A Simple Method for Determining the Flaxseed or Fish Oil Content with N, N-dimethylformamide in Microcapsules Prepared by Spray Drying

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A simple method was developed for determination of the oil content in spray-dried microcapsules by dissolving the microcapsules in N, N-dimethylformamide (DMF). Powders containing flaxseed or fish oils were prepared by homogenization and subsequent spray drying to form coarse and fine emulsions. Sodium caseinate (SC) and maltodextrin (MD) were used as an emulsifier and wall material, respectively. The oil contents in the powders were quantified using a thin-layer chromatography-flame ionization detector after hexane extraction; the results agreed well with the initial oil contents on a dry basis in the feed emulsion, regardless of the oil-droplet size in the powders. The fatty acid compositions of the flaxseed and fish oils in the powders were obtained without an extraction process, using the proposed method. After dissolving the microcapsules in DMF, the peroxide value (POV) of the encapsulated oil was determined using the acetic acid-chloroform method. Although SC, MD, and DMF slightly increased the POV, the effect of the increase on the POV was not significant. The proposed method therefore enables easy and rapid estimation of the oil content, fatty acid composition, and POV of an encapsulated oil.

Keywords: fatty acid composition, fish oil, microencapsulation, peroxide value, spray drying

1. Introduction

Microencapsulation has been widely used for the preparation of powdered edible oil products, because it can provide prolonged shelf-life by protecting oils with appropriate encapsulating substances such as milk protein, dextrin, modified cellulose, gelatin, plant gums, and modified starch [1–3]. Microencapsulation is used in the food industry to protect marine-based fish and fish oils, which are rich in ω-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These PUFAs are well known to have a variety of health benefits such as anti-inflammatory, anticancer, antioxidant, and insulin-sensitizing effects [4–6]. In the food industry, the most widely used techniques for encapsulating lipophilic compounds are based on the production of an oil-in-water emulsion, followed by either spray drying, freeze drying, molecular inclusion, enzymatic gelation, or coacervation. Among the various techniques, spray drying is the most common, because of its low cost and availability of equipment. The advantages of spray drying also include continuous operation, with the possibility of automatic control and constant product quality at high throughput rates. The disadvantage of this technique is the high-temperature conditions necessary for drying, and exposure to air. Moreover, during drying, some of the product may adhere to the surfaces of the capsules, resulting in potential oxidation of the final food products. In order to obtain a high loading efficiency, even if the wall material is suitable, optimal spray-drying conditions must be used [7–9]. The main factors in spray drying that should be optimized are the inlet and outlet air temperatures. The final product is evaluated in terms of loading efficiency [10–12], particle size [13–15], reconstituted oil-droplet size [16], density [17], morphology [13–16], fatty acid composition [11, 12], peroxide value (POV) [16–18], and thiobarbiturate value [18], when needed. In some analyses, the isolation of oil from microcapsules is required. This affects the analytical results.

The most commonly used methods for extracting and purifying lipids from foods are the Röse–Gottlieb [19], Folch [20], and Bligh and Dyer [21] methods. In most of the published studies, the loading efficiency, fatty acid composition, and POV of the encapsulated lipid were
determined after solvent extraction of the lipid using several solvent systems. Hogan et al. [22] used petroleum ester extraction, according to the Röse–Gottlieb method, for soy oil/sodium caseinate (SC) powders. Baik et al. [23] used a hexane/isopropanol (3:1, v/v) mixture to extract fish oil from microcapsules prepared with a corn syrup and SC, after washing them with hexane. Drusch et al. [24] used an ethanol/hexane/ethyl acetate mixture for extracting fish oil from microcapsules made with modified starch. Partanen et al. [25] used an isooctane/isopropanol (2:1 v/v) mixture for extracting flaxseed oil from whey protein isolate/lactose powders. Kolanowski et al. [26] used Soxhlet extraction with petroleum ester for fish oil/maltodextrin (MD) microcapsules. Although these methods are efficient for extracting lipids with a wide range of hydrophobicities, they involve many manual operations, and the analyses are time consuming. It has been shown that the use of different methods results in different lipid recoveries, as confirmed by a comparative study [27]. Thus, in the existing analysis methods, it is important to choose the optimal extraction solvent for microcapsules. Since \(N,N\)-dimethylformamide (DMF), a hydrophilic aprotic solvent, could solubilize the component of microcapsule such as oils, carbohydrates and proteins, it is expected that analysis methods using DMF for spray-dried powder containing emulsified flaxseed oil or fish oil would be developed.

