PHYSICO-CHEMICAL EVALUATION OF THE NATURAL STABILITY OF COCONUT MILK EMULSION

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ABSTRACT

The stability of coconut milk emulsion against creaming at different pH levels was evaluated in terms of the concentration of oil, protein, total phosphorus and lipid phosphorus retained in the aqueous phase after a 6-hr standing. Better stability was generally observed in the pH regions where the coconut proteins were more soluble. Two stability maxima were observed: the higher one at pH 1.5-2.0 and the lower one at pH 6.5. Minimum stability was observed in the pH range of 3.5-6.0. The correlation coefficients between the oil concentration and lipid phosphorus, total phosphorus and protein concentrations were 0.923, 0.933 and 0.820, respectively, at the pH range of 1.0 to 10. At the pH range of 1.0 to 8.5 the correlation coefficient for protein was a very high 0.971. These results tend to show that there was a relatively tight and pH independent association between the oil and the phospholipids but the oil-protein association was more pH dependent.

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KEY WORDS: Coconut milk. Natural stability. Emulsion stability index. Correlation coefficient.

INTRODUCTION

Coconut milk is an oil-in-water type of emulsion produced after filtering and pressing the ground coconut meat, with or without the addition of water. The freshly prepared coconut milk appears stable and homogenous. However, after a few minutes, the cream separates as a distinct layer above the aqueous phase. The natural stability of coconut milk is believed to be due to the presence of natural emulsifiers, generally thought to be the proteins and the phospholipids. Since these molecules have both hydrophilic and hydrophobic groups, they are

expected to be adsorbed into the oil-water interface, hence the emulsifying effect.

The isolation and characterization of coconut proteins showed that the salt-soluble globulin constitutes about two-thirds of all the proteins and, together with albumin, constitutes about 90% of the total coconut proteins (Samson *et al.*, 1971).

lysophosphatidylethanolamine comprises about 23% of all phospholipids in coconut meat. The rest of the phospholipids are composed of phosphatidylinositol, phosphatydylserine, phosphatidylchline, phosphatidylethanolamine and unknown phospholipids in about equal abundance.

Two of the important factors that affect the stability of an emulsion are the pH and the globule size. Since both proteins and phospholipids have polar groups, their intra- and inter-molecular interactions are expected to be affected by changes in pH of the emulsion. This in turn will affect their emulsifying capabilities. On the other hand, the rate of emulsion destabilization (creaming) is directly proportional to the globule size (Gopal, 1968). Processing operations which tend to produce smaller globules are expected to result to more stable emulsion (Hagenmaier, Cater and Mattil, 1972a).

This study characterized the molecular interaction between the oil globules, the proteins and the phospholipids in relation to emulsion stability against creaming.

MATERIALS AND METHODS

Preparation of Coconut Milk. — The pared meat from fully matured nuts was milled with an equal weight of distilled water in an electrically powered disc-attrition mill (Quaker City Mill model 4-E) adjusted at the minimum disc clearance. The finely comminuted meat was then filtered by using a double-layered cheese cloth and manually squeezed with a twisting motion to extract most of the milk.

Determination of Emulsion Stability at Different pH Values. — One part of coconut milk was diluted with two parts of distilled water (1:2 dilution) and the mixture was stirred briefly. The pH was adjusted to the desired value by dropwise addition of 1 N sodium hydroxide or 1 N hydrochloric acid. The milk sample was then blended for 3 min in a Waring blendor set at high speed. The method for stability determination was adapted from that of Titus *et al.* (1968). The emulsion was transferred into 30-ml test tubes, capped and then pasteurized at 65-70°C for 1 hr. The coconut milk samples were cooled in running tap water, shaken manually but vigorously for 30 sec and then finally cooled to room temperature. The emulsion was first allowed to stand for 6 hr at room temperature and then its upper half was carefully pipetted out. The remaining bottom half (or aqueous phase) was then shaken for about 30 sec in a Vortex test tube mixer. Eight ml of the

remaining milk sample (aqueous phase) was taken for oil determination (Bligh and Dyer, 1959) and another 1 ml taken was diluted appropriately for the determination of protein (Lowry *et al.*, 1951), total phosphorus and lipid phosphorus (Plesums and Bunch, 1971).

