ble. Presumably, by interpolation, one might expect the effects of certain dicarbonyl compounds as pro-oxidants to be more manifest in a bottled or bulk oil at room temp than in the same oil in a relatively thin layer at 57C.

According to Uri (21), a compound may act as a pro-oxidant if it is converted in any manner to an unstable free radical which can add oxygen to form a peroxide radical which accepts hydrogen from an unsaturated fatty acid to initiate a chain reaction.

It seems odd that the thickness of layers or AOM stabilities of oils had little effect on the time required for those oils to become rancid when the pro-oxidants were added to them. Examination of Figures 1, 2 and 3 shows that control oils decreased considerably in stability in the Schall Oven Test with decreasing layer thickness or AOM stability. However, oils containing a certain pro-oxidant had approx the same measured rate of oxygen absorption. One might expect that dependency of the pro-oxidant action of the dicarbonyl compounds on the presence of oxygen would result in a diminished pro-oxidant effect in thicker layers of oil of higher AOM stability.

We have no suggestions to offer at present to explain mechanisms of pro-oxidant activity encountered, but it was thought worth while to present this paper in order to disclose that certain compounds related in structure to dehydro reductones may act as pro-oxidants in vegetable oils at temp of 57C or less.

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Chromatographically Homogeneous Egg Lecithin as Stabilizer of Emulsions for Intravenous Nutrition[1, 2]

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Abstract

Physically stable emulsions of cottonseed and soybean oils at 20% concn were prepared with chromatographically homogeneous egg lecithin at 1% concn as the sole emulsifier, and a 2.5% solution of glycerol as the aqueous phase. The physical stability of the emulsions was a function of the pH of the product, optimum pH 6.6–6.8. Aqueous solutions of dextrose and sorbitol decreased in pH to 4.8 when autoclaved, regardless of prior adjustment of pH to as high as 8.5, and emulsions in which these solutions were used as the aqueous phase exhibited phase separation. There was no significant decrease in pH, and no phase separation, in emulsions which contained glycerol solution as the aqueous phase. It appears that glycerol is superior to dextrose as the isotonic agent in lecithin-stabilized emulsions for intravenous nutrition.

Particle sizes and their distribution in lecithin-stabilized emulsions of cottonseed and soybean oils were determined by means of a Coulter Counter. Approximately 99% of the oil in these emulsions was dispersed as particles whose diameters were no larger than 5 μ.

Introduction

FAT EMULSIONS for intravenous nutrition have been widely proposed, as may be noted in the extensive review by Geyer (3). The emulsifying systems of many of these proposed emulsions include phosphatides, the majority of which are soya phosphatides, although egg phosphatides also have received attention. Schuberth and Wretlind (5) described the use of egg phosphatides as the emulsifier for a soybean oil emulsion and present physiological results which they obtained; however, the phosphatides used were impure, since the analytical results given are not theoretical for phosphatidylcholine. In an investigation of highly purified egg phosphatides, principally lecithin, as the major component of an emulsifier system for the preparation of fat emulsions, Yeadon et al. (9) concluded that purified egg lecithin was not an efficient emulsifier, particularly with respect to autoclaving stability. These workers found that certain additives to the lecithin emulsifier system enhanced its effectiveness, but do not give any explanation for this effect.

Phospholipids are naturally occurring components of body fluids and tissues, and therefore it seems very reasonable to use a pure natural lecithin as the emulsifier for intravenous fat emulsions. Recently, a rapid method for purifying egg phosphatides by column chromatography has been published by this Laboratory (7), and chromatographically homogeneous lecithin has been investigated as the sole emulsifier in fat emulsions. This report presents the results of that investigation. It should be clearly understood that the described emulsion at this time is intended only for experimental use in animals. Such tests are underway at other institutions.

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[3] A laboratory of the So. Utiliz. Res. & Dev. Div., ARS, USDA.

Materials and Methods

Emulsifiers. The emulsifiers were 1) chromatographically homogeneous phosphatidylcholine (lecithin), which was obtained by the chromatographic fractionation of crude egg phosphatides on a column of alumina; and 2) the remainder of the crude egg phosphatides from which the lecithin had been removed. Emulsifier (2) has been termed the "cephalin" fraction, although other components were present. The chromatographic fractionation of the crude egg phosphatides is described in a previous publication (7).

