Characterization of Diacylglycerol Oil Mayonnaise Emulsified Using Phospholipase A_2-Treated Egg Yolk

Shigeru Kawai*

Health Care Products Research Laboratories, Kao Corporation, Tokyo 131-8501, Japan

ABSTRACT: Mayonnaise samples were prepared using DAG oil as the oil phase; the properties and stability were compared with mayonnaise samples prepared from TAG oil. Normal (nontreated) egg yolk and phospholipase A_2 (PLA2)-treated egg yolk were used as emulsifiers. The DAG oil mayonnaise prepared with nontreated egg yolk (DAG-M) was unstable, with cracks forming within 4 wk at 40°C. This type of fracture was not observed for TAG oil mayonnaise prepared with nontreated egg yolk (TAG-M). ^{31}P NMR and quantitative analyses of phospholipids suggested that the phospholipids of egg yolk lipoprotein were dissolved in DAG-M oil droplets, which might result in the coalescence and fracture of the DAG-M. Phospholipids were not dissolved in TAG-M oil droplets. No crack formation was observed for DAG oil mayonnaise prepared with PLA2-treated egg yolk (DAG-PLM) after storage for more than 4 wk at 40°C. ^{31}P NMR spectroscopy and quantitative analyses of phospholipids indicated that the dissolution of phospholipid molecules into the oil droplets was almost prevented in DAG-PLM. The stability of DAG-PLM is improved probably because the PLA2 treatment changes the molecular polarity of egg yolk phospholipids and prevents them from dissolution into the DAG oil droplets from oil/water interface.

Paper no. J10771 in *JAOCS 81*, 993–998 (November 2004).

KEY WORDS: Diacylglycerol, emulsion, lipoprotein, mayonnaise, phospholipase A_2, phospholipids.

The main components of common edible oils are TAG. DAG are natural minor components of edible oils (1,2) and also are commonly used as emulsifiers in a variety of food products. Since DAG molecules contain two FA and one hydroxyl group, they are more polar than TAG.

DAG oil was recently found to exhibit interesting nutritional properties. DAG oil suppresses the increase of serum TAG after meals, compared with other edible oils, and it is less likely to be converted to body fat (3–5). Because the excessive intake of oils is a major factor contributing to diseases such as obesity and diabetes, it could be nutritionally helpful to substitute DAG oils in various food products.

There are no significant differences in the cooking properties between DAG oil and TAG oil (6–9). Shimada and Ohashi (10) studied the emulsification properties of DAG oil. They reported that a water-in-oil type emulsion was easily formed using DAG oil without using emulsifiers, because DAG itself works as an emulsifier. Masui *et al.* (11) reported that margarine prepared with DAG oil showed a much better stability than conventional margarine containing TAG oil (11). On the other hand, it is difficult to form stable oil-in-water (o/w) type emulsions using DAG oil, especially in the case of products with a high oil content, such as mayonnaise (10,12).

In this study, o/w emulsions, whose formulations were close to that of commercial Japanese mayonnaise, were prepared using both DAG oil (DAG-M) and TAG oil (TAG-M). Two different types of egg yolk were used as emulsifiers: normal (nontreated) egg yolk and phospholipase A_2 (PLA2, EC 3.1.1.4)-treated egg yolk. The structures and properties of the mayonnaise samples were investigated; the important factors that govern the emulsion stability are discussed from the viewpoint of the interaction between emulsifiers and oil phases.

EXPERIMENTAL PROCEDURES

Materials. DAG oil was prepared by the esterification of glycerol with FA from rapeseed oil and soybean oil by methods described by Huge-Jensen *et al.* (13). The DAG oil was characterized by GC after silylation (14) and was composed of TAG (10.5%), DAG (88.7%), and MAG (0.8%). The TAG oil used in this study was a commercial soybean oil purchased from Nisshin Oil Mills Ltd. (Tokyo, Japan), the composition of which was more than 98% TAG with less than 2.0% DAG.

