The Role of Enzymes in Mushroom Nutrition

Optimization. Functional synergy.
Health benefits.
Biomass vs Extract.

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Role of Enzymes in Mushroom Nutrition

- Some mushrooms have been known to exhibit several medicinal properties for thousands of years in Asian cultures, mainly in Chinese and Japanese Traditional Medicines.
- In Traditional Chinese Medicine (TCM), mushrooms are used for prevention and treatment of several conditions such as fatigue, allergies, asthma, infections and autoimmune diseases. Also in degenerative conditions such as cancer.

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Role of Enzymes in Mushroom Nutrition

- In Western countries, mushroom nutrition is provided in either an **extracted** form (extracted specifically for **beta-glucan** content) or in a **biomass** form (mycelium and primordia (young fruit body).
- *Which are the differences between these two forms of mushroom nutrition?*
- Impact of digestive juices on the content of beta-glucans, enzymes and secondary metabolites.
- This presentation is based upon the work of Professor Amin Karmali published at:
  - Clinical Journal of Mycology, November 2014, vol IV. www.mycologyreseach.com

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Role of Enzymes in Mushroom Nutrition

- In general, oral administration of certain **enzymes** contributes to the efficacy of the following **functions** and **reduce chronic toxic overload** in the organism:
  - Balance pH levels: blood and extracellular matrix.
  - Removal of toxic substances.
  - Recovery of intestinal bacterial balance (biota balance).
  - Enhancement and balance of immune system
  - Improvement in cell metabolism

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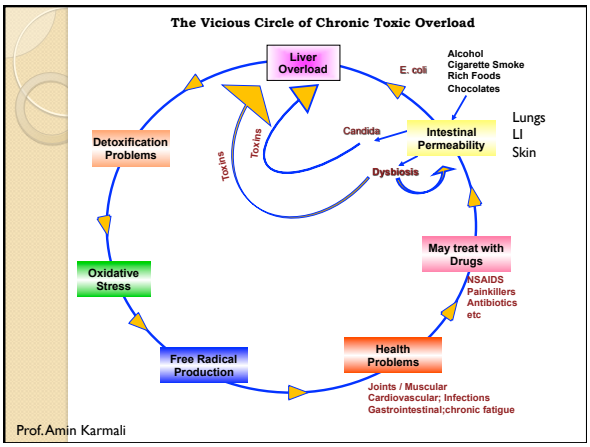

Detoxification in terms of TCM

Toxins > Fire, Yin insufficiency, Qi and/or Blood stasis, masses/Tan...

Main systems, organs and tissues involved:

Wood (LV, GB), blood > flow
Metal (L, LI, skin, mucous tissues) < cleaning
Water (K, BL) > drainage

Emunctories

Biomass: whole and complete mushroom

- ***What is biomass in terms of mushroom nutrition?***
  - Non-extracted form of edible mushrooms which consists of either the mycelium, or primordia (young fruiting body) or both.
- In this presentation, biomass will be defined as mycelium with primordia (young fruiting body -before the mushroom blooms-).
- Contents all the nutrients and active compounds, including enzymes and beta-glucanes

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Extracts

- ***What about a mushroom extract?***
- The process of hot water extraction usually requires high temperature and chemicals to precipitate the target components.
- Most of the aromatic, volatiles, and proteins (enzymes) are **lost** in the process; what **remains** are mostly complex sugars and some glyco-proteins.
- Glyco-proteins such as Beta-1,3-6,6 glucans exhibit anti-tumour and immune enhancing and immune modulating activities.

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Extracts & Enzyme Levels

- ***What are the enzyme levels in mushroom extracts?***
- Proteins, and therefore, enzymes are denatured by hot water extraction process which is conducted at temperatures above 62°C (145° F) and by chemicals used in the process, such as alcohol and sodium hydroxide

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Mushroom active components

- Beta-glucans (protein bound polysaccharides).
- Enzymes.
- Vitamins, Minerals.
- Aminoacids.
- Carbohydrates.
- Other nutrients and metabolites.

