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# Microstructural Changes of Mayonnaise during Storage

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# 마요네즈 저장 중 미세구조의 변화

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**Abstract**

The microstructural changes of mayonnaise during storage were examined by light microscopy (LM) and scanning electron microscopy (SEM). Fresh mayonnaise was composed of heterogenous population of dispersed spherical oil droplets and droplet size was normally distributed with one mode. During storage at 60 °C and -10 °C, a shift in droplet size distribution toward larger droplets was observed, as a result of coalescence of lipid droplets. Turbidimetric study also confirmed that coalescence was occurring during this accelerated aging treatments. Measurements obtained from SEM microgrphs provided better determination of smaller droplets and resulted in lower mean diameter of droplets than those obtained from LM. From these results, SEM was found to be an advantageous method of examining emulsion products as compared to LM, providing a better resolution of small droplets and a more representative view of droplet distribution, as dilution of the sample was avoided.

Key words: mayonnaise, microstructure, LM, SEM, emulsion stability

## Introduction

mayonnaise, a typical food of oil-in-water emulsions containing vegetable oil, vinegar and an emulsifying agent (usually egg yolk). Due to the high volume of oil phase (70-80%)[1] emulsion stability can become a problem in mayonnaise after prolonged storage. Changes in emulsion stability may occur through the processes of creaming, flocculation, coalescence and oiling off which represent physical changes in the dispersed droplets. It is known that droplet size and surface layer of droplets are key factors which influence emulsion stability[2,3]. Thus, several methods to determine droplet size distribution and surface layer of emulsion droplets has been developed. These include light scattering[4,5], the Coulter counter[6,7], and microscopy[8-10].

Measuremes of mayonnaise droplet size distribution using a Coulter counter is not a very satisfactory when a wide range of particle sizes is involved, since very small particles in relation to the aperture size are not adequately resolved, and large particles tend to clog the aperture[11]. Light scattering technique is rapid and reproducible, but has been reported to be only acceptable when an average droplet size rather than a size distribution[12].

Light microscopy has been used most widely to determine the droplet size distribution of emulsions. Some problems of this technique include: 1) lack of magnification hindering accurate sizing of small droplets, 2) mistaking of other particles for oil droplets[11], 3) non-uniform distribution of droplets over the counting area, 4) time consuming and tedious[12]. Scanning electron microscopy has not been employed widely in the study of emulsion products, due to difficulties encountered in fixation of samples prior to observation.

In this study, two different brands of mayonnaise were examined by LM and SEM to compare

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the information provided by these techniques. Furthermore, microstructural changes of mayonnaise stored at high and low temperature were observed by LM and SEM, which will be explained in relation to emulsion stability.

## Materials and Methods

### Materials

Two differnt brands of fresh mayonnaises were purchased from a local supermarket. To observe the changes in emulsion stability of mayonnaise during storage, accelerated aging tests (high and low temperature stress treatments) were performed; samples were stored at 60 °C and −10 °C, and picked up at 1 and 3 days of storage, respectively.

### Light microscopy

Samples (~0.5g) of mayonnaise were diluted with 20 m*l* of 10% glycerol in 0.1% sodium dodecyl sulfate (SDS) solution (to inhibit droplet flocculation) and 2 m*l* of 2% sudan IV stain, and shaken vigorously in a capped vial. After two hours, smears of the samples were examined and photographed under bright field illumination by 20X objective, using a Olympus microscope and a 35 mm camera loaded with Fuji microfilm HR II. Nine fields, covering a range of locations within the smear, were examined. Droplet size measrements were made from photographic prints. Three hundred droplets were measured representatively from the nine photographs of the sample. This procedure was repeated with three mayonnaise samples from the same jar.

### Scanning electron microscopy

Small samples of mayonnaise, approximately 3 $mm^3$ in size, were dropped into vials containing 3% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.4) at room temperature and left overnight at 4 °C. Fixation was followed by three buffer rinses, 10 min each, and post-fixed in 1% osmium tetroxide ($OsO_4$) for 10 hrs at room temperature using 0.1M sodium phosphate buffer (pH 7.4). The samples were rinsed in phosphate buffer, three times for 10 min each, followed by dehydration through a series of graded acetone solutions. The samples were critical point dried using liquid carbon dioxide in a Parr Critical Point Dryer, mounted on aluminum stubs with silver paint, gold/palladium coated in a JEOL ion sputter coating unit, and observed with an JEOL 35 SEM at an accelerating voltage of 15KV.

