Excecutive Summary

Background and Rationale

Reactive oxygen species are superoxide radicals, hydroxyl radicals, and hydrogen peroxide, often generated as byproducts of the body's metabolic process or from exogenous factors such as atmospheric pollutants. Oxygen free radical can initiate peroxidation of lipids which in sequence stimulated glycation of protein, inactivation of enzyme systems, decreased membrane fluidity and DNA mutations(Aqil et al., 2006)The oxidative damage derived from free radicals may be related to aging, degenerative and other diseases. Even though human body and other organisms have antioxidant defense and repair systems to protect them against oxidative damage, the systems are lacking to prevent all the damage. So antioxidant compounds in human diets or natural sources are of great notice as likely protective agents to help human body diminish oxidative damage. Many natural antioxidants have already been isolated from different kinds of plant materials such as oilseeds, cereal crops, vegetables, fruits, leaves, roots, spices and herbs (Mau et al., 2002). Many mushrooms are also rich sources of antioxidant compounds. Researches on mushrooms have focused mostly with polysaccharides derived from cell wall and some specific proteins, but proteins investigation aimed at creating a mushroom(s) proteome is still deficient. In this study investigates the antioxidant activity as well as proteins of some locally available edible mushroom in Phitsanulok, Loei and Trang Provinces of Thailand to provide a deeper insight in future.

Material and Methods

1. Mushrooms samples in this study

Cultivated and natural Lentinus mushrooms were collected from Phitsanulok, Loei and Trang Province. The cultivated Lentinus mushrooms, Horm (*Lentinula edodes* (Bull.) Singer), Lom (*Lentinus squarrosulus* Mont.) and Kornkaow (*Lentinus squarrosulus* Mont.) were purchased from farm in Phitsanulok and market in Loei Provinces. Natural Lentinus mushrooms, Lom mushrooms were perchased from market in Phitsanulok and Kradang *(Lentinus polychrous lev.)* mushrooms were collected from forest in Trang Province.

2. Extraction of total protein using Tris buffered phenol
3. Two-Dimensional Gel Electrophoresis
4. Preparation of mushrooms extract with methanol and hot water extract
5. Free radical scavenging assay DPPH(1,1-Diphenyl-2-picrylhydrazyl)

6. Total selenium content in mushrooms with ICP-MS
7. Ferric thiocyanate(FTC) method
8. Total phenolic compound in mushrooms with spectrophotometric method according to the Folin -Ciocalteu
9. Determination of protein by Bradford solution

Result and Conclusuin

Antioxidant activity of methanolic extracts is measured to inhibit initial stage of lipid peroxidation by FTC. The Lom and Kornkaow mushrooms in Phitsanulok showed strong antioxidant activity which is indicated by their low absorbance values. At the same concentration, the relatively upper activity was shown in the extracts of Lom followed by Kornkaow, Horm and got minimum in Kradang mushrooms when they were compared with BHA. However we may confirm lipid peroxidation inhibition with TBA method which measure the amount of lipid peroxidation in second stage. Another mechanism of antioxidants perform is scavenging of reactive oxygen and nitrogen free radicals. In this study we used DPPH assay to scavenge total free radical. This method is based on the reduction of DPPH, a stable free redical and any molecule that can donate an electron or hydrogen to DPPH can react with it and therrby bleach the DPPH absorption. As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, that is, a free radical scavenging is decreased and the resulting decolorization is stoichiometric with respect to the number of electrons captured. After mushrooms extracts were tested for the DPPH free radical scavenging ability. The methanoic extract of natural Lom mushrooms showed strongest radical scavenging activity with minimum IC_{50} at 0.5 mg/ml whereas Horm and Kornkaow mushrooms presented at higher IC_{50}. Kradang showed the poorest free radical scavenging activity. Those IC_{50} are comparable with commercial α-tocopherol and BHA as shown in Fig. 3. This should conclude that Lom mushrooms contain polyphenolics that can donate electron/hydrogen easily. We verified this hypothesis with measurement of total phenolics concentration equivalents of gallic acid. Gallic acid was the most important polyphenol in natural products in the range of 60-109 mg/g. From our result in Fig.6 high content of total phenolic compound was detected in Lom and Kornkaow mushroom, this result correlate with their potency in antioxidant activity. Total phenolic contents were rather low in Kradang and Horm mushroom and those mushrooms also showed rather poor in antioxidant activity. We should concern about a large number of phytocompound groups are implicated for antoxidant activity. Hot water Lom mushroom extracts were also exhibited strong antioxidant activity at 80% of potency free radical scavenging. The order of scavenging activity showed maximum in Lom followed by Kownkaow, Horm and Kradang mushrooms, respectively. This result correlated

with methanolic mushrooms extract. The antioxidant activity of those mushroom extracts is probable from some soluble vitamins such as vitamin C or water soluble of some phenolic compound.

Most of the protein spots in Lentinus mushrooms were concentrated in pI range 5-7 and between molecular mass 20-100 kDa, but also essential for reliable protein identification by MS or N-terminal amino acid sequencing. In this study we have modified tris buffered phenol method which is performed by Kiyotaka, H. and *et al* (2008). From Fig. 9-16, the spots of 2D gel were rather well however in future we should use longer strip 18 cm to work for MS or N-terminal amino acid sequencing to confirm our interesting proteins. We detected spot L-Seryl tRNA selenium transferase which was identified at pI ~ 8.5 and Mw ~ 51 kDa from previous study of *S. crispa* 2D gel (Kiyotaka, H. and *et al* , 2008) in Lom and KornKaow mushrooms. Selenium is an essentially required element for synthesis of selenoproteins including glutathione peroxidase(GPX) that is an important antioxidant enzyme. L-seryl tRNA selenium transferase is involved in the biosynthesis process. We also detected spot protein at pI ~ 6.43 and Mw~19 kDa which was identified as glutathione peroxidase in the same previous study. Both of the protein spots were presented in high peak of Lom and Kornkaow mushrooms by Melanie viewer 7 program supported of Swiss Institue of Bioinformmatics. Another interesting result, Se content of Lom mushrooms were also rather high in the rate of 0.42 mg/kg dry weight. These correlated data of Se content, L-seryl tRNA selenium transferase and glutathione peroxidase may be another index of antioxidant properties of Lom and Kornkaow mushrooms. However we plan to use MS or N-terminal sequencing to confirm spots of glutathione peroxidase and L-seryl-tRNA(Sec)Selenium transferase. However we should realize that there are having other groups of proteins which play role in antioxidant. We got supported data in anticancer property of Lom mushrooms by inhibition of proliferation in cancer cell lines (cholangiocarcinoma (K-100) in KKU and HT-29) by MTT-assay in precious study. In this study we characterized their antioxidant activity and detected some antioxidative proteins (glutathione peroxidase and L-seryl-tRNA(Sec)Selenium transferase). Their antioxidant activities were also supported from some selenium and phenolic compound in mushroom extracts. Their good characterizations will challenge us to study more for application in future