

Oxidation of Fat in Model Systems Related to Dehydrated Foods.^a II. Composition and Position of Dispersed Lipid Components and Their Effect on Oxidation Rates

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SUMMARY

Oxidation rates at elevated temperatures of model dehydrated systems containing dispersed lipids are influenced by concentration of the components, type of dispersing medium, and position of the lipid film with respect to the dispersing medium. Generally, the proteins decreased, and polymeric carbohydrates accelerated, oxidation rates. Arginine and lysine salts of safflower fatty acids were extremely stable. Phospholipids had a stabilizing influence when dispersed with the fat and the dispersing medium prior to freeze-drying or when applied as a film between the dry medium and the fat film. When the positions of the fat and the phospholipid films were reversed, the protective action of the phospholipids on the stability of the system decreased markedly.

INTRODUCTION

This paper continues investigations into the stability of model systems related to dehydrated foods. These simplified systems were designed to contain lipid, protein, and carbohydrate components in varying concentrations for purposes of studying the interactions occurring under accelerated storage conditions at elevated temperatures.

In a previous publication (Bishov *et al.*, 1960) we noted that dehydrated fatty emulsions dispersed with an inert polymeric carbohydrate, carboxymethyl cellulose (CMC), could be used to evaluate the role of proteins, phospholipids, and heme compounds in lipid oxidation. Other investigators have also noted that phospholipids alone or with proteins acted as antioxidants (Dutton and

Edwards, 1945; Henick *et al.*, 1958; Lea, 1939; Olcott and Mattill, 1936; Patrick and Morgan, 1944), whereas heme components acted as catalytic pro-oxidants (Brown and Tappel, 1958; Robinson, 1924; Tappel, 1953; Watts, 1954). The present paper continues these investigations with special emphasis on determining the influence of component concentration, types of dispersing media, and the position of lipid with respect to the dispersing media, on oxidation rates in dehydrated model systems. The relationships established in these oxidation studies emphasize the quantitative nature of the reactions involved. Togashi *et al.* (1959) recently noted that variations in the chemical nature at the supporting surfaces for thin fat films showed significant differences in protective action against lipid oxidation. They also observed that phospholipids were effective as inhibitors of the surface oxidative reaction. Chargaff (1949) noted that ionic charge alteration probably plays an important role in the protein and lipid interactions, and Doty and Schulman (1949) demonstrated the existence of lipid-bound protein at charged interfaces. Luck (1949) concluded that the binding of fatty acid anions by the positively charged amino acid residues is due to electrostatic attraction and van der

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Waal forces. It is probable that some of the proposed mechanisms and postulates apply to the experimental evidence presented herein. The experimental design was necessarily exploratory in nature since the reactions of fat and other food components in the newly developed freeze-dried rations have not been studied to any extent.

It is hoped that the results of these investigations may facilitate new approaches to extending the storage life of the recently developed freeze-dried rations for the Armed Forces.

EXPERIMENTAL

A) Materials and methods

The methods have been described (Bishov *et al.*, 1960). The techniques for preparation and freeze-drying the emulsions (Rolfes *et al.*, 1955), accelerated aging at elevated temperatures in the rancimeter (James, 1945; Salwin, 1954), and the measurement of oxidation products as peroxides and as volatile carbonyl and other components (Hills and Thiel, 1945; Sidwell *et al.*, 1958) were similar to those in the first paper of this series (Bishov *et al.*, 1960).

The following materials were used: carboxymethyl cellulose gum (CMC); soy oil, refined edible; soy phospholipids; hemoglobin; potato starch, 10 g boiled 10 min in 300 ml H₂O; egg white, sugar-free spray-dried; amylose starch, Stein Hill & Co., Inc., 10 g boiled 10 min in 300 ml H₂O; ovalbumin, water-soluble, de-fatted freeze-dried whole egg; gelatin, type B, pH 6.8, p.i., 4.9, Atlantic Gelatin Co.; aqueous beef extract, clear soluble fraction, freeze-dried; cottonseed oil, refined; lard, refined; L-arginine safflate and L-lysine safflate, General Mills; dialdehyde starch (Sumstar), Sumner Chemical Co.

