REFERENCES

1. Pittman, C.U., Jr., Chem. Technol. 560 (1973).
2. Bailar, J.C., Jr., Cat. Rev. Sci. Eng. 10:17 (1974).
3. Michalska, Z.M., and D.E. Webster, Chem. Technol. 117 (1975).
4. Grubbs, R.H., Ibid. 512 (1977).
5. Bruner, H., and J.C. Bailar, Jr., JAOCS 49:533 (1972).
6. Bruner, H., and J.C. Bailar, Jr., Inorg. Chem. 12:1465 (1973).
7. Pittman, C.U., Jr., B.T. Kim and W.M.J. Douglas, J. Org. Chem. 40:590 (1975).
8. Frankel, E.N., E. Selke and C.A. Glass, J. Am. Chem. Soc. 90:2446 (1968).
9. Frankel, E.N., and F.L. Little, JAOCS 46:256 (1969).
10. Frankel, E.N., E. Selke and C.A. Glass, J. Org. Chem. 34:3936 (1969).
11. Awl, R.A., E.N. Frankel, J.P. Friedrich and E.H. Pryde, JAOCS 55:577 (1978).
12. Frankel, E.N., Ibid. 47:11 (1970).
13. Holy, N.L., J. Chem. Soc. Chem. Commun. 1074 (1978).
14. Holy, N.L., J. Org. Chem. 43:4686 (1978).
15. Holy N.L., Ibid. 44:239 (1979).
16. Eisenhauer, R.A., and R.E. Beal, Anal. Chem. 35:1110 (1963).
17. Butterfield, R.O., JAOCS 46:429 (1969).
18. Evans, C.D., G.R. List, H.A. Moser and J.C. Cowan, Ibid. 50:218 (1973).
19. Davies, E.L., "The Design and Analysis of Industrial Experiments," Haftner Publ., New York, NY, 1954.

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Effect of Amino Acids on the Autoxidation of Safflower Oil in Emulsions

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ABSTRACT

Oxygen absorption rates were measured on liquid emulsions containing safflower oil and various amino acids. The antioxidant effects of the several amino acids were quite variable depending on the type of emulsifier used, the pH of the system and the presence of added sugar. Preliminary tests with Maillard reaction products obtained by heating dextrose with lysine showed little stabilizing effect. In freeze-dried emulsions, methionine, threonine, lysine and histidine all exhibited antioxidant activity. With sodium caseinate as the matrix, methionine was much better than any of the other amino acids. The inclusion of sugar enhanced the rate of oxidation. Porosity measurements on the freeze-dried powders revealed that oxygen diffusion was not rate-determining. When xanthan gum was used to replace caseinate in these dried emulsions, oxidation rates increased.

INTRODUCTION

The literature contains conflicting observations on amino acids as antioxidants for fats (1-6). Marcuse et al. tested a number of amino acids with linoleate (1,2). Tryptophan and histidine were very functional whereas glycine and alanine showed weak activity. Sliwick and Siechowsky found methionine and cysteine to have antioxidant effects in soybean oil whereas cystine was a weak prooxidant (3). Recently Farag et al. studied the effects of various amino acids in oil-in-water emulsions, in oils and in freeze-dried systems (4-6). The amino acids tested were all prooxidants in liquid emulsion and in oils. This effect was attributed to the NH_3R group. But in the dried systems the amino acids, with the exception of cysteine, all proved to be antioxidants. A number of reasons have been offered to explain these variations in behavior including differences in temperature, trace metal content, lipid concentration, emulsifier type and pH.

In our laboratory, we studied the influence of sugars and polyols on the oxidation of safflower oil in liquid emulsions (7). It was found that oxidative stability improved as the level of added sugar or polyol was increased. It was suggested that these compounds do not behave as actual antioxidants. They increase the viscosity of the aqueous phase which results in improved resistance to phase separation. A lower concentration of oxygen in the aqueous phase when sugar is present and a slower diffusion of the gas through the oil-water interface were suggested as causes for the better oxidative stability.

The objective of the work reported here was to determine the autoxidation rates of safflower oil in liquid emulsions containing added amino acids. The studies were subsequently extended to include freeze-dried emulsions with sodium caseinate or xanthan gum as matrices. Preliminary results with Maillard reaction products in liquid emulsions also are reported.