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Beta glucans content comparision

- ***What is the impact of gastric juices on the beta 1-3,-1.6 glucan content in an extracted mushroom extract compared to a biomass equivalent?***

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Impact of Digestive Enzymes

- Assessment and comparison of the impact of digestive enzymes *(in vitro)* on the constituents of two Reishi compounds, one an extract (20 x) of fruiting bodies (Myco) *versus* a biomass equivalent (MRL):
  - a) in the absence of proteolytic enzymes,
  - b) in the presence of pepsin and
  - c) in the presence of trypsin.

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Role of Enzymes in Mushroom Nutrition

	Enzymes, polysacharides and secondary metabolites per g of product	In absence of Proteolytic Enzymes	
		Reishi A MRL	Reishi B Myco
1	Protein content	44.8 mg	40.5 mg
2	b-1,3-1,6- glucans with anti-tumour activity		
2.1	Water soluble fraction	24.0 µg	117.0 µg
2.2	2.2 Hot water fraction	29.0 µg	750.0 µg
2.3	2.3 NaOH fraction	976.0 µg	2193.0 µg
2.4	2.4 KOH fraction	2213.0 µg	246.0 µg
2.5	2.5 HCl fraction	642.0 µg	378.0 µg

Biomass and Extract BEFORE ANY CONTACT with proteolytic enzymes (digestion)

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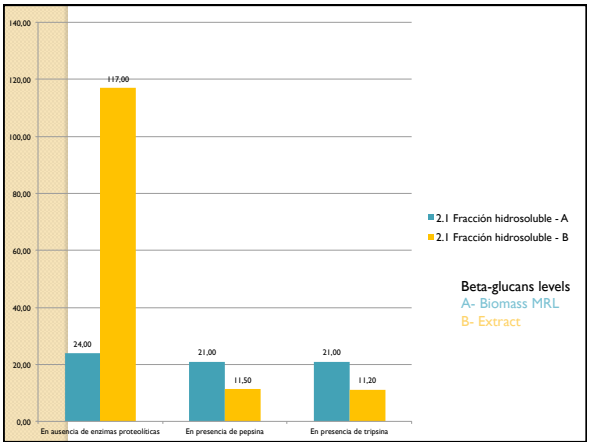
	Enzymes, polysacharides and secondary metabolites per g of product	in the presence of Pepsin	
		Reishi A MRL	Reshi B Myco
1	Protein content	35.9 mg	30.5 mg
2	b-1,3-1,6- glucans with anti-tumour activity		
2.1	Water soluble fraction	21.0 µg	11.5 µg
2.2	2.2 Hot water fraction	22.0 µg	5.0 µg
2.3	2.3 NaOH fraction	956.0 µg	21.9 µg
2.4	2.4 KOH fraction	2103.0 µg	26.0 µg
2.5	2.5 HCl fraction	632.0 µg	33.0 µg

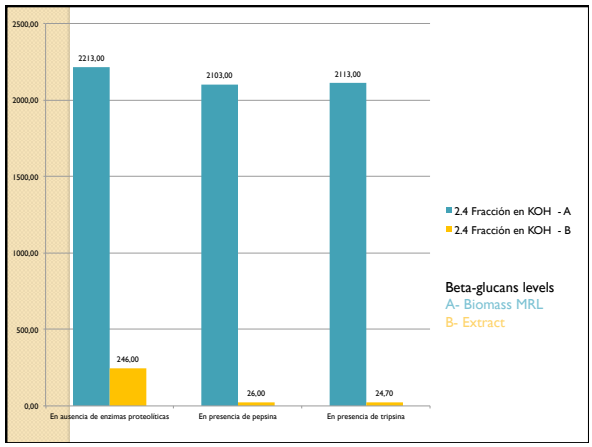
Beta glucans level **higher** in biomass AFTER "digestion enzyme" contact.

