Original Research

Syringe-dispensed omega-3 lipid injectable emulsions should be stored under airtight refrigeration: a proposal for the efficient supply of unapproved precious lipid resources

Makoto Setoguchi[1,2*]; Mitsuru Muto, M.D., Ph.D.[2,3]; Tomonori Ohata, Ph.D.[4]; Ryuichi Fukuoka, Ph.D.[2,5]; Hirohito Ikeda, Ph.D.[4]; Hatsumi Aki, Ph.D.[4]; Masaomi Haraguchi, Associate degree of Medical Technology[6]; Shota Hanjo, B.S.Pharm.[1]; Junko Arima, M.Pharm.[1]; Satoshi Ibara, M.D., Ph.D.[2,7]

[1] Department of Pharmacy, Kagoshima City Hospital, Kagoshima, Japan.

[2] Working group for Inflammatory bowel disease and Intestinal failure providing Supportive and Hearty care (WISH)

[3] Department of Pediatric Surgery, Research Field in Medical and Health Sciences, Medical and Dental Area, Research and Education Assembly, Kagoshima University, Kagoshima, Japan.

[4] Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka, Japan

[5] Department of Pharmacy, Hakuyukai Onsen Hospital, Kagoshima, Japan.

[6] Department of Clinical laboratory, Kagoshima City Hospital, Kagoshima, Japan.

[7] Department of Neonatology, Kagoshima City Hospital, Kagoshima, Japan.

***Corresponding Author:**

Makoto Setoguchi, M.Pharm.

Department of Pharmacy, Kagoshima City Hospital,

37-1 Uearata, Kagoshima, Japan 890-8760

E-mail: setoguchi-m36@kch.kagoshima.jp

Tel: +81-99-230-7000

Fax: +81-99-230-7075

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Abstract

Background: Both fish oil lipid injectable emulsion (FO-ILE) and mixed oil lipid injectable emulsion (MO-ILE) are key components of parenteral nutrition and require importation into Japan, and they are easily oxidized after opening. Given the small daily volumes of these lipids dispensed in infants and children with intestinal failure (IF), the purpose of the study was to identify the optimal storage method.

Methods: Lipids were prepared in polypropylene syringes in the following manner: air-sealing and photoprotection, air-sealing only, photoprotection only, and uncovered. Samples were stored for 14 days at 4°C or 26°C. The degree of oxidative degradation was evaluated by measuring malondialdehyde (MDA) concentration and pH compared to the values measured immediately after opening.

Results: For FO-ILE, the increase in MDA concentration for 14 days was insignificant in air-sealed samples, regardless of photoprotection (+0.45 μM, p=1.0) or no-photoprotection (+0.52 μM, p=1.0). This trend was more pronounced at 4°C than at 26°C (p<0.01). The maximum pH decrease was 0.08 at 4°C. MO-ILE exhibited an insignificant increase in MDA concentration for 14 days with air-sealed samples, regardless of photoprotection (+0.36 μM, p=0.11) or no-photoprotection (+0.33 μM, p=0.76). This

trend was more pronounced at 4°C than at 26°C (p<0.01). The maximum pH decrease was 0.12 at 4°C. For soybean oil lipid injectable emulsion (SO-ILE), the trend was similar with no considerable deterioration.

Conclusion: Syringe-dispensed FO-ILE and MO-ILE stored under airtight refrigeration remained undeteriorated for 14 days. Our results are considered clinically valuable when supplying these expensive resources for infants with IF.

Keywords: Intestinal failure, injectable lipid emulsions, lipid peroxidation, malondialdehyde, parenteral nutrition

Clinical Relevancy Statement

Fish oil lipid injectable emulsion (FO-ILE) and mixed oil lipid injectable emulsion (MO-ILE) are essential components of parenteral nutrition (PN) in children with intestinal failure (IF). Both are easily deteriorated with atmospheric oxygen and can potentially cause organ dysfunction in patients due to oxidative stress. This study revealed that the oxidative degradation of FO-ILE and MO-ILE in dispensed syringes is minimized for 14 days when maintained under airtight refrigeration. Given that FO-ILE or MO-ILE is unapproved in Japan and must be imported from overseas, a useful daily supply method is proposed.

Introduction

Children with intestinal failure (IF), where the gut cannot absorb sufficient fluids and nutrients necessary for maintaining life, require long-term parenteral nutrition (PN). Such children have the risk of cholestatic liver injury or intestinal failure-associated liver disease (IFALD) [1]. One cause of hepatotoxicity is the use of conventional soybean oil lipid injectable emulsion (SO-ILE). SO-ILE contains high amounts of omega-6 long-chain polyunsaturated fatty acids, which are sources of inflammatory eicosanoids, such as leukotrienes and prostaglandins, and phytosterols that prolong bile excretion [2,3].

In the early 2000s, fish oil lipid injectable emulsion (FO-ILE)-containing omega-3 fatty acid, metabolized to less inflammatory leukotrienes with no phytosterols, has emerged as a counterpart to SO-ILE [4] and has been used in the treatment of IFALD [5]. Mixed oil lipid injectable emulsion (MO-ILE) (30% soybean oil, 30% medium-chain triglycerides, 25% olive oil, and 15% fish oil) has emerged, and it is currently used in many nations as a standard lipid emulsion in PN for patients with IF [6,7].

Since fish oil is composed of polyunsaturated fatty acids structured with multiple carbon double bonds [8], oxidation of the emulsion immediately by atmospheric oxygen occurs after opening the vials. When these degraded and peroxidized lipids enter the body, they

oxidize the fatty acids that constitute the cell membranes via a chain reaction [9]. Functional disorders in the liver, kidney, and lungs are known to become apparent due to such oxidative stresses in experimental animal models [10,11].

Currently, SO-ILE is the sole emulsion approved in the Japanese market. FO-ILE and MO-ILE have to be imported from overseas. Therefore, these emulsions are expensive, but are valuable medicinal resources. Since the daily dose volume is often limited for infants with IF, the supply of these emulsions is in the form of pre-filled syringes. The purpose of the study was to verify the degree of lipid peroxidation over time for syringe-dispensed samples and identify the most suitable storage method needed to minimize its oxidative degradation, making it useful for clinical application in Japanese children.