For the control, a separate portion of the diluted and pH-adjusted coconut milk emulsion was blended and pasteurized as in previous treatments. However, samples for analysis were taken immediately after they had been cooled and vigorously shaken.

The emulsion stability index (ESI) was determined from the oil concentrations in both the control and the sample using the following formula (Titus *et al.*, 1968):

$$ESI = \frac{\text{oil concentration in the sample}}{\text{oil concentration in the control}} \times 100$$

An ESI of 100 would indicate a completely stable emulsion while an ESI of zero would indicate a completely unstable emulsion.

RESULTS AND DISCUSSION

pH and Emulsion Stability.

The emulsion stability was measured by the amount of oil retained in the aqueous phase after 6 hr of standing, both in actual concentrations and percentages relative to original oil concentrations. In order to approximate the oil concentration in cow's milk, the coconut milk extracted with an equal weight of distilled water was further diluted twice to its volume. This dilution gave an average concentration of 44.6 mg oil per ml emulsion.

There was minimum emulsion stability at the pH range of 3.5 to 6.0 (Fig. 1). The pH of the natural coconut milk was from 6.1 to 6.3 which is very near the instability range. This is probably the reason why even a slight microbial attack on the coconut milk results in the lowering of its pH (Fernandez and Lirio, 1970) thereby rendering the emulsion unstable.

It may be also noted that at the minimum stability range, the aqueous phase retained only about 1% of the original oil concentration in the milk. Furthermore, at this pH range the aqueous phase was practically transparent and without any precipitate settling. Coagulated proteins in the absence of fat have higher density than water (Hagenmaier, Cater and Mattil, 1972b) and are therefore expected to settle. The coagulated proteins in the coconut milk creamed with the oil on the surface of the emulsion. This was a possible indication of the affinity between the proteins and the soil droplets. This further indicated that even if the proteins coagulated, their association with the oil droplets might not have been significantly affected.

Two maxima can be seen in Fig. 1, one at about pH 1.5-2.0 and the other at pH 6.5. Even at the most stable pH (1.5-2.0) the aqueous phase retained only about 60% of the original oil. Furthermore, the pH