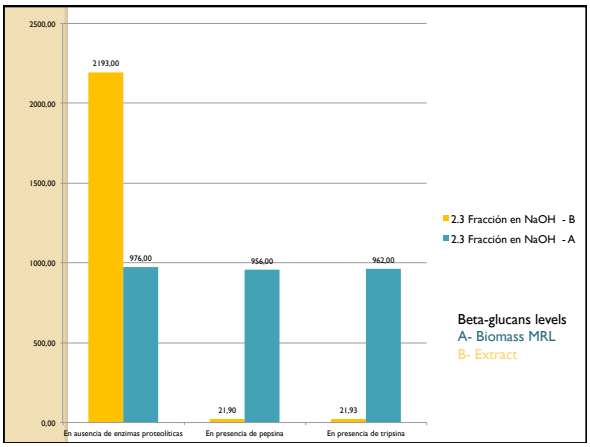
Role of Enzymes in Mushroom Nutrition

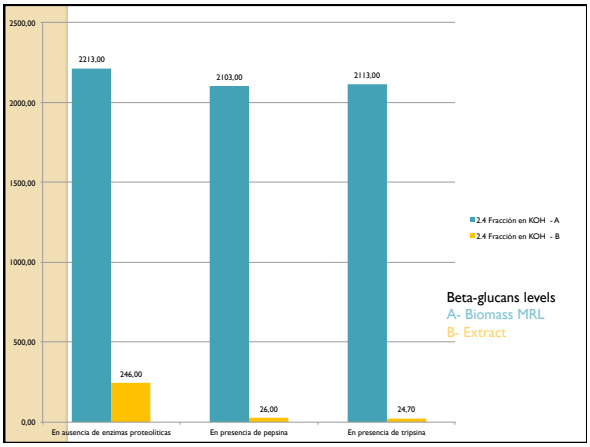
	Enzymes, polysacharides and secondary metabolites per g of product	in the presence of Trypsin	
		Reish A MRL	Reshi B Myco
1	Protein content	37.1 mg	33.2 mg
2	b-1,3-1,6- glucans with anti-tumour activity		
2.1	Water soluble fraction	21.0 µg	11.2 µg
2.2	2.2 Hot water fraction	25.0 µg	7.1 µg
2.3	2.3 NaOH fraction	962.0 µg	21.93 µg
2.4	2.4 KOH fraction	2113.0 µg	24.7 µg
2.5	2.5 HCl fraction	630.0 µg	32.0 µg

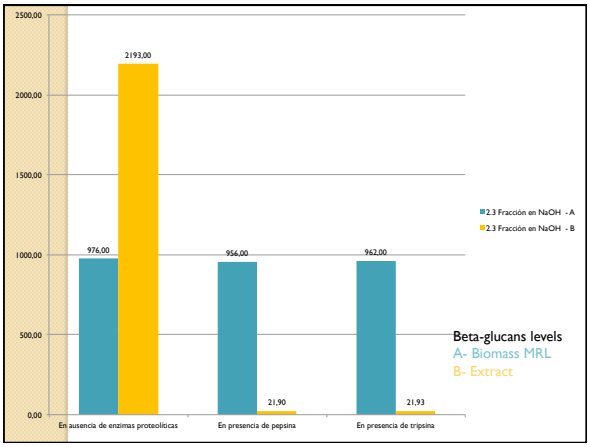
Beta glucans level **higher** in biomass AFTER "digestion enzyme" contact.











Conclusions about beta glucans

- Active beta-1,3-1,6 glucan content in both samples is similar and significant. "Before".
- *However*: situation changes when samples are in the presence of proteolitic enzymes (digestion).
- Biomass sample (MRL) shows higher level of active beta-glucans in a human digestive system emulation.
- ***Biomass sample is more resistant than the extract sample to digestive enzymes.***

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Conclusions regarding beta-glucans

- BIOMASS BETTER & GLOBAL ACTIVITY.
- ***Why is biomass more resistant?***
- The extracted form is more exposed to the action of the proteolytic enzymes since there are no physio-chemical barriers to prevent such exposure, compared to biomass equivalent.
- Nature of the mushroom cells, itself, protect the active compounds.