The aim of this study is to develop a new and rapid method for determining the loading efficiency, fatty acid composition, and POVs of the oil in spray-dried powders. Much attention has recently been paid to encapsulation efficiency and oxidation stability of spray-dried powder with nano–ordered oil–droplets [13, 24, 28]. However, it might result in lower extraction recovery of oil from spray-dried powders in solvent extractions since nano–ordered emulsions often improve the physical stability [29]. Therefore, the spray-dried powders containing large and small oil–droplet were prepared using a rotor–stator and high–pressure homogenizer as follows.

The carrier solution was prepared by dissolving MD as a wall material in distilled water at 60°C and cooling to room temperature. Flaxseed oil or fish oil, as a core material, was made up to 30 or 40 wt% with respect to solid powders that consisted of MD and SC. The oil was blended with the carrier solution to give total–oil and solid contents of 37.9 wt% or 60 wt%. The compositions of the feed emulsion were 11.4 wt% oil, 1.1 wt% SC, 25.4 wt% MD, and 62.1 wt% distilled water for flaxseed oil, and 24.0 wt% oil, 1.8 wt% SC, 34.2 wt% MD, and 40 wt% distilled water for fish oil. The different wall material systems of the individual samples are summarized in Table 1. The sample was homogenized using a rotor–stator homogenizer (PT–6100, Kinematica, Littau, Switzerland) at 8000 rpm for a total time of 3 min, with a 30 s interval between every minute of homogenization. The homogenized emulsion was further subjected to high–pressure homogenization to obtain a fine emulsion with smaller oil–droplets. The emulsion was then cooled to 15°C in a water bath.

### 2. Materials and Methods

#### 2.1 Materials

SC, containing 94% protein, was obtained from the Mitsubishi–Kagaku Foods Corp. (Tokyo, Japan). MD (Pinedex #3) with a dextrose equivalent value of 25 was purchased from the Matsutani Chemical Industry Co., Ltd. (Itami, Japan). Flaxseed oil was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Fish oil, reported to contain 6.4% EPA and 25.1% DHA, with no added antioxidant, was purchased from the NOF Corporation (Tokyo, Japan). Both oils were maintained at −80°C until use. Other chemicals used were of analytical grade and obtained from Wako Pure Chemical Industries Ltd.

#### 2.2 Preparation of feed emulsions

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### Table 1 Compositions of emulsions and spray–dried powders.

| Sample     | Composition (wt%) | Oil  | MD   | SC   | Water |
|------------|-------------------|------|------|------|-------|
| Flaxseed oil Emulsion | 11.4 | 25.4 | 1.1  | 62.1 |
| Powder     | 30.0              | 67.0 | 3.0  | -    |
| Fish oil   Emulsion  | 24.0 | 34.2 | 1.8  | 40.0 |
| Powder     | 40.0              | 57.0 | 3.0  | -    |
2.3 Spray drying of emulsions
The feed emulsions were fed to a pilot-scale spray-dryer (Ohkawara L-8, Ohkawara Kakouki Co., Ltd., Yokohama, Japan) equipped with a centrifugal atomizer. The detailed configuration of the spray dryer has been described elsewhere [30]. The feed rate was 25 mL/min for flaxseed oil powder and 30 mL/min for fish oil powder. The atomizer speed was 30000 rpm, and the air flow rate was set at 110 kg/h. In the drying process, the inlet air, at 180°C for flaxseed oil powder and 140°C for fish oil powder, took its natural course along the flow to settle between 80 and 90°C at the outlet. After cooling to room temperature, the collected powders were stored in closed glass containers at -30°C until use.

