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

Dissertation Summary

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Salmon meat is known for their distinct reddish-orange muscle coloration, which dictates its market quality and value. This pigmentation is due to the accumulation of astaxanthin in their muscles, which are acquired through their diet. Salmon meat also contains a considerably high amount of polyunsaturated lipids which make it susceptible to oxidation, which 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 of the strategies being used to control oxidative degradation. 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 several post-harvest seafood commodities.

This study was conceptualized with the aim of preserving astaxanthin-rich salmonid meat from oxidation through the supplementation of ergothioneine-rich mushroom extracts (ME). Chapter 2 aimed to determine the feasibility of preserving astaxanthin through the addition of ergothioneine-rich ME in an *in vitro* cell model. Giant-sized astaxanthin-filled liposomes were used as *in vitro* cell models mimicking the astaxanthin-pigmented cells of salmon meat to elucidate the interactions of astaxanthin and lipid compounds with ergothioneine-rich mushroom extract under oxidation-induced conditions, to provide better understanding of the agricultural and post-harvest applications of mushroom extract applications to post-harvest

commodity preservation. Azocompounds 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN) were used as hydrophilic and lipophilic radical initiators, respectively. Liposomes were added with either AAPH or AMN, incubated, and monitored for oxidative stability. Results of this study effectively demonstrated that the presence of ergothioneine or ME together with astaxanthin in the liposomes have additive synergistic antioxidant functions that could neutralize reactive radical species to control the progress of lipid oxidation and delay astaxanthin degradation. Crude mushroom extracts had higher antioxidative capacity than the equimolar concentrations of ergothioneine alone, which demonstrates the antioxidative properties of other compounds present in the extract such as the phenolics.

With the feasibility of ergothioneine to protect astaxanthin from oxidation demonstrated in the initial study, a subsequent study (Chapter 3) was conducted to determine the antioxidative effects of ME, added to astaxanthin-pigmented rainbow trout (*Oncorhynchus mykiss*) meat, during low temperature storage (-10 °C). Results showed promising effects of ME-treated 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. In addition, the feasibility of ME dietary supplementation and ergothioneine uptake in fish and its effects on the growth and pigmentation of the fish was determined by conducting a feeding trial. Prepared ME was added to the diet of the fish at 3% and 5% (w/w) prior the daily broadcast, which lasted for 2.5 months. Results of this feeding trial showed 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 (Chapter 4) was done with coho salmon (*Oncorhynchus kisutch*) to evaluate the applicability and effects of feeding supplementation strategy on other salmonid species. Different concentrations of ergothioneine-rich ME were incorporated into the 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 in the ME-supplemented coho salmon. Moreover, a positive uptake of ergothioneine from the diet was noted in the ME-supplemented group. Ergothioneine-containing meat samples from ME-supplemented group exhibited radical scavenging activities. Moreover, meat samples were collected from the cultured fish, kept at low temperature (-2 and -18 °C), and evaluated for the effects of ME dietary supplementation on the lipid hydroperoxide formation, astaxanthin content, and changes in visual coloration during storage. 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 bioinformatics studies (Chapter 5) 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 in the expression of ETT in certain tissues. Results demonstrated that ETT is expressed in *O. mykiss* blood and muscle tissues, which provided 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 extracts from edible mushroom species are efficient sources of ergothioneine as well as other potent antioxidants, which has demonstrated synergistic effects 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 grow-out culture of certain salmonid species, which have considerable effects in the mitigation of lipid oxidation during post-harvest storage. The results obtained in this study could also provide insights on the possible antioxidative properties of the other bioactive components present in edible mushrooms. Furthermore, this study could expand the understanding and application of naturally-derived antioxidants from edible mushrooms in the post-harvest preservation of oxidation-susceptible food commodities.