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The Role of Enzymes in Mushroom Nutrition

- The biomass form of mushrooms contents, not only protein-bound polysaccharide complexes (i.e. betaglucans), but also **active enzymes** responsible for:
  - A) preventing oxidative stress
  - B) inhibiting cell growth
  - C) promoting detoxification
- Synergic action

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Mushrooms enzymes actions

- A. Preventing oxidative stress,
  - i.e.: Laccase, SOD
- B. Inhibiting cell growth,
  - i.e.: Proteases, Glycosamilases.
- C. Promoting detoxification,
  - i.e.: Peroxidases, cytochrome P450, glucose-2-oxidase.

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Role of Enzymes in Mushroom Nutrition

- *A. Enzymes that prevent oxidative stress*

Laccase: catalyses the reduction of di-oxygen to water and the oxidation of a wide range of phenolic or related compounds.

- Laccase is a powerful reactive oxygen species (ROS) scavenger and may prevent oxidative damage in mammalian tissues.
- Improves detoxification.

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Role of Enzymes in Mushroom Nutrition

- Cell damage is induced by reactive oxygen species (ROS) which can be either free radicals or molecules containing oxygen atoms, i.e., Peroxides. Oxidant species produce free radicals or are activated chemically by them.
- Natural killer (NK) cells are susceptible to ROS and lose their activity due to the effects of ROS.

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Role of Enzymes in Mushroom Nutrition

- *A. Enzymes that prevent oxidative stress*

Superoxide dismutase (SOD)

- SOD and SOD mimicking substances can restore the NK cell activity by protecting them from oxidative damage.

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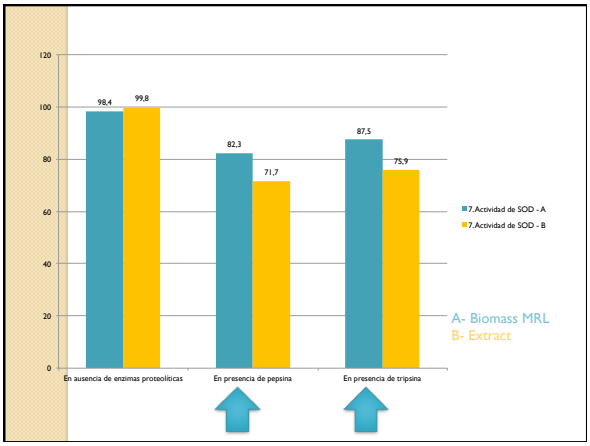
Role of Enzymes in Mushroom Nutrition

Enzymes, polysacharides and secondary metabolites per g of product	In absence of Proteolytic Enzymes		in the presence of Pepsin		in the presence of Trypsin	
	Reishi A MRL	Reishi B Myco	Reishi A MRL	Reishi B Myco	Reishi A MRL	Reishi B Myco
Superoxide dismutase (SOD) activity	98.4 m U	99. 8 m U	82.3 m U	71.7 m U	87.5 m U	75.9 m U



Role of Enzymes in Mushroom Nutrition

- B. *Enzymes that inhibit cellular growth*
- **Protease enzymes** have been shown to degrade cancer cells as well as toxins.
- **Glucoamylase** is an enzyme that break down large starch molecules in the human body into the useful energy compound of glucose. This is accomplished by removing the alpha-1 and 4-glycosidic linkages from the non-reducing end of the starch molecule.

