The distribution of fatty acids in the position of glycerides hydrolyzed from fish oil by lipase

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Abstract: In order to determine the position and the content of fatty acids attached to glycerides and the migration degree of fatty acids in the migration reaction, hydrolysis of fish oil was carried out with lipolase-100T derived from *Aspergillus oryzae*. The content of fatty acids in the glyceride mixture was analyzed and compared with that of fish oil. The amounts of fatty acid in 2-position and the migration degree of the fatty acid in 2,3-DG (diglyceride) and 2-MG (monoglyceride) were calculated. The results showed that approximately 95% (w/w) of DHA (docosahexaenoic acid) and 65% of EPA (eicosapentaenoic acid) was attached to the 2-position of glycerides in the fish oil. Approximately 87% (w/w) of DHA and 75% of EPA remained in 2,3-DG and 88% of DHA and 65% of EPA in 2-MG were not involved in the migration reaction.

Introduction

Enzyme reactions for the processing of PUFA-containing oil have drawn much attention since they proceed efficiently at ambient temperature and pressure. However, there has been no report that can quantitatively determine the amount of free fatty acid bonded to 2-position carbon of glycerides in fish oil and the migration degree of each kind of fatty acid in 2,3(1)- diglyceride and 2- monoglyceride. In our previous paper, we determined the amount of free fatty acid produced during hydrolysis from triglyceride (TG), diglyceride (DG), or monoglyceride (MG) [1)] . In this study, lipase derived from *Aspergillus oryzae*, a 1,3-postional specific lipase, was used to hydrolyse the fish oil. The material balance on each component in the reaction system was obtained. Also the weight percent of various fatty acids at the 2-glyceryl position of glycerides in fish oil and the migrated fraction of each type of fatty acid in 2,3(1)-DG and 2-MG were calculated.

Materials and Methods

Lipolase-100T from *Aspergillus oryzae* was purchased from NOVO Nordisk Corporation. Fish oil refined by LIPRO AS Corporation of Norway was used. The DHA content of fish oil was 8.6%(w/w) of total fatty acid content. The methods for fish oil hydrolysis and lipid compositions analysis were same as those in our previous paper [1].

Results and Discussion

The compositions of fatty acid in MG, and FFA are shown from Fig.1 and Fig. 2 and are compared with the fatty acid composition of the starting fish oil. Other fatty acids present in fish oil were not quantified due to the unavailability of fatty acid standards. As shown in Fig. 1 , the content of fatty acids-except EPA and DHA in the MG are lower than in the fish oil. The weight percent of DHA in MG was 23.5%, which is about three times higher than in the fish oil. However, the content of DHA in FFA was lower than that of other fatty acids (Fig. 2). The reason could be that DHA is poor substrates for the lipolase-100T and thus DHA is concentrated as glyceride forms in the reaction mixtures.

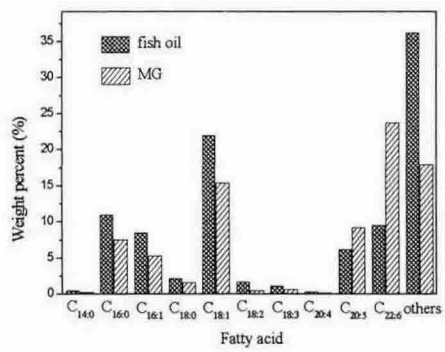

Fig. 1. The fatty acid compositions of MG and fish oil

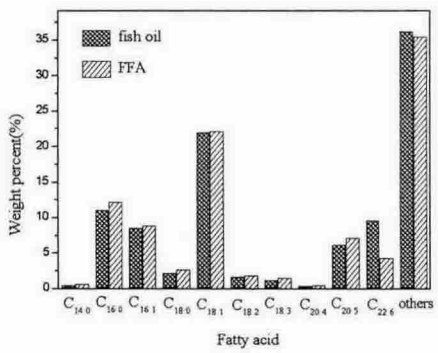

Fig. 2. The fatty acid compositions of FFA and fish oil

Fig. 3 shows the weight percent of each fatty acid at 2-position in the fish oil. Ninety five percent (w/w) of DHA in the fish oil was bonded to the 2-position of glycerides, while the composition of

