Comparative Differences in ß-1,3-1,6 Glucan content between *Ganoderma lucidum* (Reishi) mushrooms (Biomass vs Extracted) in the Presence of Proteolytic Enzymes

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INTRODUCTION

Some mushrooms have been known to exhibit several medicinal properties for thousands of years in Japanese and other Asian cultures. The Reishi mushroom, also known as *Ganoderma lucidum*, is well characterized (in Traditional Chinese Medicine) for the prevention and treatment of several disease states such as cancer, allergies and asthma.

In the West, *Reishi* is sold in an extracted form (extracted specifically for β-glucan content) or in a biomass form (mycelium and primordial (young fruit body)). The biomass form contains several substances of clinical interest such as enzymes, secondary metabolites and β-glucans. The specific quantification of β-glucans in mushrooms (extracted or biomass forms) with anti-tumour activity is of great clinical importance[1]

There are several types of β-glucans in mushroom species such as β-1,3 glucans and β-1,3-1,6 glucans[2]. As far as anti-tumour immune enhancing and modulating activities, these three activities are attributed to β-1,3-1,6 glucans which exhibit a triple helix as their tertiary structure [3,4].

METHODS

Two samples (1g) of Reishi MRL and Reishi Myco were compared to detect and to quantify enzymes, β-glucans and secondary metabolites. These two samples are different since the former is a biomass that contains mycelia and primordia whereas the latter is a concentrated extract (20x) of fruiting bodies.

The β-1,3-1,6 glucan content was determined by a colorimetric method recently established in Germany [2]. The enzyme and secondary metabolite contents were determined by methods commonly used.

In order to assess the impact of digestive enzymes on the constituents of each sample, both samples were compared *in vitro*; **a)** in the absence of proteolyic enzymes, **b)** in the presence of pepsin and **c)** in the presence of trypsin.

DISCUSSION

The data obtained reveal that, in the absence of proteolytic enzymes, both forms of Reishi contain significant levels of β 1,3-1,6 glucans with anti-tumour activity with Reishi Myco exhibiting higher values (hot water fraction and NaOH fraction).However, in the presence of pepsin and trypsin, Reishi-MRL (biomass form) exhibited higher β1,3-1,6 glucan values than Reishi Myco (extracted form) (See Table I and Fig. 7-11).

When comparing the enzyme values, in the absence of, and presence of, proteolytic enzymes, between the two forms of Reishi, (See Table II and Figures 1-6), there was an absence of important immune-enhancing enzyme activity, such as peroxidase, glucoamylase and protease activities in Reishi Myco (extracted form) when compared to Reishi-MRL (biomass form). The Reishi biomass form demonstrated a greater overall enzyme activity level over the extracted form of Reishi.

It has been known for over a century that some enzymes can be used in the prevention and even treatment of several clinical conditions. These enzymes are divided into the following activities::

Enzymes that prevent oxidative stress:

Superoxide dismutase

Enzymes that prevent cellular growth:

Protease

Glucoamylase

Enzymes that promote detoxification:

Cytochrome P-450

Peroxidase

Glucose 2 oxidase

Reishi Myco exhibits low levels of secondary metabolites compared with the Reishi-MRL product.

CONCLUSIONS

The differences in β-1,3-1,6 glucan content between both samples may be due to differences in biological material in these samples since one contains mycelia and primordia whereas the other consists of concentrated (20x) extract of fruiting bodies. In fact, immunonutrients in mycelia and primordia (young fruiting bodies) in MRL product are more resistant to proteolytic enzymes (i.e simulation of digestive tract) since it is in a biomass form and not on cell extract. Therefore, the concentrated extract of the fruiting bodies is more exposed and available to the action of proteolytic enzymes (i.e simulation of digestive tract) since there are no physio-chemical barriers to prevent such exposure.

In addition to the importance of β-1,3-1,6 glucan content, one should assess the advantages of enzyme supplementation afforded by the biomass form of mushroom nutrition.