2.4 Analysis of oil-droplet and powder-particle diameters
The size distributions of the oil-droplets in the reconstituted emulsions and of the spray-dried powder-particles were determined using a laser-diffraction particle size analyzer (SALD-7100, Shimadzu Corporation, Kyoto, Japan) equipped with a batch sample cell by the method from Soottitantawat et al. [28]. The reconstituted emulsions were obtained by dissolving the spray-dried powders in distilled water. The reconstituted emulsions were pipetted directly into the cell, which contained distilled water. The particle size distributions of the spray-dried powders were determined by dispersing the powders in 2-methyl-1-propanol in the cell. The volume-average diameter, D_43, was regarded as the mean diameter for all measurements.

2.5 Moisture content
The moisture content of each sample (1.5 g) was determined from the weight loss in an infrared moisture analyzer (HB43, Mettler-Toledo AG, Grifensee, Switzerland) at the drying temperature of 160°C until the weight was stable for 50 s.

2.6 Surface-oil
The spray-dried powders were washed with hexane by dispersing 0.3 g of the powders in 5 mL of hexane and vortexing for 15 min (Vortex Gene2, Scientific Ind. Inc., New York, USA). The post-wash hexane (1 μL) was spotted on S-III chromarods (Mitsubishi Chemical Medience, Tokyo, Japan). Quantification of the oil contents on the rods was performed using an Iatroscan MK-5 TLC-FID.

2.7 Oil content
2.7.1 DMF method
DMF (1 mL) was added to the spray-dried powder (30 mg) in a glass bottle, to dissolve the powder completely. Then hexane (1 mL) was added and the glass bottle was shaken with a vortex mixer. After dissolving the powders with a sonicator (Branson 1510, Yamato Scientific Co., Ltd., Tokyo, Japan) for 15 min, the sample was centrifuged for 10 min at 3000 rpm (average centrifugal force, 654×g) with a centrifugal separator (2010, Kubota Co., Ltd., Tokyo, Japan). A small aliquot (1 μL) of sample from the upper part (hexane layer) of the mixture was spotted on S-III chromarods, developed with diethyl ether, and oven-dried. Quantification of the oil contents of the developed rods was performed using an Iatroscan MK-5 TLC-FID.

2.7.2 Röse–Gottlieb method
Another method for quantification of the total-oil content was also used to confirm the results obtained using the DMF method. The method was based on the Röse–Gottlieb method [19], which is commonly used for quantitative measurement of fat in milk and milk powders. An aliquot (9 g) of spray-dried powder was dispersed in 180 mL of water heated at 65°C. The mixture was stirred gently, and then 25% ammonia solution (18 mL) was added, and the dispersion was heated at 65°C for 15 min in a shaking water bath. The solution was then cooled to room temperature, and the oil was extracted, using three liquid-liquid extractions, as follows: first, 30 mL of ethanol, 75 mL of hexane, and 75 mL of diethyl ether; second and third, 15 mL of ethanol, 45 mL of hexane, and 45 mL of diethyl ether. The organic layer was separated, and dried with anhydrous sodium sulfate. After filtration, the solvent was evaporated using a rotary evaporator at 40°C, and the extracted oil was cooled under vacuum and weighed.

2.8 Fatty acid composition
DMF (200 μL) was added to spray-dried powder (10 mg) in a glass bottle, to dissolve the powder completely. Methylated fatty acids were obtained using a fatty acid methylation kit (Nacalai Tesque, Kyoto, Japan), according to the manufacturer’s instructions. The fatty acid compositions of the flaxseed and fish oils in the powders were determined using a capillary gas chromatograph–mass spectrometer (GCMS–QP5050, Shimadzu).
Corporation) equipped with a fused-silica Ulbon HR-1 capillary column (30 m × 0.25 mm × 0.25 μm film thickness, Shinwa Chemical Industries, Ltd., Kyoto, Japan). The oven temperature was programmed to increase from 50 to 280°C at 3°C/min, and kept isothermally for 34 min. The injector and transfer line temperatures were both 250°C. The flow rate of the carrier gas (He) was 1.0 mL/min. Split injection with a ratio of 1:50 was used. The sample volume injected was 1 μL. The electron impact ionization mass spectrometer was operated as follows: ionization voltage, 70 eV; ion source temperature, 200°C; and scan mode, 40.0–500.0 m/z.