EXPERIMENTAL PROCEDURES

Materials

Refined safflower oil was obtained from the Pacific Vegetable Oil Co., San Francisco, CA. This oil contained no added antioxidants. Sodium stearoyl-2-lactylate (Artodan SP50), distilled monoglycerides (Dimodan TH), polyglycerol esters of fatty acids (Triodan 55) and citric acid esters of monodiglycerides (Acidan BC) were obtained from Grindsted Products, Inc., Kansas City, MO. Sodium caseinate was a product of the New Zealand Dairy Board. Xanthan gum (Keltrol) was from Kelco Co., Clark, NY. Sucrose, dextrose monohydrate and lactose were all from Mallinkrodt, Inc., St. Louis, MO. L-methionine, and L-threonine were from ICN Pharmaceuticals, Inc., Cleveland, OH. L-histidine was obtained from Nutritional Biochemicals Co., Cleveland, OH, and L-lysine hydrochloride was from J.T. Baker, Phillipsburg, NJ. Crystal Springs Water, Pine Hill Crystal Spring Co., Bronx, NY was used.

Methods

Oil-in-water emulsions were prepared by dissolving amino acids and sugar (where indicated) in spring water, dispersing anionic surfactant and adjusting pH in some cases with 5% NaOH or 0.1 N HCl. Dimodan TH or Triodan 55 were dissolved in safflower oil at 50 C and added to the water phase. The mixture was homogenized for 2 min using a Tekmar Probe at maximal speed (Model SD45N from Tekmar Co., Cincinnati, OH). Emulsion temperatures increased to 50-60 C during homogenation.

Oxygen absorption rates were measured on 20-g samples (5 g oil) in duplicate at room temperature following the

method of Bishov with a modification as previously described (9). Values for duplicates showed good agreement until oxidation had become extensive. Emulsion stability measurements were made using pulsed NMR (10,11).

Freeze-dried emulsions were prepared by dissolving amino acids in spring water and adjusting pH to 7.0. A mixture of protein or gum, sugar and/or Artodan SP50 was dispersed in the water phase, safflower oil was added, and the emulsions containing 60-90% water were homogenized using the Tekmar probe (with proteins as matrix) or a Waring Blender (with gum as matrix). The emulsions were frozen immediately in shallow trays on dry ice and then freeze-dried. A period of ca. 72 hr was necessary to reach a moisture level of <1%. Moisture contents were determined by the Karl Fischer technique (12). Porosity of dried powders was measured using an Aminco Porosimeter (American Instrument Co., Silver Springs, MD). Maillard reaction products were prepared by dissolving 2 g dextrose and 2 g lysine in 10 ml spring water and incubating in capped bottles at 50 C for 4 hr, 8 hr, 17 hr and 24 hr. Liquid emulsions were prepared with the reaction products at the 1% level basis lysine following the procedure just outlined.

RESULTS AND DISCUSSION

Amino Acids in Liquid Emulsions

Liquid emulsions containing 1% added amino acids were prepared and their oxidation rates determined by measuring the amount of oxygen consumed during storage at room temperature. The composition of the emulsions is shown in Table I. In Table II are tabulated the results of storage tests as relative oxidation rates (relative to an emulsion without added amino acid). The pH of these emulsions was not adjusted. From this table it appears that most of the amino acids showed prooxidant activity with relative oxidation rates greater than 1.0. From this list, 4 amino acids were chosen for further studies. They were histidine, threonine, lysine and methionine.

TABLE I

Liquid Emulsion Composition (%)

25	Safflower oil
1	Sodium stearoyl lactylate (SSL)
1	Distilled monoglycerides
1	Amino acid
72	Water phase[a]

[a]Water with and without sugar.

TABLE II

Relative Oxidation Rate (ROR) of Amino Acids

Amino acid	ROR[a]
Valine	6.2
Isoleucine	6.0
Leucine	5.0
Cysteine	4.1
Alanine	4.0
Threonine[b]	3.4
Serine	2.8
Tyrosine	2.1
Phenylalanine	1.2
Proline	1.1
Asparagine	1.3
Histidine[b]	1.0
Tryptophane	0.8
Methionine[b]	0.8
Arginine	0.8
Lysine hydrochloride[b]	0.8

[a]Relative to control (amino-acid-free) emulsion which after 6 days at RT had absorbed 39.3 meq O_2/Kg of oil. The pH of emulsions is not adjusted.

[b]Chosen for further study.

