 C for 15 sec, and* ● — ● *repasteurized at 94 C for 15 sec.*

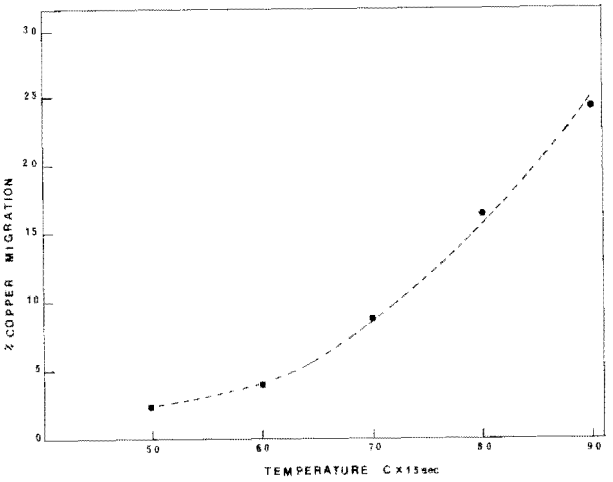

Figure 3. *The influence of heat treatment on the migration of copper from the serum to the fat phase of cream.*

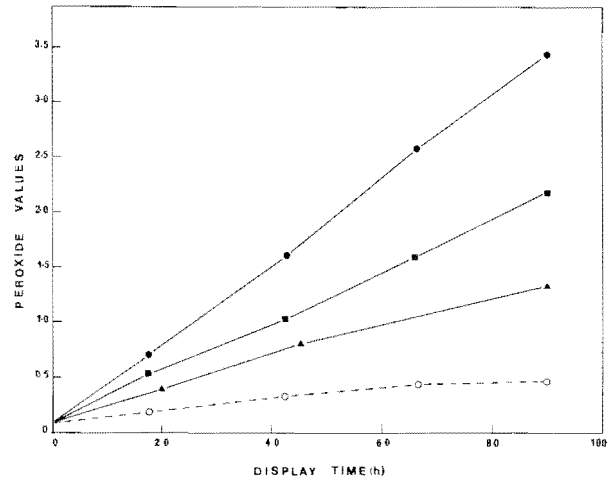

Figure 4. *The influence of addition of EDTA on the peroxide values of cream.* O — O *raw cream,* ● — ● *cream pasteurized at 82 C for 15 sec.* ■ — ■ *Cream pasteurized at 82 C + 0.05% EDTA, and* ▲ — ▲ *Cream pasteurized at 82 C + 0.2% EDTA.*

Figure 4 shows the effect on peroxide values of addition of ethylenediaminetetraacetic acid (EDTA), a water soluble copper complexing agent, to cream before heat treatment. The EDTA (0.05 and 0.2%) was added 2 h before heat treatment and the sample well stirred at intervals to permit complexing of copper to take place. Addition of EDTA proved effective in retarding oxidation of heat treated cream. It would appear that the EDTA, by complexing copper, prevented its migration to the fat globule surfaces, and made it less available as an oxidation catalyst. In another experiment EDTA was added to cream after heat treatment. Its antioxidant influence was only very slight when compared with the effect shown in Fig. 4.

The influence of free sulfhydryl groups in photocatalyzed oxidation was studied by adding sulfhydryl containing compounds to pasteurized cream (72 C for 15 sec) and measuring the peroxide values after

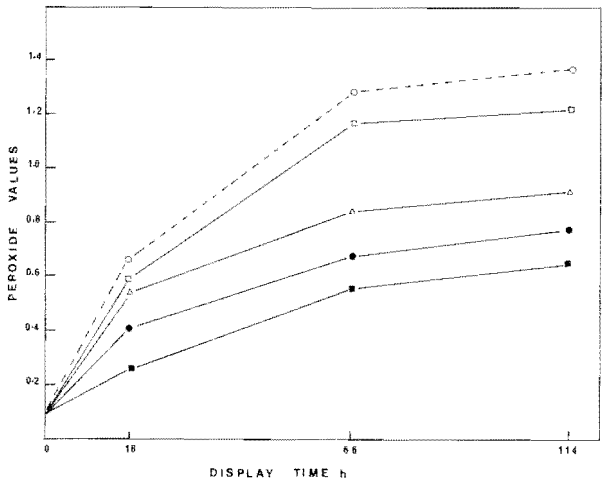

Figure 5. *The influence of cysteine hydrochloride on the peroxide values of cream pasteurized at 72 C for 15 sec.* O — O *control,* □ — □ *0.01% cysteine hydrochloride.* △— △*0.05% cysteine hydrochloride,* ● — ● *0.10% cysteine hydrochloride, and* ■ — ■ *1.0% cysteine hydrochloride.*

different holding times. The following compounds were used: thioglycolic acid, cysteine hydrochloride, glutathione, and mercapto-ethanol. The control and test samples were pasteurized at 72 C for 15 sec and held in the display cabinet to render them more susceptible to oxidation and thus permitting easier detection of the effect of the added compounds.

Cysteine hydrochloride had a marked antioxidant effect over the concentration range of 0.01 to 1.0% (Fig. 5). Further experiments showed that cysteine hydrochloride had antioxidant activity at concentrations down to 0.001%. The compounds thioglycolic acid, glutathione, and mercapto-ethanol also inhibited photocatalyzed oxidation of cream when used at concentrations ranging from 0.001 to 0.1%.