Materials and Methods

Storage conditions for filled lipid emulsion samples

It has been reported that maintaining airtight [12] and protecting from light [13] are beneficial in preventing oxidative deterioration of all-in-one parenteral admixtures. We focused on the exposure to oxygen and light, and examined whether the oxidative degradation of the lipid emulsions in filled syringes can be minimized by blocking these factors.

Three lipid emulsions of 2 mL each, FO-ILE 10% (Omegaven 10%; Fresenius Kabi, Kriens, Switzerland), MO-ILE 20% (SMOFlipid 20%; Fresenius Kabi, Kriens, Switzerland), and SO-ILE 20% (Intralipos 20%; Otsuka Pharmaceutical Factory, Tokushima, Japan), were filled individually into polypropylene 2.5-mL Luer-lock syringes (Terumo Corporation, Tokyo, Japan) and packaged in the following four ways (Fig. 1).

Fig. 1

Package 1: Applied vacuum packing with an oxygen scavenger and covering with a photoprotection film after filling (air-sealing + photoprotection).

Package 2: Vacuum packing with oxygen scavenger after filling (air-sealing only).

Package 3: Covering with a photoprotection film after filling (photoprotection only).

Package 4: Without packing and wrapping after filling (none)

A pharmacist prepared all samples under an ISO Class 5 environment with a Biological Safety Cabinet (BSC) in a sterile room with a front room separated by an air shower as specified in USP Chapter <797>. The filled syringes were placed in a plastic bag with an oxygen scavenger (Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan) and then air-sealed using a vacuum device (Wide Systems Inc., Yamaguchi, Japan). Photoprotection

films (Terumo Corporation, Tokyo, Japan) that block most of the ultraviolet and visible lights in the 350–500-nm range were used to prevent direct exposure to light and denaturation of vitamins in PN solution.

Each sample was placed under two different temperature conditions mimicking the normal environment in daily clinics: (1) inside a refrigerated cabinet maintained at approximately 4°C and protected from light except when the door was opened, and (2) inside a room adjusted to 26°C with air conditioning and away from direct sunlight. The temperature was continuously measured using a data logger RX-350TH (AS ONE, Osaka, Japan), and the degree of lipid peroxidation was evaluated for 14 days for samples kept in a refrigerator and those kept in an air-conditioned room. The color change, agglomeration, and oil layer separation associated with creaming denaturation of lipid samples were monitored in each syringe.

Evaluation of the degree of lipid peroxidation in dispensed samples

Malondialdehyde (MDA), an end product of oxidative degradation in a peroxide reaction of the lipid emulsion, was measured to evaluate the oxidation state of each sample. MDA is widely used as an indicator of lipid peroxidation [14]. A malondialdehyde assay kit (Northwest Life Science Specialties LLC, Washington, USA) was used for the

measurements. Thiobarbituric acid (TBA) reagent, which is a luminescent reagent, followed by an acid reagent (for the reaction of MDA with TBA under acidic conditions) and butylated hydroxytoluene (BHT) reagent (to prevent sample oxidation) were added to 250 μL of each sample. After incubation at 60°C for 60 min, 1-butanol (FUJIFILM Wako Pure Chemical, Osaka, Japan), 1 N NaOH solution (Kishida Chemical, Osaka, Japan), and 3.7 M phosphoric acid (FUJIFILM Wako Pure Chemical, Osaka, Japan) were added to remove impurities, as reported by Badcock et al. [15]. The concentration of MDA in the samples was quantified by measuring the excitation light at 535 nm and 572 nm using an absorption spectrophotometer (Eppendorf Bio Spectrometer, Eppendorf Corporate, Hamburg, Germany).

For each lipid emulsion vial, the day of opening was set as day 0, and data measured on day 0 were used as control. MDA concentration measurement in samples stored in a refrigerator and those stored in an air-conditioned room was performed on days 3, 7, and 14. The number of samples measured at each evaluation point was nine per target lipid emulsion.

Measurement of pH in dispensed samples

The pH value of each sample was measured simultaneously during the MDA

measurement. A pH meter (LAQUA twin®, AS ONE, Osaka, Japan) was used, and standard buffer solutions of pH 4.0, 7.0 and 9.0 (Nacalai Tesque, Kyoto, Japan) were also used for calibration. For each lipid emulsion vial, the day of opening was set as day 0, and data measured on day 0 were used as control.

Bacterial culture tests for the confirmation of aseptic operability

Microbiological tests were performed on the lipids stored in the refrigerated cabinet as a representative of all dispensed samples to ensure that aseptic manipulation had been accomplished through the experiments. Each vial was opened on day 0, and samples on days 0, 3, 7, and 14 were dispensed into aerobic and anaerobic bottles (Bac T/ALERT® FN Plus and Bac T/ALERT® FA Plus, bioMérieux, Marcy-l'Etoile, France). Further, all the samples were incubated for 14 days, and observation was done using a blood culture detection system (BACT/ALERT VIRTUO®, bioMérieux, Marcy-l'Etoile, France). The number of samples measured at each evaluation point was nine per target lipid emulsion.

Measuring droplet size and density

The droplet size and density of each injectable lipid sample was evaluated using the previously reported method with Nanosight (Malvern Panalytical, Worcestershire, United Kingdom) [16]. Briefly, samples of each lipid emulsion diluted 10,000-fold with distilled

water were used for the measurement. The nanoparticle tracking analysis was applied to calculate the Stokes-diameter in the solutions, and then evaluated the lipid droplet particle size and density.

Statistical analysis

Statistical analyses were performed using BellCurve for Excel version 3.20 (Social Survey Research Information Co., Ltd., Tokyo, Japan). Data are presented as means ± standard deviation (SD). A two-way ANOVA, followed by Tukey-Kramer post-hoc test, was used to compare the difference between each control value and each measured value over time, as well as the difference between each storage condition. Statistical significance was set at $P < 0.05$.

Results

The difference between the maximum and minimum temperatures in each storage environment through the experimental period was 0.4°C (3.8°C–4.2°C, average 4.0°C) in the refrigerated cabinet and 4.3°C (23.2°C–27.5°C, average 26.0°C) in the air-conditioned room.

