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Stability and Uniformity of Oil Droplets in Preparation of O/W Emulsion Agar Gel

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We investigated the stability of a monodispersed o/w emulsion and the uniformity of oil droplet distribution in an agar-based emulsion gel during successive stages of gel preparation. At each stage of the emulsion-sol preparation, almost no differences in the droplet diameter and number in excess of the range of measurement error were found. These results directly indicate that the monodispersity of the emulsion was maintained before and after heating of the agar sol. To evaluate oil droplet uniformity in the emulsion gel, sections of 5 mm in height were cut from the top, middle and bottom of the emulsion gel. No differences were found in the number of oil droplets and the coefficient of variation of droplet sizes in top, middle and bottom sections of the gels with oil volume fractions ranging from 0.01 to 0.27 in any of the emulsion gels.

Keywords: emulsion gel, agar, stability, uniformity, monodispersed o/w emulsion

An emulsion gel containing oil droplets is a good food model. Many authors writing on gel formation have studied proteins such as soybean (Yamano *et al.*, 1981) and gelatin (Hinode & Kawamura, 1994), and polysaccharides such as starch (Kawabata, 1993), agar (Shiraki & Kainuma, 1977) and pectin (Watase, 1982). Oil droplet size and oil volume fraction have important effects on the texture and rheological properties of the emulsion gels (Matsumura *et al.*, 1993; McClements *et al.*, 1993; Yilmazer & Kokini, 1991). Most studies, however, have reported the behavior of polydispersed o/w emulsions. To discuss the basic rheology of the emulsion gel, it is necessary to prepare a monodispersed emulsion. In a previous paper (Chen *et al.*, 1993), we already reported a membrane emulsification method to prepare a monodispersed o/w emulsion. It may be predicted that it would be difficult to obtain a monodispersed o/w emulsion with controlled stability because during preparation, while undergoing heating and other procedures, uniformity of oil globule distribution may be lost by creaming and coalescence before the gel gelatinizes. Our purpose in the present study was to examine the stability of oil droplets in the monodispersed o/w emulsion and the uniformity of oil droplet distribution in the emulsion gels during emulsion and gel preparation. We employed agar as a gelling agent because of its ability to gel at low concentration.

Materials and Methods

A monodispersed o/w emulsion was prepared using an aqueous solution containing 1% (w/v) emulsifier (polyglycerine fatty acid) and corn oil by a membrane emulsification method (Chen *et al.*, 1993). Agar emulsion gel was prepared by the gelling method of Isozaki *et al.* (1976) and Odake *et al.* (1990) with modifications. The preparation procedure for producing emulsion agar gel is shown in Fig. 1. Several samples with an oil volume fraction ranging from 0.01 to 0.27 were produced by either dilution or concentration of an emulsion with an initial volume fraction of about 0.1. The concentration of the emulsion was performed by centrifugation at $1000\times g$ for 10 to 30 min. No change was found in emulsion droplet diameter following centrifugation. Agar powder (2%) was then dispersed in the emulsion. These dispersions were stirred gently at 20°C for 60 min, then heated, first at 70°C for 30 min, and then at 90°C for 30 min. After being degassed for 15 min, these emulsions were poured into cylindrical cells (inside diameter 24 mm, height 38 mm) to a height of about 24 mm, cooled for 1 h and stored at 3°C in a refrigerator for 18 h to obtain sufficiently hard gels.

Measurements of oil droplet size distribution and number of oil droplets in emulsion At each stage of preparation, a small volume of the sample was taken from the emulsion agar sol and diluted with water. The droplet number per 0.01 mm^2 on a hollow slide glass was determined by image analyzing optical microphotography using an image analyzer (Luzex IIIU: NIREKO Co., Ltd.). To express the monodispersity of the o/w emulsion, a coefficient of variation (CV) was calculated as follows;

$$CV\ (\%)=(SD/D)\times 100$$

where SD is the standard deviation of the oil droplet size distribution in the emulsion and D is the mean diameter of the droplets.