Oils. Cottonseed oil (free fatty acid (FFA) content 0.05%, I.V. 105.6) was a refined, bleached, winterized and deodorized oil, specially selected for resistance to oxidation, obtained from the Southern Cotton Oil Co. The same type of oil has been used in an emulsion which is described in a previous publication (8).

Alkali-refined soybean oil (FFA content 0.03%, I.V. 129.1), was obtained from Archer Daniels Midland Co. This oil was deodorized at 210C for 0.5 hr at a pressure of 1 mm; citric acid (0.005%) was added as a stabilizer after deodorization.

Preparation of Emulsions. The concn of oil in all of the prepared emulsions was 20 w/v. A solution of the emulsifier in a minimum volume of absolute ethanol was added to the required amount of oil, and the ethanol then was removed at reduced pressure by warming the oil phase to ca. 50C. Traces of ethanol remaining would cause no difficulty. This method was preferred to the direct dispersion of lecithin in oil by heating the oil phase to ca. 90C, and rapidly stirring.

The isotonic aqueous phases of the experimental emulsions were 2.5% solutions of glycerol, or 5% solutions of dextrose. The aqueous phase was warmed to 50–60C, and the oil phase, warmed to approx the same temp, was added to the aqueous phase. The system was stirred with a Series 2000 Standard Premier Laboratory Dispersator for 5 min at 3700 rpm to produce a crude emulsion. During the stirring period, 0.1*N* NaOH was added to the crude emulsion to adjust the pH to 6–7. The crude emulsion was homogenzied at an applied pressure of 3500 psi in a 2-stage Superhomo, Cherry-Burrell Co. After the fourth cycle of the emulsion through the homogenizer, 0.1*N* NaOH was added if necessary to readjust the pH to between 6.5 and 6.8. The emulsion then was cycled through the homogenizer four more times, and the pH was adjusted to 6.8. The particle sizes of the emulsion were observed microscopically with an oil immersion lens (950x), and homogenization was considered to be completed if essentially all of the observed particles were less than 1 μ in diam, and there were no particles over 7 μ in diam.

As an example of the changes in the pH of an emulsion during a typical preparation, the pH of a crude emulsion was 5.4, and was adjusted to 7.0; after four cycles of homogenization, the pH had decreased to 6.1, and was readjusted to 6.8. The pH remained constant at 6.8 with additional homogenization.

After homogenization, the emulsion was bottled in 500 cc intravenous solution bottles, and sterilized in a steam autoclave at 110C for 10 min, or at 117C for 15 min. The bottles of emulsion were removed from the autoclave and cooled at 4C on a mechanical roller.

Stability Test. The relative physical stability of the various emulsions was determined by mechanical shaking at room temp, followed by measurement of the amount of phase separation. This shaking procedure is described in a previous publication (8).

Particle Size Distribution. A Coulter Counter was used to determine the distribution of particle sizes, as described in a previous publication (6).

Surface Tension. A Cenco-duNuoy Direct Reading Tensiometer was used to measure the surface tension at 25C of emulsions.

Results and Discussion

Effect of pH on Physical Stability of Lecithin-Stabilized Emulsions. The initial emulsion in which lecithin was used as the sole emulsifier contained 1.2 w/v of the latter, 20% of soybean oil, and an isotonic solution of dextrose as the aqueous phase. No attempt was made to control or adjust the pH of this system, which was 5.5. Upon homogenizing this emulsion, a very foamy product was formed, and the majority of oil particles were fairly large in diam (ca. 2 μ). Phase separation occurred during autoclaving, and the pH of the separated emulsion was 4.9.

When a 2.5% solution of glycerol was used as the aqueous phase of an emulsion which was identical in all other components to that above, with no adjustment of pH, the emulsion did not exhibit phase separation upon autoclaving, but there was some coalescence of oil particles which resulted in a general increase in particle sizes.