2.9 Peroxide value

The POV was determined using the AOCS Cd 8–53 acetic acid–chloroform procedure [31]. The spray-dried powders (1 g) were dissolved in DMF (9 mL) under sonication, followed by mixing with chloroform–acetic acid mixture (3:2, v/v; 80 mL). In the Röse–Gottlieb method, the oil (0.2 g) extracted from the spray-dried powders was poured into chloroform–acetic acid mixture (3:2, v/v; 80 mL), and then a saturated solution of potassium iodide (1 mL) was added. The mixture was shaken by hand for 1 min and then kept in the dark for 5 min. After addition of distilled water (80 mL), the mixture was titrated against sodium thiosulfate (0.002 mol/L) using a potentiometric titration system (East Ox Titrator, Mettler-Toledo International Inc., Switzerland). A blank mixture was also analyzed under similar conditions. The POV (meq/kg) was calculated using Eq. (1):

POV = \left( \frac{V_s - V_b}{M} \right) C

where C is the concentration of sodium thiosulfate (mol/L); V_s and V_b are the volumes of sodium thiosulfate exhausted by the sample and the blank, respectively (mL); and M is the mass of flaxseed oil or fish oil in the spray-dried powder (g). The POV was expressed as milliequivalents (meq) of active oxygen per kilogram of oil.

DMF, MD, and SC are present in the test sample in the DMF method, although they are not present in the reference analytical method. The effects of added amounts of DMF, MD, and SC to the POV titration on POV determination were therefore examined. The effect of dimethyl sulfoxide (DMSO), which can also be used as a solvent for dissolving the powders, on the POV was also examined for comparison with DMF. The effect of the solvent was examined by adding 2–36 mL of DMF or DMSO to the measurement system. MD (100–1000 mg) or SC (10–100 mg) in DMF (9 mL) was also added to the measurement system and solubilized by sonication for 15 min, to determine whether or not MD and SC affected the POV determination.

2.10 Statistical analysis

All values from duplicate or triplicate determinations were expressed as mean value ± standard deviation.

3. Results and Discussion

3.1 Characterization of emulsified spray-dried powders

Four types of spray-dried powders were prepared, with various combinations of the core material and oil-droplet size in the emulsion, to investigate the applicability of the proposed DMF method. The physicochemical properties of the spray-dried powders, such as oil-droplet size in the reconstituted emulsion, powder size, moisture content, and surface oil content are summarized in Table 2. The oil-droplet diameters in the spray-dried powders were changed by the rotor–stator homogenization and subsequent pressure homogenization at 100 MPa. Two passes through the pressure homogenizer

| Oil | Emulsion condition (High-pressure homogenization) | Powder diameter (μm) | Reconstituted emulsion | Moisture content (wt%) | Surface-oil content (wt%) |
|-----|-----------------------------------------------|----------------------|-----------------------|-----------------------|--------------------------|
|     | [High-oil-droplet diameter]                   | [Standard deviation] |                       |                       |                          |
|     | Large oil-droplet (Low-shear / 0 cycle)        | 23.1                 | 4.21                  | 0.54                  | 1.39                     | 4.56                     |
|     | Small oil-droplet (High-shear / 2 cycles)     | 20.0                 | 0.22                  | 0.13                  | 1.73                     | 1.35                     |
| Flaxseed oil |                                                |                       |                       |                       |                          |
| Fish oil | Large oil-droplet (Low-shear / 0 cycle)        | 41.2                 | 1.48                  | 0.47                  | 1.86                     | 9.77                     |
|       | Small oil-droplet (High-shear / 2 cycles)     | 48.9                 | 0.17                  | 0.14                  | 1.55                     | 0.0730                   |
decreased the averaged oil-droplet diameter in the reconstituted emulsion to below 1 \( \mu \text{m} \). Oil-droplets of mean size 1.48–4.21 \( \mu \text{m} \) in the reconstituted emulsions were categorized as large oil-droplets, and those of mean size 0.17–0.22 \( \mu \text{m} \) were categorized as small oil-droplets.