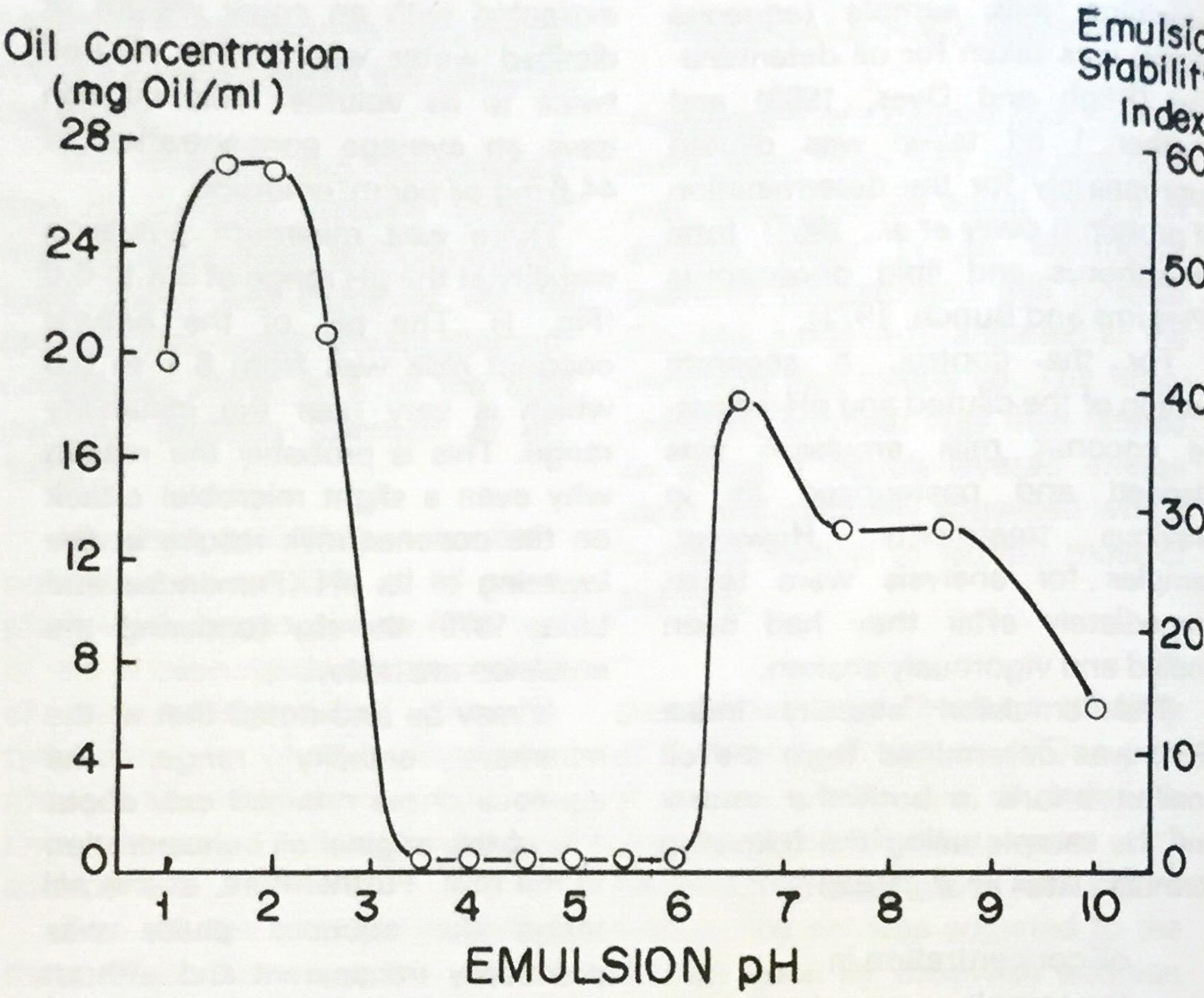

Fig. 1. Oil concentrations and emulsion stability indices (ESI) at different pH values.

range of maximum stability was too low for food uses. The other stability (pH 6.5) although relatively lower has the potential food applicability since it falls within the normal pH range of food products.

The above results showed that, although pH alone could not stabilize the coconut milk emulsion, the stability peaks could be used as initial basis for further development of methods of improving emulsion stability.

Protein and Emulsion Stability.

The coconut milk obtained through the described extraction and dilution contained an average of 5.37 mg protein per ml. This is only about one-sixth of the protein concentration in cow's milk (FNRC, 1968). For better nutritional and stabilizing effect, it may be necessary to fortify the emulsion with more proteins.

There was a high correlation coefficient of 0.971 between the oil concentration and the protein concentration in the aqueous phase of the emulsion at the pH range of 1.0 to 8.5. At pH 10, there was a significant separation between the oil globules and the proteins (Fig. 2). This may indicate a relatively tight association between the oil and the