The effect of adjustment of the pH of lecithin-stabilized emulsions on their physical stability was determined by preparing two emulsions, one of which contained a 5% solution of dextrose, and the other a 2.5% solution of glycerol, as the respective isotonic aqueous phases. The pH of portions of these emulsions was adjusted either with 0.1*N* NaOH or with 0.1*N* HCl, to provide a series of emulsions in which the pH values varied from 3–8. The emulsions in which the pH was decreased to less than 5.0 separated into two phases within a matter of minutes, without regard to the composition of the aqueous phase. There was no phase separation in those emulsions in which the pH was 5.0 or above, and these emulsions then were autoclaved at 110C for 10 min. All of the emulsions in which dextrose solution was the aqueous phase showed oil separation when removed from the autoclave, regardless of the pH of the emulsion prior to autoclaving. The pH of the unstable emulsions decreased to approx the same value, 5.0 $\pm$ 0.2.

Those emulsions with pH values of 5.0 and above, with glycerol solution as the aqueous phase, showed no separation after autoclaving, and there were no significant differences between the pH values of the emulsions before and after autoclaving.

The decrease in the pH of autoclaved lecithin-stabilized emulsions in which solutions of dextrose were the isotonic aqueous phases was investigated to determine whether the decrease was due to the characteristics of dextrose, to the hydrolysis of lecithin, or to an interaction between lecithin and the oil phase of the emulsion. Solutions of 5% dextrose, of 5% dextrose buffered with 10% phosphate solution, of 2.5% glycerol and of 5% sorbitol were prepared. Also, two emulsions were prepared, one emulsion stabilized with 1.2% of lecithin, with distilled water as the aqueous phase, and the other with 1.2% of the "cephalin" fraction, with a 5% solution of dextrose as the aqueous phase. The pH of the solutions and of the lecithin-stabilized emulsion was adjusted to 6.4–7.0, and the various samples then were autoclaved at 110C for 10 min. When cooled to 25C, the pH was measured. The results are given in Table 1. The pH of both the dextrose and sorbitol solutions

TABLE I

Change of pH of Various Samples After Autoclaving

Composition of sample	Original pH	Adjusted pH	pH after autoclaving at 110C
Sol. of 5% dextrose (under nitrogen)	5.2	6.4	4.8
Sol. of 5% dextrose, phosphate buffered	7.0		6 3
Sol. of 2.5% glycerol	5.5	6.8	6.5
Sol. of 5% sorbitol	5.3	7.0	4.8
1.2% lecithin 20% soybean oil to 100% with distilled water	5.1	6.4	6.4
1.2% "cephalin" 20% soybean oil to 100% with 5% sol. of dextrose	7.8		7.1

decreased approximately to the same value, whereas the glycerol and buffered dextrose solutions, and the emulsion with distilled water as the aqueous phase, showed but little change in pH. There was no phase separation in the latter emulsion. Also, the cephalin-stabilized emulsion, which contained a solution of dextrose as the aqueous phase, showed no phase separation. The cephalin, because of its basic nature, apparently buffered the emulsion, similar to the buffering action of phosphate in the solution of dextrose, and prevented a large decrease of pH, and phase separation, such as occurred in emulsions which contained lecithin and dextrose.

The effect of the time of heating as one of the factors responsible for the decrease in pH of solutions of dextrose was determined by preparing a 5% aqueous solution of dextrose, heating the solution to 100C for pre-determined times, and measuring its pH after cooling to 25C. The pH of the solution, which originally was 5.8, decreased in the following manner:

Min at 100C	pH
0	5.80
2	5.32
4	5.02
6	4.90
8	4.90
10	4.88

The maximum decrease in pH of a solution of dextrose apparently required several minutes at an elevated temp, such as would be the conditions during the autoclaving of an emulsion.

