Results

Antioxidant activity

FTC assay: Antioxidants activity of Lentinus mushrooms in Phitsanulok , Loei and Trang Provinces

From Table 1, mushroom samples were extracted with methanol, natural Lom and Kornkaow mushrooms showed high yield. The lowest yield was Kradang and cultivated mushrooms. The methanolic crude extract of three wild and two cultivated mushrooms from Phitsanulok, Loei and Trang Province were screened for their antioxidant activity and free radical scavenging properties using α-tocopherol and butylated hydroxyanisol(BHA) as standard antioxidants. Antioxidant activity or potency of linoleic acid peroxidation of methanolic mushrooms were measured by ferric thiocyanate (FTC) assay and potency of free radical scavenging activity was determined using DPPH assay. The FTC method was used to measure the amount of peroxide at the beginning of the lipid peroxidation. Peroxidation of the linoleic acid reacts with ferrous chloride and form ferric ion. Ferric ion combines with ammonium thiocyante and produce ferric thiocyanate. The reaction is red. The higher the absorbance can assume that the lesser potency linoleic peroxidation inhibition. The mushroom extracts tested showed low absorbance values, which indicated a high level of antioxidant activity. None of the mushroom extracts showed absorbance values greater than the negative controls at the end point of both methods, indicating the presence of antioxidant activity. From result, all the mushroom extracts exhibited strong antioxidant as determined by FTC, comparing with the activity of the standard commercial antioxidants, alpha-tocopherol, and BHA. The overall potency lipid peroxidation inhibition of *Lentinus spp.* were rather good and not different as similar with positive control manage BHA by FTC assay as present in Fig 1. From Fig. 2, there was interesting from FTC result that natural and cultivated Lom mushroom had almost similar antioxidant properties. However, from potency free radical scavenging assay in Fig. 4, IC_{50} of natural Lom mushrooms were lower than cultivated Lom mushrooms. Graph in Fig. 3, the highest potency of free radical scavenging was performed in Lom mushrooms with lowest IC_{50}. The lower potency was obtainable in Kornkaow, Horm mushrooms, respectively. The buck potency of free radical scavenging was Kradang mushrooms.

The corresponding result of potency free radical scavenging was shown in Fig. 5. The highest potency free radical scavenging of hot water mushroom extracts were Lom and showed lower in Kornkaow, Horm and lowest in Kradang mushrooms.

Table 1. Yield of methanolic extracts from local mushrooms in local area of Phitsanulok Loei and Trang Province

Mushrooms	Amount[1](g)	Extraction yield[2](%,w/v)
เห็ดลมธรรมชาติ จ.พิษณุโลก (Natural Photsanulok Lom mushroom)	0.83	8.3
เห็ดลมเพาะ จ.พิษณุโลก (Cultivated Photsanulok Lom mushroom)	0.1	1
เห็ดขอนขาว จ.พิษณุโลก (Phitsanulok Kornkaow mushroom)	0.85	8.5
เห็ดกระด้าง จ. ตรัง (Trang Kradang mushroom)	0.1	1
เห็ดหอม จ.เลย (Loei Horm mushroom)	0.49	4.9

Extracted from dried medicinal mushrooms(10g). Each value is expressed as mean (n=3).

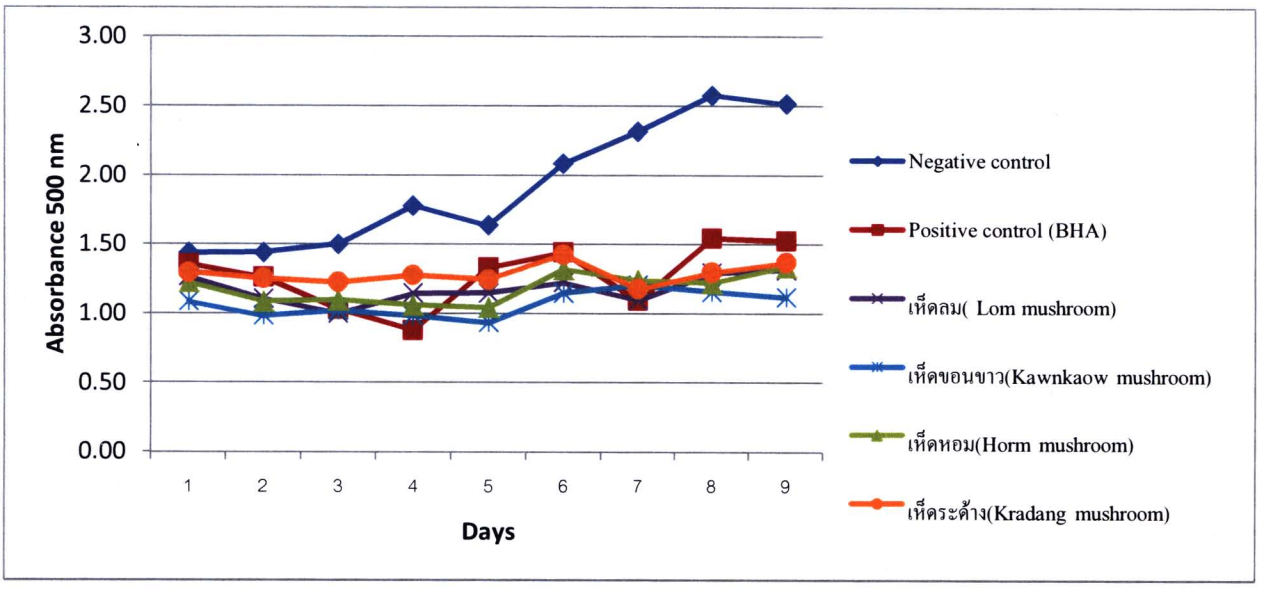

Fig. 1 Potency of linoleic acid peroxidation inhibition activity of methanolic mushroom extracts. The result are shown as means±SD (n=3).

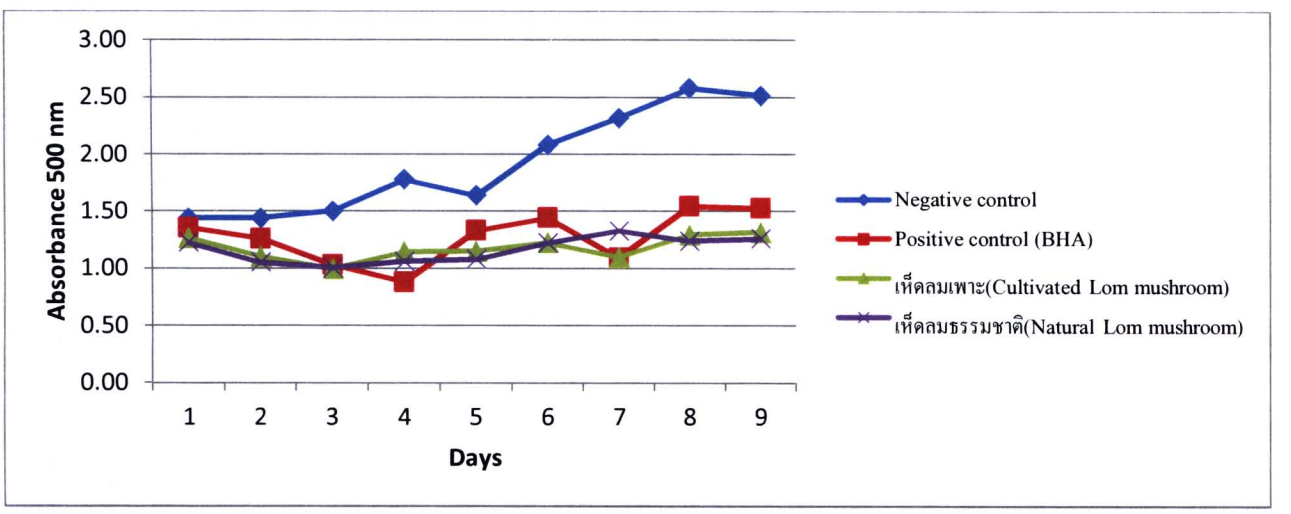

Fig. 2 Potency of linoleic acid peroxidation inhibition activity of cultivated and natural Lentinus extracts with methanol. The result are shown as means±SD (n=3).