The degree of lipid peroxidation evaluated with MDA

1) FO-ILE

The control of MDA concentration was 2.19 ± 0.10 μM. In the refrigerated cabinet, the MDA concentration in air-sealing samples remained stable compared to the control throughout the complete 14 days (Package 1, range 2.18–2.64 μM [p=0.69–1.0]; Package 2, range 2.16–2.71 μM [p=0.58–1.0]) (Fig. 2A). The MDA concentration in air-sealing samples was also stable compared with that in the control for 14 days in the air-conditioned room (Package 1, range 2.62–3.29 μM [p=0.58–0.96]; Package 2, range 2.77–3.18 μM [p=0.65–0.91]) (Fig. 2B).

Fig. 2

In contrast, the MDA concentration in samples stored without air-sealing increased up to +290.4% compared with that in the control (Package 3, range 2.40–6.37 μM; Package 4, range 2.40–6.31 μM) over 14 days under the refrigerated cabinet storage and up to +1563.2% under the air-conditioned room storage (Package 3, range 6.52–30.95 μM; Package 4, range 10.97–34.29 μM). A significant increase in oxidants was observed from day 7 in the refrigerated samples and day 3 in the samples stored in the air-conditioned room compared with that in the control (Fig. 2A, p=0.003–0.005 [day 7]; 2B, p<0.001 [day 3]).

Considering the temperature, the increase in oxidants on day 14 was significantly

suppressed during storage under refrigeration in all packages compared with that during storage at air-conditioned room temperature (Package 1, p=0.008; Package 2, p=0.002; Package 3, p<0.001; Package 4, p<0.001) (Fig. 2A vs. 2B). Interestingly, the increase in MDA concentration was significantly suppressed by photoprotection in the samples stored at air-conditioned room temperature (Fig. 2B package 3 vs. package 4, p<0.001).

2) MO-ILE

The control MDA concentration was 0.96 ± 0.08 μM. In the refrigerated cabinet, the MDA concentration in air-sealing samples remained stable compared to the control for 14 days (Package 1, range 1.11–1.32 μM [p=0.060–0.11]; Package 2, range 1.10–1.29 μM [p=0.084–0.76]) (Fig. 3A). In the air-conditioned room storage, the increase in oxidants tended to be suppressed by air-sealing (Package 1, range 1.30–1.72 μM [p=0.050–0.66]; Package 2, range 1.48–1.87 μM [p=0.011–0.28]) (Fig. 3B).

Fig. 3

In contrast, the MDA concentration in samples stored without air-sealing increased up to +202.1% compared with that in the control (Package 3, range 1.20–1.95 μM; Package 4, range 1.20–1.92 μM) for 14 days in the refrigerated cabinet storage and up to +1339.6% in the air-conditioned room storage (Package 3, range 1.63–8.21 μM; Package 4, range 2.28–12.9 μM). A significant increase in oxidants was observed from day 7 in the

refrigerated storage and day 3 in the air-conditioned room storage compared with that in the control (Fig. 3A, $p<0.001$ [day 7]; 3B, $p<0.001$ [day 3]).

Considering the temperature, the increase in oxidants on day 14 was significantly suppressed under refrigeration in all packages compared with that under storage at air-conditioned room temperature (Package 1, $p=0.003$; Package 2, $p< 0.001$; Package 3, $p<0.001$; Package 4, $p<0.001$) (Fig. 3A vs. 3B). The increase in MDA concentration was significantly suppressed by photoprotection in the samples stored at air-conditioned room temperature (Fig. 3B package 3 vs. package 4, $p<0.001$).

3) SO-ILE

The control MDA concentration was 0.69 ± 0.15 μM. Although the increase in oxidants was smaller than that of FO-ILE and MO-ILE, the increase in MDA concentration tended to be suppressed when the samples were air-sealed and stored in a refrigerated cabinet (Fig. 4A, B).

Fig. 4

Changes in pH

1) FO-ILE

A pH of 8.12 ± 0.07 was identified as control. In the refrigerated condition, the pH of the air-sealing samples ranged from 8.04–8.10 in package 1 and 8.04–8.11 in package 2 over 14 days. The decrease seen compared to the control ranged from just 0.01–0.08 throughout the experimental period, which was statistically negligible until day 7 (Fig. 5A, p=0.48–0.70 [day 7]). When stored at air-conditioned room temperature, the pH of the air-sealing samples ranged from 7.92–8.06 in package 1 and 7.92–8.03 in package 2. There was a 0.06–0.20 decrease compared to the control, which was statistically significant throughout the experimental period (Fig. 5B, p=0.023 [day 3]).

Fig. 5

In samples without air-sealing, the pH ranged from 7.82–8.01 in package 3 and 7.82–7.96 in package 4 under refrigerated conditions. The pH was lower at air-conditioned room temperature, i.e., 7.30–7.98 in package 3 and 7.26–7.93 in package 4. There was a 0.11–0.86 decrease compared to the control, which was statistically significant throughout the experimental period (Fig. 5, p<0.001).

Considering the temperature, the decrease in pH on day 14 was significantly smaller in the refrigerated storage for all packages (p<0.001) than in the air-conditioned room storage (Fig. 5A vs. 5 B).

2) MO-ILE

A pH of 7.90 ± 0.01 was identified as a control. In the refrigerated condition, the pH of the air-sealing samples ranged from 7.79–7.88 in package 1 and 7.78–7.88 in package 2 over 14 days. The decrease compared to the control was 0.02–0.12 throughout the experimental period, and this was statistically negligible until day 3 (Fig. 6A, p=0.79–0.84 [day 3]). During storage at air-conditioned room temperature, the pH of air-sealed samples ranged from 7.74–7.86 in package 1 and 7.73–7.85 in package 2. There was a 0.04–0.17 decrease compared to the control, which became statistically significant after day 7 (Fig. 6B, p<0.001 [day 7]).