The inability of lecithin to stabilize autoclaved emulsions in which the aqueous phase was a solution of dextrose confirmed the results obtained with a similar emulsifier system and with a similar aqueous phase, as reported by Yeadon et al. (9). The phase separation observed in the present investigation was due to the pH of the emulsion system as influenced by the dextrose solution which comprised the aqueous phase, and not to a lack of emulsifying efficiency of lecithin. One explanation of the inability of lecithin to prevent a decrease in the pH of the emulsion may be found in the report of Jukes, which states that solutions of lecithin have no buffering power within the physiological range (4). This is accounted for by the fact that lecithin is internally neutralized through reaction between a strongly acidic group and a strongly basic group. Lecithin, with its quaternary choline group (strongly basic) has no pK_A value on the alkaline side. Cephalin, on the other hand, with its ternary ammonia group, does have a pK_A value on the alkaline side (ca. pH 9). Thus cephalin has buffering ability against H^+ in the physiological range. Cephalin-stabilized emulsions did not decrease in pH to a great extent, even though prepared with a solution of dextrose as the aqueous phase. It appears that solutions of dextrose cannot be used as the aqueous phase of emulsions for intravenous nutrition in which egg lecithin is the emulsifier, but that a solution of glycerol is entirely satisfactory.

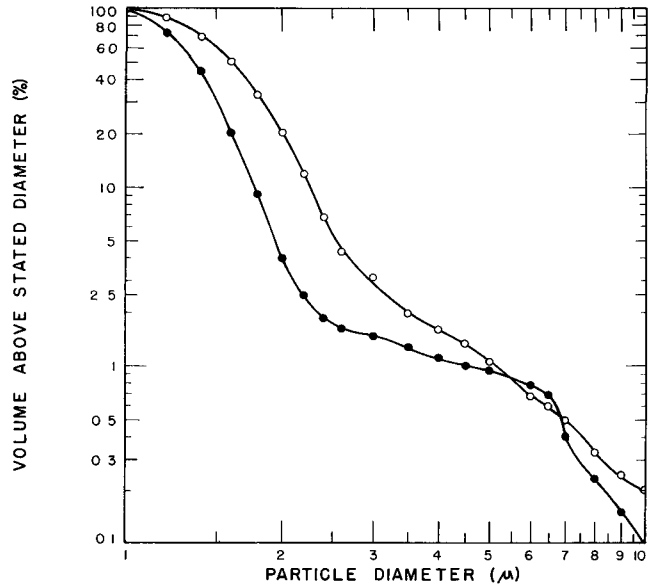

FIG. 1. Relative volume of dispersed oil (%) in emulsions of cottonseed oil (open circles) and of soybean oil (closed circles) above the stated particle diam from 1–10 μ.

Optimum pH. The optimum adjusted pH of lecithin-stabilized emulsions was determined by preparing a series of emulsions in which the pH was adjusted in the range 6–8, and measuring the surface tension of these emulsions. The results are as follows:

pH of emulsion, adjusted	surface tension at 25C, dynes/cm
6.0	42.05
6.5	32.20
6.6	30.80
6.8	30.00
7.0	40.40
8.0	41.60

The surface tension of these emulsions decreased sharply within a narrow range of pH, then just as sharply increased. It would appear that as the pH value approached 6.8 the lecithin was more hydrophilic, which accounted for the lower surface tension. The lowest surface tension occurred at a pH of 6.6–6.8, which may have significance with the isoelectric point of lecithin of 6.7 ± 0.2, as reported by Chain and Kemp (1). The theoretical isoelectric point of lecithin is 7.5, as reported by Fischgold and Chain (2). The measured value of 6.7 is attributed by Chain and Kemp to the rapid and spontaneous decomposition of lecithin, by which fatty acids are liberated.

Emulsifier Concentration. Emulsions with a soybean oil content of 20 w/v were prepared with lecithin as the emulsifier at concn of 0.4, 0.6, 0.8, 1.0 and 1.2 w/v, with isotonic solutions of glycerol as the aqueous phase, and with the pH adjusted to 6.6–6.8. These emulsions were shaken mechanically for 15 min, then observed for physical stability. Phase separation occurred in those emulsions in which the content of lecithin was 0.8% or less, with the least amount of separation in the emulsion with 0.8% of lecithin. There was no phase separation in the emulsions which contained 1.0 and 1.2% of lecithin. A lecithin content of 1.0% seemed to be adequate as an emulsifier for the preparation of emulsions of 20% of oil.