Pasteurized and frozen egg yolk that contained 10% NaCl was obtained from Taiyo Kagaku Co., Ltd. (Mie, Japan). PLA2 was obtained from Novozymes Japan Ltd. (10,000 unit/mL; Tokyo, Japan). Salt, sugar, monosodium glutamate, mustard powder, and vinegar were purchased from Ako Kaisui Co., Ltd. (Hyogo, Japan), Dai-Nippon Meiji Sugar Co., Ltd. (Tokyo, Japan), Ajinomoto Co., Inc. (Tokyo, Japan), KIS Co., Ltd. (Tokyo, Japan), and Mitsukan Co., Ltd. (Aichi, Japan), respectively.

Preparation of emulsions. The basic composition of mayonnaise samples is shown in Table 1. The mayonnaise samples were prepared by two-step emulsification processes. The first process, premixing, was carried out in 2-kg batches using a vertical vacuum mixer (5DMV-r; Dalton Corporation, Tokyo, Japan). After de-aeration, the powdered ingredients and water phase were mixed for 2 min. The oil phase was then gradually added over 3 min, and mixed for 2 min more to finish the premixing process. The premixed products were then thoroughly

*To whom correspondence should be addressed at Health Care Products Research Laboratories, Kao Corporation, 2-1-3, Bunka, Sumida-ku, Tokyo, 131-8501, Japan. E-mail: kawai.shigeru@kao.co.jp

TABLE 1
Basic Composition of Mayonnaise

Ingredient	(%)	Ingredient	%
Oil	70.0	Salt	1.5
10% Salted egg yolk	15.0	Sugar	1.0
Vinegar	6.0	Monosodium glutamate	0.5
Water (balance)	5.5	Mustard powder	0.5

emulsified using a colloid mill (MM-2; Nihonseiki Kaisha Ltd., Tokyo, Japan), operated at 5,000 rpm, 0.35 mm clearance and 0.7 L/min flow rate, to produce the final mayonnaise samples.

The stability of emulsions is sometimes affected by the hydrophilic or hydrophobic surface properties of their packaging materials (15). Affinity of the continuous phase of emulsions and surface properties of the container are important; an o/w emulsion may exhibit better stability when stored in a container whose surface is hydrophilic. Therefore, two types of containers were used to compare stability; a laminated commercial tube (the surface of which is low-density polyethylene layer) and a glass jar.

The diameter of the oil droplets and viscosity of emulsion were measured immediately after preparation. For the droplet diameter measurement, the emulsion sample was dispersed in 0.5% SDS solution. The particle diameters and their SD were measured using a laser diffraction-type particle size analyzer (SALD-2100; Shimadzu Corporation, Tokyo, Japan). Viscosity was measured with a Brookfield RVDV-I+ viscometer (Brookfield Engineering Laboratories Inc., Middleboro, MA) using rotor No.6. Viscosity was measured after 30 s at a rotation speed of 4 rpm at 20°C. The diameters of the oil droplets and their SD, and the viscosity of the mayonnaise samples are listed in Table 2. Variations in the diameters of emulsion droplets and viscosities of emulsions between different experiments are within about 10%. It is interesting that emulsion samples having small droplet diameters exhibited large viscosity. Small droplet diameter gives a large droplet boundary area where slippage occurs during shear; this may be one of the factors responsible for the observed diameter–viscosity relation.

Preservation test. The samples were stored at 40 or 20°C, and the appearance of the sample was evaluated after 2 and 4 wk. For evaluation of the reproducibility of the results, five different samples obtained from the same sample preparation process were tested for each emulsion.

Cryo-scanning electron microscope (SEM) observation. The mayonnaise sample was quickly frozen in liquid nitrogen and mounted on a sample stage. The sample was fractured using a cold knife held at about –150°C. The sample temperature was raised to about –85°C for surface sublimation to expose surface details. The sample temperature was then decreased to –120°C and maintained during the SEM observation at 1 kV using a Hitachi S-4300 SE instrument (Tokyo, Japan).

^{31}P NMR spectroscopy. ^{31}P NMR measurements were carried out using an EX-270 spectrometer (JEOL Ltd., Tokyo, Japan) operating at 109.25 MHz, using a 45° plus (6.5 μs), with 16 K data points and a 20,000 Hz spectral width. Mayonnaise samples were introduced into a 10-mm diameter NMR tube.