Evaluation of oil droplet uniformity in the emulsion gel After removal from the cell, the sample cylinder was reduced to 24 mm in diameter and 19 mm in height by cutting off the top 5 mm to remove the effects of evaporation and the meniscus. Sections of 5 mm in height were cut from the top, middle and bottom of the gels after removal from the cylindrical cells and were further subdivided into samples of 1 g each. Each sample was then dissolved in distilled water in a water bath at 90°C for 30 min. The resulting solution was poured onto a hollow slide glass and warmed by steaming over a water bath. Oil droplet uniformity was measured by the image analysis described above.

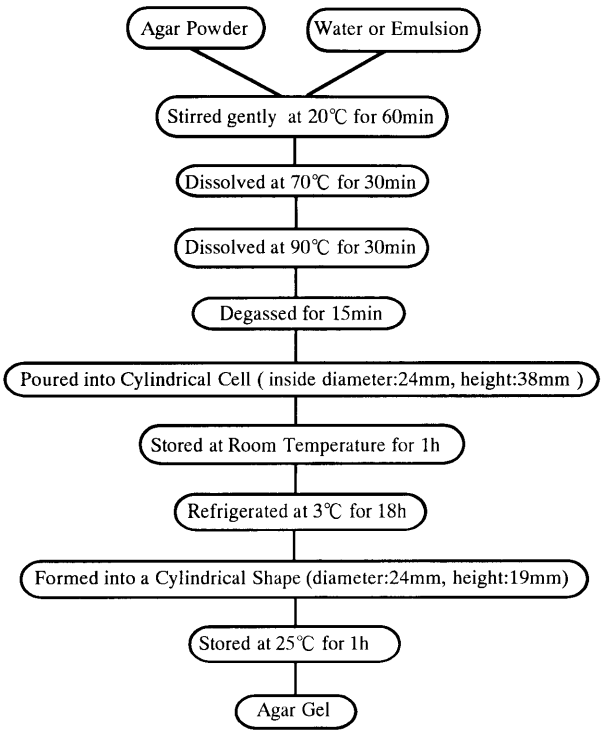

Fig. 1. Preparation of agar gel.

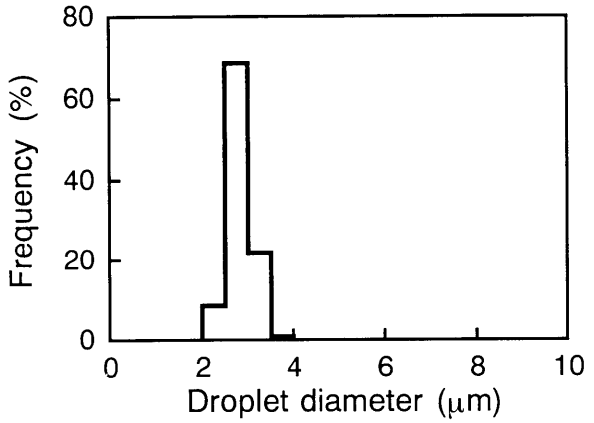

Fig. 2. Distribution of droplet size in emulsion prepared by membrane emulsification.

Table 1. Size and number of oil droplets in emulsion containing agar.

Oil volume fraction		Emulsion in agar sol		
		20℃ 1 h	20℃ 1 h +70℃ 30 min	20℃ 1 h+70℃ 30 min +90℃ 30 mmin
0.05	Size (μm)	3.1± 0.0	3.1± 0.1	3.2± 0.0
	Number	1630.0±59.9	1613.6±33.6	1632.0±47
0.20	Size (μm)	3.2± 0.1	3.3± 0.1	3.3± 0.0
	Number	1493.8±25.0	1503.0±58.8	1491.6±57.2

Results and Discussion

The droplet size distribution of the emulsion which was prepared using a membrane of pore size diameter 0.73 μm is illustrated in Fig. 2. Mean droplet size was found to be 3.22 μm. The diameter and the number of droplets in the same emulsion-agar sol at successive stages of preparation are shown in Table 1. At each stage, almost no differences in the droplet size or number in excess of the range of measurement error were found. Droplet size distributions of emulsions before and after heating are shown in Fig. 3. In emulsions heated at 90℃ for 30 min, the coefficient of variation increased slightly but the monodispersity of the emulsion was proved to be maintained because the coefficient of variation was below 10%. From these results, it can be concluded that