Professor Amin Karmali

Fig. 1- Peroxidase

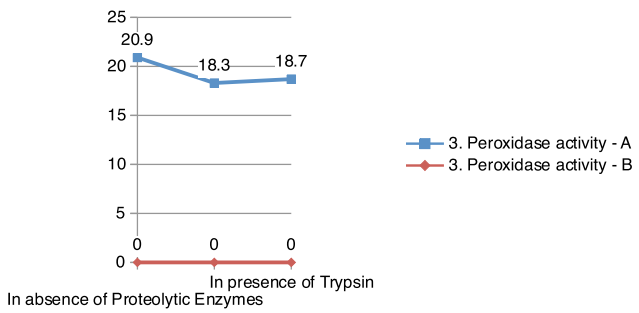

Fig. 2- Glucoamylase

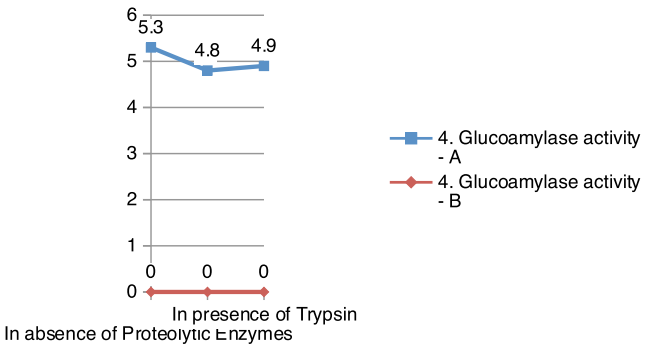

Fig. 3 - Protease

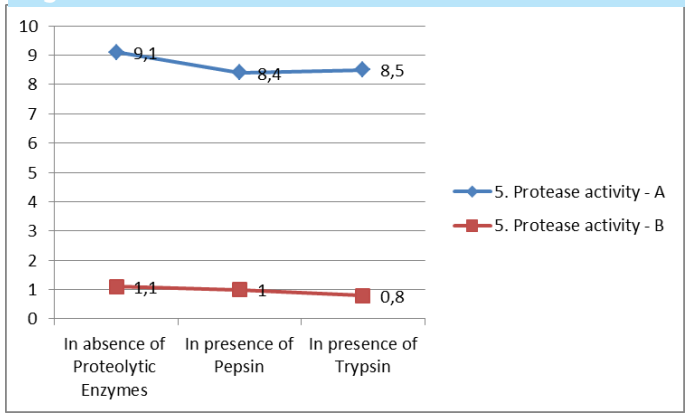

Fig. 4 - Glucose 2-Oxidase

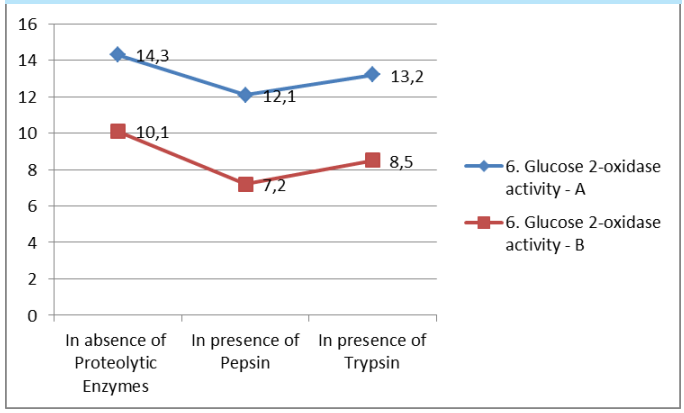

Fig. 5 - SOD

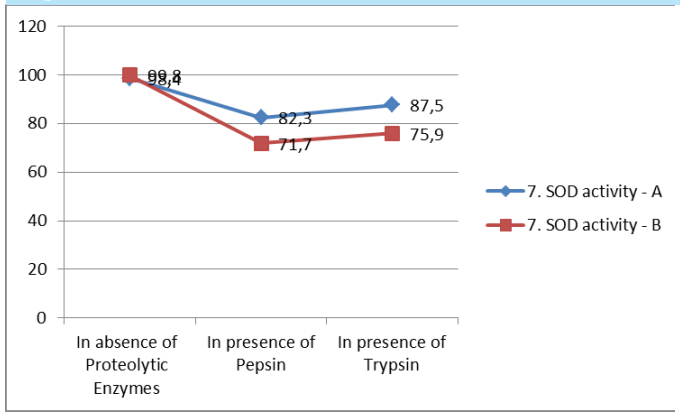

Fig. 6 - Cytochrome 450

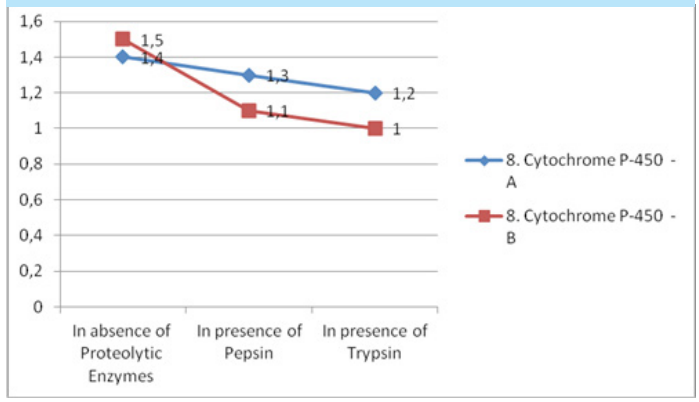

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Fig. 7 - Water Soluble Fraction