### Turbidity measurements

Samples of mayonnaise wer diluted with 0.1% SDS to achieve a final dilution of 1:1000. The absorbance of the diluted emulsions were measured at 500 nm using a Spectronic 20 digital spectrophotometer[4]. The measured absorbance is related to the interfacial area of emulsion, and decreases in absorbance with time and temperature provide an indication of emulsion stability.

### Centrifugation

Emulsion stability is commonly measured in terms of the amounts of oil separation from an emulsion during centrifugation. In this study, samples of mayonnaise were centrifuged in 50 m*l* polypropylene tubes in a Sorval centrifuge at 11,000g for one hour, both before and after high and low temperature stress treatments. Semi-quantitative measurements of the amounts of released oil were made immediately after the tubes were removed from the centrifuge.

## Results and Discussion

### Light microscopy

A typical light micrograph of fresh mayonnaise A is shown in Figure 1a. Dur to the non-uniform distribution of droplet sizes throughout the smear, the entire range of droplet sizes found in this sample cannot be identified in this one micrograph. Measurements of nine hundred droplets resulted in the droplet size distribution shown in Figure 2. The histogram revealed that the droplet size distributions were normally distributed with one mode, typical of emulsion droplet size distributions. Difficulties were experienced in making droplet size measure-

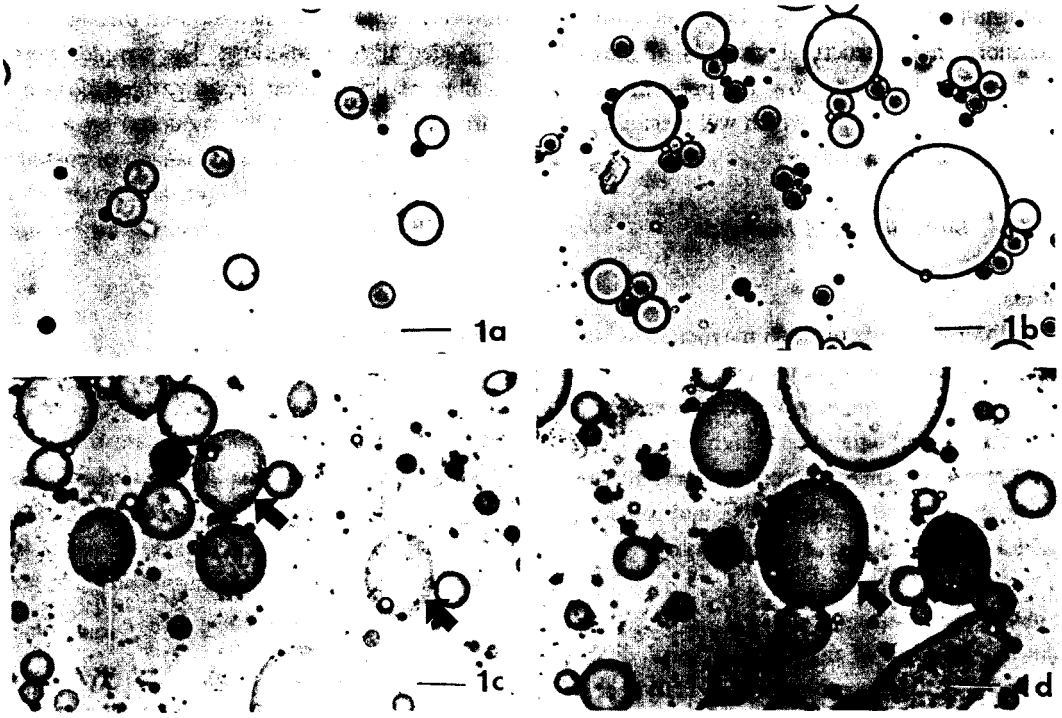


Fig. 1. Light micrographs of mayonnaise A. 1a. Fresh mayonnaise A 1b. Mayonnaise A stored at −10°C for 1 day. 1c and 1d. Mayonnaise A stored at −10°C for 3 days. Separated oil droplets without surface layer were seen (arrows) (bar = 10 μm)

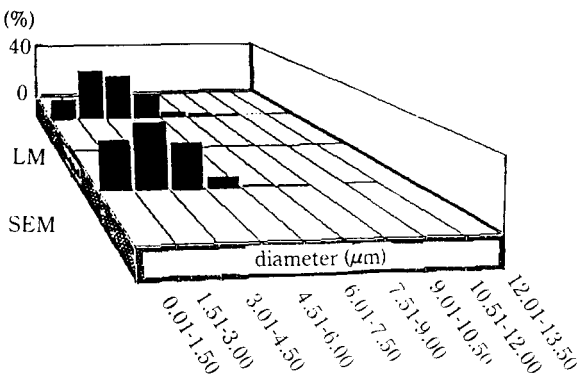


Fig. 2. Frequency distribution of lipid droplets sizes determined by LM and SEM of mayonnaise A

ments. Small droplets (<0.5 μm) were difficult to identify and measure, which may contribute to the overestimation of droplet size by LM.