Fig. 6

In samples without air-sealing, the pH ranged from 7.63–7.83 in package 3 and 7.65–7.85 in package 4 under refrigerated conditions. The pH was lower at air-conditioned room temperature, with 7.47–7.75 in package 3 and 7.38–7.75 in package 4. There was a 0.05–0.52 decrease compared to the control, which was statistically significant throughout the experimental period except for package 4 stored in refrigerated conditions on day 3 (Fig. 6, p=0.004).

Considering the temperature, the decrease in pH on day 14 was significantly smaller in the refrigerated storage for all packages (p=0.032) than in the air-conditioned room storage (Fig. 6A vs. 6B).

3) SO-ILE

The control pH of Intralipos was 7.21 ± 0.25. Although FO-ILE and MO-ILE showed a significant decrease in pH even under air-sealing, SO-ILE showed no significant decrease in pH compared with the control throughout the 14 days under air-sealing, irrespective of storage in a refrigerator cabinet or an air-conditioned room (Fig. 7, p=0.28–1.0).

Fig. 7

Microbiological test

Neither aerobic nor anaerobic bacteria were detected in any of the samples stored in a refrigerated cabinet throughout the 14 days.

Discussion

It is of great concern that the only approved injectable lipid emulsion in Japan is an SO-ILE. The usefulness of FO-ILE, a pure fish oil, is widely known in the treatment of cholestatic liver disease [17,18]. In addition, the European Society for Pediatric Gastroenterology, Hepatology and Nutrition and the North American Society For Pediatric Gastroenterology, Hepatology & Nutrition state that MO-ILE is recognized as the most suitable balanced composite injectable lipid emulsion for daily PN in children with IF [19,20]. In Japan, the prevalence of pediatric IF is estimated at approximately 15.8

in 1 million [6,21,22]. However, these emulsions need to be imported into Japan to benefit these patients. Hence, they are a very expensive and valuable medical resources when compared to conventional soybean lipid. Both lipids are commercially available in air-sealed 100 or 250 mL glass bottles. Considering the total daily requirement for pediatric patients, much of the emulsions are not used for a single patient and there is significant wastage after opening the vial. Our practice has been to dispense the lipid in pre-filled syringes to minimize waste. Therefore, we recognized the urgent need for determining an appropriate storage method for these injectable lipid emulsions in daily clinical practice.

This study revealed that the most desirable storage method of FO-ILE and MO-ILE in a dispensed syringe is to maintain airtight refrigeration to minimize oxidative deterioration. To the best of our knowledge, there are no practical reports on storage methods for syringe-dispensed injectable lipid emulsions focusing on oxidative deterioration. Commercial-based injectable lipid emulsions are designed for single-use once opened and are not intended to be stored in syringes for a certain period. Watrobska et al. investigated the effect of changes in zeta potential on the surface of dispensed lipid droplets of FO-ILE and MO-ILE in polypropylene syringes. The potential was shown to be invariant at 4°C and 25°C for 30 days and 40°C for 14 days. No lipid droplet aggregation was reported [23]. They also investigated the size of MO-ILE droplets in an

admixed PN preparation and reported no changes at 4°C for 21 days [24]. In our study, no creaming or signs of agglomeration were observed in any of the dispensed lipid samples for 14 days at 4°C and air-conditioned room temperature. Additionally, the lipid droplet size and density in all dispensed samples stored at 4°C and air-conditioned room temperature showed no change after 14 days (Table S1). However, the oxidative deterioration of injectable lipid emulsions progressed over time without air-sealing of the dispensed samples.

Lipid peroxides produced in oxidatively degraded injectable lipid emulsions have the potential to cause chain oxidation reactions in the fatty acids that make up cell membranes *in vivo* [9]. In addition, various reactive oxygen species generated by chain oxidation reactions are known to increase cytokine secretion and activate inflammatory transcription factors [25]. Furthermore, it has been reported that reactive oxygen species are involved in carcinogenesis by reacting with biopolymers such as DNA and affecting protein composition *in vivo* [26]. Given that pediatric IF mostly occurs in neonates whose biological functions are underdeveloped [27], it is especially important to minimize the production of lipid peroxides when providing injectable lipid emulsions.

We chose MDA, the end product of the lipid peroxide reaction [28,29], as an indicator to

measure the degree of peroxidation in our study. As expected, FO-ILE showed the largest increase in MDA concentration compared to other lipids (Fig. 2–4). The MDA concentration was lowered by storage in a refrigerator rather than storage at air-conditioned room temperature (Fig. 2–4). In addition, the MDA concentration could be minimized significantly by applying air-sealing (Fig. 2–4). We conducted similar experiments under the harsh conditions of a car exposed to direct sunlight during the day at temperatures of up to 55.4°C. As predicted, oxidative degradation was most pronounced under this condition in all samples compared to the results mentioned above (Table S2).

The pH value of dispensed FO-ILE and MO-ILE processed by air-sealing and photoprotection and stored in a refrigerator varied by 0.08 and 0.11, respectively, for 14 days (Fig. 5A, 6A). Even though statistical differences were detected in these changes, they were considered negligible. Compared to the control pH of SO-ILE (7.21 ± 0.25), measured immediately after opening, the lowest pH observed in FO-ILE (8.04) and MO-ILE (7.79) was high. We have not experienced progressive drug-induced acidemias with SO-ILE even in premature babies in our daily clinical practice. Further, some antibiotics (e.g., vancomycin hydrochloride) and cardiovascular drugs (e.g., dopamine hydrochloride) at pH levels of around 4.0 exhibit no effects on patients' acid-base

equilibrium. The pH value *in vivo* is normally maintained in the range of 6.8–7.8 by the buffering system [30,31]; hence, a decrease in pH ranging between 0.1–0.2 from around 8.0, as seen in FO-ILE and MO-ILE is considered to be within the acceptable buffering range when they are administered.

Regarding the effect of photoprotection processing, a significant difference in MDA concentration in samples stored in an air-conditioned room was observed (Fig. 2B, 3B, 4B, package 3 vs. 4). The samples stored in a refrigerator were not exposed to light except when the door was opened; hence, there was little difference in the change in MDA concentration. However, assuming the daily home parenteral nutrition management, samples can be kept under sunlight for a while; photoprotection processing can also be useful.