TABLE 2
Average Droplet Diameter and Viscosity of Mayonnaise Samples

Sample	Droplet diameter (μm)		Viscosity
	Average	SD[a]	(Pa·s)
TAG-M	3.4	1.2	87
DAG-M	2.6	0.8	116
TAG-PLM[b]	2.1	0.6	126
DAG-PLM[b]	3.8	1.7	79

[a]SD of the distribution measured by light scattering.
[b]Reaction rate of the phospholipase A_2 (PLA2)-treated egg yolk is 62%. TAG (DAG)-M, TAG (DAG) oil mayonnaise prepared with untreated egg yolk; TAG(DAG)-PLM, TAG (DAG) oil mayonnaise prepared with PLA2-treated egg yolk.

^{31}P NMR spectra were collected using a one-pulse sequence applied proton decoupling. A total of 1,000 scans was collected for each sample with a 0.41 s acquisition time and a 1.59 s relaxation delay time. Chemical shifts are given relative to 85% phosphoric acid as the external reference (0 ppm). All measurements were conducted at 24°C.

Quantitative analyses of phospholipids. The phospholipid concentration of the oil phase of the emulsion was measured immediately after emulsification and following storage at 20°C for 0.5, 1, 3, 6, and 24 h. The phospholipid concentrations were obtained from the average of three different samples for each emulsion.

Samples were centrifuged for 30 min at 18,000 × *g* rpm at 20°C to separate the oil phase. The separated oil phase (DAG or TAG) was then weighed (15 mg), and the phospholipid concentrations therein were measured using a commercial kit (C-testwako; Wako Pure Chemical Industries, Ltd., Tokyo, Japan): a combined enzymatic method using phospholipase D, choline oxidase, and peroxidase (16). The estimated error of this method was within the range of about 10%. PE cannot be detected by this technique, therefore, the analyzed phospholipids were estimated to be about 85% of the total amount of phospholipids in the egg yolk (17).

Preparation of PLA2-treated egg yolks. PLA2-treated egg yolks, having different reaction rates (from 25 to 100%) were prepared by incubation at 50°C. The incubation conditions were as follows: i) reaction rate of 25%, 100 unit/g egg yolk and 0.5 h; (ii) rate of 44%, 400 unit/g egg yolk and 1 h; (iii) rate of 62%, 200 unit/g egg yolk, and 3 h; (iv) rate of 77%, 500 unit/g egg yolk and 3 h; and (v) rate of 100%, 2,000 unit/g egg yolk, and 20 h. Since the calcium content of egg yolk was sufficient to give an optimal reaction rate for PLA2, we did not add further calcium into egg yolk. The DAG-PLM and TAG-PLM samples were prepared using PLA2-treated egg yolk having different reaction rates. The preservation test was carried out for all the emulsion samples to clarify the relationship between the reaction rate and emulsion stability. Detailed analyses of the structures and properties of DAG-PLM and TAG-PLM were carried out for the emulsions using PLA2-treated egg yolk whose reaction rate of 62%.

The reaction rate of the PLA2 treatment was determined by the titration of FA. Precisely 2 g of egg yolk was dispersed in 60 g of water, and the solution was titrated with 0.02 N alco-

holic KOH to pH 8.5. The reaction rate was calculated by difference in the titration value between the nontreated (used as a background, defined reaction rate of 0%) and perfectly reacted (reaction rate of 100%) egg yolks.

RESULTS AND DISCUSSION

In the preservation test, crack formation was always observed after 4 wk for the DAG-M samples stored at 40°C (Fig. 1). The crack was sometimes observed after 2 wk for the DAG-M samples stored at 40°C. Cracks were observed for samples filled in both laminated tubes and glass jars. Crack formation was observed only for the DAG-M samples stored at 40°C; DAG-M stored at 20°C, TAG-M, TAG-PLM, and DAG-PLM (at different PLA2 reaction rates) showed no fracture in the experimental preservation conditions used here. The results described above were basically reproduced for different preservation tests (for different sets of emulsion samples).