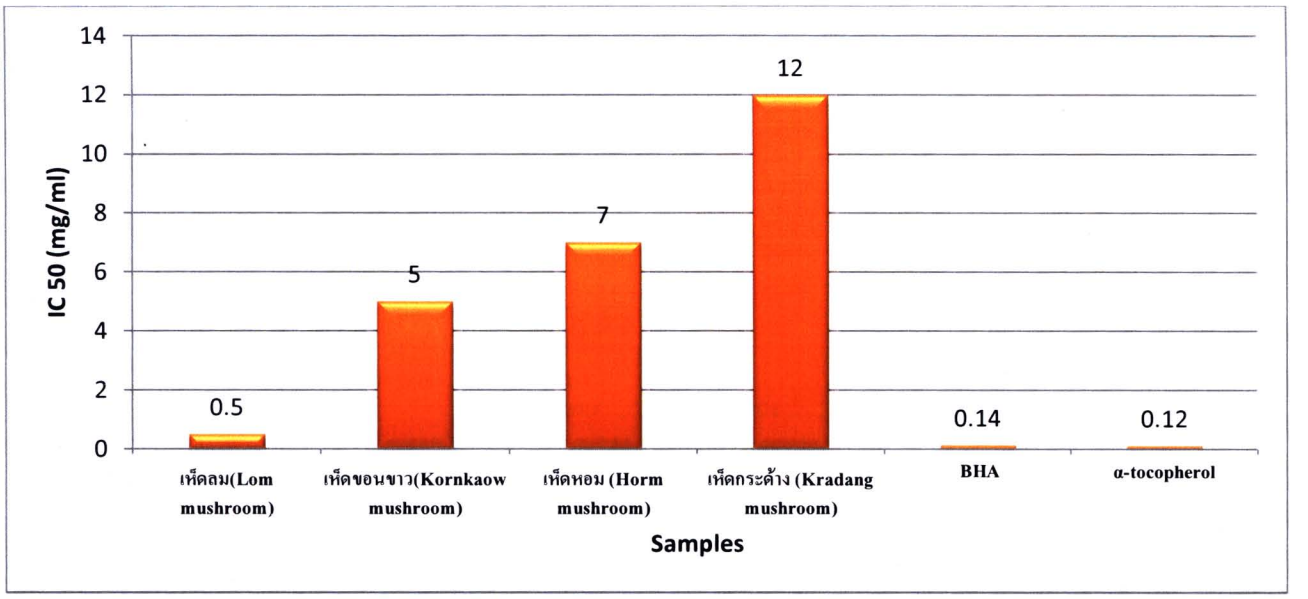

Fig. 3 Potency of free radical scavenging activity of methanolic mushrooms extract in Phitsanulok, Loei and Trang Provinces were determined with the DPPH method. The result are shown as means (n=3).

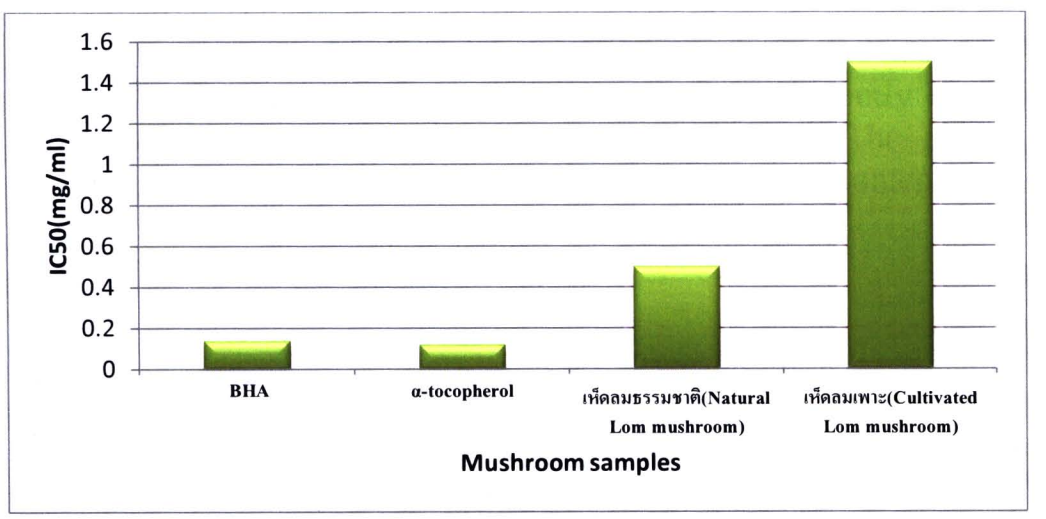

Fig.4 Potency of Free radical scavenging activity of methanolic natural and cultivated Lom mushrooms extract in Phitsanulok, Trang Provinces were determined with the DPPH method. The result are shown as means (n=3).

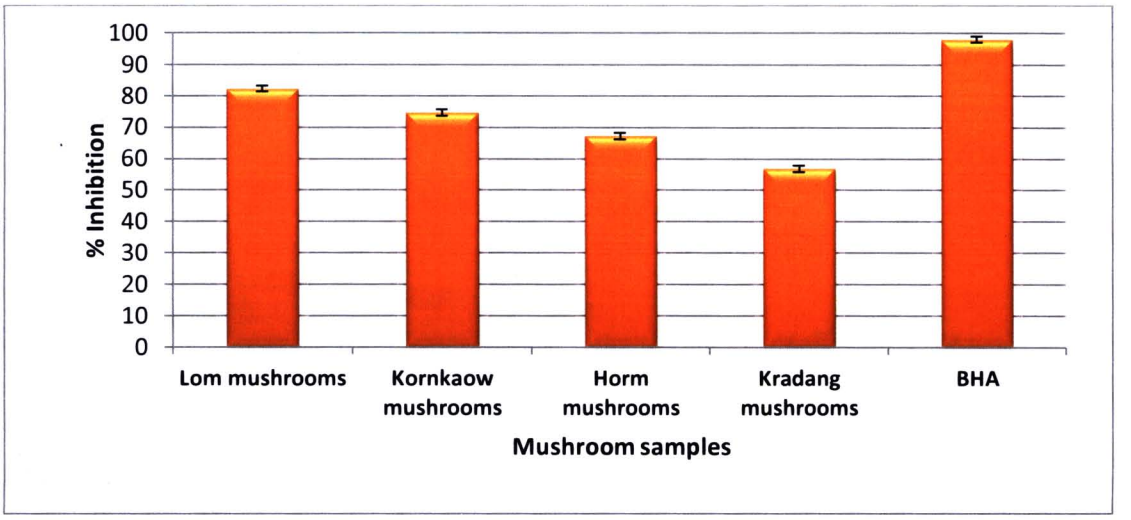

Fig.5 Potency of free radical scavenging activity or % inhibition of hot water mushrooms extract in Phitsanulok, Loei and Trang Province: neutralization of DPPH radicals of samples in the free radical scavenging activity assay. Butylated hydroxyanisol(BHA) was used as positive control. The result are shown as means±SD (n=3).