The ideal storage method shown in this study that minimizes the increase in MDA concentration after dispensing FO-ILE and MO-ILE will be useful in supporting children with IF. The extent to which the increase in MDA concentration and decrease in pH of injectable lipid emulsions will lead to adverse events when administered for a long therapeutic period is still unclear. Further investigation is required to verify these questions.

Conclusion

Both FO-ILE and MO-ILE can be provided for 14 days under the same conditions immediately after opening if filled into syringes by pharmacists on a clean bench in a sterile room with processed air-sealing and stored in a refrigerator immediately while protecting them from light. The results of this study show that a small amount of dispensed FO-ILE and MO-ILE, which are not approved for use in Japan but are important medical resources for children with IF, can be administered safely.

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Statement of Authorship

Makoto Setoguchi, Mitsuru Muto, Ryuichi Fukuoka, Hirohito Ikeda, Hatsumi Aki, Junko Arima, and Satoshi Ibara contributed to the study design. Makoto Setoguchi, Tomonori Ohata, Masaomi Haraguchi, and Shota Hanjo participated in data collection. Makoto Setoguchi performed the statistical analysis and drafted the manuscript. Mitsuru Muto

and Ryuichi Fukuoka provided technical support and advice. All authors critically revised the manuscript and approved the final manuscript.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section (Table S1, S2) at the end of the article.

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Figure legends

Figure 1. Differences in the packaging of syringe-filled lipid emulsions.

Syringes filled with each lipid emulsion were packaged and processed in four different ways, as shown in the figure.

Figure 2. Changes in MDA concentration in FO-ILE under various storage conditions.

Trends in the refrigerated cabinet (A) and air-conditioned room (B) are shown (mean ± SD). Significant differences ($p<0.05$, $p < 0.01$) between packages are shown as †, ††, and significant differences from day 0 are shown as *, **.

Figure 3. Changes in MDA concentration in MO-ILE under various storage conditions.

Trends in the refrigerated cabinet (A) and air-conditioned room (B) are shown (mean ± SD). Significant differences ($p< 0.05$, $p < 0.01$) between packages are shown as †, ††, and significant differences from day 0 are shown as *, **.

Figure 4. Changes in MDA concentration in SO-ILE under various storage conditions.

Trends in the refrigerated cabinet (A) and air-conditioned room (B) are shown (mean ± SD). Significant differences ($p<0.05$, $p < 0.01$) between packages are shown as †, ††, and

significant differences from day 0 are shown as *, **.

Figure 5. The pH changes in FO-ILE at each storage condition.

The pH immediately after opening was set as the baseline. Trends in the refrigerated cabinet (A) and air-conditioned room (B) (mean±SD) are shown. Significant differences (p<0.05, p<0.01) between packages (†, ††) and differences from day 0 (*, **) were observed.

Figure 6. The pH changes in MO-ILE at each storage condition.

The pH immediately after opening was set as the baseline. Trends in the refrigerated cabinet (A) and air-conditioned room (B) (mean±SD) are shown. Significant differences (p<0.05, p<0.01) between packages (†, ††) and differences from day 0 (*, **) were observed.

Figure 7. The pH changes in SO-ILE at each storage condition.

The pH immediately after opening was set as the baseline. Trends in the refrigerated cabinet (A) and air-conditioned room (B) (mean±SD) are shown. Significant differences (p<0.05, p<0.01) between packages (†, ††) and differences from day 0 (*, **) were observed.

Fig. 1

Package 1

Package 2

Package 3

Package 4

Fig. 2

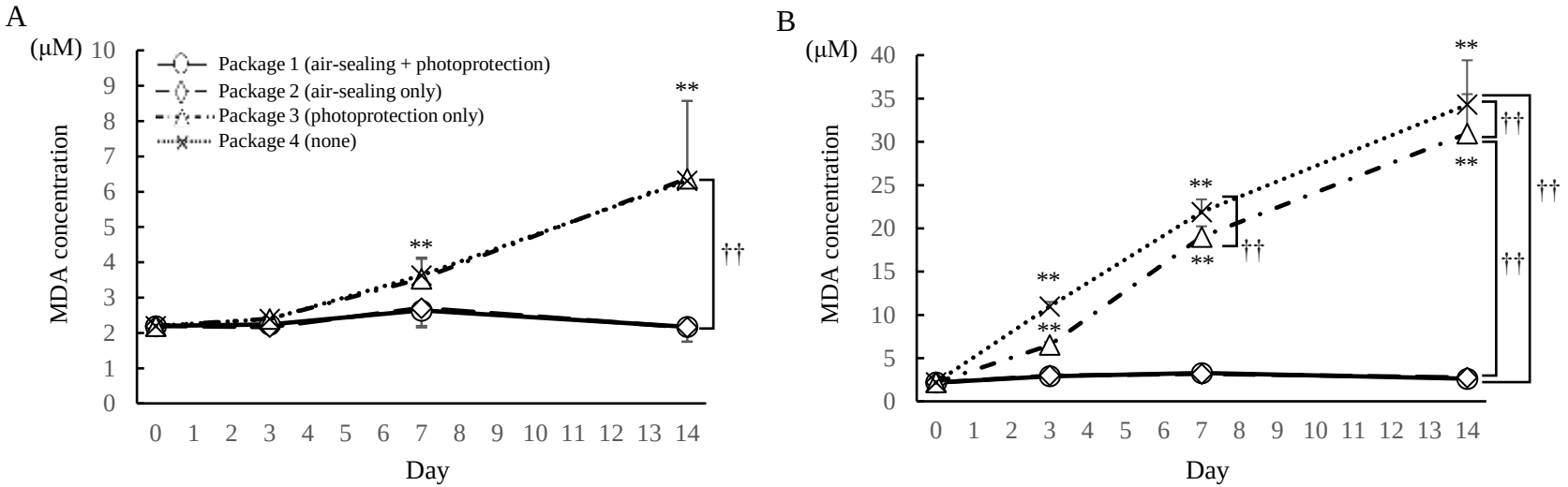
A
(μM)
MDA concentration
Package 1 (air-sealing + photoprotection)
Package 2 (air-sealing only)
Package 3 (photoprotection only)
Package 4 (none)
**
††
Day
B
(μM)
MDA concentration
**
††
Day