Figure 2 shows the cryo-SEM images of TAG-M, DAG-M, and DAG-PLM emulsions. For TAG-M and DAG-PLM, the boundaries of the individual emulsion droplets are clearly observed. On the other hand, boundaries are not clearly observed for DAG-M.

Figure 3 shows the results of the ^{31}P NMR measurements. In the spectrum of TAG-M, only a broad signal was observed at 1.1 ppm (Fig. 3A). For DAG-M, the same broad signal was observed at 1.1 ppm along with additional sharp signals at 0 and 0.8 ppm (Fig. 3B). Spectrum C is egg yolk lecithin dissolved in DAG oil. Two sharp signals were observed at 0 ppm and 1.0 ppm (Fig. 3C). The main signal at 0 ppm in the spectrum of DAG-M (B) and egg yolk lecithin (C) had the same chemical shift and half-height width. The main signal observed at 0 ppm and the other minor signal at 0.8 ppm were assigned to PC and PE, respectively, based on comparisons with authentic specimens. In the spectrum of DAG-PLM (Fig. 4D), three components were observed, but the intensity of the signal at 0 ppm was smaller than that in the DAG-M spectrum.

^{31}P NMR spectra of DAG-PLM prepared with PLA2-treated egg yolks having different reaction rates (from 25 to 77%) are shown in Figure 4. The intensity of the sharp signals at 0 ppm decreased with increasing reaction rate.

The phospholipid concentrations in the oil phase are shown in Figure 5. As already mentioned, PE cannot be detected by this technique. Therefore, the analyzed phospholipids were estimated to be about 85% of the total phospholipids in egg yolk (17). The dissolution of phospholipids in the DAG oil started immediately after the preparation of the emulsion and reached a constant value after 3 h. The phospholipid concentration in the oil phase at 24 h was 5.0 mg/g-oil for DAG-M and 0.8 mg/g-oil for DAG-PLM. Little phospholipid dissolution was observed for TAG-M. The total content of phospholipids in egg yolks is about 10% (18). If all the phospholipids were dissolved in the oil phase, the phospholipid concentration would be estimated to be 18 mg/g-oil (16 mg/g-oil except PE). The measured phospholipid concentration in the oil phase of DAG-M was 5.0 mg/g-oil, which corresponds to about 30% of the total phospholipids in egg yolk.

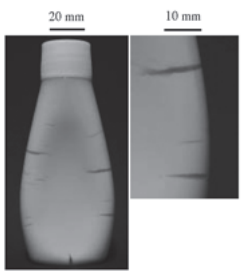

FIG. 1. Crack formation in the DAG oil mayonnaise prepared with nontreated egg yolk (DAG-M) sample stored at 40°C for 4 wk.

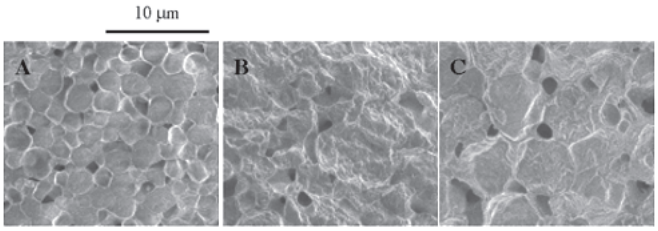

FIG. 2. Cryo-scanning electron microsopy micrographs of (A) TAG oil mayonnaise prepared with nontreated egg yolk (TAG-M), (B) DAG-M, and (C) DAG oil mayonnaise prepared with phospholipase A_2 (PLA2)-treated egg yolk (DAG-PLM) (reaction rate of 62%). For other abbreviation see Figure 1.