From result in Fig.6, graph represented total phenolic content in *Lentinus spp.* was rather high. The highest content was shown in Kornkoaw and slighter in Lom, Horm and Kradang mushrooms, respectively. The phenolic content and percentage of inhibition by DPPH assay were correlated in Kornkaow mushrooms while in Lom mushroom were not. Total phenolic content also showed differently in cultivated and natural Lom mushrooms as shown in fig. 7. The natural Lom mushroms represented higher content of phenolic content than the cultivated one.

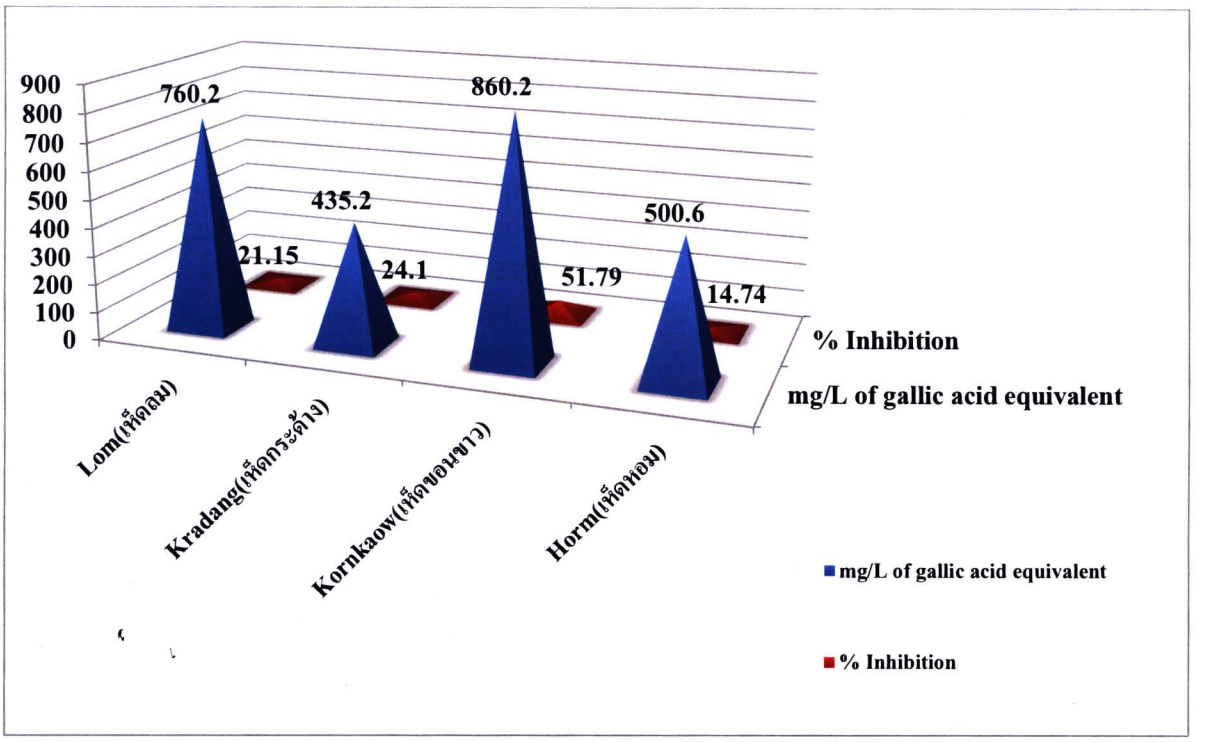

Fig.6 Comparison of total polyphenol content and percentage of potency of free radical scavenger activity by DPPH assay in Lentinus mushrooms. The result are shown as means (n=3).

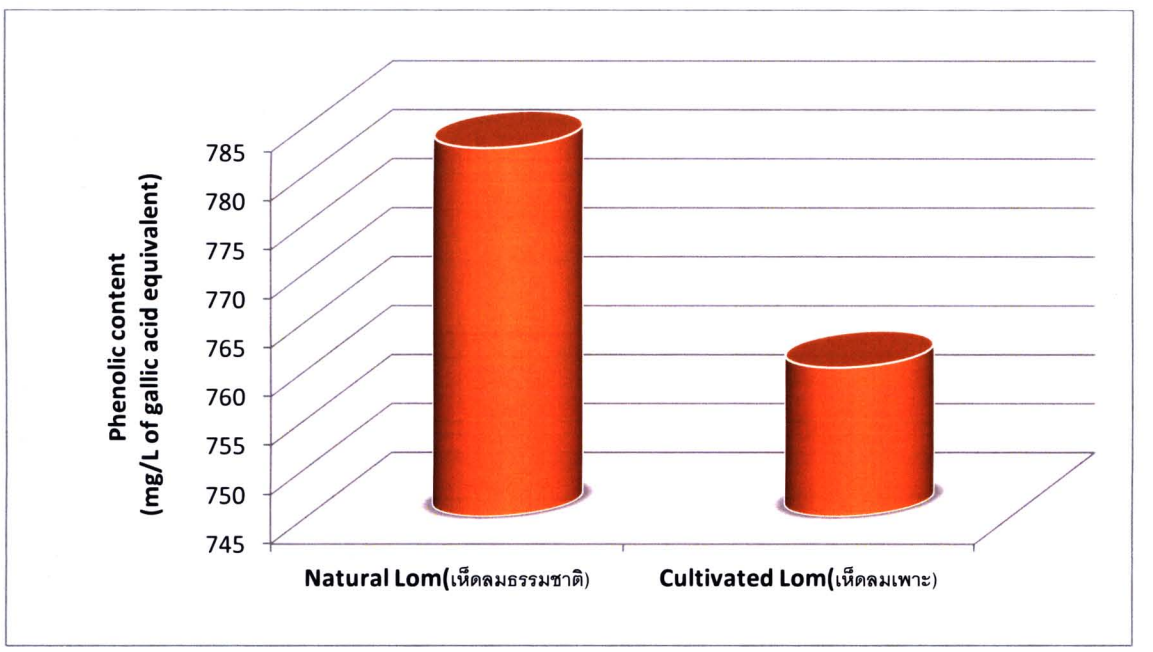

Fig.7 Total polyphenol content in natural and Lom mushrooms. The result are shown as means (n=3).

After extraction of proteins from *Lentinus* mushrooms with Bradford solution, the concentration of proteins of Kornkaow mushrooms showed maximum, lesser in Horm, and minimum in Lom and Kradang. From SDS-PAGE 12% or 1D in Fig.8, proteins in Lentinus mushrooms were extracted with tris buffered phenol. Profile of proteins in natural Lom, cultivated mushrooms, Kradang and Kornkaow were almost similar. The major difference molecular weights of proteins in natural and cultivated Lom mushroom were shown in about 20 and 50 kDa as pointed by arrow. Proteins of Horm mushrooms in lane 6 were rather different from other *Lentinus spp.* in lane 1-4. 2D gels of natural and cultivated Lom mushrooms were in Fig. 9 and 10. From pI and molecular weight of spot proteins in natural and cultivated Lom mushrooms, the interesting proteins were glutathione peroxidase (pI 6.43, Mw = 19 kDa) and L-seryl-tRNA(Sec) selenium transferase (pI = 8.5, Mw = 51 kDa) Fig. 9 and 10, we found that natural Lom mushrooms showed higher peak of those proteins than cultivated Lom mushrooms. In Fig. 11 and 12., we compared peak of the proteins, glutathione peroxidase and L-seryl-tRNA(Sec) selenium transferase, in natural Lom mushrooms and Kradang mushroom. Both of proteins expressed lower in Kradang mushrooms. Kornkaow and natural Lom mushrooms were also compared of the proteins in Fig. 13, 14, the lower peak was shown in Kornkaow mushrooms but not significantly different. Another mushroom showed lower peaks of glutathione peroxidase and L-seryl-tRNA(Sec) selenium transferase when compared with natural Lom mushrooms was shown in Fig. 15 and 16.