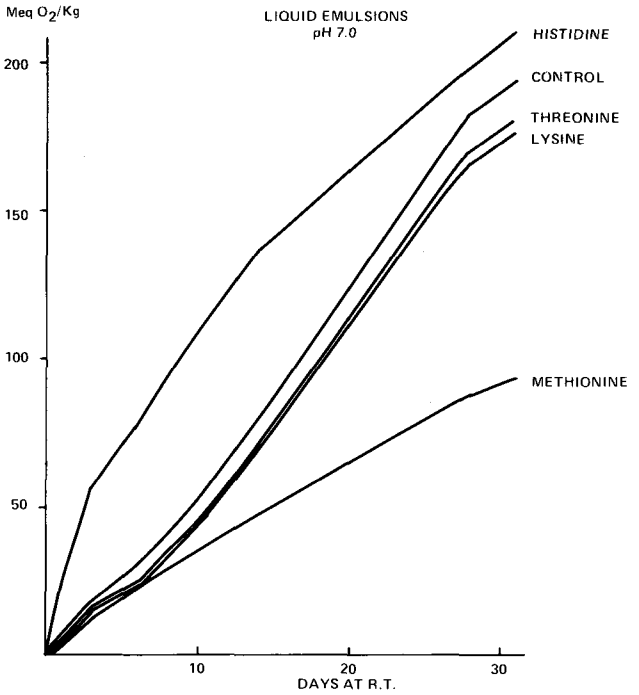

FIG. 1. Oxygen absorption of amino-acid-containing liquid emulsions.

In Figure 1 are shown oxidation rates for oil-in-water emulsions containing these 4 amino acids and for a control emulsion without amino acid. In each case, the pH was adjusted to 7.0 before the storage test was begun. Histidine showed a significant prooxidative effect whereas threonine and lysine were weak antioxidants. In this test, methionine behaved as a fairly strong antioxidant. In a previous paper (13) evidence was presented that the antioxidative effect of methionine may be attributed to small amounts of methional present in the methionine or generated from it. Methional was demonstrated to be an effective antioxidant with a strong cabbage-like odor, which in most cases was observed with the emulsions to which methionine had been added.

Influence of pH

In a previous study with emulsions containing sugar (7), the stability toward oxidation was increased as the pH was raised over the range 6.2-8.2. This effect was attributed to a decreased rate of creaming and phase separation of the emulsion caused by a greater negative charge on the oil droplets as more anion migrated to the oil-water interface at the high pH. Other workers also have documented this pH dependency in the presence of anionic surfactants (14). If diffusion of oxygen through the oil-water interface is the rate-determining step in autoxidation, then there should be a positive correlation between oxidative stability and stability to phase separation.

In a similar series of experiments, the influence of pH on the oxidation rates of emulsions with and without amino acids was measured. In a simple oil-in-water emulsion containing no amino acid or sugar (Fig. 2, upper curves) this effect of improved oxidative stability with increasing pH is confirmed. But in the presence of 10% dextrose

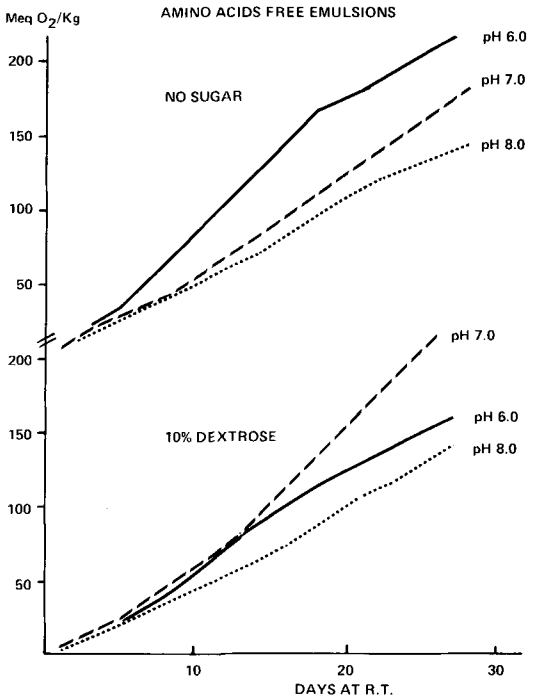

FIG. 2. Effect of pH on liquid emulsion oxidation.

(lower curves) a reversal was obtained. The emulsion at pH 6 appears to be more stable to oxidation than the one at pH 7.

The situation with 1% lysine is even more confusing. Here there was a complete reversal so that an inverse relationship between stability and pH apparently holds (Fig. 3a). When 10% dextrose was added, the effect was to minimize the stability differences between samples, but the same inverse order of oxidation rate still held. There is no obvious explanation for this reversal. Some other unknown factor must override the effect of pH.

With methionine (Figure 3b), the results in emulsions containing no sugar again follow the expected stability sequence showing better stability if pH is raised. The addition of dextrose results in a leveling effect. Emulsions at pH 6 and pH 7 oxidize at approximately the same rate whereas the one at pH 8 oxidizes somewhat faster.

In the interest of brevity, the oxidation curves for emulsions containing threonine and histidine have been omitted. With threonine, the emulsion at pH 8 was the most stable when dextrose was present, but it was of intermediate stability in the absence of sugar. With histidine the emulsions at pH 8 were of intermediate stability with or without added sugar. We can offer no adequate explanation for the reversals of the stability sequence with pH.