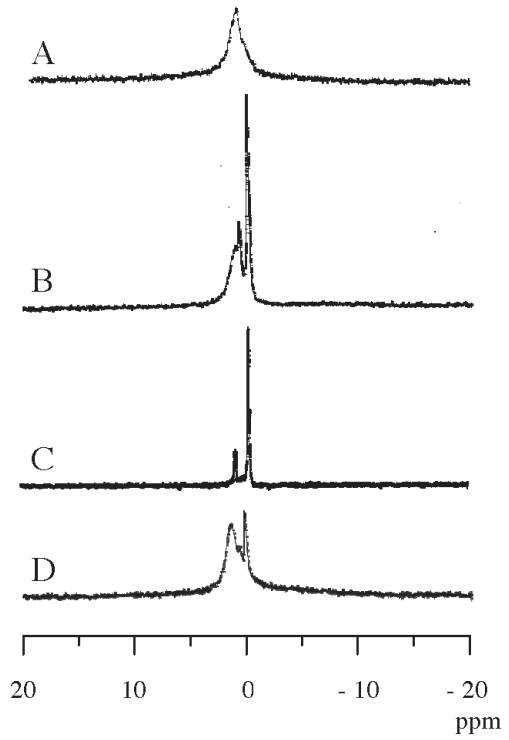

FIG. 3. ^{31}P NMR spectra of (A) TAG-M, (B) DAG-M, (C) egg yolk lecithin dissolved in DAG oil, and (D) DAG-PLM (reaction rate of 62%). For abbreviations see Figures 1 and 2.

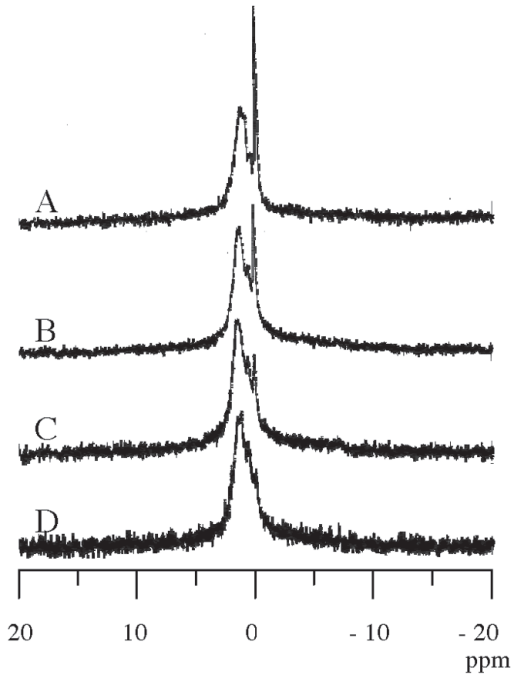

FIG. 4. ^{31}P NMR spectra of DAG-PLM prepared using PLA2-teated egg yolk at different reaction rate: (A) 25, (B) 44, (C) 62, and (D) 77%. For abbreviations see Figure 2.

Long-term stability is one of the important requirements for mayonnaise emulsions. DAG-M samples stored at 40°C showed crack formation (Fig. 1), whereas DAG-M samples stored at 20°C, TAG-M, TAG-PLM, and DAG-PLM (at different PLA2 reaction rates) showed no fracture under the preservation conditions used in these experiments. In general, emulsion instability processes are accelerated by storage at high temperatures. Therefore, the results at 40°C should enable us to predict instability over longer storage intervals at room temperature. Since the fracture of DAG-M was observed for samples filled in both laminated tubes and glass jars, this is not caused by the surface properties (hydrophilicity or hydrophobicity) of the containers.

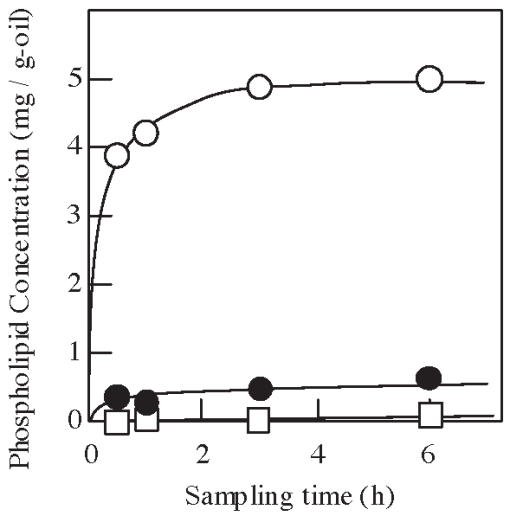

FIG. 5. Phospholipid concentrations (except for PE) of TAG-M (□), DAG-M (○), and DAG-PLM (reaction rate of 62%) (●) in the oil phase. The average of three different samples is plotted. Zero sampling time corresponds to the point at which the oil phase was added to the vacuum mixer in the premix process.