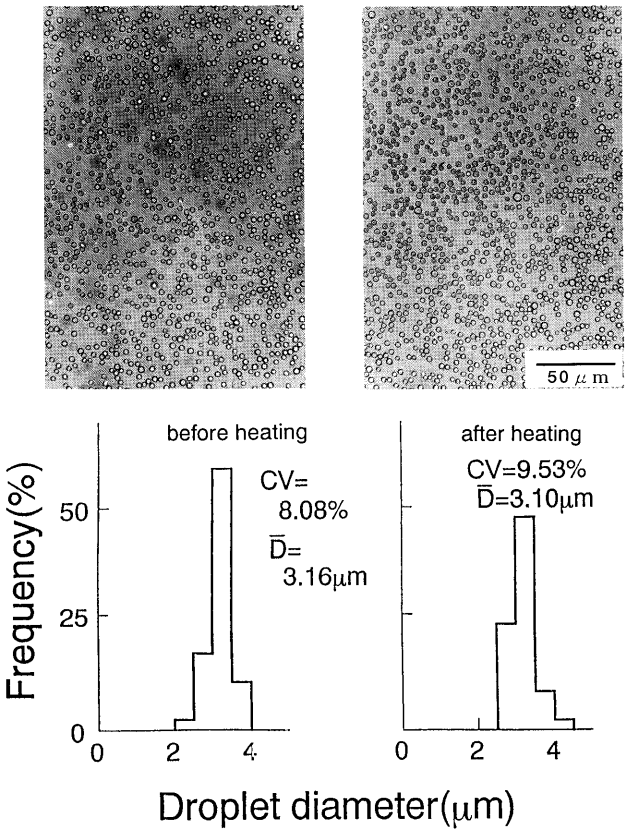

Fig. 3. Distribution of droplet size in emulsions.

Table 2. Number of oil droplets in emusion gel.

Oil volume fraction	Top	Middle	Bottom
0.01	1683	1764	1751
0.02	2469	2550	2600
0.13	1590	1614	1660
0.17	2331	2313	2331
0.18	1561	1600	1597
0.27	2250	2320	2327

Droplet number was estimated in an area of 0.1 mm^2 under a microscope.

Table 3. Droplet size and coefficient of variation of oil droplet distribution in emulsion gel (ϕ: 0.05).

	Top	Middle	Bottom
Mean diameter (μm)	3.34	3.35	3.29
Coefficient of variation (%)	11.2	12.9	8.03

the stability of oil droplets can be reliably maintained throughout the preparation of emulsion gels. We think the monodispersity of the emulsion droplets imparted high stability to the emulsion.

Table 2 shows the number of oil droplets in the top, middle and bottom sections of gels with oil volume fractions ranging from 0.01 to 0.27. There was no difference in the number of droplets in the top, middle or bottom sections of any of the emulsion gels. Mean droplet size and the coefficient of variation in an emulsion gel with 0.05 of oil volume fraction are presented in Table 3. There was no significant difference in the mean droplet size or number of oil droplets between sections of the same emulsion gel. Similar results were obtained for the other oil fractions. Agar is a gelling agent which can gelate at temperatures below 30°C and can form a gel at concentrations as low as 0.1–0.2%. It is also well known that agar gel is a three-dimensional network consisting of long molecular chains, with junctions partially supported by cross-linking and containing bound water molecules. Xiong *et al.* (1991) reported that slow heating formed a stronger gel matrix than rapid heating. In the present study, the preparation period for emulsion gels of 4 h is considered sufficient to form an effective gel matrix. Fat globules reinforce agar gels by playing the role of 'filler' in the voids in the gel matrix (Aguilera & Kessler, 1989). Stability of the emulsion was established by using protein gels such as whey protein isolate (McClements *et al.*, 1993), milk protein (Xiong *et al.*, 1991), soy protein fraction (Rivas & Sherman, 1983), whey protein (Rolf *et al.*, 1986) and alfalfa leaf protein (Barbeau & Kinsella, 1987), etc. These authors noticed that oil droplets were stabilized by protein which incorporated the oil droplets into the three-dimensional structure of the gel. We believe the emulsion uniformity was maintained by the gel matrix of agar.

In the present investigation, we could prove the stability and uniformity of oil droplets of emulsion gels in a polysaccharide gel prepared with agar.

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