OXIDATION STABILITY OF EICOSAPENTAENOIC AND DOCOSAHEXAENOIC ACID INCLUDED IN CYCLODEXTRINS

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ABSTRACT

We have proposed a novel method for complexing of the ω-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic and docosahexaenoic acids, with cyclodextrin as dry powders by a twin-screw kneader. The effect of various oxidation conditions on powdery PUFA were investigated. Further, the powdery PUFA developed was employed in the preparation of fish meal as a functional sea food paste.

1. INTRODUCTION

The ω-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic and docosahexaenoic acids have important physiological functions. They are believed to have antithrombotic, cholesterol depressant, and antiallergenic properties, among others. These PUFAs are chemically quite reactive, requiring proper encapsulation in a powder form to protect against autoxidation[1]. We have developed a novel method for complexing of PUFAs with cyclodextrin as dry powders by a twin-screw kneader[2]. In the present study the effect of various oxidation conditions on powdery eicosapentaenoic ester and docosahexaenoic acids were investigated. It was also observed that carbohydrates could be used to further protect the PUFA cyclodextrin inclusion complexes. Further, the powdery docosahexaenoic acid developed was employed in the preparation of fish meal as a functional sea food paste.

2. MATERIALS AND METHODS

2.1 Materials

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Reagent grade of α-, β-, and γ-cyclodextrin(CD) were from Ensuiko Sugar Refining Co. and Wacker Chemicals. Eicosapentaenoic acid ethyl ester (EPA: purity 82%) was gift from Nippon Suisan Co., and docosahexaenoic acid oil:triglyceride form (DHA: purity 45%) was from Maruha Co. Maltodextrin was gift from Nippon Starch Chemical Co. Other chemicals were of analytical grade.

2.2 Preparation of inclusion complex

The inclusion complex powders of EPA or DHA with cyclodextrin were prepared by a twin-screw kneader in a nitrogen atmosphere. Twenty to thirty grams of α-, β- or γ-CD were weighed and mixed with EPA or DHA, followed by adding distilled water to an initial moisture content of 30 or 50% on dry basis. Carbohydrate such as maltose, maltodextrin or pullulan was also added when the coating effect of carbohydrate were investigated. The mixture was kneaded in a twin screw kneader (KRC-S1, Kurimoto Steel Ltd.) at 60 to 50 °C for 30 min. The wet slurry was washed with diethyl ether and dried *in vacuo* at 20 to 40 °C for 15 hr.

2.3 Autoxidation of inclusion powders

About 0.1-3 g of the complex powder was placed in a glass bottle or plate, and stored at 50°C and a relative humidity of 75%. The effect of humidity was measured with a humidity-controlled gas flowing through the glass bottle by connecting plastic tubes. The powder was sampled at certain intervals for the determination of the residual EPA and the peroxide value (POV) of DHA. EPA was analyzed by gas chromatography (GC-14A, Shimadzu). POV value of DHA was quantified by iodometric titration method.

2.4 Preparation of fish meal paste ("Kamaboko") involved DHA powder

Surimi from walleye pollack was chopped with NaCl, water and DHA, by means of a speed cutter at 10°C for 5min. The salt-ground paste was stuffed into a plastic vessel with a cover, and heated at 90°C for 20 min in a water bath to prepare "heating gel". After cooling the gel with ice water, and stored in a refrigerator. POV of DHA in the gel was measured periodically by extracting DHA into chloroform.

3. RESULTS AND DISCUSSION

3.1 Autoxidation of powdery PUFA

The time courses of autoxidation of powdery PUFA are illustrated in Fig.1. As shown in Fig.1(a), the washed powdery EPA in α- or β-CD was quite stable to oxidation, and most EPA remained unoxidized. However, the amount of EPA included per unit mass of α- or β-CD was quite small, about 0.05 and 0.01, respectively. The resistances against autoxidation of the unwashed powders are also improved in comparison with liquid EPA ester, but are inferior to the washed powder. Powdery EPA in α-CD was rapidly

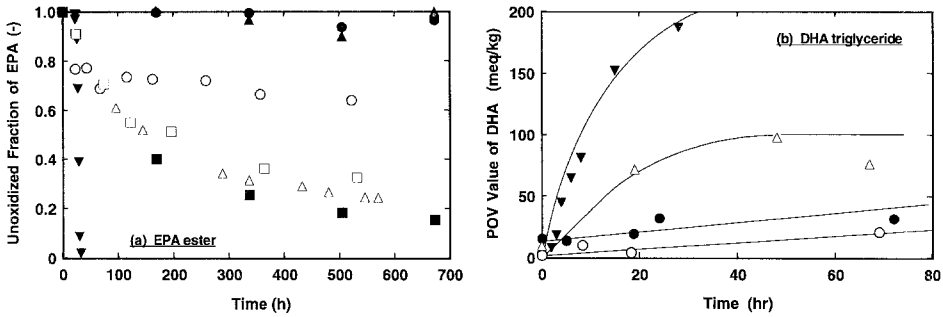

Fig. 1 Retardation of the autoxidation of powdery EPA ester and DHA triglyceride. (▼: Liquid EPA ester or DHA triglyceride. ○,●: in α-CD, △,▲: in β-CD, □,■: in γ-CD. Open symbol are unwashed powder. Closed symbols are washed powder.)