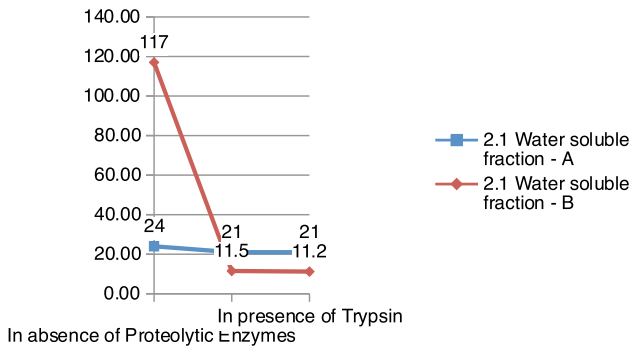

Fig. 8 - Hot Water Fraction

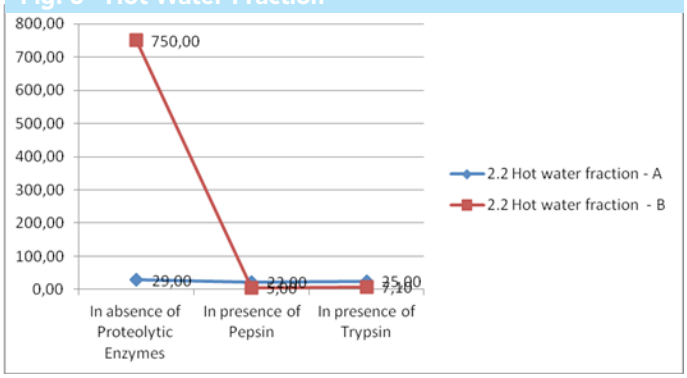

Fig. 9 - NaoH Fraction

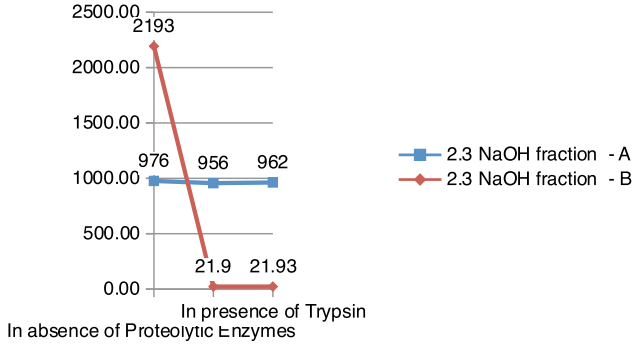

Fig. 10- KOH Fraction

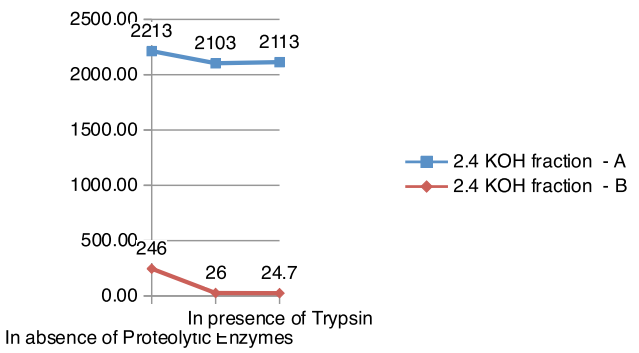

Fig. 11 - HCL Fraction

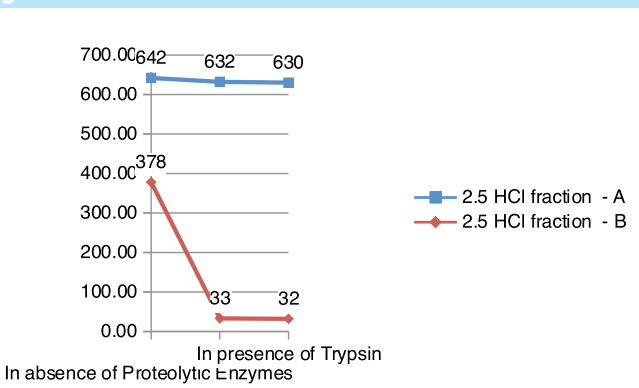

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Table I: Comparision of Impact of Proteolytic Enzymes on Beta 1,3-1,6 Glucan Activity between Reishi-MRL (biomass) and Reishi Myco (extract) (one gram of product in powder form)

	I. IN ABSENCE OF PROTEOLTIC ENZYMES (per gram of product)		
	In absence of Proteolytic Enzymes	Reish A MRL	Reshi B Myco
2.1	Water soluble fraction	24.0µg	117.0µg
2.2	Hot water fraction	29.0µg	750.0µg
2.3	NaOH fraction	976.0µg	2193.0µg
2.4	KOH fraction	2213.0µg	246.0µg
2.5	HCl fraction	642.0µg	378.0µg

	II IN THE PRESENCE OF PEPSIN (per gram of product)		
		Reish A	Reshi B
2.1	Water soluble fraction	21.0µg	11.5µg
2.2	Hot water fraction	22.0µg	5.0µg
2.3	NaOH fraction	956.0µg	21.9µg
2.4	KOH fraction	2103.0µg	26.0µg
2.5	HCl fraction	632.0mg	33.0mg

	III IN THE PRESENCE OF TRYPSIN (per gram of product)		
		Reish A	**Reshi B**
2.1	Water soluble fraction	21.0µg	11.2µg
2.2	Hot water fraction	25.0µg	7.1µg
2.3	NaOH fraction	962.0µg	21.93µg
2.4	KOH fraction	2113.0µg	24.7µg
2.5	HCl fraction	630.0µg	32.0µg

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Table II: Comparision of Impact of Proteolytic Enzymes on Beta 1,3-1,6 Glucan , Enzyme and Secondary Metabolite Activity between Reishi-MRL (biomass) and Reishi Myco (extract) in one gram of powder.