Homogenization of cream inhibited photocatalyzed oxidation. The antioxidant influence of homogenization was apparent at pressures as low as 100 psi and increased with increasing pressures up to 2500 psi the relative benefit declining as the pressure was raised (Fig. 6).

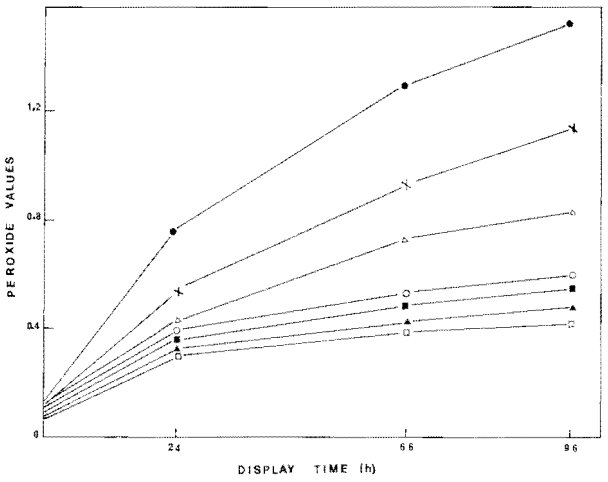

Figure 6. *The effect of different pressures of homogenization on the peroxide values of cream.* ● — ● *unhomogenized control,* ×—×*100 psi,* △ — △ *500 psi,* O — O *750 psi,* ■ — ■ *1,000 psi,* ▲ — ▲ *1,500 psi, and* □ — □ *2,500 psi.*

When the homogenized creams were subsequently heated to 80 C for 15 sec and then held under fluorescent light, the antioxidant influence of homogenization, though somewhat diminished, was still evident even at the lower pressures used.

DISCUSSION

Heat treatment of cream increased its propensity to photocatalyzed oxidation as measured by peroxide values. The higher the temperature up to 94 C for 15 sec, the greater the peroxide values after exposure to fluorescent light. This appears to conflict with the widely held opinion that liberation of free sulfhydryl groups when the serum proteins, β-lactoglobulin in particular, are denatured by heat confers antioxidant properties on many dairy products.

In a critical literature review, Wilkinson (*13*) noted that there was evidence both to suggest that free -SH

groups acted as catalysts in the oxidation of lecithin and certain fatty acids and also to support the view that -SH groups acted as antioxidants. He suggested that at low concentrations -SH groups have a pro-oxidant effect, whereas at high concentrations they have antioxidant properties. Aurand et al. (*1*) added various levels of cysteine hydrochloride to raw milk before heating and found no inhibitory effect on oxidized flavor development. The sulfhydryl compounds cysteine hydrochloride, glutathione, thioglycolic acid, and mercapto-ethanol when added to pasteurized cream in this study all showed definite antioxidant activity which increased with increasing concentration over the range of 0.001 to 0.1%.

Despite the antioxidant influence of sulfhydryl groups it is clear from this study that the net effect of heat treatment of cream is pro-oxidant. Smith and Dunkley (*11*) concluded that pasteurization increased the susceptibility of milk to oxidized flavor development. Samuelsson (*9*) noted that the TBA values of heated buttermilk increased with increase in heat treatment. In a previous study in this laboratory Phelan (*6*) showed that when the temperature of repasteurization of cream was raised from 80 to 105 C the TBA values, after holding, increased.

While relatively high treatment of cream leads to increased peroxide values, this pro-oxidant effect does not necessarily hold for other milk products. Thus we have found, in this laboratory, that high heat treatment of milk for drying gave lower peroxide values in both nitrogen and non-nitrogen packed full cream milk powder held at 10 C.

The antioxidant influence of free sulfhydryl groups released during heat treatment of cream is apparently overshadowed by another change which is decidedly pro-oxidant. Copper migrates from the serum phase to the surfaces of the fat globules where it acts as an oxidation catalyst (Fig. 3). If the migration of copper from the serum phase is inhibited by addition of certain chelating agents before heat treatment, the pro-oxidant influence is diminished.

When cream is heat treated before addition of a copper chelating agent, the role of the chelating agent in inhibiting oxidation is less. Presumably, the copper which had already migrated to the fat globule surfaces is not readily complexed by the chelating agent.

The view that singlet oxygen is involved in the primary initiation of lipid oxidation is gaining in favor. Singlet oxygen can be formed through photochemical reactions in the presence of a sensitizer. Light, particularly in the ultra violet region is a particularly effective initiator of oxidation when sensitizers such as copper and bound copper complexes are present. Heat treatment of cream may perhaps alter the copper-protein complexes and thus increase their pro-oxidant effect.

Hill (*2*) has recently demonstrated the presence in milk of the antioxidant enzyme superoxide dismutase and has suggested that the small amounts of dismutase normally present in milk might play a part in the control of lipid oxidation. Although little is yet known about the role of superoxide dismutase in milkfat oxidation, the heat treatment given to cream in this study may have inactivated the enzyme thus allowing the superoxide anion and its products to catalyze oxidation.

The physical change in the fat phase brought about by homogenization inhibits development of oxidized flavor in pasteurized milk possibly through the formation of copper chelates (*11*). Likewise, homogenization of pasteurized cream (72 C for 15 sec) at pressures from 100 up to 2500 psi reduced the tendency of cream to photocatalyzed oxidation. The anti-oxidant effect, which increased with increasing pressures up to 1500 psi, was relatively greater at the lower pressures used.

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