Influence of Sugar Type and Level

Emulsions with amino acids and one of 3 different sugars were tested. The sugars were adjusted at 2 levels, 10 and 20%. Dextrose and maltose were chosen as examples of reducing mono- and disaccharides whereas sucrose served as an example of a nonreducing disaccharide.

Figure 4a shows the oxidation rate of control emulsions with no amino acid. The lower part of the figure shows that there is no difference resulting from the type of sugar at the 10% level. At 20%, dextrose accelerates oxidation substantially. Since this was not observed in the oxidation of emulsions containing amino acids, its validity is questionable.

When amino acids were used, little effect was noted as a result of either the type or the level of sugar. Threonine was an exception (Fig. 4b). It appears from the figure that lactose accelerated oxidation at both 10 and 20% levels. The reasons for these differences are unknown at the present time.

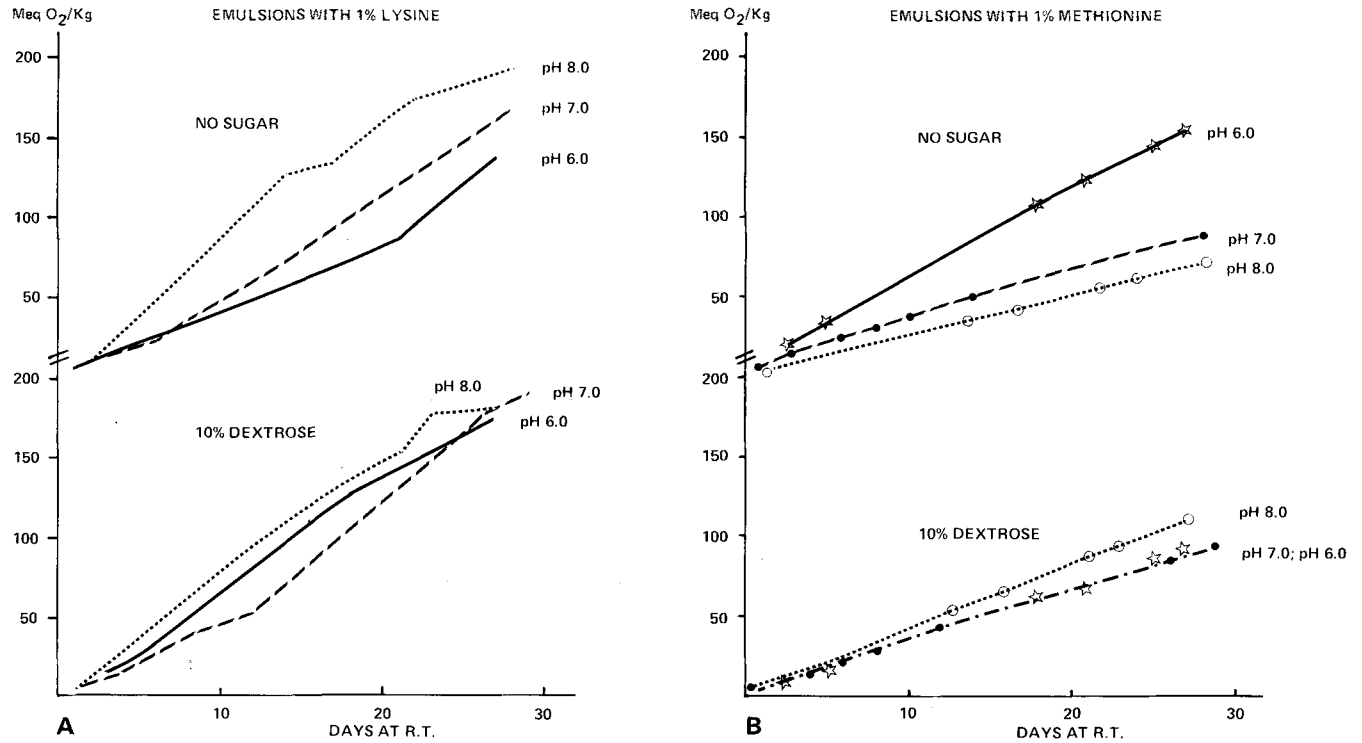

FIG. 3. (A) Lysine emulsions and oxidative stability—effect of sugar and pH; (B) methione emulsions and oxidative stability—effect of sugar and pH.