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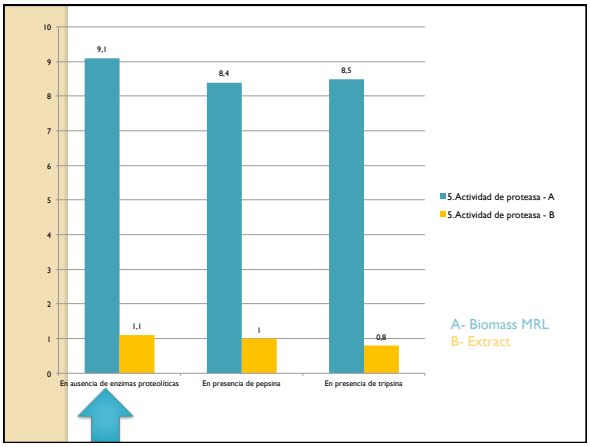
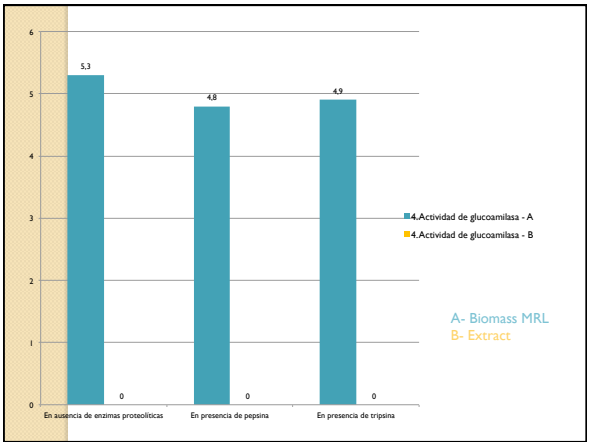
The Role of Enzymes in Mushroom Nutrition

Enzymes, polysaccharides and secondary metabolites per g of product	In absence of Proteolytic Enzymes		in the presence of Pepsin		in the presence of Trypsin	
	Reishi A MRL	Reshi B Myco	Reishi A MRL	Reshi B Myco	Reishi A MRL	Reshi B Myco
Protease activity	9.1 m U	1.1 m U	8.4 m U	1.0 m U	8.5 m U	0.8 m U



Role of Enzymes in Mushroom Nutrition

Enzymes, polysaccharides and secondary metabolites per g of product	In absence of Proteolytic Enzymes		in the presence of Pepsin		in the presence of Trypsin	
	Reishi A MRL	Reshi B Myco	Reishi A MRL	Reshi B Myco	Reishi A MRL	Reshi B Myco
Glucoamylase	5.3 U	0.0 U	4.8 U	0.0 U	4.9 U	0.0 U
Beta-glucanasase activity						



Role of Enzymes in Mushroom Nutrition

- C. *Enzymes that promote detoxification*

Peroxidase

- Peroxidase enzymes catalyse hydrogen peroxide-dependent one-electron oxidation of a wide range of phenolic and related compounds which result in the formation of arylcation radicals.
- Their various benefits and actions are widely distributed in the body.

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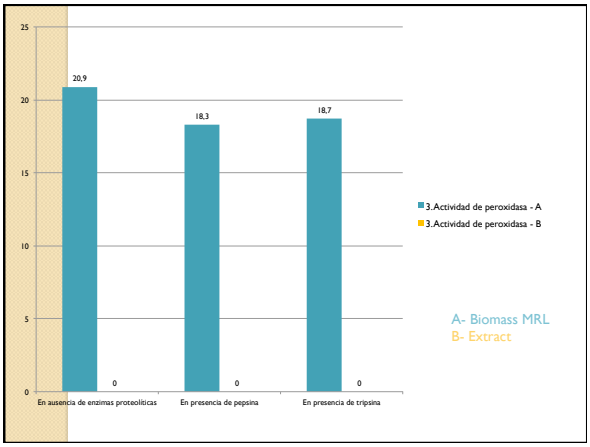
Role of Enzymes in Mushroom Nutrition

Enzymes, polysaccharides and secondary metabolites per g of product	In absence of Proteolytic Enzymes		in the presence of Pepsin		in the presence of Trypsin	
	Reishi A MRL	Reshi B Myco	Reishi A MRL	Reshi B Myco	Reishi A MRL	Reshi B Myco
Peroxidase activity	20.9 m U	0.0 m U	18.3 m U	0.0 m U	18.7 m U	0.0 m U



Role of Enzymes in Mushroom Nutrition

- C. *Enzymes that promote detoxification*

Cytochrome P450

- A very diverse group of naturally occurring and synthetic compounds are metabolized by cytochrome P450 (=CYP), a wide family of **mono-oxygenases** in the liver. These CYP substrates range from endogenous compounds such as steroids and cholesterol to drugs and carcinogens such as phenobarbital (PB) and aromatic hydrocarbons.
- The oxidized products are more polar, and the result is generally an easier detoxification.