The mean droplet diameter determined for fresh mayonnaise A from LM micrographs was 3.45 μm and mayonnaise B was 5.07 μm, which is significantly different at $P<0.01$. This may be due to the different formulars of mayonnaises, types of homogenizers used and the intensities at which they operate.

### Scanning electron microscopy

The emulsions of mayonnaise were composed of a heterogenous population of dispersed spherical oil droplets, as shown in Figure 3a. Chemically fixed, dehydrated and critical point dried samples of mayonnaise revealed that structure could be visualized only in the regions of the sample that were both well-fixed by osmium tetroxide and undisturbed by physical actions during procecessing. In interior regions of the samples, where the fixatives had not penetrated adequately, the lipid was solublized during dehydration, resulting in the empty network of continuous phase proteinaceous solids found in Figure 3b.

Droplet size measurements of mayonnaise were obtained from SEM micrographs at a total magnifi-

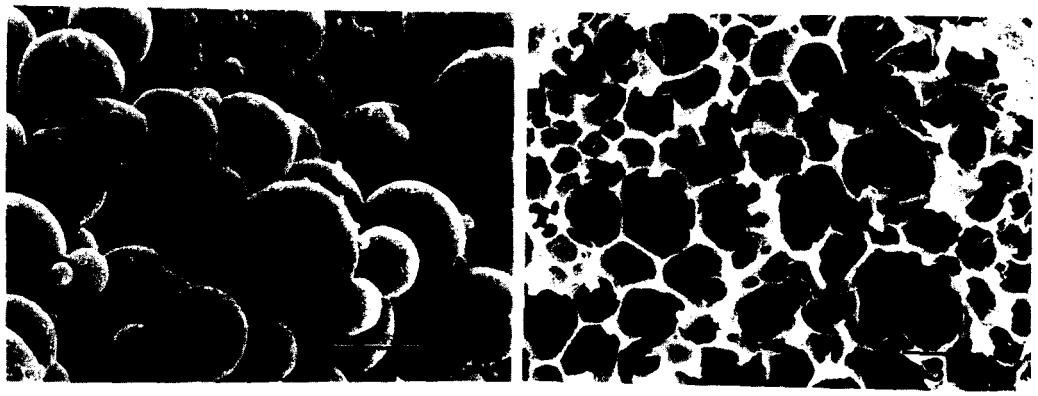

Fig. 3. Scanning electron micrographs of mayonnaise (bar = 10 um)

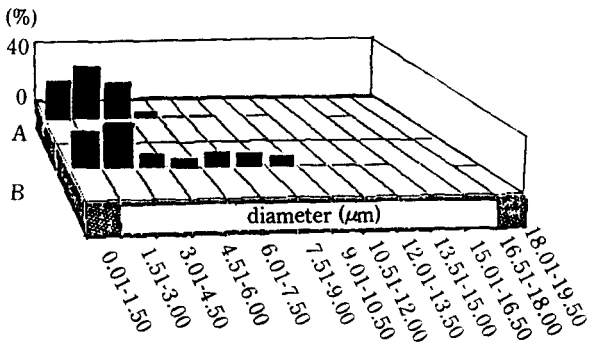


Fig. 4. Frequency distribution of lipid droplets sizes determined by SEM of mayonnaise A and B

cation of 2000X. Nine hundred droplets were measured from nine well preserved regions of the sample. By measuring the diameter of droplets, histograms of droplet size distribution for each mayonnaise were prepared. Mayonnaise A was observed to contain more very small droplets and less heterogenous as compared to mayonnaise B, as shown by comparing the range of droplet sizes of 0.5 $\mu$m to 14.5 $\mu$m for mayonnaise A to the range of 0.7 $\mu$m to 20.0 $\mu$m for mayonnaise B (Figure 4). The mean droplet diameters for two brands (A:2.75 $\mu$m, B: 4.31 $\mu$m) were found to be significantly different at $p<0.01$. This confirms the result obtained from LM micrographs.