Table 2 Protein concentration of mushrooms extracts with tris buffered phenol by Bradford(1976)

Mushrooms	Protein concentration (mg/ml)
เห็ดลมธรรมชาติ จ.พิษณุโลก (Natural Photsanulok Lom mushroom)	2.57
เห็ดลมเพาะ จ.พิษณุโลก (Cultivated Photsanulok Lom mushroom)	2.66
เห็ดขอนขาว จ.พิษณุโลก (Phitsanulok Kornkaow mushroom)	11.14
เห็ดกระด้าง จ. ตรัง (Trang Kradang mushroom)	2.49
เห็ดหอม จ.เลย (Loei Horm mushroom)	5.58

One dimensional Gel Electrophoresis and two dimensional Gel electrophoresis

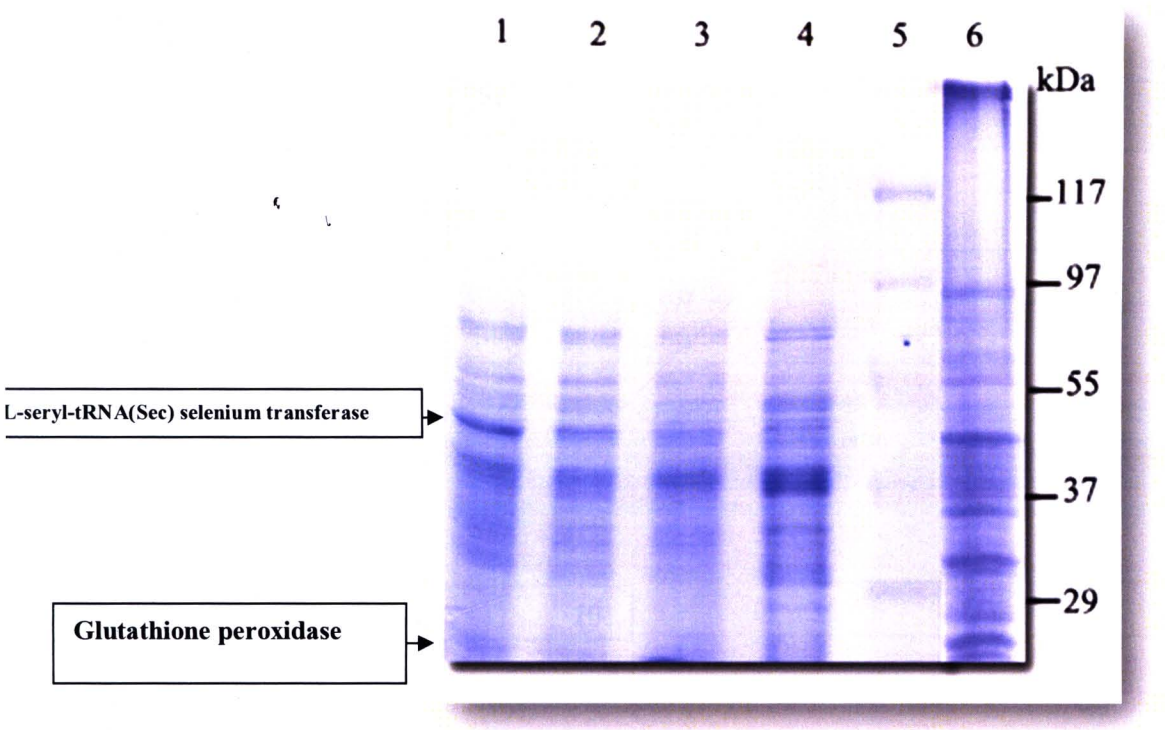

Fig.8 1D gel profile of local area mushrooms in Phitsanulok Loei and Trang Province (lane 1: Natural Lom mushroom, 2: Cultivated Lom mushroom, 3: Kradang mushroom, 4: Kornkaow mushroom, lane 5: marker proteins, 6: Horm mushroom). Total proteins were separated on 12% SDS-PAGE.

2D-PAGE of mushrooms

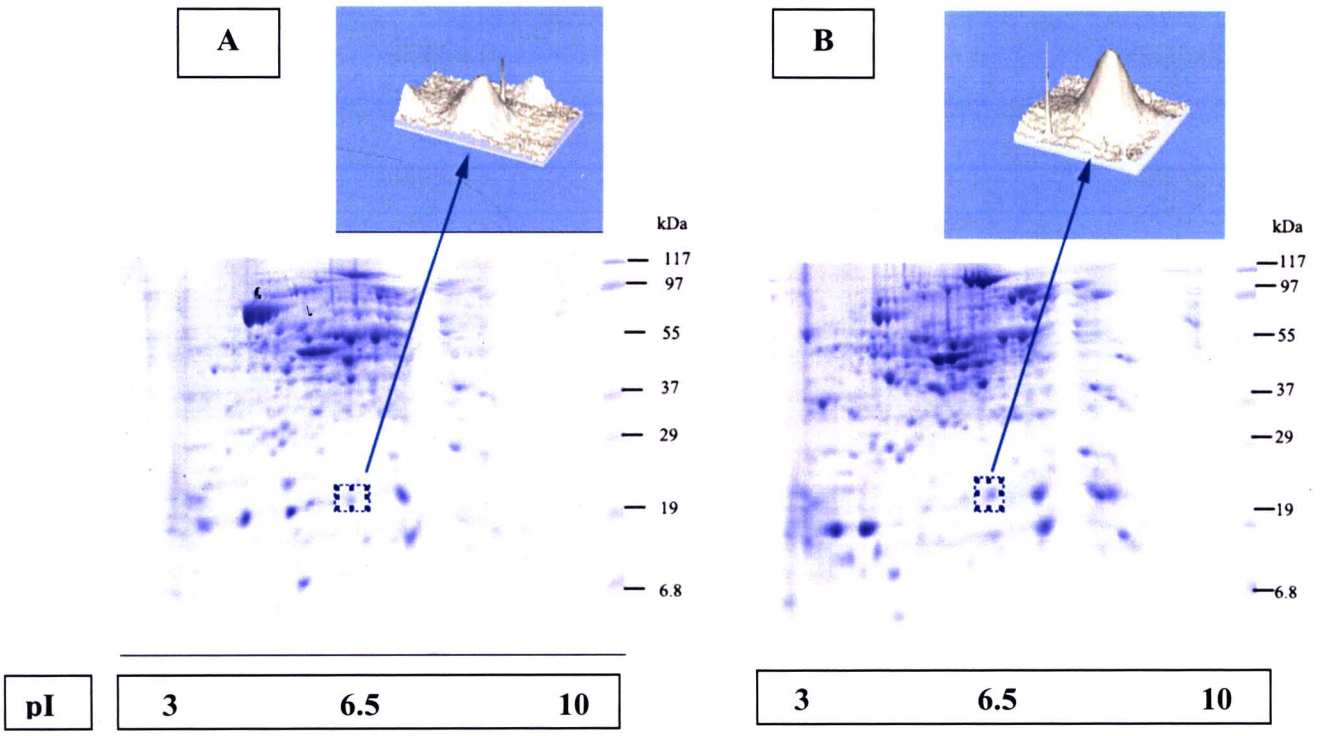

Fig.9 Development of 2D gels. The total soluble proteins were separated on precast IPG strips(7 cm, pH 3-10 NL) in the first dimension followed by 12% SDS-PAGE in the second dimension. Molecular masses were determined by running standard protein markers (2.5µl/gel; Bio-Rad), and separated proteins stained with colloidal CBB G-250). A: Cultivated Lom mushrooms, B: Natural Lom mushrooms. The arrow represented glutathione peroxidase (pI 6.43, Mw = 19 kDa)