B) Preparation of dehydrated emulsions

The emulsions used to obtain data for the graphic material in the following figures are briefly described. Detailed descriptions appear in the earlier paper.

Five grams of the dispersing medium were suspended in 300 ml water for one minute in a Waring Blendor. The other components were added in the sequence as follows: heme compounds, phospholipids (50 mg/g fat), and fatty materials (10 g), blended for one minute after each addition. The emulsions were shell-frozen in a 5-L boiling flask and freeze-dried. The dried emulsions were ground in a sharp-bladed Waring Blendor, and refrigerated at -20°C until needed.

Samples that involved film studies were prepared as follows: (a) One gram of soy oil was dissolved in twenty ml acetone. This was then adsorbed

on 500 mg freeze-dried CMC dispersing medium. The ratio of fat to dispersing medium was 2:1 for all experiments. (b) 50 mg of phospholipids were dissolved in 20 ml petroleum ether and adsorbed on the CMC dispersing medium. (c) Phospholipids were placed on the oil film of (a) from a solution containing 50 mg of phospholipids in a 3:1 methyl alcohol-petroleum ether mixture in which the oil was sparingly soluble. (d) The oil was placed on the phospholipid film by proceeding as in (a). In all cases above, the solvent was evaporated by air-drying in a beaker.

C) Accelerated storage

Samples containing 1.0 g of fatty material and the other components, as described, were heated in a stream of air in rancimeter tubes for accelerated aging at 95°C (James, 1945). These samples were heated continuously for the periods indicated, and the volatile components were collected during each 150 minutes. Unless otherwise indicated, the data are plotted in integrated form as shown.

RESULTS AND DISCUSSION

I. Effects of Variations of Fat and Other Food Substances on Rate of Oxidation in Dehydrated Emulsions

It was previously shown (Bishov *et al.*, 1960) that carboxymethyl cellulose gum could be used as a dispersing medium in investigating oxidation characteristics of dehydrated emulsions containing fat, phospholipids and heme compounds. The present studies include investigation of several polymeric carbohydrates besides carboxymethyl cellulose, such as potato, starch, and dialdehyde starch; and proteins, such as gelatin, egg white, and ovalbumin as dispersing

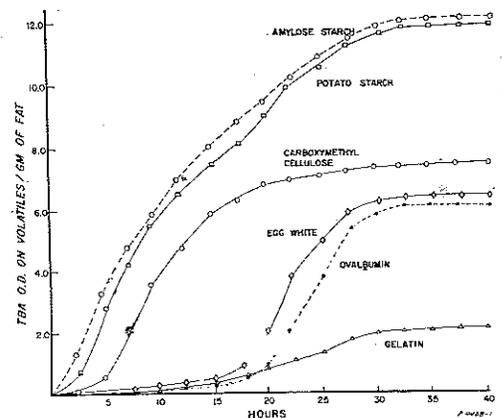


FIG. 1. Effect of some dispersing media on fat oxidation in dehydrated emulsions.

media. These are shown in Fig. 1. The data indicate that oxidation was significantly higher and the induction periods shorter for the carbohydrates than for the proteins. As shown in Fig. 2 and discussed in the first paper, heme substances, such as myoglobin from beef extract when added to CMC soy oil in dehydrated emulsions, also appear to accelerate the oxidation rate of fat-containing emulsions. This probably results in the reduced acceptability of some of the freeze-dried meat items. Increasing quantities of the extract significantly accelerated the oxidative reaction with shorter induction periods, resulting in a more rapid formation of