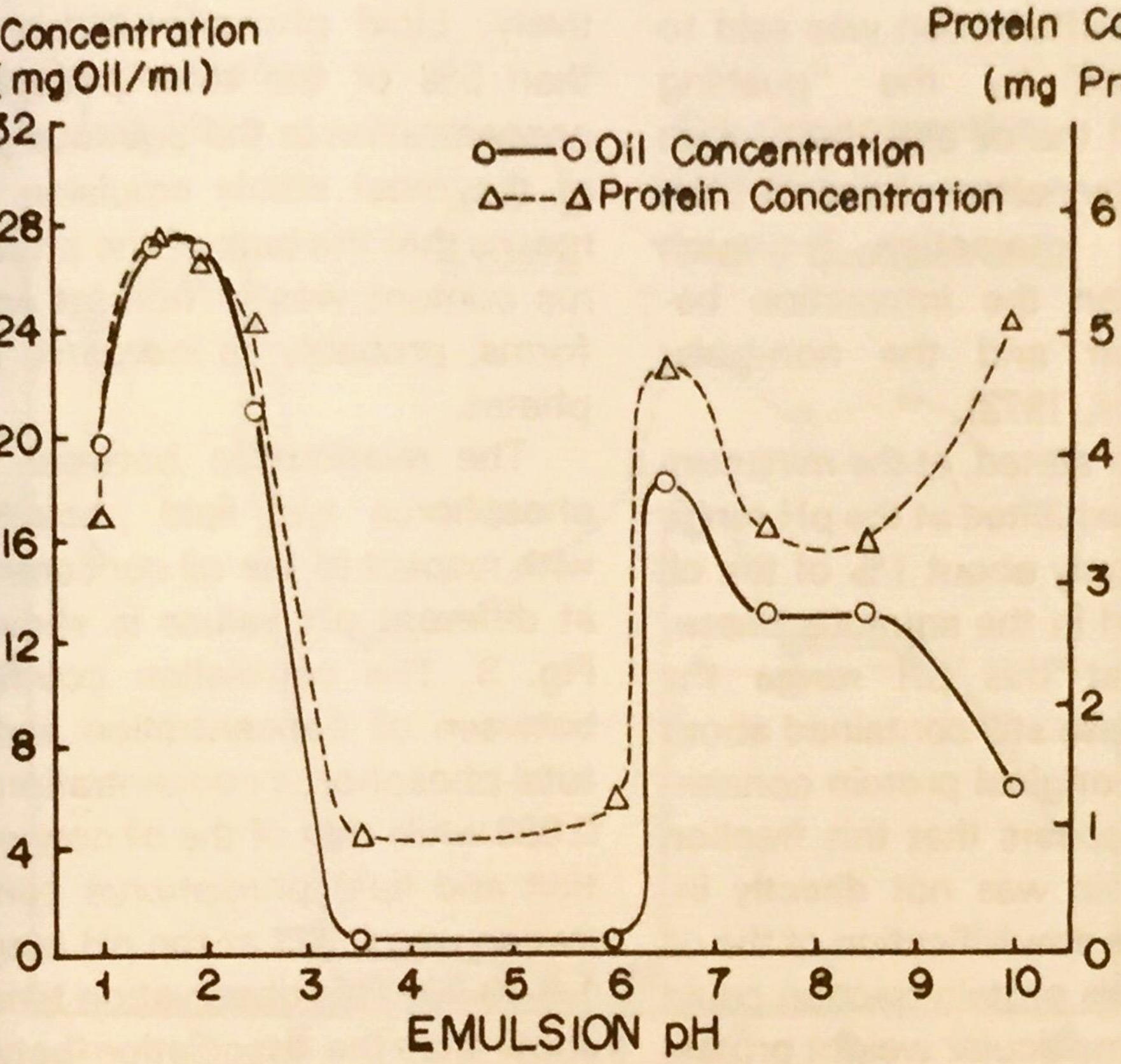

Fig. 2. Relationship between the oil concentration and the protein concentration at different pH values.

Correlation coefficients, r:

r_c = 0.820 for pH 1.0 to 10
= 0.971 for pH 1.0 to 8.5

$r_{(0.01, 20)}$ = 0.537

$r_{(0.01, 18)}$ = 0.561

protein which apparently separated only at the pH range where the proteins were expected to exhibit high negative charges. This was consistent with the assumption (Karel, 1973) that the oil globules exhibit a net negative charge which resulted in a very significant repulsion with the protein molecules at pH 10.

The shape of the pH vs protein concentration curve was also observed to be similar to the pH vs protein solubility (isolate) curve reported by Samson *et al.* (1971). This might be an indication that the protein-lipid association might not have altered to a great extent the behavior of such proteins at the different pH values of the solution. This further supported the proposal (Chou and Morr, 1979) that most of the ionic and polar groups of the protein molecules are projected outward and in direct contact with water. Hence they are also directly affected by the pH of the solution. The intermolecular forces that might have enhanced the adsorption of the protein molecules into the oil globules could be hydrophobic bonding of the non-polar groups.

This type of interaction was said to be enhanced by the "pushing together" of the oil and the protein molecules by water because the water-water interaction is much stronger than the interaction between water and the non-polar groups (Karel, 1973).