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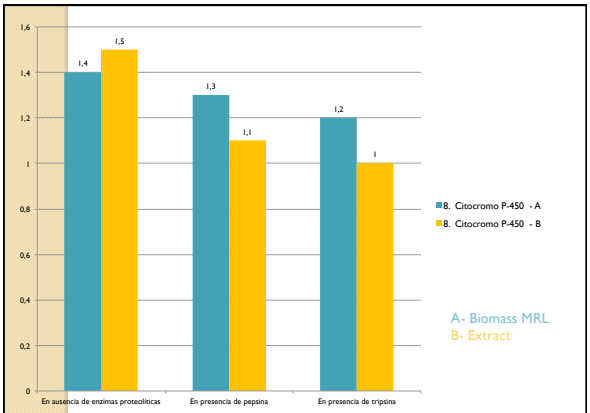
Role of Enzymes in Mushroom Nutrition

Enzymes, polysacharides and secondary metabolites per g of product	In absence of Proteolytic Enzymes		in the presence of Pepsin		in the presence of Trypsin	
	Reishi A MRL	Reshi B Myco	Reishi A MRL	Reshi B Myco	Reishi A MRL	Reishi B Myco
Cytochrome "P-450"	1.4 nmoles	1.5 nmoles	1.3 nmoles	1.1 nmoles	1.2 nmoles	1.0 nmoles



Role of Enzymes in Mushroom Nutrition

- C. *Enzymes that promote detoxification*

Glucose-2-oxidase

- Catalyses the oxidation of several aldopyranoses producing hydrogen peroxide and 2-keto-D-glucose.
- This activity has been found to exhibit anti-tumor activity (*in vitro*) against Ehrlick ascites tumour cells by inhibiting cell proliferation.

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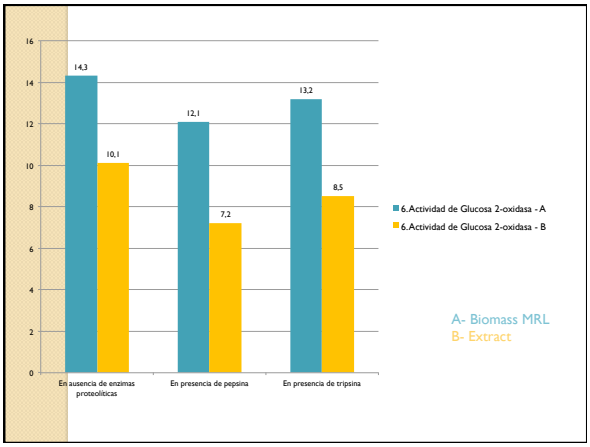
Role of Enzymes in Mushroom Nutrition

Enzymes, polysacharides and secondary metabolites per g of product	In absence of Proteolytic Enzymes		in the presence of Pepsin		in the presence of Trypsin	
	Reishi A MRL	Reishi B Myco	Reishi A MRL	Reishi B Myco	Reishi A MRL	Reishi B Myco
Glucose 2-oxdase activity	14.3 m U	10.1 m U	12.1 m U	7.2 m U	13.2 m U	8.5 m U



Conclusions regarding enzyme content

- When exposed to proteolytic enzymes such as pepsin and trypsin the differences in enzyme content between both samples (biomass and extract) may be due to differences in biological material in these samples.
- Biomass form is more resistant to proteolytic enzymes (gastric juices) than the extracted form.
- Concentrated extract form is more exposed to the action of the proteolytic enzymes since there are no physio-chemical barriers to prevent such exposure.