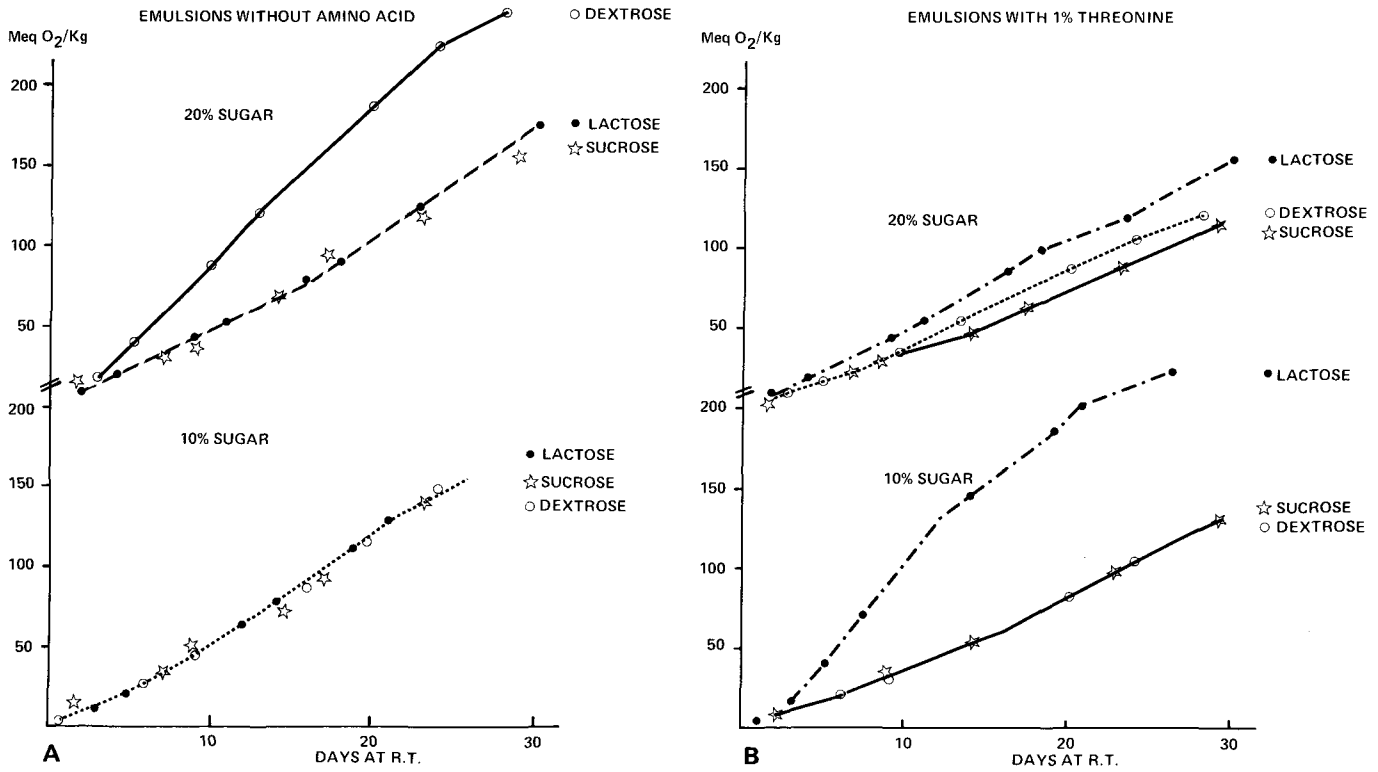

FIG. 4. Effect of sugar on emulsion oxidative stability–A: amino-acid-free emulsions; B: 1% threonine emulsion.

Influence of Emulsion Stability

Three series of emulsions were prepared with different emulsifier systems, i.e., distilled monoglycerides (DGMS)/SSL, citric acid esters of monoglycerides (Acidan) and polyglycerolesters (Triodan). In this case, emulsion composition is the same as in Table I, except that the water phase contained 10% dextrose and the total emulsifier level was 1%. Storage tests at room temperature and O_2 absorption measurements revealed that the emulsions prepared with Acidan proved more stable to oxidation than the emulsions prepared with DGMS/SSL, (Fig. 5). Varying antioxidant activity was exhibited by all 4 amino acids. In general, emulsions prepared with Triodan also show greater oxidative stability than the corresponding emulsions prepared with DGMS/SSL.

Emulsion stability was followed by pulsed nuclear magnetic resonance (PNMR) (10). At zero time the emulsions with Acidan and Triodan were more uniform than the emulsions prepared with DGMS/SSL,except the lysine and histidine emulsions prepared with Triodan, which for unknown reasons, separated immediately after preparation.

PNMR measurements revealed that the emulsions creamed gradually for the first 4 days after preparation, after which no further changes were observed. The emulsions with Acidan and DGMS/SSL show almost equal creaming after 4 days, whereas the control emulsion with Triodan apparently is more stable to separation.