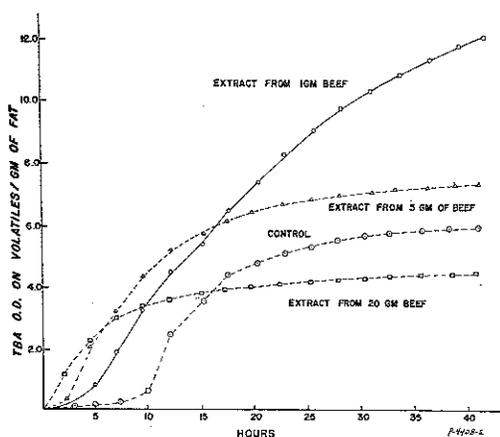


FIG. 2. Effect of myoglobin from beef extract on fat oxidation in dehydrated emulsions.

carbonyl compounds. The lower levels of total thiobarbituric acid (TBA) reactive volatile substances at the end of aerating at 95°C suggest that with increasing beef extract concentration, increasing proportions of peroxides break down into compounds other than carbonyls.

The sequence of formation and breakdown of peroxides in the model dehydrated fat emulsions is shown in Fig. 3. The data show that, initially, in oxidation of fat, peroxides are formed at a very rapid rate, reaching a maximum after five hours at 95°C. The groups assayed by the TBA test did not reach their maximum for more than 20 hr at that temperature. These data suggest that the volatile substances measured with the TBA reagent are peroxide breakdown products. A similar sequence of reactions with

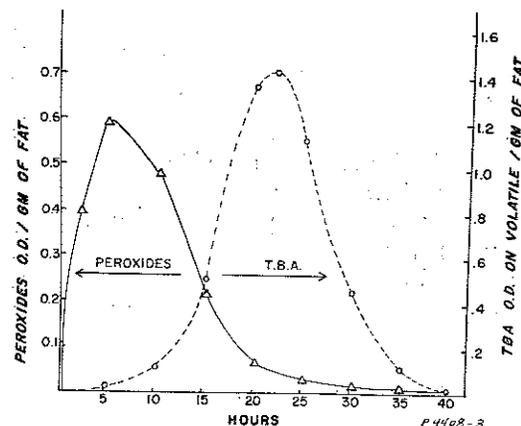


FIG. 3. Oxidation at elevated temperatures of fat in dehydrated emulsions as measured by peroxides and TBA.

increasing temperatures at a constant exposure interval was reported in the earlier paper.

Oxidation rates are significantly influenced by the nature of the fatty substances dispersed in the dehydrated emulsions. Oxidation rates of several fatty substances in dehydrated CMC emulsions are shown in Fig. 4. The data indicate that L-arginine- and L-lysine-safflates were extremely stable. Data not presented here indicate that even in the presence of oxidation-promoting hemoglobin the safflates retained significantly low rates of oxidation, whereas fats such as lard, soy oil, and cottonseed oil were oxidized very

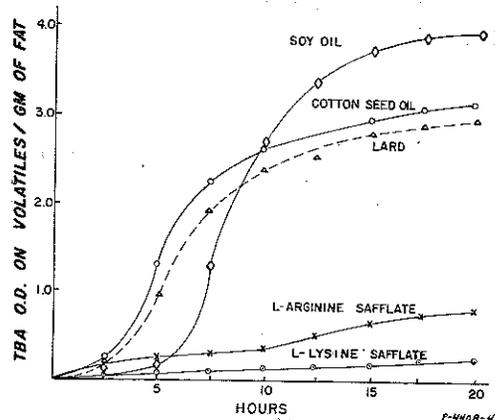


FIG. 4. Oxidation of some fatty substances in dehydrated carboxymethyl cellulose CMC emulsions.

rapidly. These safflates were, however, normal substrates for lipoxidase-catalyzed oxidation in aqueous suspension.