However, the mean droplet diameters determined by SEM (A: 2.75 $\mu$m, B: 4.31 $\mu$m) was lower than those obtained by LM (A: 3.45 $\mu$m, B: 5.07 $\mu$m). It would appear from the histogram that a significant number of droplets (less than 1.5 $\mu$m in diameter) were missed in the LM measurements (Figure 2). This may be due to the greater resolution and depth of field afforded by SEM comparing to LM. Thus, small droplets (<1.5 $\mu$m) can be easily visualized under SEM, as shown by comparing the ratio of small droplets (<1.5 $\mu$m) of 28% by SEM to the ratio of 14% by LM. From the data gathered in this experiment, it appeared that SEM method was more appropriate for emulsions with small droplets (less than 1.0 $\mu$m of diameter) and more useful technique for determining droplet size distributions of emulsions.

### Accelerated aging tests

Storage of mayonnaise at 60 °C and −10 °C would nocommonly occur in commercial practice, however, these high and low temperature stress conditions were employed to accelerate the destabilization of mayonnaise.

Following high had low temperature stress treatments, changes in emulsion stability with time was observed as the tendency for the droplet size to increase with time, resulting in more irregularly shaped, larger droplets (Figure 5b, 5c, 6b and 6c). Droplets of separated oil were often observed in LM micrographs of stored mayonnaise (Figures 1c and 1d). Combined histograms obtained for fresh and stored mayonnaise A at 60 °C are shown in Figure 7. The droplet size distribution of the stored sample has longer tail to the right than fresh sample, which indicates the coalescence of lipid dro-