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Aplications of mushroom nutrition in different conditions

MUSHROOM NUTRITION: PROGRAM SUGGESTIONS

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Treatment suggestions

- Applications for **Reishi-MRL** in Hayfever
- Supplementation for Hayfever*
  - Acute phase – 6-9 tabs / day
  - Maintenance – 3 tabs/day
  - *Nutrition Practitioner-October 2003

Treatment suggestions

Alcohol Liver Steatosis

- ***Cordyceps sinensis*** -Mycology News #8

Week	Tabs/ Day	Tabs/Week	Tubs of 90 Tabs
Week 1	6	42	
Week 2	6	42	
Week 3	6	42	
Week 4	6	42	
Week 5	6	42	
Week 6	6	42	
Week 7	6	42	
Week 8	6	42	
Week 12	6	42	
Total		504	5.0

Treatment suggestions

Fibromyalgia

- ***Coriolus versicolor*** -Mycology News #6

Week	Tabs/ Day	Tabs/Week	Tubs of 90 Tabs
Week 1	6	42	
Week 2	6	42	
Week 3	6	42	
Week 4	6	42	
Week 5	6	42	
Week 6	6	42	
Week 7	6	42	
Week 8	6	42	
Total		336	4.0

Treatment suggestions

- **Radiotherapy*** – **Six week course in stage III cancer patients**
  - ***Coriolus versicolor*** - Mycology News #4

Week	Tabs/ Day	Tabs/Week	Tubs of 90 Tabs
Week 1	6	42	
Week 2	9	63	
Week 3	12	84	
Week 4	12	84	
Week 5	12	84	
Week 6	12	84	
Week 7	9	63	
Week 8	6	42	
Total		546	6.0

- * Depending on the cancer and affected area, combinations could be necessary with Reishi, Blazel...

Treatment suggestions

- **Leaky Gut**
  - ***Coriolus versicolor*** - Mycology News #10

Week	Tabs/ Day	Tabs/Week	Tubs of 90 Tabs
Week 1	9	63	
Week 2	9	63	
Week 3	9	63	
Week 4	9	63	
Week 5	9	63	
Week 6	9	63	
Week 7	9	63	
Week 12	9	63	
Total		756	8.0

Treatment suggestions

- **LSIL HPV infection**
  - ***Coriolus versicolor*** - Clinical Journal of Mycology Vol 2

Week	Tabs/ Day	Tabs/Week	Tubs of 90 Tabs
Week 1	6	42	
Week 2	6	42	
Week 3	6	42	
Week 4	6	42	
Week 5	6	42	
Week 6	6	42	
Week 7	6	42	
Week 26	6	42	
Total		1092	12.0

Treatment suggestions

- **Gout**
  - **Reishi (*Ganoderma lucidum*)** - Mycology News #10

Week	Taps/ Day	Taps/Week	Tubs of 90 Tabs
Week 1	6	42	
Week 2	6	42	
Week 3	6	42	
Week 4	6	42	
Week 5	4	24	
Week 8	4	24	
Week 9	2	14	
Week 32	2	14	
Total		717	8.0

Treatment suggestions

- **Protocol-Dementia-**
  - (Coriolus-MRL /Hericium-MRL)
    - *Coriolus versicolor* -Clinical Journal of Mycology Vol 4

Week	Tabs/ Day	Tabs/Week	Tubs of 90 Tabs
Week 1	6	42	
Week 2	6	42	
Week 3	6	42	
Week 4	6	42	
Week 5	6	42	
Week 6	6	42	
Week 7	6	42	
Week 52	6	42	
Total		2184	24.0

Treatment suggestions

- **Protocol-Dementia-**
  - (Coriolus-MRL /Hericium-MRL)
    - *Hericium erinaceus* -Clinical Journal of Mycology Vol 4

Week	Tabs/ Day	Tabs/Week	Tubs of 90 Tabs
Week 1	6	42	
Week 2	6	42	
Week 3	6	42	
Week 4	6	42	
Week 5	6	42	
Week 6	6	42	
Week 7	6	42	
Week 52	6	42	
Total		2184	24.0

Role of Enzymes in Mushroom Nutrition

Conclusions

- There is strong evidence suggesting that important antioxidant and cytoprotective enzymes are present in various edible fungi.
- This points out the importance of a therapeutic strategy based on nutritional interventions with mushroom supplementation to prevent and limit the deleterious consequences associated with high oxidation damage in oxidant disorders such as coronary heart disease, asthma, chronic and neurodegenerative disorders and cancer.

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Thank you!
Dank Je Wel

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