II. Effects of Concentration of Lipid Films and Dispersions on Oxidation in Dehydrated Emulsions

The data presented thus far indicate that oxidation interactions in the dehydrated-food-like model-system emulsions are significantly influenced by the composition of all the components that come into contact with each other. The data that follow demonstrate that concentration and position of the lipid components are also significant factors. The phenomena discussed here are probably those of surface interactions. The structures in the model systems are similar to those of freeze-dehydrated food in that the solids remaining after crystallized ice is sublimed, have a flaky structure whose volume is equivalent to that of the wet food. These structures provide tremendous areas for spreading lipid materials as films or dispersions. Oxidation reactions similar to those with thin lipid films probably take place, as suggested by the work of Togashi *et al.* (1959).

A. Effect of Concentration of Lipids as Dispersions and as Added Films on Oxidation in Dehydrated Emulsions

The data in Fig. 5 indicate that, for a given quantity of dispersed fat, when emulsified with CMC prior to dehydration, oxidation increases with increasing quantity of the dispersing (CMC) medium. The higher

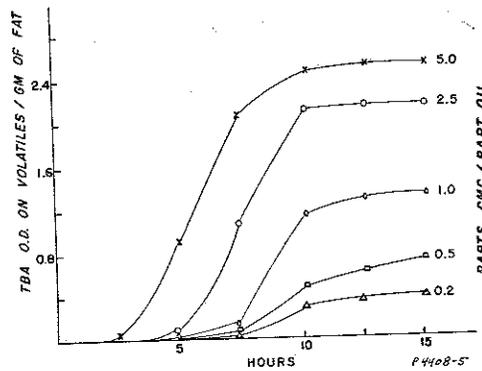


FIG. 5. Effect on oxidation of concentration of fat dispersed with CMC in dehydrated emulsions.

oxidation levels are accompanied by decreasing induction periods. This suggests that the greater the dispersion of the available fat the more rapid is the oxidation. As shown by the TBA values in Fig. 5 the oxidation levels increased fivefold with a twenty-fivefold increase in the quantity of the dispersing medium. The data in Fig. 6 show that when fat was applied as a film on the freeze-dried carboxymethyl cellulose by evaporation from petroleum ether, the oxidation rate did not vary significantly among the emulsions for the first 10 hours of aera-

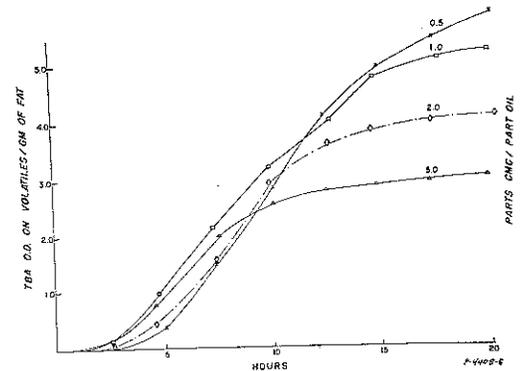


FIG. 6. Effect on oxidation of concentration of fat film added on dehydrated CMC.

tion. For aeration periods of more than 10 hours, the extent of oxidation decreased with increasing dispersion provided by the increasing quantities of the CMC.

Comparison of Figs. 5 and 6 indicates that induction periods for fat film applied on freeze-dried CMC (Fig. 6) are significantly shorter than for those when fat and CMC were dispersed before freeze-drying. The diminution in the total accumulated TBA reactants with increasing dispersion may, in part, be attributed to the physical protection afforded some of the oil by the capillarity of the freeze-dried CMC. The degree of this protection was constant and independent of the amount applied, therefore increasing the percentage of the oil protected as the ratio of CMC to oil was increasing. Further examination of the data in Fig. 6 suggests that the extent of oxidation was greater probably because of the mobility of the increasingly thinner fat film

as quantities of the dispersing medium decreased. This observation is in agreement with those of Togashi *et al.* (1959) in their investigations of thin films.