These results correlate well with the O_2 absorption data. The more stable emulsions with Acidan and Triodan are also more stable to oxidation. The histidine emulsion with Triodan, which separated immediately after preparation, oxidizes faster than the other histidine emulsions, whereas the lysine emulsion with Triodan, which also separated, is remarkably stable to oxidation. The reason for this is unknown.

In emulsions prepared with citric acid esters of mono-

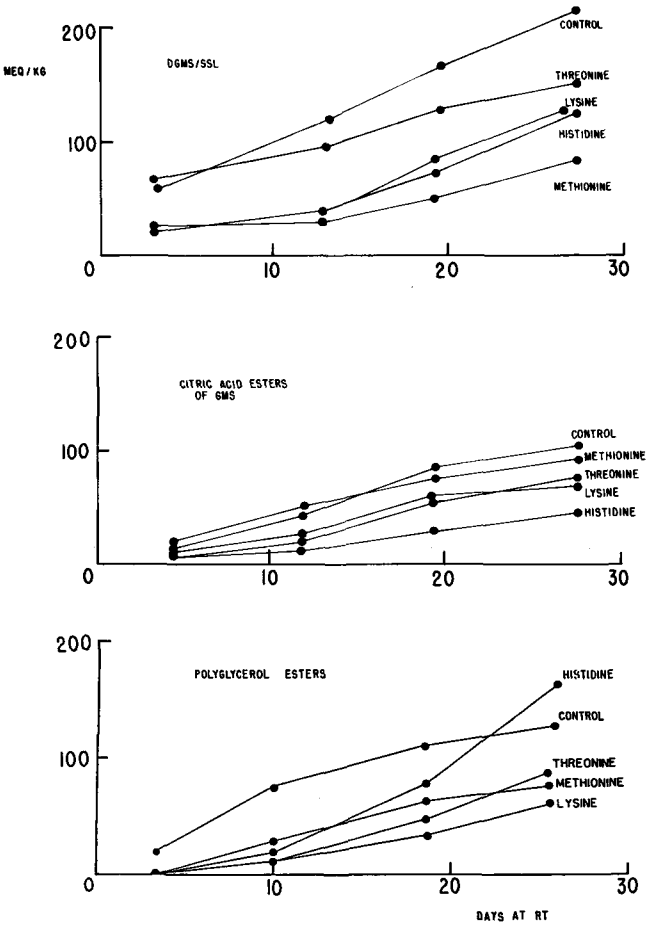

FIG. 5. Emulsifiers and oxidative stability of emulsions.

TABLE III

Composition of Freeze-dried Emulsions

Ingredient (%)	Composition (%)[a]			
	1	2	3	4
Safflower oil	63.3	84.7	61.0	80.6
Sodium caseinate	6.3	8.5	—	—
Xanthan gum	—	—	6.1	8.1
Sucrose	25.0	—	24.4	—
SSL	2.5	3.4	6.1	8.1
Amino acid	2.5	3.4	2.4	3.2
Moisture	0.35	0.36	0.85	0.70
(Porosity[b])	11	100	3	150

[a]On dry basis.
[b]m^2/g.

glycerides (Acidan), the stability to oxidation may have been further enhanced by small amounts of citric acid, which is a well known trace metal deactivator and antioxidant synergist. This may also explain the somewhat different pattern of the curves observed with the Acidan emulsions.

Freeze-dried Emulsions

The stabilities of freeze-dried emulsions containing amino acids were studied in 2 types of systems; one with sodium caseinate as the matrix and one with xanthan gum. Table III gives the composition of the dried emulsions. The samples were stored at 60 C to accelerate the oxidation.

Figure 6a shows the oxidation rates of freeze-dried emulsions with sodium caseinate. This figure shows all the amino acids to be antioxidants, methionine being far superior to the others. When sucrose is present (Fig. 6b) the oxidation rate is substantially higher but the trend is unchanged.

Porosities of these powders are given in Table III and they show the freeze-dried emulsions without sugar to be more porous by a factor of about 10 to 1. This great difference may be a result of the introduction of air into the sugar-free emulsions during preparation, whereas the sugar-containing emulsions did not foam as much.

From the porosity data one would expect that the freeze-dried emulsions without sugar would oxidize faster because of greater diffusion rates of oxygen. The results obtained contradict this. It is suggested that the casein in the freeze-dried emulsions without sugar binds the oil to a greater extent and protects it from oxidation. In the powders containing sugar, the oil has to compete with the sugar for binding sites on the protein and a greater portion of the oil is accordingly exposed and thus much more amenable to oxidation.