As earlier stated, at the minimum oil stability exhibited at the pH range of 3.5-6.0, only about 1% of the oil was retained in the aqueous phase. However, at this pH range the aqueous phase still contained about 20% of the original protein concentration. It appears that this fraction of the protein was not directly involved in the emulsification of the oil globules. This protein fraction could be the low molecular weight protein (MW = 24,000 daltons) which was shown to be soluble throughout the tested pH range (Hagenmaier, Cater and Mattil, 1972b).

Eighty-four per cent of the coconut proteins was reported to have a molecular weight of 150,000 daltons (Hagenmaier, Cater and Mattil, 1972b). Since it was also shown that the solubility of this protein fraction varies with the pH, it is possible that this protein fraction contributed mainly to the stability of the emulsion.

Phosphorus and Stability.

The total phosphorus and lipid phosphorus concentrations in the diluted coconut milk were found to be 121 and 3.73 ug per ml, respectively. Lipid phosphorus was less than 5% of the total phosphorus concentration in the aqueous phase of the most stable emulsion. This means that the bulk of the phosphorus content was in non-fat soluble forms, probably as inorganic phosphates.

The relationship between total phosphorus and lipid phosphorus with respect to the oil concentration at different pH values is shown in Fig. 3. The correlation coefficient between oil concentration and the total phosphorus concentration was 0.933 while that of the oil concentration and lipid phosphorus concentration was 0.923 at the pH range of 1.0-10.00. This observation tends to show that the association between the total globules and the phosphorus-containing molecules was pH independent and relatively tight.

Based on the low concentration of the lipid phosphorus and its relatively tight association with the oil globules, it might be logical to suggest that fortification of the emulsion with phospholipids would significantly increase its stability.

Results of the study have revealed the pH values where the natural coconut with emulsion has stability optima for use as bases for further emulsion stabilization. These also suggest that since both the proteins and phospholipids are tightly associated with the oil globules, emulsification may be further improved upon addition of proteins and/or phospholipids into the emulsion.