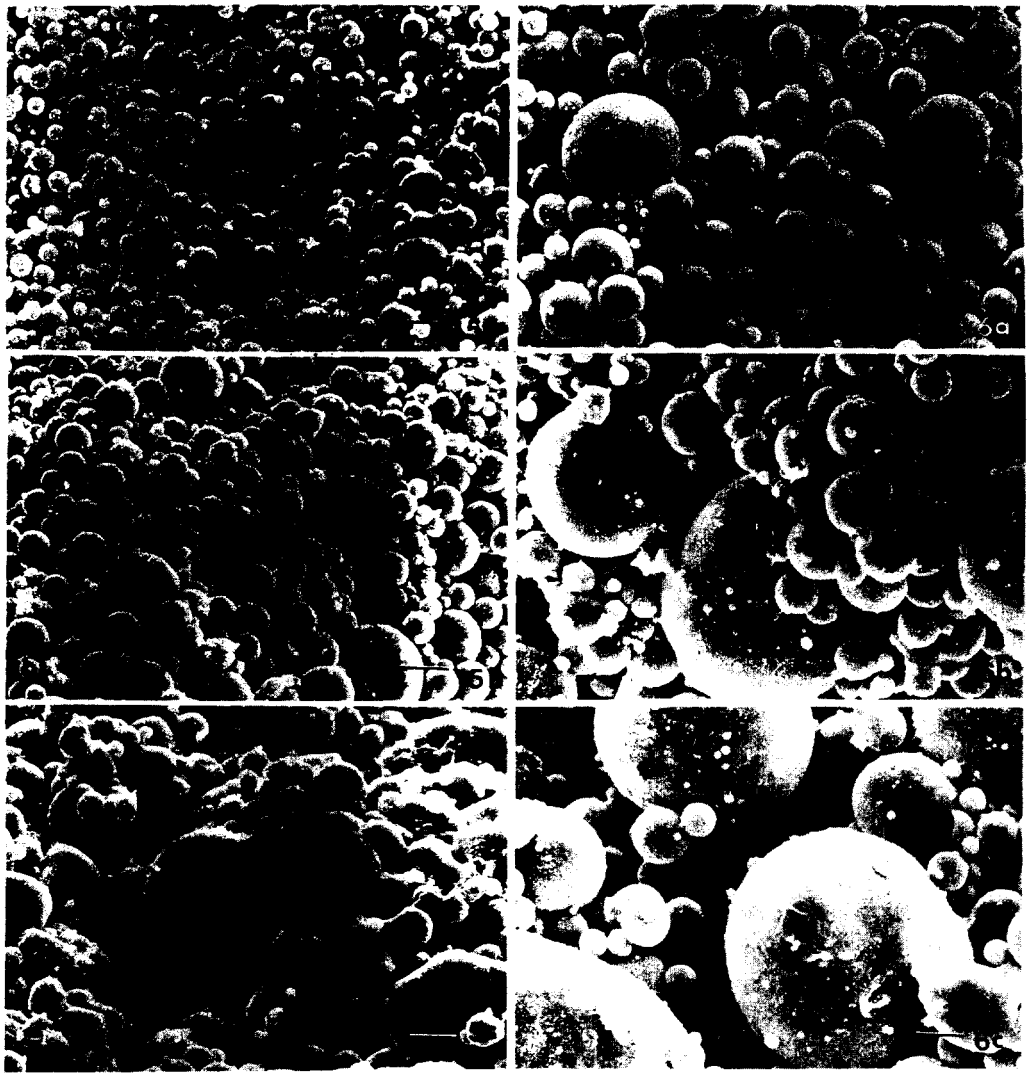

Fig. 5. Scanning electron micrographs of mayonnaise A. 5a. Fresh mayonnaise A. 5b. Mayonnaise A stored at 60 °C for 1 day. 5c. Mayonnaise A stored at 60 °C for 3 days (bar = 10 $\mu$m)
Fig. 6. Scanning electron micrographs of mayonnaise B. 6a. Fresh mayonnaise B. 6b. Mayonnaise B stored at −10 °C for 1 day. 6c. Mayonnaise B stored at −10 °C for 3 days (bar = 10 $\mu$m)

plets with time during storage at 60 °C. The mean droplet diameter for fresh mayonnaise A was 2.75 $\mu$m, as compared to 3.26 $\mu$m and 3.50 $\mu$m for the mayonnaise stored for 1 and 3 days, respectively. The mean droplet diameter of mayonnaise B increased from 4.31 $\mu$m to 4.80 $\mu$m after 3 days of storage at 60 °C. This increase of droplet diameter may be due to the increases in Brownian motion[2,3], increased solubilization of the surfactant, melting of the fat crystals and possibly changes in the electrical double layer around lipid droplets at high temperature.[11]

Data were also gathered from mayonnaise A and B stored at −10 °C for 3 days (Table 1). The mean droplet diameter of mayonnaise A increased from 2.75 $\mu$m to 3.65 $\mu$m after 3 days of storage at −10 °C, whereas mayonnaise B from 4.31 $\mu$m to 4.97 $\mu$m in the same period. At freezing temperature, possibly

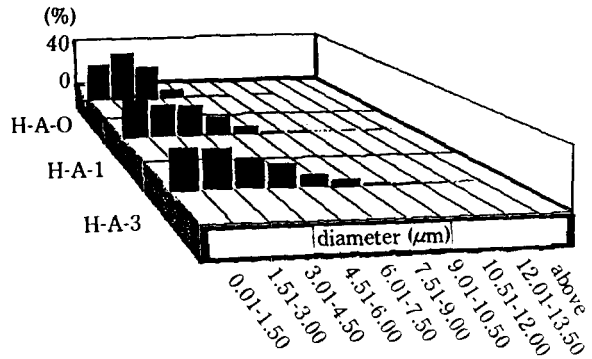


Fig. 7. Frequency distribution of lipid droplets sizes determined by SEM of mayonnaise A stored at 60 °C. H-A-O: fresh mayonnaise A. H-A-1: mayonnaise A stored for 1 day. H-A-3: mayonnaise A stored for 3 days

the changes of fat crystallization may contribute to the increased rates of droplet coalescence[13].

Results obtained from turbidimetric and centrifugation studies also indicated that during storage at 60 °C and −10 °C for 3 days, two different mayonnaise became increasingly unstable dut to droplet coalescence. The measurements of absorbance at 500 nm, which is related to the interfacial area, were significantly higher in fresh than in stored mayonnaises (Table 1). As the droplets coalesce, the total interfacial area of a given weight of sample decreases, since larger droplets have less surface area than an equivalent volume of smaller droplets.[5]

The significant differences in absorbance values for mayonnaise A and B suggest that there is a larger interfacial area in mayonnaise A. As presented in Figure 4, 5a, 6a and Table 1, mayonnaise A contained more very small droplets and had lower mean diameter than mayonnaise B did. Thus, mayonnaise A has larger surface area and shows higher absorbance at 500 nm, even though other ingredients, such as sugar, salt, and egg yolk may also influence the turbidity of the diluted emulsions. Therefore, conclusions regarding significant turbidity differences between two brands can be interpreted from the differences between mean droplet diameters.

