Comparative Enzyme Analysis of *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos*

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Introduction

Inflammation is a natural immune response that takes place in response to trauma, infection, tissue injury or noxious stimuli. During this process, activated inflammatory cells such as neutrophils, eosinophils, mononuclear phagocytes and macrophages increase the secretion of nitric oxide (NO), prostaglandin E2 (PGE2) and cytokines.

Macrophages cells have three main functions in inflammation: antigen presentation, phagocytosis, and immunomodulation through the production of several cytokines as well as growth factors, therefore, they play a crucial role in the initiation, maintenance, and resolution of inflammation[1].

Oxidative stress occurs when the equilibrium shifts in favor of reactive oxygen species (ROS) as a result of a depletion of anti-oxidant agents. Such overproduction of ROS can cause oxidative damage to biomolecules (eg. Lipids, proteins, DNA) which may be responsible to chronic diseases such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, chronic inflammation, stroke, aging and other degenerative diseases in humans.

Oxidative damage is prevented by a defensive system that includes non-enzymatic and enzymatic anti-oxidants. Enzymatic anti-oxidants are widely used as markers of oxidative stress such as superoxide dismutase, catalase, and glutathione peroxidase[2]. Humans have extensively consumed mushrooms for several millennia. Mushrooms are a very rich source of enzymes, secondary metabolites, vitamins, minerals, proteins, polysaccharide, have high fiber content and low fat levels. Mushrooms contain several bioactive molecules such as terpenoids, steroids, phenols, nucleotides, glycoprotein derivatives, peptides, and free and protein-bound polysaccharides. Therefore, they have been considered as potential source of antioxidant and anti-inflammatory activity[3].

As discussed in Clinical Journal of Mycology Vol IV, it has been known for over a century that some enzymes can be used in the prevention and even treatment of several clinical conditions. Important immune-enhancing enzyme activity is found in the biomass form of mushroom nutrition and these enzymes are divided into the following activities:

a) Enzymes that prevent oxidative stress:

Superoxide dismutase

b) Enzymes that prevent cellular growth:

Protease, Glucoamylase

c) Enzymes that promote detoxification:

Peroxidase, Cytochrome P-450

Objective

The aim of the present work was to investigate the levels of enzymes and several secondary metabolites involved in coagulation as well as anti-oxidant agents present in the biomass form of *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos* in the presence and absence of proteolytic enzymes.

The following anti-oxidant parameters were investigated: peroxidase, glucoamylase, glucose2-oxidase, superoxide dismutase, cytochrome P-450, cytochrome P-450 reductase, catalase, protease, glutathione peroxidase, glutathione levels and secondary metabolites in all mushroom fractions[1].

Discussion

The data obtained reveal that all tested fungi products contain significant levels of several different enzymes and secondary metabolites including thrombin inhibitor.

The simulation of the gastro-intestinal tract was carried out in the presence and absence of pepsin and trypsin which revealed that the presence of these proteases did not significantly reduce the levels of several different enzymes and secondary metabolites.

Concerning to secondary metabolites, all tested mushroom products exhibited high levels of thrombin inhibitors. Results now obtained suggest that these mushrooms are a rich source of anti-oxidant agents which can protect the organism from the harmful effects of ROS.

Conclusions

The data presented in this study shows that the tested mushroom products are a rich source of enzymes and secondary metabolites including anti-oxidant agents which inactivates ROS. Moreover, immunonutrients in mycelia and primordia (young fruiting bodies) present in the biomass form of of *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos* are resistant to proteolytic enzymes (i.e simulation of digestive tract) since it is in a biomass form and not on cell extract.

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	Inonotus obliquus (Chaga)	*Auricularia auricula*	*Poria cocos*
Protein Content	49,5 mg	54,8 mg	59,3 mg
Peroxidase	35,8 mU	31,5 mU	39,5 mU
Glucoamylase	5,9 U	4,3 U	4,1 U
Glucose 2-oxidase	7,2 mU	4,9 mU	6,9 mU
Superoxide Dismutase	975 U	487,5U	446,9 U
Cytochrome P-450	2,9 nmole	2,1 nmole	2,5 nmole
Cytochrome P-450 reductase	10,2 mU	8,5 mU	9,8 mU
Catalase	18,1 mU	15,3 mU	21,5 mU
Protease	29,5 mU	27,1 mU	31,3 mU
Glutathione peroxidase	25,6 mU	18,5 mU	25,9 mU
Glutathione leves (ug/g)	30,3	32,5	19,1
Secondary metabolite (Thrombin inhibitors %)	7,80%	5,70%	6,30%

Table I -Comparative Differences in Enzyme Content between *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos*. (Absence of trypsin and pepsin.)

	Inonotus obliquus (Chaga)	*Auricularia auricula*	*Poria cocos*
Protein Content	41,4 mg	48,9 mg	52,6 mg
Peroxidase	30,9 mU	27,7 m U	35,1 m U
Glucoamylase	4,8 U	3,9 U	3,7 U
Glucose 2-oxidase	5,9 m U	4,2 m U	5,1 m U
Superoxide Dismutase	965,8 U	479,3 U	440,1 U
Cytochrome P-450	2,4 nmole	1,8 nmole	2,1 nmole
Cytochrome P-450 reductase	8,9 m U	7,1 m U	7,1 m U
Catalase	16,8 m U	12,8 m U	17,5 m U
Protease	23,9 m U	25,7 m U	28,1 m U
Glutathione peroxidase	21,8 m U	15,1 m U	21,1 m U
Glutathione leves (ug/g)	29,5	30,1	18,1
Secondary metabolite (Thrombin inhibitors %)	6,90%	4,70%	5,90%

Table II - Comparative Differences in Enzyme Content between *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos* with presence of pepsin

	Inonotus obliquus (Chaga)	*Auricularia auricula*	*Poria cocos*
Protein Content	43,7 mg	51,3 mg	53,1 mg
Peroxidase	32,7 mU	28,9 m U	32,9 m U
Glucoamylase	5,3 U	4,2 U	3,9 U
Glucose 2-oxidase	6,3 m U	4,4 m U	6,2 m U
Superoxide Dismutase	871,8 U	485,3 U	442,5 U
Cytochrome P-450	2,6 nmole	1,9 nmole	2,3 nmole
Cytochrome P-450 reductase	9,4 m U	7,5 m U	7,9 m U
Catalase	15,4 m U	13,8 m U	19,8 m U
Protease	25,8 U	24,8 m U	28,8 m U
Glutathione peroxidase	23,1 m U	16,7 m U	22,5 m U
Glutathione leves (ug/g)	31,1	30,9	19,5
Secondary metabolite (Thrombin inhibitors %)	7,10%	5,30%	6,10%

Table III Comparative Differences in Enzyme Content between *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos* with presence of trypsin

Note: One enzyme unit (U) is defined as the amount of enzyme required to convert one micromole of substrate to product per minute under certain experimental conditions. One milli-enzyme unit (mU) is defined as the amount of enzyme required to convert one nanomole of substrate to product per minute under certain experimental conditions

The the biomass form of *Inonotus obliquus* (Chaga), *Auricularia auricula* and *Poria cocos* was supplied by Mycology Research Laboratories Ltd.-United Kingdom. (www.mycologyresearch.com)