Effects of ergothioneine-rich mushroom extract supplementation on the oxidative stability of astaxanthin in salmonids

[課程博士・論文博士共通]

博士学位論文内容要旨
Abstract

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論文題目 Title	Effects of ergothioneine-rich mushroom extract supplementation on the oxidative stability of astaxanthin in salmonids (エルゴチオネインに富むきのこ抽出物の給与がサケ科魚類のアスタキサンチンの酸化安定性に及ぼす影響)		

Certain salmonid species are known for their distinct reddish-orange muscle coloration, and the same characteristic also dictates the commodity's market quality. This pigmentation is due to the accumulation of astaxanthin in the fish muscles. Fish and other animals cannot biosynthesize carotenoids including astaxanthin but can be acquired through their diet. Salmonid meat also contains a considerably high amount of polyunsaturated lipids which makes it susceptible to oxidation. This could lead to quality deterioration due to discoloration, loss of nutritional value, production of unhealthy by-products, and compromised organoleptic properties. The supplementation of naturally-derived products with high antioxidative properties is one strategy being used to control oxidative degradation. (2*S*)-3-(2-Sulfanylidene-1,3-dihydroimidazol-4-yl)-2-(trimethyl-azaniumyl)propanoate (ergothioneine) is a potent hydrophilic antioxidant abundantly found in several edible mushroom species and has been widely used to control oxidation and quality deterioration in seafood commodities.

This study was conceptualized with the aim of preserving astaxanthin-rich salmonid meat from oxidation by the supplementation of ergothioneine-rich mushroom extracts (ME). Specifically, this study initially aimed to determine the feasibility of preserving astaxanthin through the addition of ergothioneine-rich ME in an *in vitro* cell model. With the feasibility of ergothioneine to protect astaxanthin from oxidation demonstrated in the initial study, a subsequent study was conducted to determine the antioxidative effects of ME, added to fish meat, during low temperature storage. In addition, the feasibility of ME dietary supplementation and ergothioneine uptake in fish was determined by conducting a feeding trial. Moreover, another feeding trial was conducted to evaluate the feasibility of ME dietary supplementation with other salmonid species and evaluate its antioxidative effects in fish meat during post-harvest low temperature storage. Furthermore, another study was carried out with the aim to determine the most probable gene sequences encoding for ergothioneine transporter proteins (ETTs) in certain *Oncorhynchus* spp. through bioinformatics.

Liposomes were used as *in vitro* cell models to evaluate the effects of ME in preserving astaxanthin-containing cells under oxidation-induced conditions. Results of this study effectively demonstrated that the presence of ergothioneine or ME together with astaxanthin in the liposome have additive synergistic antioxidative functions that could neutralize reactive radical species and control the advancement of lipid oxidation and delay astaxanthin degradation. The evaluation of the antioxidative properties of the crude mushroom extract was shown to have significantly higher activities than pure authentic ergothioneine standard. These findings suggest the presence of other compounds in crude ME with potent antioxidative properties as well.

The positive confirmation of the hypotheses in the *in vitro* experiment led to the application of ergothioneine-rich ME to astaxanthin-pigmented rainbow trout (*Oncorhynchus mykiss*) meat to evaluate its

effects against lipid oxidation and astaxanthin degradation during storage. Results showed positive effects of ME-treatments in meat in controlling astaxanthin degradation and lipid oxidation. Subsequently, a 10-week feeding trial on rainbow trout was done to evaluate feed acceptability and ergothioneine uptake using different concentrations of ME, as well as to assess the effects on the growth and pigmentation of the fish. Results of this feeding trial showed a positive response of fish towards the acceptability of the different concentrations of ME-supplemented feeds. Considerable ergothioneine uptake was confirmed and was correlated with the decrease in the levels of lipid hydroperoxides in blood. Moreover, no adverse effects were observed on the growth, lipid content, and pigmentation of the fish, as a response to the dietary supplementation of the ME concentrates.

The next ME-supplemented feeding experiment was carried out with coho salmon (*Oncorhynchus kisutch*) to evaluate the applicability and effects of feeding supplementation on other salmonid species. Different concentrations of ergothioneine-rich ME were incorporated into astaxanthin-rich commercial diets of coho salmon and were administered for 8 weeks. Results of the supplemented feeding showed no adverse effects on the growth, pigmentation, and fat deposition of the ME extract supplemented fish. Moreover, a positive uptake of ergothioneine from the diet was noted in the ME-supplemented group. Meat samples were collected from the cultured fish and kept at low temperature storage and were evaluated for the effects of ME-supplementation on lipid hydroperoxide formation, astaxanthin content, and changes in visual coloration. Results showed mitigation of lipid oxidation and discoloration in the meat of ME-supplemented fish. The findings of this study demonstrated the feasibility of incorporating ergothioneine into the diet of fish during grow-out culture as a strategy to preserve the quality of the fish as well as to provide added value to the commodity.

To be able to further understand the effects of dietary ergothioneine supplementation on the fish, further studies were conducted to elucidate the mechanism of ergothioneine absorption, transport, and accumulation in fish. The SLC22 gene family to which ergothioneine and carnitine transporters belong is one example of a relatively large gene group that shares a considerable amount of homology among its members but also exhibit distinct and specific functions for each unique homolog. Based on the various bioinformatics analyses conducted for salmonid SLC22 homologs, a clearer picture of the most appropriate candidates for salmonid ergothioneine transporter gene was thus obtained. The candidate genes determined through evolutionary phylogeny, sequence analysis, and topology comparison should, therefore, be the subject of future studies related to ergothioneine uptake in salmonids. This candidate gene for *O. mykiss* was used to evaluate the effects of ergothioneine-rich ME dietary supplementation on the expression of ETT in certain tissues. Results demonstrated that ETT is expressed in *O. mykiss* blood and muscle tissues and that ETT expression was actually downregulated with ME supplementation, despite increased ergothioneine accumulation in these tissues. This provides stronger evidence of the feasibility of dietary supplementation of ergothioneine-rich mushroom extracts in maintaining salmonid flesh quality against oxidative damage and degradation.

In summary, the crude hydrophilic mushroom extracts are an efficient source of ergothioneine as well as other potent antioxidants, which was demonstrated to have a synergistic effect with astaxanthin, as tested in both *in vitro* and *in vivo* applications. The utilization of underutilized commercial food processing waste such as mushroom cuttings or spent culture media is a rich and economical source of ergothioneine and crude mushroom extracts that can be used as feed additives and dietary supplements in the grow-out culture of certain salmonid species.