	Enzymes, polysacharides and secondary metabolites per gram of product	In absence of Proteolytic Enzymes		In presence of Pepsin		In presence of Trypsin	
		Reish A MRL	Reshi B Myco	Reish A MRL	Reshi B Myco	Reish A MRL	Reshi B Myco
1	Protein content	44.8mg	40.5 mg	35.9 mg	30.5mg	37.1mg	33.2mg
2	b-1,3-1,6- glucans with anti-tumour activity						
2.1	Water soluble fraction	24.0mg	117.0mg	21.0mg	11.5mg	21.0mg	11.2mg
2.2	Hot water fraction	29.0mg	750.0mg	22.0mg	5.0mg	25.0mg	7.1mg
2,3	NaOH fraction	976.0mg	2193.0mg	956.0mg	21.9mg	962.0mg	21.93mg
2.4	KOH fraction	2213.0mg	246.0mg	2103.0 mg	26.0mg	2113.0mg	24.7mg
2.5	HCl fraction	642.0mg	378.0mg	632.0mg	33.0mg	630.0mg	32.0mg
3	Peroxidase activity	20.9mU	0.0mU	18.3mU	0.0mU	18.7mU	0.0mU
4	Glucoamylase/Beta-glucanasase activity	5.3 U	0.0 U	4.8 U	0.0mU	4.9 U	0.0mU
5	Protease activity	9.1mU	1.1mU	8.4mU	1.0mU	8.5mU	0.8mU
6	Glucose 2-oxdase activity	14.3mU	10.1mU	12.1mU	7.2mU	13.2mU	8.5mU
7	Superoxide dismutase (SOD) activity	98.4mU	99.8mU	82.3mU	71.7mU	87.5mU	75.9mU
8	Cytochrome "P-450"	1.4 nmoles	1.5nmoles	1.3nmoles	1.1nmoles	1.2nmoles	1.0nmoles
9	Cytochrome P 450 reductase	15.5mU	12.5mU	12.6mU	8.7mU	13.3mU	6.2mU
10	Secondary metabolites (Thrombin inhibitors)	4.9%	1.1%	4.6%	1.0%	4.7%	1.0%

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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

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