Evidence that coalescence was occurring during storage was also obtained from centrifugation of fresh and stored samples. The amount of oil separating from the samples during centrifugation is related to the degree of oil droplet coalescence. In fresh samples, only a thin film of oil formed on the top of all samples after 1 hr of centrifugation at

Table 1. Mean droplet diameter of mayonnaises determined by LM and SEM (simple arithmetic mean) and turbidity measurements at various treatment stages

| Treatment | SEM (um) Mean ± S.D. | LM (um) Mean ± S.D. | O.D. 500 nm Mean ± S.D. |
|---|---|---|---|
| H-A-O [a)] | 2.75 ± 1.41 [a, 1b), c)] | 3.45 ± 1.71 [a, 2] | 0.997 ± .010 [c, d)] |
| (H-B-O) | (4.31 ± 3.53)$^{A,1}$ | (5.07 ± 3.00)$^{A,2}$ | (0.689 ± .018)$^{C}$ |
| H-A-1 | 3.26 ± 2.26 $^{b,1}$ | 3.57 ± 2.50 $^{b,2}$ | 0.740 ± .021 $^{b}$ |
| (H-B-1) | (4.60 ± 3.76)$^{B,1}$ | (5.26 ± 3.50$^{B,2}$ | (0.577 ± 0.26)$^{B}$ |
| H-A-3 | 3.50 ± 2.52 $^{c,1}$ | 4.05 ± 4.43 $^{c,2}$ | 0.665 ± .011 $^{a}$ |
| (H-B-3) | (4.80 ± 5.14)$^{B,1}$ | (6.70 ± 4.54)$^{C,2}$ | (0.431 ± .015)$^{A}$ |
| F-A-O | 2.75 ± 1.41 $^{a,1}$ | 3.45 ± 1.71 $^{a,2}$ | 0.997 ± .010 $^{c}$ |
| (F-B-O) | (4.31 ± 3.53)$^{A,1}$ | (5.07 ± 3.00)$^{A,2}$ | (0.689 ± .018)$^{C}$ |
| F-A-1 | 3.37 ± 3.01 $^{b,1}$ | 3.33 ± 4.76 $^{a,1}$ | 0.701 ± .017 $^{b}$ |
| (F-B-1) | (4.56 ± 3.38)$^{B,1}$ | (4.90 ± 4.06)$^{A,2}$ | (0.528 ± .071)$^{B}$ |
| F-A-3 | 3.65 ± 3.71 $^{c,1}$ | 4.58 ± 5.60 $^{c,2}$ | 0.575 ± .078 $^{a*}$ |
| (F-B-3) | (4.97 ± 5.45)$^{C,1}$ | (7.32 ± 9.19)$^{C,2}$ | (0.367 ± .024)$^{A}$ |

[a)] H and F means heat and freezing treatments, respectively. A and B are brands of mayonnaises, 0, 1, 3 is period of storage (day)

[b)] Values with different superscript letters (a, b, c and A, B, C) within a column are significantly different at $p<0.05$, (Z test)

[c)] Values with different superscript numbers (1, 2) within a row are significantly different at $p<0.05$ (student's t test)

[d)] $n = 5$

12000g. After 3 days at 60°C and −10°C, however, 3 to 8% (w/w) of the emulsion separated out as oil after centrifugation. There was no significant difference observed between mayonnaise A and B in the degree of oil separation.

## 요 약

마요네즈 저장 중 미세구조의 변화를 광학현미경과 주사 전자현미경으로 관찰하였다. 신선한 마요네즈는 다양한 크기의 지방구로 이루어져 있었으며 지방구의 크기 분포는 정규분포를 보였다. 60°C와 −10°C에서 저장하는 동안 지방구의 合一에 의해 지방구가 커지는 경향을 보였으며, 탁도에 의한 실험결과 또한 이러한 지방구의 合一현상을 확인시켜 주었다. 전자현미경은 광학현미경에 비해 크기가 작은 지방구를 측정하기가 용이하였으며, 따라서 지방구의 평균 입경이 작았다. 이것은 전자현미경의 높은 해상력과 심도 때문이며, 더우기 시료를 희석할 필요가 없어 균일한 지방구의 분포를 보여주는 전자현미경 방법은 광학현미경에 비해 유화제품의 지방구 분포를 측정하기에 유리한 방법이라 하겠다.

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