From Figure 6c, the same general trend is discernible in the xanthan-containing dried emulsions. Methionine again is a far better antioxidant than the other amino acids. In this case, the position between lysine and histidine is reversed. Sugar again shows a prooxidant effect although not as pronounced as with sodium caseinate. The porosity data of Table III again shows the sugar-free emulsion to be far more porous than the sugar-containing emulsion. Thus, the xanthan gum is able to protect the oil toward oxidation although the effect is not as great as with casein as matrix.

Emulsions with Maillard Reaction Products

It is well known that the reaction products formed when reducing sugars react with amino acids (Maillard reaction) have antioxidative effects. It has been suggested that the stabilization might result from colorless products formed early in the process rather than from the brown pigments normally associated with Maillard reactions (15). In an extension of the work with amino acids in liquid emulsions, Maillard reactions were induced between dextrose and lysine in water at 60 C, and the resulting products were tested in liquid emulsions.

Table IV gives the relative oxidation rates of 4 emulsions with Maillard products between lysine and dextrose. The Maillard products were applied at the 1% level calculated on the basis of lysine, and they had been reacted at 60 C for 4 hr, 8 hr, 17 hr and 24 hr, respectively. The 4-hr products had a very light brown color, and the color

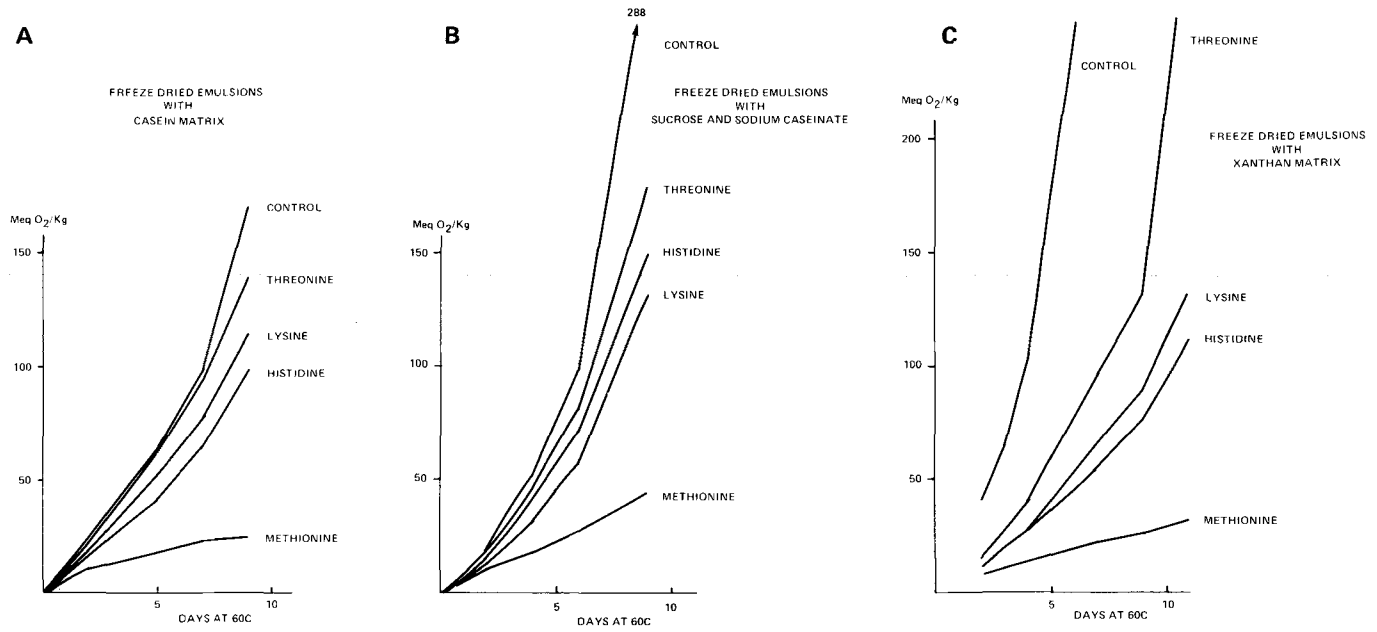

FIG. 6. Effect of amino acids on freeze dried emulsions; (A) casein matrix; (B) casein-sugar matrix; (C) xanthan gum matrix.

TABLE IV

Relative Oxidation Rate (ROR) of Emulsion with Maillard Products

Sample[a] (hr)	ROR[b]
4	0.82
8	0.84
17	0.77
24	0.76
Lysine/no dextrose	1.04

[a]Maillard reactions between lysine and dextrose in water solution at 60 C for the time indicated. Applied at 1% level calculated on lysine basis.