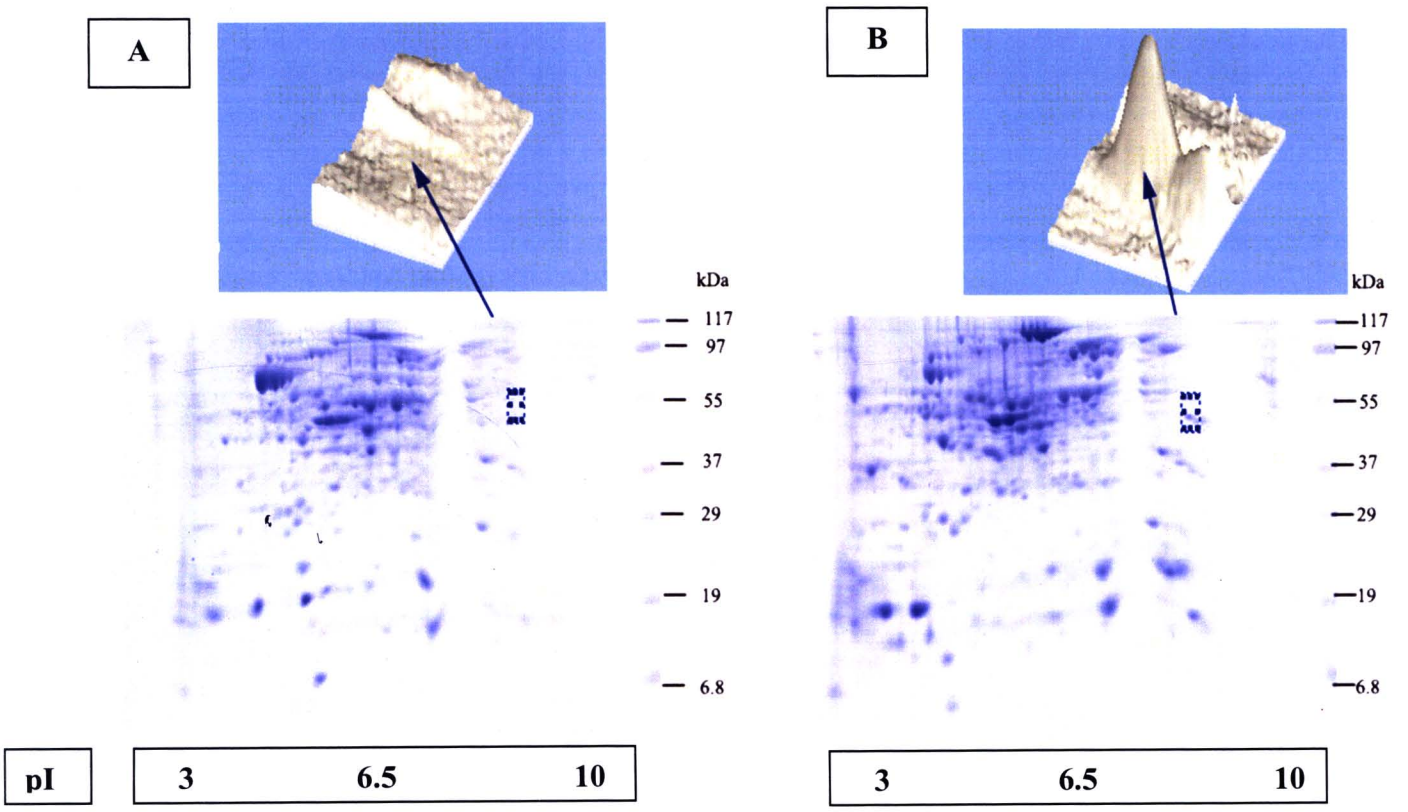

Fig. 10 Development of 2D gels. The total soluble proteins were separated on precast IPG strips(7 cm, pH 3-10 NL) in the first dimension followed by 12% SDS-PAGE in the second dimension. Molecular masses were determined by running standard protein markers (2.5µl/gel; Bio-Rad), and separated proteins stained with colloidal CBB G-250). A: Cultivated Lom mushrooms, B: Natural Lom mushrooms. The arrow represented L-seryl-tRNA(Sec) selenium transferase (pI = 8.5, Mw = 51 kDa)