The cryo-SEM observations suggest that the stable samples (TAG-M and DAG-PLM) show clear droplet boundaries in their images (Fig. 2). For the case of unstable DAG-M samples, the droplet boundaries are collapsed. It is difficult to conclude that the collapse occurs spontaneously in the mayonnaise sample or during the SEM sample preparation (freeze-fracture processes). In any case, changing the emulsifier from nontreated egg yolk to a PLA2-treated egg yolk improves the stability of the droplet boundaries of DAG-M. The droplet diameter of the DAG-PLM evaluated from the SEM image is somewhat larger than that obtained from the particle size analyzer (see Table 2). This suggests the coalescence of emulsion droplets during preservation. The diameter measurement using the particle size analyzer was carried out immediately after sample preparation, but the SEM observation was carried out almost 2 wk after the preparation.

In the mayonnaise samples, phosphorus atoms (from egg yolk) occur in two different chemical species, phospholipids and protein components. The chemical shift of the phosvitin is about 1 ppm at pH = 4 (19), which nearly overlaps with the main signal of phospholipids. In addition, the observed NMR spectra have relatively broad peak widths. Therefore, it is difficult to separate the peak of phosvitin from the whole spectrum. However, more than 80% of phosphorus atoms are included in phospholipids (20), so the ^{31}P NMR spectra shown in Figures 3 and 4 could reflect mainly the chemical states of the phosphorus atoms in phospholipid molecules.

In general, the spectral widths of NMR signals are a function of the mobility of the molecules: A low mobility results in a broad signal and a high mobility a sharp signal. The broad signal at 1.1 ppm observed in the spectra of mayonnaise samples indicates that the mobility of the phospholipid molecules is rather restricted, presumably because the phospholipid molecules are located in the lipoprotein structure and/or are adsorbed onto the emulsion droplet surfaces. The sharp and symmetrical signal observed at 0 ppm in ^{31}P NMR spectra of DAG-M and DAG-PLM suggests that these phospholipid molecules have a high mobility. According to the measurement of egg yolk lecithin dissolved in DAG oil and authentic PC, this signal is assigned to the phospholipids dissolved in the DAG oil droplets. We cannot explain the fact that the chemical shift of PC (0 ppm) is the same as that of inorganic phosphoric acid (external reference); it is possibly due to the solvent effect of the DAG oil (21).

The results of ^{31}P NMR measurements and the quantitative analyses of phospholipids suggest that the phospholipids in the DAG-M are dissolved in the DAG oil droplets, which may be one of the main causes of the low stability. For the case of

TAG-M, phospholipids are not dissolved in the oil droplets but rather exist in the lipoprotein and/or on the emulsion droplet surfaces, resulting in a stable emulsion. These different behaviors of phospholipids may be due to the differences in the polarity of TAG oil *vis-à-vis* DAG oil; the relatively high polarity of DAG oil can be attributed to the presence of a hydroxyl group in the molecules, which increases the solubility of the phospholipids in the oil droplets. The PLA2 treatment of egg yolk selectively hydrolyzed the ester bond at the *sn*-2 position of 1,2-diacyl-*sn*-glycero-3-phosphatide, which increased the molecular polarity of phospholipids in the egg yolk. This effect greatly reduced the amount of phospholipids that dissolved in the DAG oil, which could be one of the major reasons for the high stability of the DAG-PLM samples.

In this study, we mainly focused on the chemical states of the phospholipids of egg yolk in order to discuss the stability of emulsions of mayonnaise samples. However, a number of studies have suggested that the high emulsification ability of egg yolk is not due to phospholipids alone; the characteristic structure of egg yolk lipoproteins (complex of the phospholipids and proteins) makes a large contribution to the emulsification properties (22). We did not directly observe the effect of PLA2 treatment of egg yolk on the structure and properties of egg yolk lipoproteins. However, we estimate that changing the molecular polarity of phospholipids by the enzyme treatment could affect the structure and emulsification properties of lipoproteins. For example, conformational change of proteins induced by the PLA2 treatment could affect the water-holding capacity and accelerate the coagulation of apoproteins, which may result in the unique fracture phenomenon (crack formation) observed here.