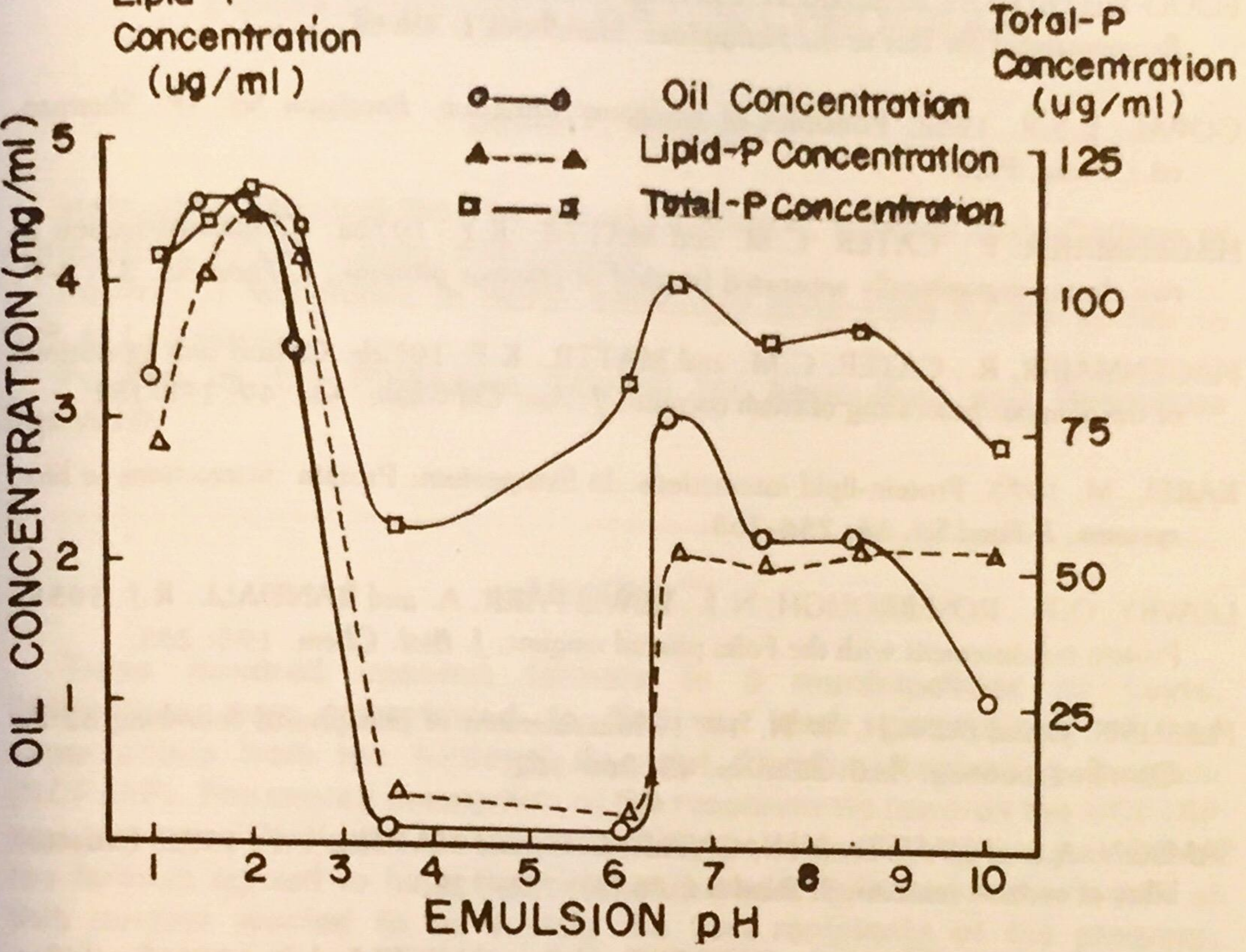

Fig. 3. Relationships between the concentrations of oil, lipid phosphorus and total phosphorus at different pH values.

Correlation coefficients (r) between:

oil conc. and lipid-P conc: 0.923

oil conc. and total-P conc: 0.933

$^{r}(0.01, 20) = 0.573$

LITERATURE CITED

BLIGH, E.G. and DYER, W.J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Phys.* 37(8): 911-7.

CHOU, D.H. and MORR, C.V. 1979. Protein-water interactions and functional properties. In Symposium: Functionality of proteins. *J. Am. Oil Chem. Soc.* 56: 53A-61A.

FERNANDEZ, W.L. and LIRIO, V.M. 1970a. Themophilic, mesophilic and psychrophilic bacterial plate count of ''gata'' (coconut milk) stored for 24 hours at 55°C, 30°C, and 10°C. *Phil. Agric.* 54(5-6): 202-9.

FOOD NUTRITION RESEARCH CENTER (FNRC). 1968. *Food Composition Table Recommended for Use in the Philippines*. Handbook I. 4th ed.

GOPAL, E.S.R. 1968. Principles of emulsion formation. *Emulsion Sci.* (P. Sherman, ed.). Acad. Press.

HAGENMAIER, R., CATER, C.M. and MATTIL, K.F. 1972a. Characteristization of two chromatographically separated fraction of coconut proteins. *J. Food Sci.* 37: 4-7.

HAGENMAIER, R., CATER, C.M. and MATTIL, K.F. 1972b. Critical unit operations of the aqueous processing of fresh coconut. *J. Am. Oil Chem. Soc.* 49: 178-181.

KAREL, M. 1973. Protein-lipid interactions. In Symposium: Protein interactions in biosystems. *J. Food Sci.* 38: 756-763.

LOWRY, O.H., ROSEBROUGH, N.J., LEWIS PARR, A. and RANDALL, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265.

PLESUMS, J. and BUNCH, W.H. 1971. Measurement of phosphorus following 32 P. Cerenkov counting. *Anal. Biochem.* 43: 360-362.

SAMSON, A.S., KHAUND, R.N., CATER, C.M. and MATTIL, K.F. 1971. Extractability of coconut proteins. *J. Food Sci.* 36 (5): 725-728.

TITUS, T.C., WIANCO, N.M., BARBOUR, H.P. and MICKLE, J.B. 1968. Emulsifier efficiency in model systems of milk fat or soybean oil and water. *Food Technol.* 22: 1449-1451.