oxidized during an initial period, followed by a stable region against oxidation. This indicates that EPA truly included in α-CD may be quite resistant to oxidation. On the other hand, powdery EPA in β- or γ-CD were gradually oxidized as time increased. The oxidation time courses of DHA in α- and β-CD are shown in Fig.1(b), in which the oxidation degree was measured by the POV value. The powdery DHA in α-CD has about 8 times smaller POV value than liquid DHA. β-CD was also useful for elongation of DHA shelf-life. Though the unwashed DHA powder in α-CD has slightly higher initial POV value than the washed powder, oxidation did not progress abruptly.

3.2 Protecting autoxidation by adding carbohydrates

Since the mass fraction of PUFA truly included in CDs is small, much PUFA remained unincluded and adsorbed onto CD's molecules[2]. To protect these PUFA molecules

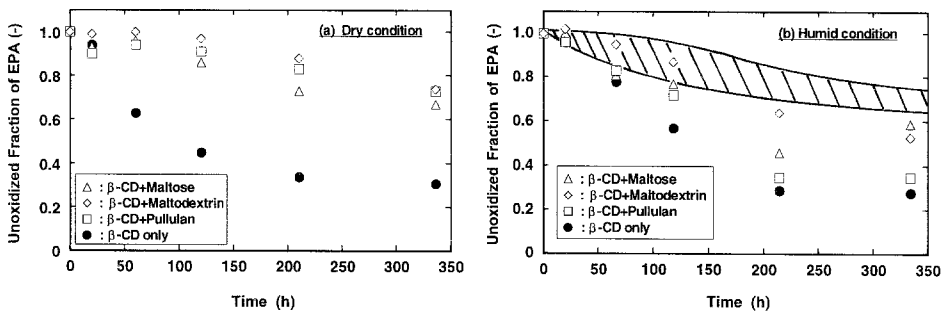

Fig. 2 Effectiveness of the coating with carbohydrate to the retardation of autoxidation of the complex powder between EPA ester and β-CD.

from oxidation, carbohydrates such as maltose, maltodextrin, and pullulan, were added for encapsulation. Figure 2(a) shows the effect of the carbohydrates on the resistance

against autoxidation in β-CD. The hybrid powder had equivalent stability to the washed β-CD complex powder, though EPA content was much higher (0.08 g/g). The similar effects were observed for other CD and carbohydrate combination. The addition of a carbohydrate was especially helpful in the case of β-CD inclusion complexes. As shown in Fig.2(b), the oxidation was accelerated in humid condition. From Figs.2(a) and (b), it is found that carbohydrates were less effective under humid condition. Similar results were obtained for α- and γ-CD complex powder.

3.3 Oxidation of DHA powder involved in fish meal paste

Figure 3 shows the oxidation of DHA in the fish meal paste, which was stored at 10°C. Three different forms of DHA was used in the fish paste. In a liquid form DHA was oxidized rapidly, and the POV value exceeded 40 after 4 days of storage. POV value of

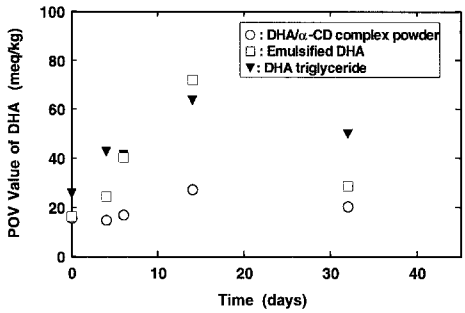

Fig.3 Shelf-life of DHA triglyceride of various forms in the fish meal paste.

the DHA emulsified with distilled water increased markedly during an initial period of oxidation. In the contrary, the powdery DHA in α-CD was very stable against oxidation, and the POV value did not increase above 30 during one month storage. This implied that the powdery DHA in α-CD was useful for a functional fish meal paste.

4. CONCLUSION

Both the powdery EPA ester and DHA triglyceride exhibited a marked resistance against autoxidation. It was also observed that carbohydrates could be used to further protect the PUFA cyclodextrin inclusion complexes from oxidation. The POV of powdery DHA in fish meal paste remained virtually unchanged for 20 days without any antioxidants.

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