### 3.2 Oil content

Quantification of the total-oil content in the powders is important in assessing the oil retention and encapsulation capacities of the encapsulating agents. The oil contents of the powders were determined using the DMF and Röse–Gottlieb methods. We assumed that all the initial oil was retained in the powders, because the amounts of MD and SC to that of the oil were sufficient. Figure 1 shows that there was almost no difference between the oil contents in the powders using the two methods for the flaxseed oil powders. These values of the oil contents were comparable to the initial oil content on a dry basis in the feed emulsion. In contrast, for the fish oil powders, the oil content obtained using the DMF method was about 40 wt%, regardless of the oil-droplet size in the powders. However, the oil contents obtained using the Röse–Gottlieb method were lower than those obtained using the DMF method; the oil contents in the powders were 19.8 and 3.9 wt% for large and small oil-droplets, respectively. These results suggest that the extraction yield in the Röse–Gottlieb method is influenced by the oil composition and oil-droplet size in the powders.

It is known that the Röse–Gottlieb method is suitable for powdered milk. However, Manirakiza et al. [32] reported that the extraction yields of milk powders and fish flour obtained using this method were only 82%. Le et al. [33] indicated that phase separation was difficult in liquid–liquid extraction for some buttermilk powders containing high amounts of proteins. However, in the DMF method, it is easy to separate the two phases, and the method is suitable for analysis of the oil contents in spray-dried powders, because DMF can solubilize protein emulsifiers such as SC. This phase-separation behavior of fish oil powders would affect the extraction of fish oils from the spray-dried powders. In the Röse–Gottlieb method, separation of the water and solvent phases for the fish oil powders was significantly less than for the flaxseed oil powders.

### 3.3 Fatty acid composition

PUFAs are highly prone to accelerated oxidative rancidity, because of their high unsaturation. Choo et al. [34] reported that the fraction of \( \alpha \)-linolenic acid in flaxseed oil rapidly decreased to 51.0% from 56.5% on heating in a frying pan at 150°C for 6 min. Wang et al. [35] showed that the PUFAs content in fish oil decreased to about 25% from 60% during storage at 30°C for 66 d. For this reason, several studies [7–10,13,14,16–18,23–26] have examined the effects of factors such as the wall material, oil content, oil-droplet size, and spray-drying conditions on the protection of PUFAs in flaxseed oil and fish oil powders.

Figure 2 shows the fatty acid compositions of flaxseed oil and fish oil in the spray-dried powders obtained using the DMF and Röse–Gottlieb methods. As can be seen from Fig. 2(A), for flaxseed oil powders, \( \alpha \)-linolenic acid (18:2, \( n \)-3) was detected as the main PUFAs. Although sample preparation was carried out without a hexane extraction process in the DMF method, the chromatograms agreed well with those for the Röse–Gottlieb method. For the fish oil powders, EPA (20:5, \( n \)-3) and DHA (22:6, \( n \)-3) were detected as the main PUFAs. Although sample preparation was carried out without a hexane extraction process in the DMF method, the chromatograms agreed well with those for the Röse–Gottlieb method. For the fish oil powders, EPA (20:5, \( n \)-3) and DHA (22:6, \( n \)-3) were detected as the main PUFAs. Although sample preparation was carried out without a hexane extraction process in the DMF method, the chromatograms agreed well with those for the Röse–Gottlieb method. For the fish oil powders, EPA (20:5, \( n \)-3) and DHA (22:6, \( n \)-3) were detected as the main PUFAs.

![Fig. 1 Total-oil contents in spray-dried powders analyzed using (□) DMF and (■) Röse–Gottlieb methods. Values are means ± standard deviation of two measurements.](image)

### 3.4 POV

In POV measurements, the extracted and concentrated oil is usually used for the titration of sodium thiosulfate solution in the reaction with potassium iodide in a chloroform-acetic acid mixture. In the DMF method, it is not necessary to extract and concentrate the oil. However,
the sample contains SC and MD as the emulsifier and carrier matrix, respectively. These compounds might influence the titration volume of sodium thiosulfate solution in the POV measurements, because compounds with redox components might react with potassium iodide in DMF. DMSO can also dissolve the spray-dried powders. The effects of DMF and DMSO as the solvents and of SC and MD in the spray-dried powders on the titrant volume in the POV measurements were examined. The addition of DMF to the measurement system had little effect on the titrant volume. DMF is therefore a solvent suitable for dissolving the spray-dried powders to determine the POV of the oil in the powders. In contrast, the addition of 4 mL of DMSO greatly increased the titrant