Fig. 3

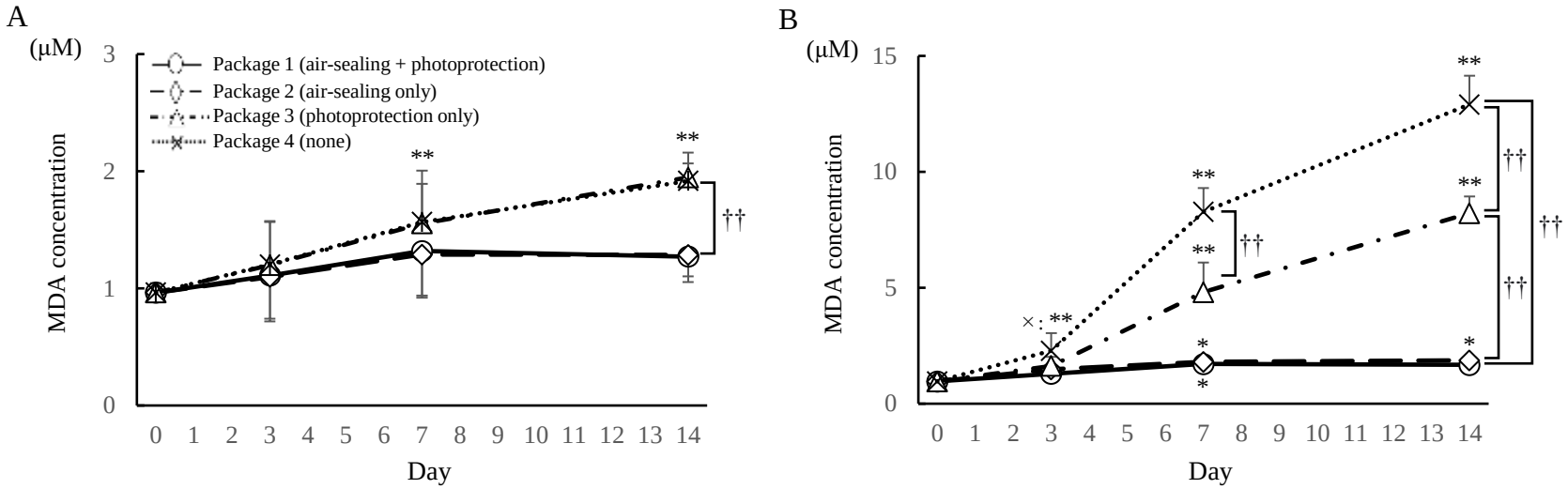
A
(μM)
MDA concentration
Package 1 (air-sealing + photoprotection)
Package 2 (air-sealing only)
Package 3 (photoprotection only)
Package 4 (none)
Day
B
(μM)
MDA concentration
Day

Fig. 4

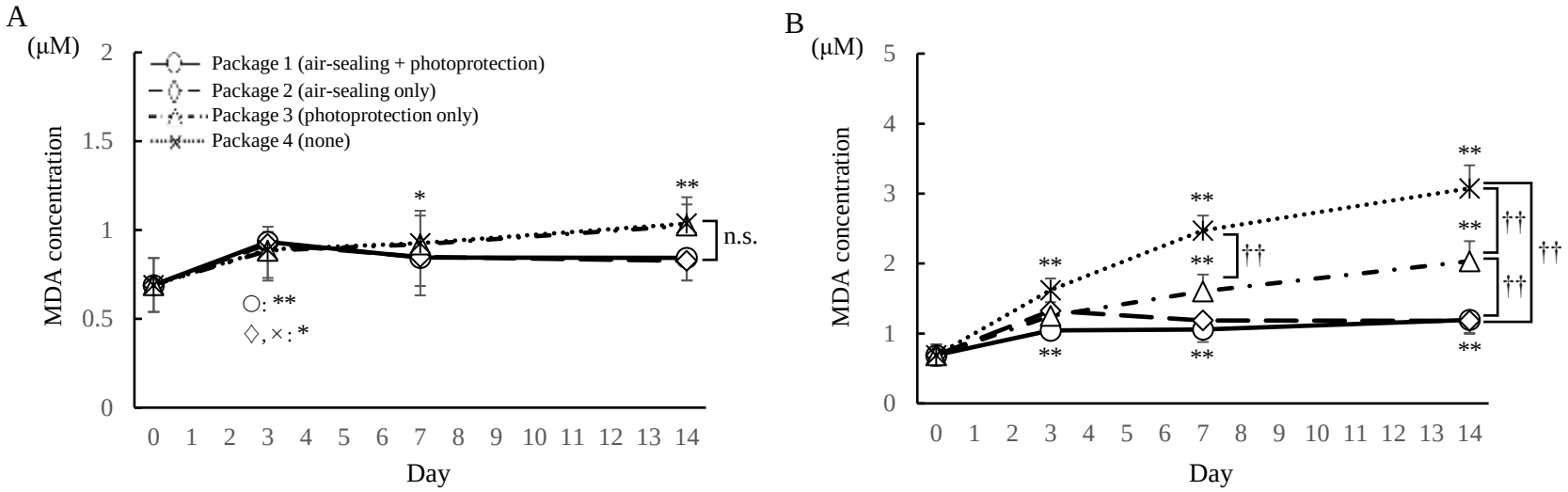
A
(μM)
MDA concentration
Package 1 (air-sealing + photoprotection)
Package 2 (air-sealing only)
Package 3 (photoprotection only)
Package 4 (none)
○: **
◇, ×: *
n.s.
Day
B
(μM)
MDA concentration
Day