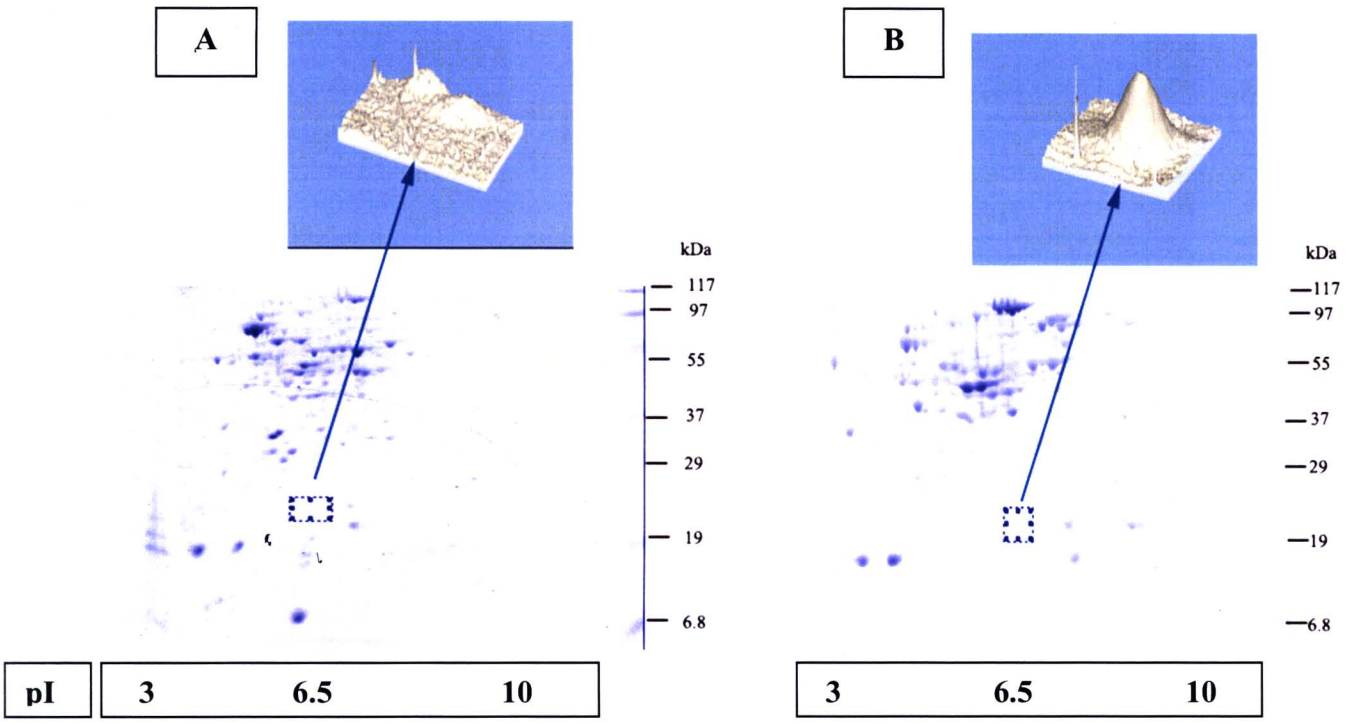

Fig.11 Development of 2D gels. The total soluble proteins were separated on precast IPG strips(7 cm, pH 3-10 NL) in the first dimension followed by 12% SDS-PAGE in the second dimension. Molecular masses were determined by running standard protein markers (2.5µl/gel; Bio-Rad), and separated proteins stained with colloidal CBB G-250). A: Kradang mushrooms, B: Natural Lom mushrooms. The arrow represented glutathione peroxidase (pI 6.43, Mw = 19 kDa)

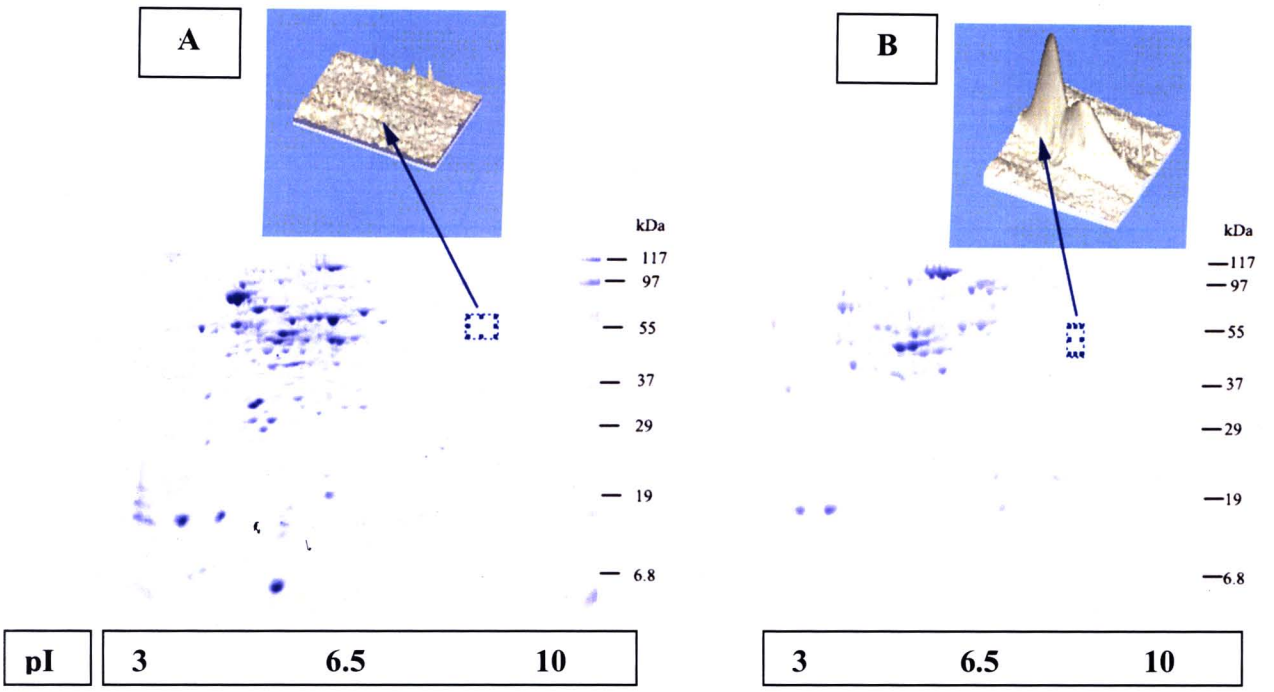

Fig.12 Development of 2D gels. The total soluble proteins were separated on precast IPG strips(7 cm, pH 3-10 NL) in the first dimension followed by 12% SDS-PAGE in the second dimension. Molecular masses were determined by running standard protein markers (2.5µl/gel; Bio-Rad), and separated proteins stained with colloidal CBB G-250). A: Kradang mushrooms, B: Natural Lom mushrooms. The arrow represented L-seryl-tRNA(Sec) selenium transferase (pI = 8.5, Mw = 51 kDa)