Fig. 2 Fatty acid compositions of oils in (A) spray-dried flaxseed oil powders and (B) spray-dried fish oil powders.
volume, and further addition of DMSO gradually decreased the volume, although the reason for this peculiar effect of DMSO on the titrant volume remains unclear. Figure 4 shows the effect of MD or SC on the volume of sodium thiosulfate solution. The titrant volume increased with increasing amount of SC or MD added. The DMF contained 670 mg of MD and 30 mg of SC for the flaxseed oil spray-dried powders, and 570 mg of MD and 30 mg of SC for the fish oil spray-dried powders. It was calculated that these components would increase the POVs by 3.66 and 3.40 meq/kg-oil for microencapsulated flaxseed oil and fish oil, respectively. The POVs of the flaxseed and fish oils in the spray-dried powders were estimated using Eq. (2)

$$\text{POV} = \frac{(V_s - V_0 - V_w) C}{M}$$

where $V_0$ and $V_w$ are the volumes (mL) of sodium thiosulfate exhausted by the blanks for DMF, and both MD and SC, respectively.

Table 3 Peak areas of $\alpha$-linolenic acid methyl ester, EPA methyl ester or DHA methyl ester in spray-dried powders containing flaxseed or fish oils on fatty acid analysis by using DMF and Röse–Gottlieb methods. Values are means±standard deviation of two measurements.

| Oil          | Emulsion condition (High-pressure homogenization) | Analytical method | Peak area ($\times 10^5$) |
|--------------|---------------------------------------------------|-------------------|---------------------------|
|              | Large oil-droplet (Low-shear / 0 cycle)            | Röse–Gottlieb     | 4.03±0.23                 | -                          | -                          |
| Flaxseed oil | Large oil-droplet (High-shear / 2 cycles)          | Röse–Gottlieb     | 4.79±0.53                 | -                          | -                          |
|              | Small oil-droplet (Low-shear / 0 cycle)            | DMF               | 3.85±1.13                 | -                          | -                          |
|              | Small oil-droplet (High-shear / 2 cycles)          | DMF               | 4.27±1.08                 | -                          | -                          |
| Fish oil     | Large oil-droplet (Low-shear / 0 cycle)            | Röse–Gottlieb     | -                         | 0.231±0.129                | 1.60±0.03                  |
|              | Large oil-droplet (High-shear / 2 cycles)          | DMF               | -                         | 0.345±0.031                | 1.67±0.12                  |
|              | Small oil-droplet (Low-shear / 0 cycle)            | Röse–Gottlieb     | -                         | 0.335±0.001                | 1.71±0.04                  |
|              | Small oil-droplet (High-shear / 2 cycles)          | DMF               | -                         | 0.309±0.031                | 1.66±0.02                  |

Figure 5 shows a comparison of the POVs of the spray-dried powders analyzed using the DMF and Röse–Gottlieb methods. For the flaxseed oil in the powders, the POVs obtained using the DMF method were very...
close to those obtained using the Röse–Gottlieb method. In contrast, for the microencapsulated fish oil, the POVs obtained using the two methods were different. This difference in the POV might be the result of lower recovery of fish oil from the powders by extraction with the hexane/diethyl ester/ethanol mixture, as shown in Fig. 1.

4. Conclusions

Spray-dried powders were dissolved in DMF to determine the oil contents of the powders, fatty acid compositions, and POVs. The oil contents in the spray-dried powders could be accurately measured, regardless of the oil-droplet size in the powders. The fatty acid compositions were rapidly determined without extracting the oil. The POV of the microencapsulated oil was determined without extracting the oil, although the effects of wall materials on the titrant volume had to be corrected. The POV estimated using the proposed method is more reliable than that estimated using the conventional Röse–Gottlieb method.