Fig. 5

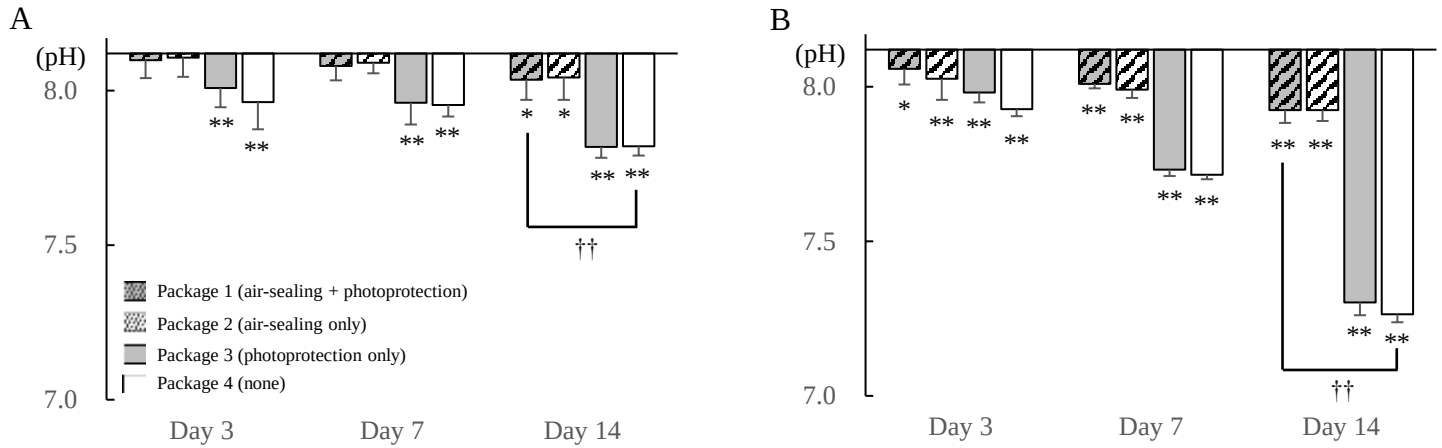
A
(pH)
8.0
7.5
7.0
Package 1 (air-sealing + photoprotection)
Package 2 (air-sealing only)
Package 3 (photoprotection only)
Package 4 (none)
Day 3
Day 7
Day 14
B
(pH)
8.0
7.5
7.0
Day 3
Day 7
Day 14

Fig. 6

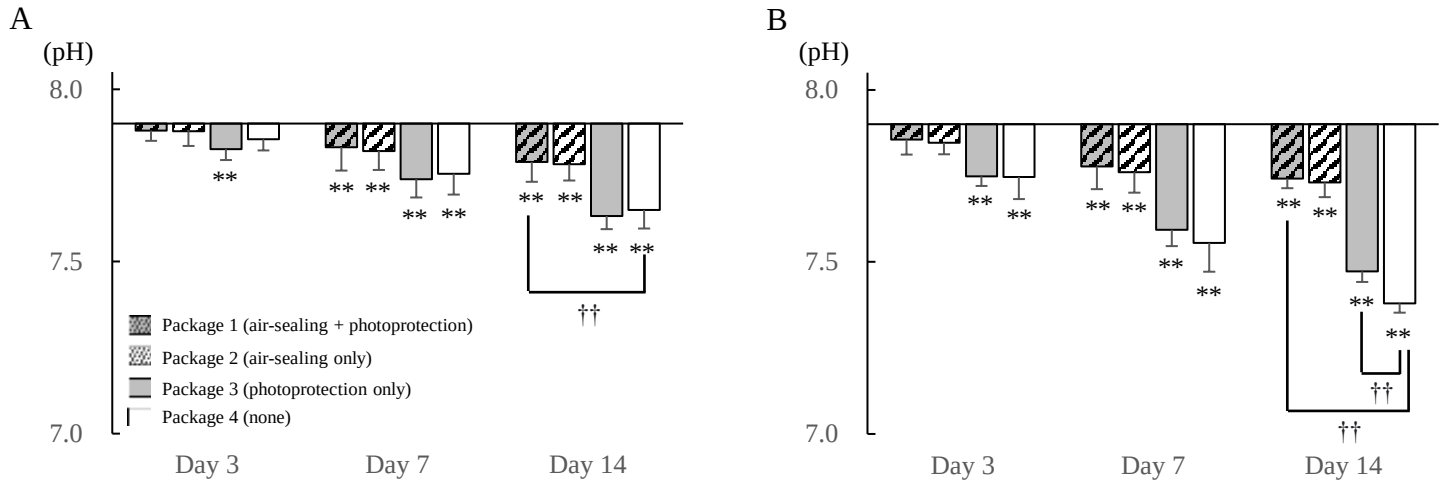
A
(pH)
8.0
7.5
7.0
Package 1 (air-sealing + photoprotection)
Package 2 (air-sealing only)
Package 3 (photoprotection only)
Package 4 (none)
Day 3
Day 7
Day 14
B
(pH)
8.0
7.5
7.0
Day 3
Day 7
Day 14

Fig. 7

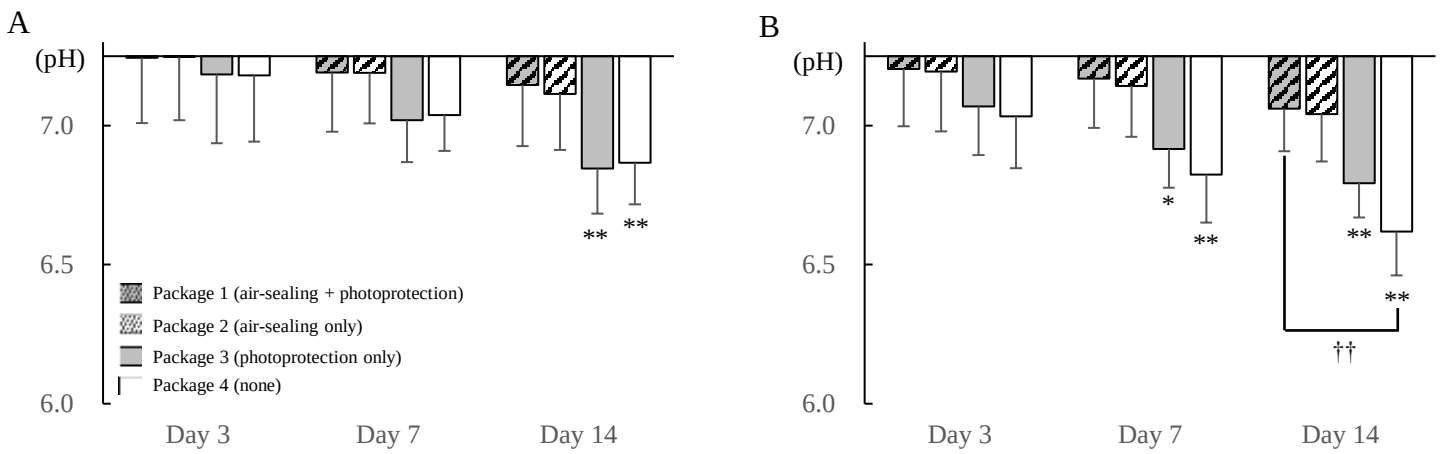
A
(pH)
7.0
6.5
6.0
Package 1 (air-sealing + photoprotection)
Package 2 (air-sealing only)
Package 3 (photoprotection only)
Package 4 (none)
**
**
Day 3
Day 7
Day 14
B
(pH)
7.0
6.5
6.0
*
**
**
**
††
Day 3
Day 7
Day 14