Dutilh and Groger (23) reported that mayonnaise prepared with PLA2-treated egg yolk exhibited a high heat stability, which was attributed to structural changes in lipoproteins as the result of PLA2 treatment (24). Kumar and Mahadevan (25) reported that the lipoprotein structure of the yolk plasma is maintained by the phospholipids, since treatment with phospholipase C induces gelation. These facts indicate that the treatment of phospholipids introduces the alteration of protein conditions and/or lipoprotein structure. Therefore, the PLA2 treatment carried out in this study may also change the emulsification characteristics of proteins in egg yolk and/or lipoprotein structure, such as adsorption conformation and/or elastic properties of protein layers at the oil/water interface, which could affect the emulsion stability. Further investigations, focused on the structures and properties of lipoproteins before and after the enzyme treatment, will be required to understand the stabilization mechanisms associated with DAG mayonnaise.

ACKNOWLEDGMENTS

The author is grateful to Shinji Yamada, Kenji Masui, and Yoshinobu Nakajima for useful suggestions, to Yoko Mikawa for preparation and evaluation of samples, to Tomohito Ikeda for performing the quantitative analyses of the phospholipids, and to Kao Corp. for permission to publish this paper.

REFERENCES

1. Abdle-Nalby, A.A., A.A.Y. Sheheta, M.H. Ragab, and J.B. Rossell, Glycerides of Cotton Seed Oils from Egyptian and Other Varieties, *Riv. Ital. Sostanze Grasse 69*:443–447 (1992).
2. D'Alonzo, R.P., W.J. Kozarek, and R.L. Wade, Glyceride Composition of Processed Fats and Oils as Determined by Glass Capillary Gas Chromatography, *J. Am. Oil Chem. Soc. 59*:292–295 (1988).
3. Maki, K.C., M.H. Davidson, R. Tsushima, N. Matsuo, I. Tokimitsu, D.M. Umporowicz, M.R. Dicklin, G.S. Foster, K.A. Ingram, B.D. Anderson, *et al.,* Consumption of Diacylglycerol Oil as Part of a Reduced-Energy Diet Enhanced Loss of Body Weight and Fat in Comparison with Consumption of a Triacylglycerol Control, *Am. J. Clin. Nutr. 76*:1230–1236 (2002).
4. Nagao, T., H. Watanabe, N. Goto, K. Onizawa, H. Taguchi, N. Matsuo, T. Yasukawa, R. Tsushima, H. Shimasaki and H. Itakura, Dietary Diacylglycerol Suppresses Accumulation of Body Fat Compared to Triacylglycerol in Men in a Double-Blind Controlled Trial, *J. Nutri. 130*:792–797 (2000).
5. Taguchi, H., H. Watanabe, K. Onizawa, T. Nagao, N. Gotoh, T. Yasukawa, R. Tsushima, H. Shimasaki, and H. Itakura, Double-Blind Controlled Study on the Effects of Dietary Diacylglycerol on Postprandial Serum and Chylomicron Triacylglycerol Responses in Healthy Humans, *J. Am. Coll. Nutr. 19*:789–796 (2000).
6. Fukuda, H., T. Kimura, T. Sugimoto, and N. Iritani, Studies of Triacylglycerols and Diacylglycerols for Cookery, *J. Cookery Sci. Jpn. 35*:172–179 (2002).
7. Ohno, Y., Deep-frying Oil Properties of Diacylglycerol-rich Cooking Oil, *J. Oleo Sci. 51*:275–279 (2002).
8. Ogawa, H., S. Okushima, and H. Kodama, The Cooking Characteristics of Diacylglycerol-Rich Cooking Oils When Preparing Dishes, *J. Integrated Study Diet. Habits 12*:100–108 (2001).
9. Nakatsugawa, K., K. Ohashi, and A. Shimada, Comparison of Oxidation Stability of Diacylglycerol and Triacylglycerol, *Nippon Shokuhin Kagakku Kogaku Kaishi 48*:429–436 (2001).
10. Shimada, A., and K. Ohashi, Interfacial and Emulsifying Properties of Diacylglycerol, *Food Sci Technol Res. 9*:142–147 (2003).
11. Masui, K., K. Yasunaga, T. Nishide, Y. Nakajima, and T. Yasukawa, Physicochemical and Frying Characteristics of Oil High in Diacylglycerol, *Proceedings of the 2001 PIPOC International Palm Oil Congress*, Kuala Lumpur, 2001, pp. 65–73.
12. Ohashi, K., and A. Shimada, Emulsifying Properties of Diacylglycerol in a Concentrated Oil-in-water Emulsion System, *J. Cookery Sci. Jpn. 35*:132–1138 (2002).
13. Huge-Jensen, B., D.R. Galluzzo, and R.G. Jensen, Studies on Free and Immobilized Lipases from *Mucor miehei*, *J. Am. Oil Chem. Soc. 65*:905–910 (1988).
14. Watanabe, T., M. Shimizu, M. Sugiura, M. Sato, J. Kohori, N. Yamada, and K. Nakanishi, Diacylglycerol Using Immobilized 1,3-Regiospecific Lipase Lipozyme RM IM, *Ibid. 80*:1201–1207 (2003).
15. Kobayashi, Y., Mayonnaise, in *Handbook of Interface*, edited by Y. Iwasawa, Y. Umezawa, T. Sawada, and K. Tsujii, N.T.S., Tokyo, 2001, pp. 1074–1079.
16. Takayama, M., S. Itoh, T. Nagasaki and I. Tanimizu, A New Enzymatic Method for Determination of Serum Choline-Containing Phospholipids, *Clin. Chim. Acta 79*:93–98 (1977).
17. Shigematsu, Y., Egg Yolk Lecithin, in *Research and Developments of Functional Lipids*, edited by K. Sato, Y. Yamane, M. Iwashita, and H. Mori, CMC Publishing, Tokyo, 2001, pp. 245–258.
18. Nishimoto, K., and Y. Asano, Chemistry of Egg, in *Egg*, edited by Y. Asano and R. Ishihara, KOHRIN, Tokyo, 1985, pp. 89–91.