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ジメチルホルムアミドを用いた乳化噴霧乾燥粉末中の含油量の迅速測定法

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近年、噴霧乾燥法による魚油の粉末化が盛んに検討されている[10-18]。作製した粉末の特性の評価において、粉末の含油量とその油分の脂肪酸組成および過酸化物値（POV）などが重要である。しかし、これらの分析には溶媒抽出法による油分の抽出が必須であるため、多くの時間と労力を要することが検討の妨げとなっている。そこで本研究では、含油量、脂肪酸組成、POVの迅速な分析を目的として、N,N-ジメチルホルムアミド（DMF）を用いた新たな分析法（DMF法）の開発について検討した。

PUPA含有油として、亜麻仁油および魚油を用いた。乳化剤（カゼインNa（SC））と賦形剤（マルトデキストリン（MD））の混合水溶液に、亜麻仁油または魚油を加え、ホモジナイザーで攪拌し、エマルションを作製した。これを微細エマルションにする場合は、さらに、高圧乳化機を用いて100 MPaで2回の処理を行った。ついで、乳化液を噴霧乾燥機を用いて乾燥し、各種の噴霧乾燥粉末を作製した。

作製した噴霧乾燥粉末の含油量は、DMF法で測定するとともに、粉末油脂の分析に広く用いられているRöse-Gottlieb法[19]によりその妥当性を検証した。

DMF法による手順は以下の通りである。

1. DMF 1 mLに粉末300 mgを加え、15 min超音波で処理して粉末を完全に溶解し、ヘキサン1 mLを添加してボルテックミキサーで攪拌した。ついで、3000 rpmで10 min遠心分離を行い、ヘキサン層1 μLを薄層クロマトグラフィ検出装置で分析した。

一方、Röse-Gottlieb法による手順はヘキサン/ジエチルエーテル/エタノールによる抽出操作を3回行って、重量法により含油量を決定した。

また、粉末中の油分の脂肪酸組成およびPOVは、DMF粉末を溶解したのち、脂肪酸メチル化キットを用いたエステル化法および酢酸-クロロホルム法[31]による電位差滴定によりそれぞれ分析した。

DMF法により得られた亜麻仁油および魚油粉末の含油率は、それぞれ噴霧溶液中の添加量に相当する30 wt%および40 wt%であった。一方、Röse-Gottlieb法によると亜麻仁油粉末の含油率はDMF法によるそれとよく一致したが、魚油粉末では、Röse-Gottlieb法によるSC含有粉末の分析において、液-液抽出時に油層と水層の間中に中間層が形成され、界面の判別が困難になることが報告されているが[32]、DMF法においてはSC存在下でも中間層の形成がなく、簡単に分析が行えた。

脂肪酸組成は、GC-MSクロマトグラムを比較したところ、亜麻仁油および魚油粉末のいずれも、DMF法およびRöse-Gottlieb法で分析した結果にほとんど違いはみられなかった。このように、DMF法を用いることにより、油脂の抽出工程を経ずに迅速に分析が可能であることが示された。

Röse-Gottlieb法によるPOVの分析では、粉末から抽出した油分のみを用いるが、DMF法では油分に加えて、粉末を溶解する溶媒であるDMFと粉末化の基剤であるSCとMDが含まれる。そこで、POV分析値に及ぼすこれらの影響について検討したところ、DMF、SC、MDのいずれもわずかではあるが分析値が増大した。粉末化基剤によるPOVの増加傾向はそれぞれの粉末を構成する成分の組成比に基づいて、POVの増加量を推算したところ、亜麻仁油粉末では3.66 meq/kg-oil、魚油粉末では3.40 meq/kg-oilであった。この補正により、DMF法で得られた亜麻仁油粉末のPOVは、粉末中の油滴径に係わらず、Röse-Gottlieb法によるとよく一致したが、魚油粉末では一致しなかった。魚油粉末で結果が異なった要因として、Röse-Gottlieb法による油分の抽出効率が極めて低く、正確な分析値が得られなかったことが考えられる。

噴霧乾燥粉末の含油率、脂肪酸組成、POVの迅速な分析を目的として、粉末をDMFに溶解して分析する新たな方法を開発した。亜麻仁油および魚油粉末の含油率は、油滴径によらず実際に測定できることを示した。また、粉末中の油分の脂肪酸組成を、抽出工程を経ずに簡単な操作で決定できた。粉末中の油分のPOVは、DMFおよび粉末化基剤の影響によりわずかに変動が生じたが、これらの成分の影響を補正することにより妥当なPOVの値が得られることができた。