Table S1. Variation in size and number of lipid particles in each storage method (mean ± SD)

		Refrigerated cabinet								Room							
		Package1 (air-sealing+photoprotection)				Package4 (none)				Package1 (air-sealing+photoprotection)				Package4 (none)			
		Mode diameter 1 (nm)	Mode diameter 2 (nm)	Mean (nm)	Particle number ($\times 10^{11}$ particles/mL)	Mode diameter 1 (nm)	Mode diameter 2 (nm)	Mean (nm)	Particle number ($\times 10^{11}$ particles/mL)	Mode diameter 1 (nm)	Mode diameter 2 (nm)	Mean (nm)	Particle number ($\times 10^{11}$ particles/mL)	Mode diameter 1 (nm)	Mode diameter 2 (nm)	Mean (nm)	Particle number ($\times 10^{11}$ particles/mL)
Omegaven	Day0	210	-	230±3.2	150±4.4	210	-	230±3.2	150±4.4	210	-	230±3.2	150±4.4	210	-	230±3.2	150±4.4
	Day7	200	-	230±3.7	170±6.5	210	-	240±3.7	180±12	200	-	230±3.2	160±7.3	210	-	230±1.6	170±4.5
	Day14	210	-	240±3.8	180±9.2	210	-	240±4.2	170±12	210	-	240±2.9	180±8.2	210	-	230±3.6	170±8.8
SMOF lipid	Day0	240	440	260±25	110±20	240	440	260±25	110±20	240	440	260±25	110±20	240	440	260±25	110±20
	Day7	250	430	290±3.1	120±7.1	250	430	280±3.8	120±9.7	250	430	290±5.9	120±5.2	250	440	290±3.0	120±12
	Day14	260	440	290±6.4	120±4.3	250	420	290±4.9	120±4.4	250	440	290±11.3	110±6.1	250	460	300±1.6	110±8.7
Intralipos	Day0	240	440	260±13	250±14	240	440	260±13	250±14	240	440	260±13	250±14	240	440	260±13	250±14
	Day7	220	420	240±4.9	270±13	210	440	240±5.5	260±9.9	210	420	230±4.8	210±4.6	210	390	240±2.2	210±6.0
	Day14	210	440	240±5.2	180±22	210	420	240±4.8	220±9.0	220	420	240±5.8	220±5.1	220	410	250±5.8	230±4.4

Table S2. Changes in MDA concentration and pH value in samples stored in a car

		Package1 (air-sealing+photoprotection)		Package2 (air-sealing only)		Package3 (photoprotection only)		Package4 (none)	
		MDA (μM)	pH	MDA (μM)	pH	MDA (μM)	pH	MDA (μM)	pH
Omegaven	Day0	2.19 ±0.10	8.12 ±0.07	2.19 ±0.10	8.12 ±0.07	2.19 ±0.10	8.12 ±0.07	2.19 ±0.10	8.12 ±0.07
	Day3	6.08 ±2.04 *	7.78 ±0.17	5.95 ±1.65 *	7.80 ±0.18	24.63 ±6.20 **	7.30 ±0.39 **	26.36 ±5.00 **	7.27 ±0.34 **
	Day7	6.55 ±1.80 **	7.71 ±0.17	6.98 ±1.97 **	7.70 ±0.18 *	32.91 ±3.75 **	6.43 ±0.74 **	37.17 ±1.30 **	6.16 ±0.83 **
SMOF lipid	Day0	0.96 ±0.08	7.90 ±0.01	0.96 ±0.08	7.90 ±0.01	0.96 ±0.08	7.90 ±0.01	0.96 ±0.08	7.90 ±0.01
	Day3	2.30 ±0.15	7.71 ±0.08	3.00 ±0.44 *	7.70 ±0.08	10.24 ±1.57 **	7.53 ±0.03 **	16.18 ±2.24 **	7.38 ±0.03 **
	Day7	3.91 ±1.31 **	7.43 ±0.22 **	4.49 ±0.65 **	7.46 ±0.24 **	17.54 ±2.20 **	6.76 ±0.50 **	25.06 ±4.55 **	6.43 ±0.61 **
Intralipos	Day0	0.69 ±0.15	7.21 ±0.25	0.69 ±0.15	7.21 ±0.25	0.69 ±0.15	7.21 ±0.25	0.69 ±0.15	7.21 ±0.25
	Day3	2.06 ±0.37 **	6.94 ±0.22	2.18 ±0.41 **	6.93 ±0.24	3.27 ±0.57 **	6.70 ±0.19 **	5.48 ±0.95 **	6.49 ±0.18 **
	Day7	2.06 ±0.38 **	6.83 ±0.16 **	2.77 ±0.12 **	6.74 ±0.24 **	4.62 ±0.73 **	6.30 ±0.26 **	8.02 ±0.58 **	5.65 ±0.38 **

Each value is expressed as mean ± SD. Significant differences from day 0 are indicated by * when $P < 0.05$, and ** when $P < 0.01$.
MDA, malondialdehyde