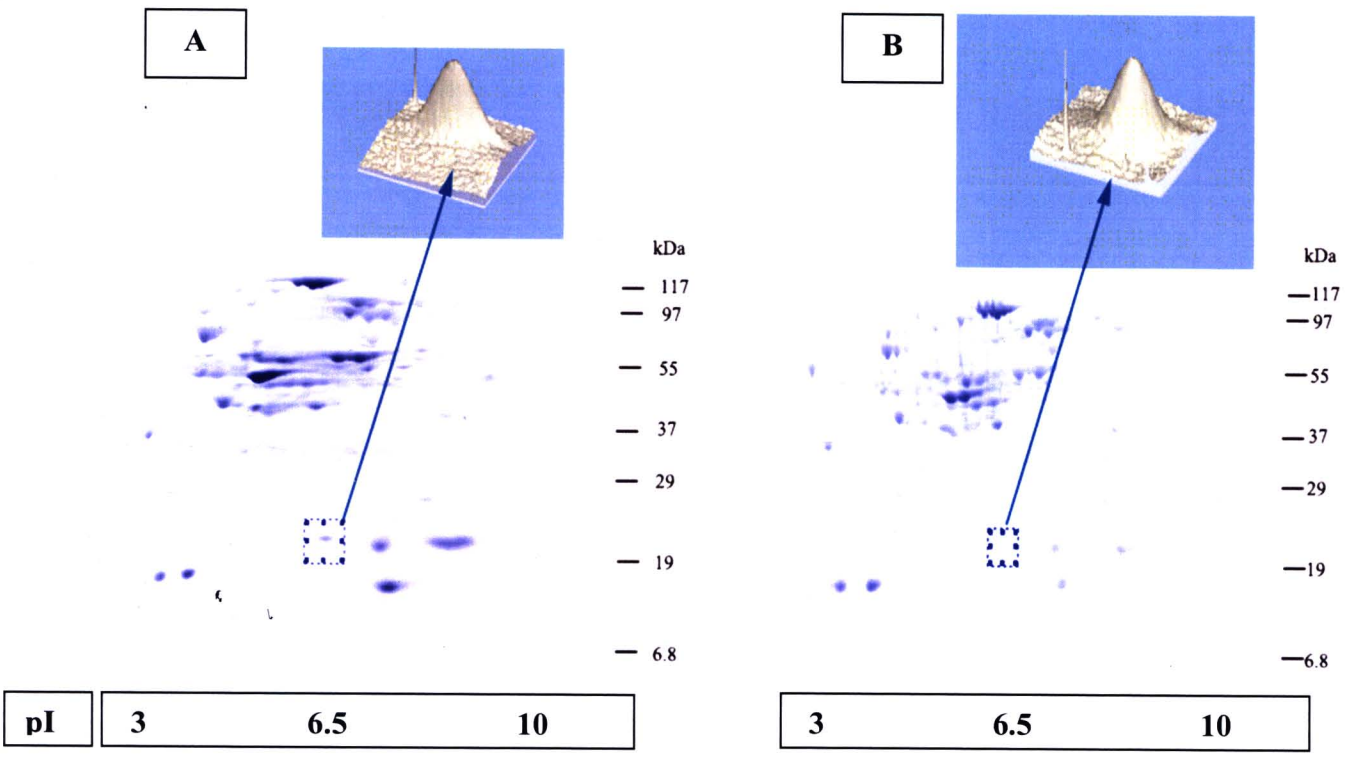

Fig.13 Development of 2D gels. The total soluble proteins were separated on precast IPG strips(7 cm, pH 3-10 NL) in the first dimension followed by 12% SDS-PAGE in the second dimension. Molecular masses were determined by running standard protein markers (2.5µl/gel; Bio-Rad), and separated proteins stained with colloidal CBB G-250). A: Kornkaow mushrooms, B: Natural Lom mushrooms. The arrow represented glutathione peroxidase (pI 6.43, Mw = 19 kDa)

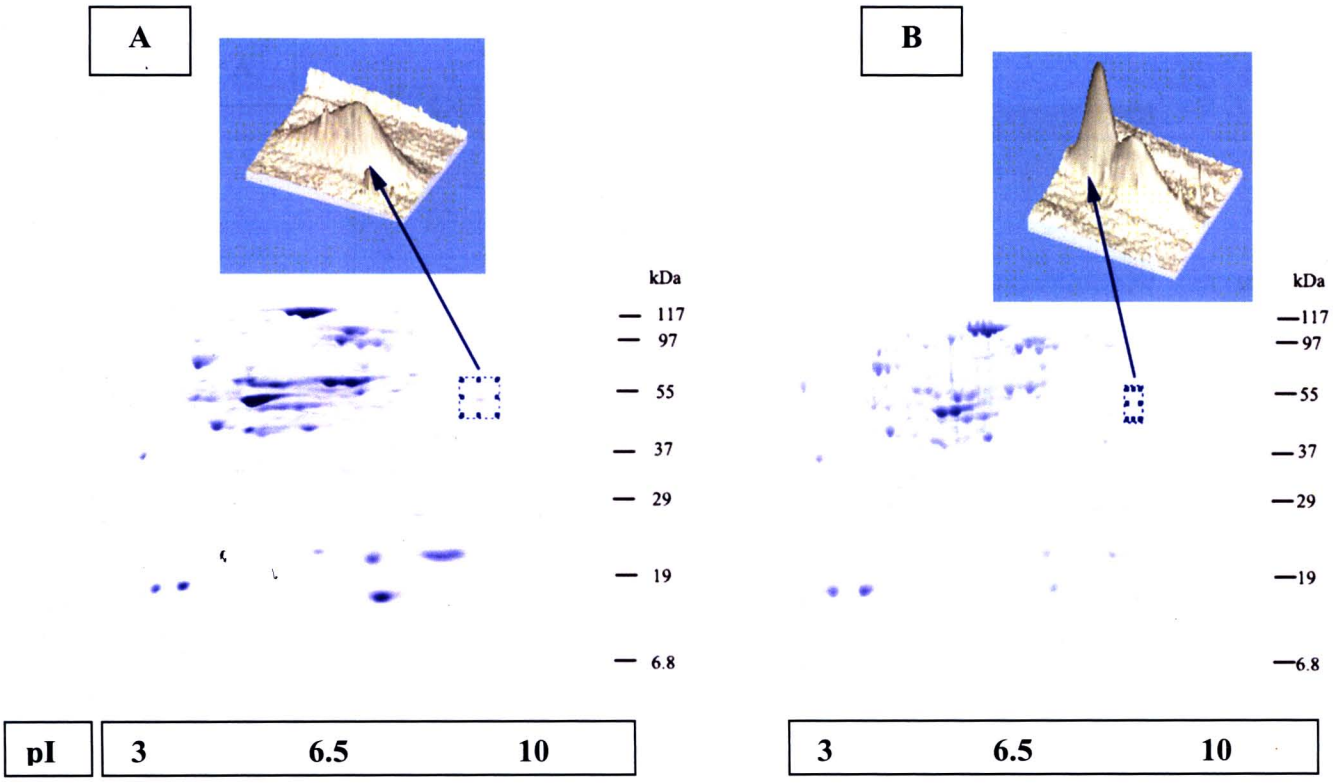

Fig.14 Development of 2D gels. The total soluble proteins were separated on precast IPG strips(7 cm, pH 3-10 NL) in the first dimension followed by 12% SDS-PAGE in the second dimension. Molecular masses were determined by running standard protein markers (2.5µl/gel; Bio-Rad), and separated proteins stained with colloidal CBB G-250). A: Kornkaow mushrooms, B: Natural Lom mushrooms. The arrow represented L-seryl-tRNA(Sec) selenium transferase (pI = 8.5, Mw = 51 kDa)