Particle Size and Size Distribution. Emulsions of

cottonseed or of soybean oils at 20% concn were prepared in which lecithin at 1% concn was the emulsifier, and a 2.5% solution of glycerol was the aqueous phase. The pH of these emulsions was adjusted to 6.8. For a comparison between the two emulsions, the number of dispersed oil particles of stated diameters from 1–10 μ were determined, and the volume of dispersed oil in the form of particles of stated diameter was calculated. These calculated volumes are relative rather than absolute, since particles of less than 1 μ in diam have not been taken into account. They do provide, however, a basis for the comparison of emulsions. The results of the particle size distributional analysis are plotted in Figure 1. In both emulsions, only 1% of the relative volume of dispersed oil was in the form of particles whose diam was more than 5 μ, and ca. 0.5% of the volume consisted of particles more than 7 μ in diam. The soybean oil emulsion appeared to have a somewhat larger volume of oil in the form of particles of 1–2 μ in diam than did the cottonseed emulsion, although both emulsions were desirably low in the volume of oil dispersed as particles of large diameters (7–10 μ).

Animal Testing. The described emulsion has been experimentally evaluated in animals only in a preliminary manner, and results are not conclusive as yet. Limitations to use of the emulsion in experimental animals only is strongly recommended.

Four rats received 4 ml of the emulsion/100 g of body wt, daily for 15 days, administered by intravenous injection through the tail vein. Results of these injections were as follows: tail necrosis, none; hemoglobin, −6.2%; red blood cells, −7.7%; body wt, +11.9%; food intake, −17%; physical activity, good; mortality, none. Complete results of more extensive testing will be reported by others at a later date.

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Determination of the Glyceride Structure of Fats: Anomalous Features of the Seed Fat of Bitter Gourd *(Momordica charantia)*[1]

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Abstract

Glyceride composition of the seed fat of *Momordica charantia* has been determined by gas liquid chromatography (GLC) of oxidized esterified glycerides. Stearodiunsaturated glycerides form ca. 80% of the total glycerides. Values obtained by the above method agree closely with those obtained by Youngs' (8) method. However, there is a wide variation between the present results and those calculated according to Vander-Wal (5) from lipase hydrolysis data. Possible reasons for this anomalous glyceride pattern are discussed.

Introduction

CHAKRABARTY ET AL. (1) studied the fatty acid composition of some seed fats of the Cucurbitaceae family, and distinguished two definite trends. Some members such as the melons and pumpkins had a simple fatty acid composition containing only oleic and linoleic acids as unsaturated components, while others such as snake gourds and bitter gourd (*Momordica charantia*) contained, in addition, considerable proportions of conjugated triene acids. Verma and Aggarwal (6) also reported the presence of ca. 50% α-eleostearic acid besides stearic, oleic and linoleic acids in the seed fat of *Momordica charantia.* During the course of an investigation on the glyceride structure of fats both by the methods of Youngs (8) and Youngs and Subbaram (9), we had occasion to examine the seed fat of *Momordica charantia.* In the present paper results of glyceride analysis of the fat are presented. The percentages of glyceride types and isomeric forms calculated according to Vander-Wal (5) from lipase hydrolysis data are also given and compared with the values obtained experimentally.

Experimental

Extraction of the Oil. The oil was extracted from the seeds by the method described by Troeng (4). The extract was filtered through a bed of celite, the residue washed with Skellysolve F and the solvent removed under reduced pressure. The yield of oil was 29.6%. The oil was preserved as a 0.1% solution in Skellysolve F in the dark.

Preparation of Methyl Esters. Ca. 20 mg of the oil was added to 5 ml of a solution containing 250 mg potassium hydroxide in 80% v/v ethanol. The mixture was kept at room temp for 12 hr after which it was diluted with 95 ml water. The soap solution was transferred to a separatory funnel and extracted twice with ethyl ether to remove unsaponifiable matter. It was then acidified with 5 *N* hydrochloric acid. After saturating the aqueous solution with NaCl, the fatty acids were extracted with three 50-ml portions of ethyl ether. Ether was removed under vacuum in a rotary evaporator. The residual fatty acids were esterified with an ethereal solution of diazomethane.

Determination of Fatty Acid Composition. The methyl esters were analyzed by GLC using both fluorinated silicone and polyester columns. An F & M, Model 500, temp programmed gas chromatographic unit with thermal conductivity detectors was used to analyze the esters on the silicone column. The methyl esters were separated into C_{16}, C_{18} and eleostearates

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