B. Effect of Added Phospholipid Film on Oxidation Rates of Dehydrated Emulsion Containing Fat or Insoluble Fat Residue

In a previous paper it was noted that phospholipids had protective effects against fat oxidation in dehydrated model systems containing fat. Fig. 7 illustrates this protective

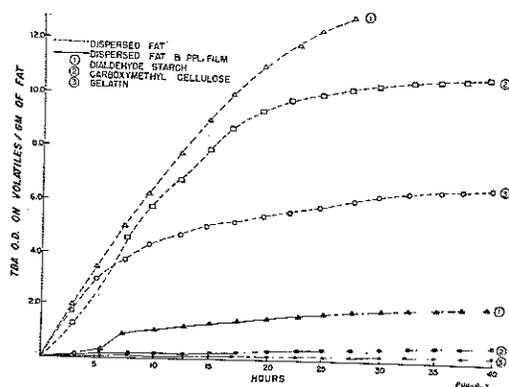


FIG. 7. Effect of added phospholipid film on fat oxidation in dehydrated emulsions containing whole fat.

action on fat with three different types of dispersing media. The protective action of phospholipids is shown by the drop in TBA

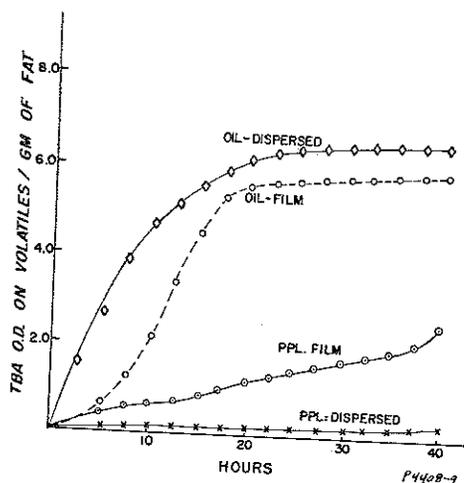


FIG. 8. Oxidation of fat and phospholipids as films and as dispersions in dehydrated emulsions.

levels to less than a fifth of that attained without phospholipid protection.

Fig. 8 shows that the oxidation of fat is less rapid when applied as a film than when dispersed with CMC, while oxidation of phospholipids is more rapid when it is applied as a film than when dispersed. The differences may be due to structural and position differences of the fat and phospholipids under investigation.

III. Effect of Phospholipid and Fat Film Positions on Oxidation Rates in Dehydrated Emulsions

Significant differences in the protective action of phospholipids on fat oxidation were noted when the position of each was reversed in relation to the dispersing medium CMC. (This is sufficient evidence that the two exist as separate films). These observations confirmed those made by Togashi *et al.* in investigations into the chemical nature of surfaces and their effect on fat oxidation.

Fig. 9 indicates that when the oil film was placed between CMC and the phospholipid film (as in curve A) the phospholipid appeared to lose a great deal of the

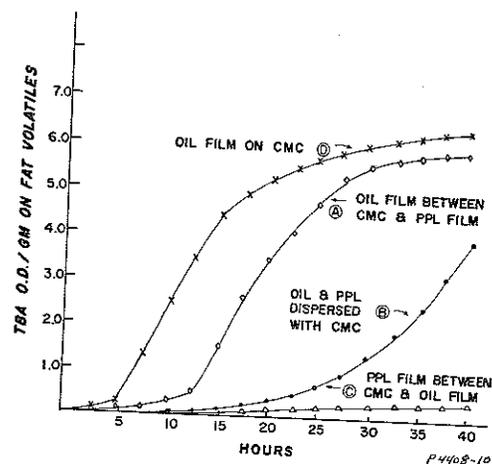


FIG. 9. Effect of phospholipid and fat film positions on oxidation in dehydrated emulsions.

protective action it had when the positions of phospholipid and oil films were the reverse (curve C).

The protective action of phospholipid and oil dispersed with aqueous CMC (curve B) falls between that of curve A and curve C.

Oxidation rates in oil film alone, curve D, without the antioxidant action of either dispersed or film phospholipids, are significantly higher accompanied by shorter induction periods.

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