[b]Relative to control (amino-acid-free) emulsion which after 25 days at RT had absorbed 148.2 meq O_2/Kg of oil.

gradually grew darker with time. It appears from the table that there is virtually no difference in the oxidation rates of these emulsions and they are only slightly more stable than control emulsions with unreacted lysine and without amino acid.

These results are preliminary. More work should be done in this area to understand better the influence of Maillard products on the autoxidation of oils, and to evaluate the potential of these active components for commercial use.

REFERENCES

1. Marcuse, R., JAOCS 39:97 (1962).
2. Marcuse, R., Fette Seifen Anstrichtsm. 63:940 (1961).
3. Sliwiok, J., and J. Siechowski, Riv. Ital. Sost. Grasse 1970:47.
4. Farag, R.S., S.A. Osman, S.A.S. Hallabo and A.A. Nasr, JAOCS 55:703 (1978).
5. Farag, R.S., S.A. Osman, S.A.S. Hallabo, A.M. Girgies and A.A. Nasr, Ibid. 55:708 (1978).
6. Farag, R.S., S.A. Osman, S.A.S. Hallabo and A.A. Nasr, Ibid. 55:613 (1978).
7. Sims, R.J., J.A. Fioriti and J. Trumbetas, Ibid. 56:742 (1979).
8. Bishov, S.J., and A.S. Henick, JAOCS 43:477 (1966).
9. Fioriti, J.A., M.J. Kanuk and R.J. Sims, Ibid. 51:219 (1974).
10. Trumbetas, J.F., J.A. Fioriti andR.J. Sims, Ibid. 54:433 (1977).
11. Trumbetas, J.F., J.A. Fioriti and R.J. Sims, Ibid. 55:248 (1978).
12. Official Methods of Analysis, 4th Edition, Assoc. Off. Anal. Chem., 1975, Method 31.069-071, p. 577.
13. Sims, R.J., and J.A. Fioriti, Ibid. 54:4 (1977).
14. Saleeb, F.Z., C.J. Cante, T.J. Streckfus and J.R. Frost, JAOCS 52:208 (1975).
15. Rhee, C., and D.H. Kim, J. Food Sci. 40:460 (1975).

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Determination of Phosphorus in Oils Using Oxygen Bomb Ashing

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ABSTRACT

A fast method for phosphorus determination in an oil matrix is described. The principle of the method is similar to that of the AOCS method, but the ashing of the oil is accelerated using an oxygen bomb. In addition, a rapid molybdovanadate reagent is used for colorimetry rather than the molybdenum blue reagents specified in the AOCS method. Agreement with the air ashing procedure averages less than 5% difference above 10 μg/g. The detection limit is in the order of 1-2 μg/g.

INTRODUCTION

The official American Oil Chemists' Society (AOCS) method (1) for the determination of phosphorus in oil has been in use for many years. The colorimetric measurement of phosphate as molybdenum blue is often replaced by the more stable yellow molybdovanadate phosphate complex (2-5), which is more tolerant to pH variations and has fewer anionic interferences. Also, the molybdovanadate procedure is faster and has fewer steps. Even though the sensitivity is not quite as high as that of the molybdenum blue procedure, it is adequate for most applications.

The hot plate/muffle furnace combination is still the most commonly used ashing procedure. The major disadvantage of this method is that it is time-consuming. Attempts to speed up the ashing by wet digestion with nitric and perchloric acids (6) are not popular because of the hazards of heating these acids in the presence of oil.

Physical methods offer a possible way to achieve faster analysis. These are being investigated by several researchers. Prevot (7, 8) obtained sensitivity in the 1 μg/g range using atomic absorption spectrophotometry with graphite furnace atomization. Belcher and coworkers (9) developed a technique called Molecular Emission Cavity Analysis (MECA) to study molecular emission using a special sample chamber. Both of these methods are relatively fast because they require little or no sample preparation. But, they are not developed enough for routine use as yet.

Since ashing of the oil is the most time-consuming part of the official AOCS method, any improvement here would be the most beneficial in terms of speed of analysis. An oxygen bomb can be used to greatly accelerate the ashing of the oil (10). The sample is ignited in a pressure vessel filled with oxygen to 22-25 atmospheres by melting a thin wire with an electric current. The oxygen bomb reduces the ashing time from one day on a hot plate and muffle furnace to ca. 15 min.

The combination of the oxygen bomb and the molybdovanadate reagent has been found to provide a simple, rapid, method for the determination of phosphorus in oil.

EXPERIMENTAL

Apparatus

The oxygen bomb apparatus used in this study was the Parr Instrument Co. Model 1901. The stainless steel capsules supplied with the apparatus were used as sample holders. Any spectrophotometer capable of measuring absorbance to 0.001 units is suitable. In this report, a CARY 118 was used.

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