Short Paper

A Technique to Enrich $\alpha$-tocopherol in Chlorella and Marine Rotifers

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Abstract: $\alpha$-Tocopherol (TOC) and various fatty acids were supplemented into the Chlorella vulgaris strain K-22 culture at 28°C for 24 h, and TOC content of the Chlorella cells was determined. The addition of TOC alone into the Chlorella culture medium did not give an increase of the cellular TOC content. However, the TOC contents in the Chlorella cells supplemented with TOC and various fatty acids were much higher than those supplemented with TOC alone. Moreover, the TOC content in the cells supplemented with a mixture of TOC and oleic acid was higher than those supplemented with a mixture of TOC and docosahexaenoic acid (DHA). The TOC content of the rotifers fed on the TOC-enriched Chlorella was much higher than rotifers fed on unenriched Chlorella. On the other hand, rotifers could be easily enriched with TOC by feeding the TOC-enriched Chlorella cells.

Key words: Chlorella vulgaris; Rotifer; $\alpha$-tocopherol; Oleic acid

Chlorella is a microalgae widely used as a feed for rotifers in commercial hatcheries in Japan. Chlorella does not contain highly unsaturated fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA). So when rotifers fed on Chlorella as the sole feed, they lack essential fatty acids necessary for marine fish larvae. Therefore, the rotifers fed on Chlorella are usually enriched with DHA prior to feeding them to fish larvae. Recently, DHA and fat soluble vitamins enriched Chlorella has been developed in Japan. The rotifers fed on DHA-enriched Chlorella cells contain significant amount of DHA without nutritional enrichment procedure, thus saving labor requirement in hatcheries.

The effect of $\alpha$-tocopherol (TOC) on the population growth of rotifers has already been studied. It was also reported that feeding of TOC-fortified Chlorella to rotifers enhanced the population growth. Moreover, it was suggested that TOC is also important for the growth and survival of larval cod and yellow tail. However, techniques for enrichment of rotifers with TOC-fortified Chlorella have never been developed, despite the important roles of dietary vitamins in fish larvae. In this study, enrichment techniques of TOC for Chlorella and rotifers were developed. Furthermore, the effects of fatty acids on the enrichment process of Chlorella with TOC were also studied.

Chlorella vulgaris strain K-22 that was isolated from a pond in Saga Prefecture, Japan, was used in this study. C. vulgaris K-22 was grown in a medium containing 2% glucose, 0.15% urea, 0.15% KH$_2$PO$_4$, 0.06% MgSO$_4$, 5 mg/l of EDTA-Na-Fe, and 2 ml/l A5 mineral solution for 72 h in the dark at 28°C. TOC was DL-$\alpha$-tocopherol (purity; 99%) purchased from Nakarai Tesque, Co. Ltd. To insure dispersion in the culture medium, TOC was dissolved first in 0.1% of ethanol (purity; 99%), or mixed with fatty acid before addition to the Chlorella culture. The concentrations of TOC and fatty acid were 0.02 and 0.2%, respectively. For the control cells, 0.1% of ethanol alone was supplemented into the culture medium. After the addition of TOC with/without fatty acid into the culture, cultivation was continued for 24 h more at 28°C. The cells were harvested by centrifugation, washed three times with distilled water, and kept at -30°C until analysis. The TOC contents in the cells were measured by high performance liquid chromatography (HPLC) after extraction and saponification of the total lipid.

Rotifer, Brachionus rotundiformis, was cultivated by feeding normal Chlorella (Chlorella V-12, Chlorella Industry, Co. Ltd.), and stocked in 1-liter plastic beakers at a density of 1000 individuals/ml. Then the Chlorella cells enriched with TOC were fed to the rotifers for 6 h at 28°C. The feeding rate of the Chlorella cells was 400 mg/l for each rotifer culture. Then, the rotifers were harvested by plankton net, and their TOC contents were measured by HPLC after extraction and saponification of the total lipid.

The data, presented as means ± SD, were subjected to one-way analysis of variance (ANOVA). Statistical significance ($P<0.01$) of the differences between means was determined by Tukey's multiple comparison procedure.

Figure 1 shows TOC contents in the Chlorella cells supplemented with TOC and each of the fatty acids. The TOC content of the control cells was 7.8 mg/100g dry cells, while, the TOC content of the cells supplemented with 0.02% of TOC without fatty acid was 8.1 mg/100g dry cells. However, the TOC contents of the cells were greatly increased by the supplementation of TOC with fatty acids such as oleic, linoleic, eicosapentaenoic, or docosahexaenoic acid. The TOC contents in the cells supplemented with a mixture of TOC and oleic or linoleic acid were significantly ($P<0.01$) higher than those of the lean process.
cells supplemented with a mixture of TOC and a highly unsaturated fatty acid. This showed that supplementation of TOC with fatty acid improved the TOC uptake of the Chlorella cells, and the enrichment of Chlorella with TOC depended on the degree of unsaturation of fatty acids. The other homologue tocopherols, such as $\beta$-, $\gamma$-, or $\delta$-tocopherol, were not detected in Chlorella.

TOC contents of rotifers fed on unenriched or TOC-enriched Chlorella cells were shown in Fig. 2. Content of TOC in rotifers fed on Chlorella, supplemented with a mixture of TOC and DHA, slightly increased compared with those fed on unenriched Chlorella. But the content of TOC in the rotifers, fed on the Chlorella supplemented with a mixture of TOC and oleic acid, was significantly ($P<0.01$) higher than that in rotifers fed on the cells supplemented with a mixture of TOC and DHA.

These results showed that Chlorella cells could be easily fortified with TOC by the supplementation of a mixture of TOC and fatty acids for 24 h. The rotifers also could be easily enriched with TOC by feeding the TOC-enriched Chlorella for several hours. However, the fatty acid supplementation greatly affected the amount of TOC uptake by Chlorella. Importantly, the amount of TOC uptake into the Chlorella supplemented with a mixture of TOC and DHA was less than that of the cells supplemented with a mixture of TOC and oleic acid. The higher unsaturated fatty acid may have consumed the more TOC, thereby preventing their oxidation. However, further studies are required for the evaluation of relationship between the degree of unsaturation of fatty acid and TOC consumption.

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References