19. Grogan, J., A. Shirazi, and G. Taborosky, Phosphorus Nuclear Magnetic Resonance of Diverse Phosvitin Species, *Comp. Biochem. Physiol. 96B*:655–663 (1990).
20. Schmidt, G., M.J. Bessman, M.D. Hickey, and S.J. Thannhauser, The Concentrations of Some Constituents of Egg Yolk in Its Soluble Phase, *J. Biol. Chem. 223*:1027–1031 (1956).
21. Meneses, P., and T. Glonek, High Resolution ^{31}P NMR of Extracted Phospholipids, *J. Lipid Res. 29*:679–689 (1988).
22. Mizutani, R., and R. Nakamura, Emulsifying Properties of Egg Yolk Low Density Lipoprotein (LDL): Comparison with Bovine Serum Albumin and Egg Lecithin, *Lebensm. Wiss. Technol. 17*:213–216 (1984).
23. Dutilh, C.E., and W. Groger, Improvement of Product Attributes of Mayonnaise by Enzymic Hydrolysis of Egg Yolk with Phospholipase A_2, *J. Sci. Food Agric.* 32:451–458 (1981).
24. Mine, Y., Structure and Functional Changes of Hen's Egg Yolk Low-Density Lipoproteins with Phospholipase A_2, *J. Agric. Food and Chem. 45*:4558–4563 (1997).
25. Kumar, S.A., and S. Mahadevan, Physicochemical Studies on the Gelation of Hen's Egg Yolk Delipidation of Yolk Plasma by Treatment with Phospholipase-C and Extraction with Solvent, *Ibid. 18*:666–670 (1970).

[Received December 18, 2004; accepted November 8, 2004]