the other fatty acids except DHA and EPA was less than 40%. This indicates that the other fatty acids in the glycerides are evenly distributed in the fish oil. Yadwad *et al.*[2] claimed that the retention of DHA in the monoglyceride fraction is due to a combination of two facts: lower specificity toward DHA than other fatty acids, and the enrichment of DHA at the 2-glyceryl position of triglycerides in fish oil.

It was reported that both 2,3-DG and 2-MG are chemically unstable species and undergo acyl group migration to produce 1-MG and 1,3-DG respectively [3]. However, there has not been any report that can quantitatively determine the migration degree of each fatty acid in the acyl-rearranged reaction. Fig. 4 shows the weight percent of fatty acids migrated and remained in 2,3-DG. The migration degree of each kind of fatty acid was different: 87% of the DHA and 75% of the EPA in 2,3-DG remained, which are higher than other fatty acids. It was found that 88% of the DHA and 65% of the EPA kept in 2-MG (Fig.5). These results indicate that DHA and EPA are so difficult to migrate that the content of DHA and EPA will be increased in glyceride form. Fig. 6 compares the weight percent of each kind of fatty acids for migration in 2,3-DG with that in 2-MG. Most fatty acids in 2-MG were easier to migrate than those in 2,3-DG.

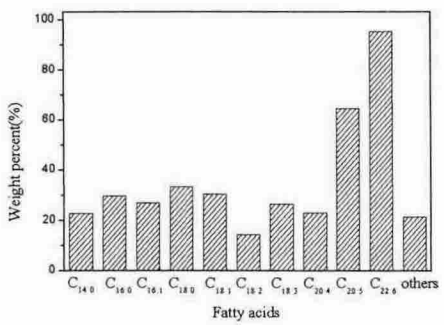

Fig. 3. Compositions of fatty acids attached to the 2-position in the fish oil remained

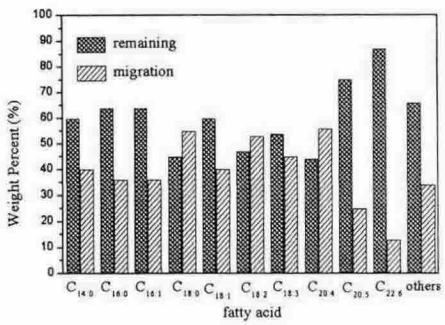

Fig. 4. The content of fatty acids migrated and in 2,3-DG

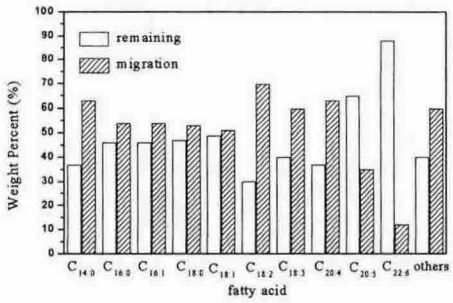

Fig.5. The content of fatty acids migrated and remained in 2-MG

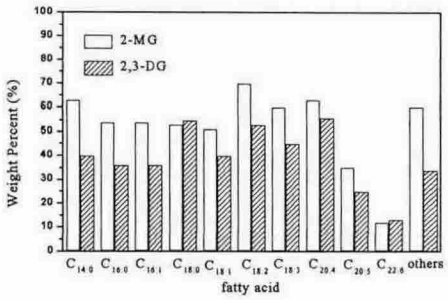

Fig.6. Comparison of the weight percent of fatty acids migrated in 2,3-DG with that in 2-MG

References

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3. Holmberg, K. and Osterberg E. Enzyme preparation of monoglycerides in microemulsions. (1988). *J. Am. Oil. Chem. Soc.* 65,1544-1548.