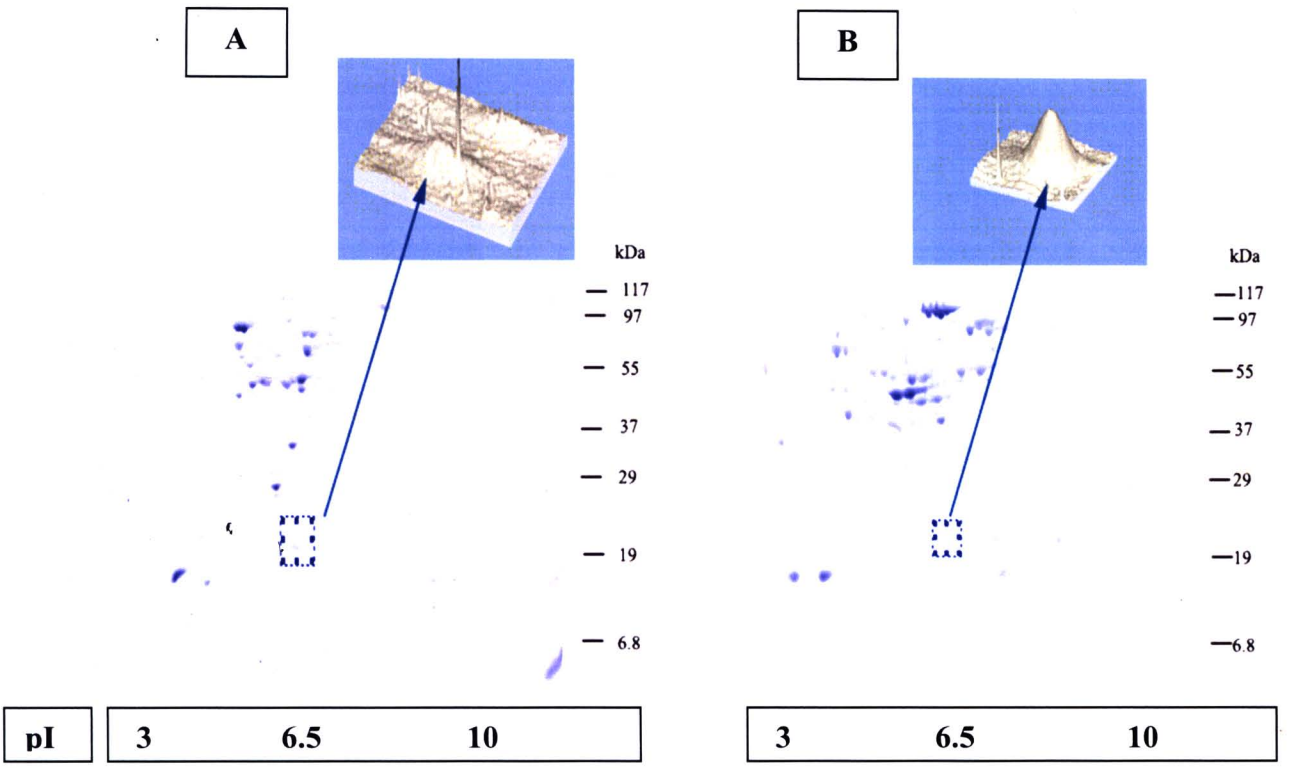

Fig.15 Development of 2D gels. The total soluble proteins were separated on precast IPG strips(7 cm, pH 3-10 NL) in the first dimension followed by 12% SDS-PAGE in the second dimension. Molecular masses were determined by running standard protein markers (2.5µl/gel; Bio-Rad), and separated proteins stained with colloidal CBB G-250). A: Horm mushrooms, B: Natural Lom mushrooms. The arrow represented glutathione peroxidase (pI 6.43, Mw = 19 kDa)

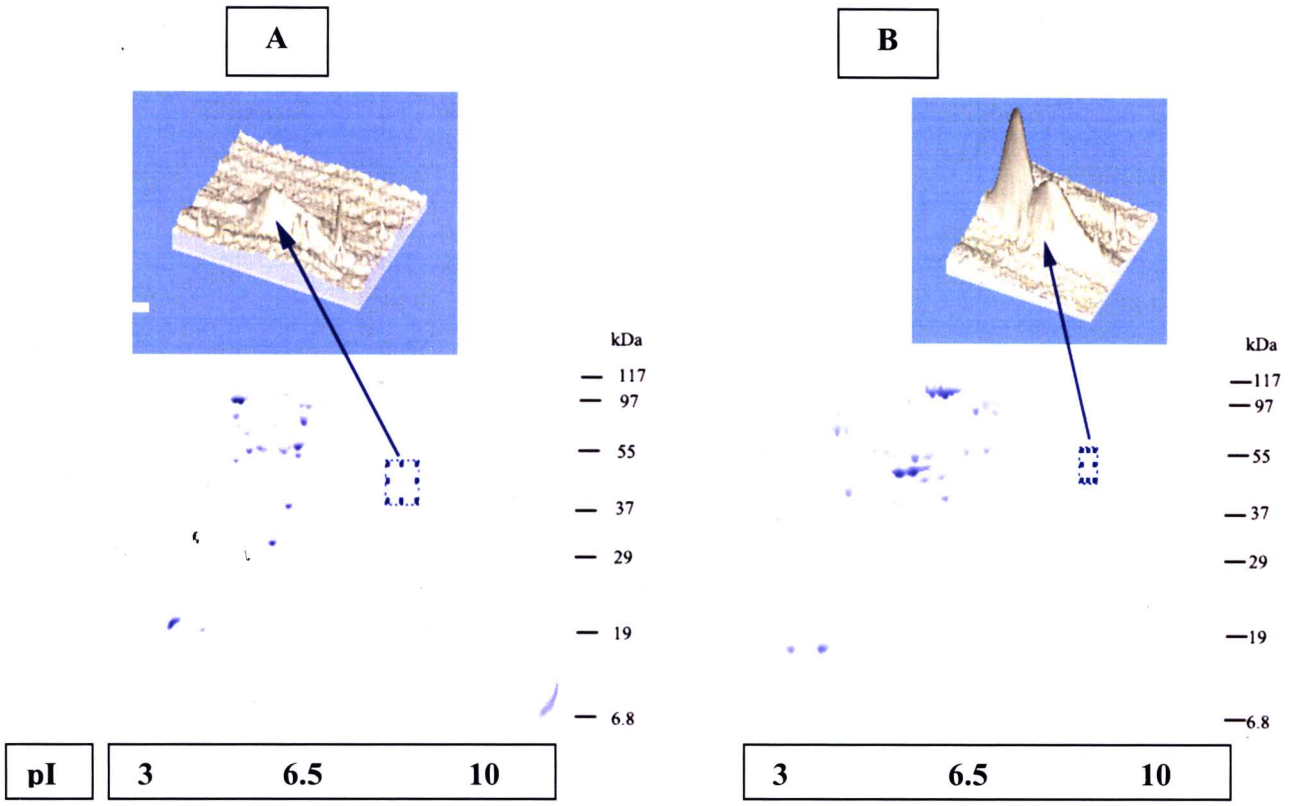

Fig.16 Development of 2D gels. The total soluble proteins were separated on precast IPG strips(7 cm, pH 3-10 NL) in the first dimension followed by 12% SDS-PAGE in the second dimension. Molecular masses were determined by running standard protein markers (2.5µl/gel; Bio-Rad), and separated proteins stained with colloidal CBB G-250). A: Horm mushrooms, B: Natural Lom mushrooms. The arrow represented L-seryl-tRNA(Sec) selenium transferase (pI = 8.5, Mw = 51 kDa)

Se content in mushrooms with ICP-MS

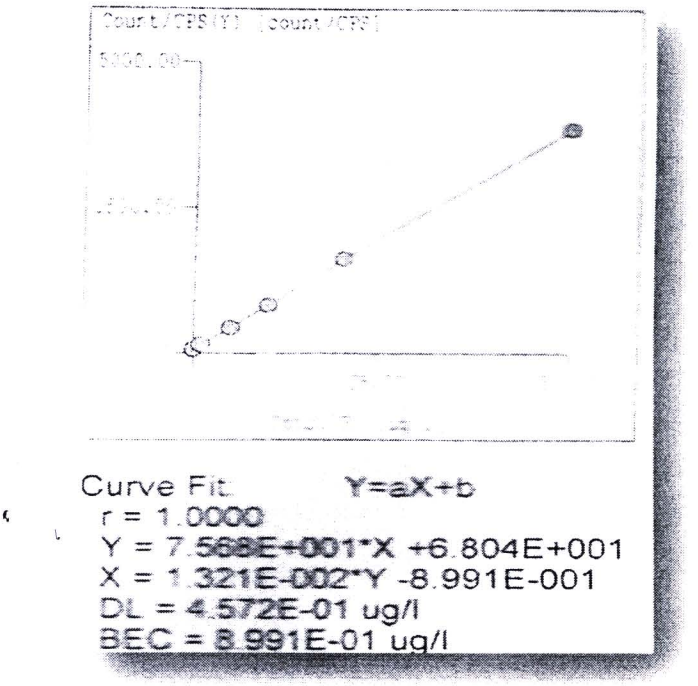

Fig 17. Calibration curve of total selenium concentration was determined by ICP-MS monitoring ^{82}Se isotopes.

Table 3. Total Se content by ICP-MS

Mushrooms	**Amount[1](mg/kg)**
เห็ดลมเพาะ (Cultivated Lom mushroom)	<0.12
เห็ดขอนขาว (Kornkaow mushroom)	0.24
เห็ดกระด้าง (Kradang mushroom)	ND
เห็ดหอม (Horm mushroom)	< 0.12
เห็ดลมธรรมชาติ Natural Lom mushroom)	0.42