

Full Length Research Paper

Quality characteristics of rice inoculated with *Inonotus obliquus* mycelia and incubated under different cultivating conditions

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This study was performed to investigate the quality characteristics of *Inonotus obliquus* mycelia rice incubated with *I. obliquus* (KCTC 256152). Different volumes of mycelial culture of *I. obliquus* were used for production of *I. obliquus* mycelia rice. Twenty percent of the mycelial culture was added to rice and designated as IOR-20, 30% as IOR-30 and 40% as IOR-40. Using the *I. obliquus* mycelia rice, the contents of β -1,3-glucan, betulin, total polyphenol and 1,1-diphenyl-2-picrylhydrazol (DPPH) radical scavenging activity, Hunter's color values, free amino acid content, and mineral content were investigated. The β -1,3-glucan content in *I. obliquus* mycelia rice were in the order of IOR-30(729.7 μ g/g) > IOR-20(716.0 μ g/g) > IOR-40(690.5 μ g/g) and those of betulin content were OR-30(1273.7 μ g/g) > IOR-40 (1247.81 μ g/g) > IOR-20 (1209.82 μ g/g). The highest content of total polyphenols (353.6 μ g/g) and activity of DPPH radical scavenging were observed in the IOR-30. The higher contents for β -1,3-glucan, betulin, polyphenol, and DPPH radical scavenging were found in 30% mycelial culture treatment, showing the best condition for the production of *I. obliquus* mycelia. Aspartic acid (10.28 to 29.44 μ g/100 g), threonine (5.43 to 11.00 μ g/100 g), serine (8.84 to 14.53 μ g/100 g), glutamic acid (31.01 to 53.61 μ g/100 g), glycine (4.77 to 10.26 μ g/100 g), valine (2.93 to 6.57 μ g/100 g), and lysine (5.20 to 14.41 μ g/100 g) contents were determined in the *I. obliquus* mycelia rice and the order was as follows; IOR-20>IOR-30>IOR-40. The IOR-30 sample exhibited the highest levels of K(899.1 \pm 8.1 mg/kg), Mg(427.3 \pm 9.3 mg/kg), Ca(480.2 \pm 6.9 mg/kg), Na(296.1 \pm 9.2 mg/kg).

Key words: Rice, *Inonotus obliquus* (KCTC 256152), β -1,3-glucan, betulin, quality characteristics.

INTRODUCTION

Rice has been, throughout history, one of the most important foods in the human diet and one of the most extended cereal crops (9% of the total cultivated soil). In fact, rice has probably fed more people in history than any other crop. Even today, rice grains sustain two-thirds

of the world's population, approximately 2.5 billion people. Rice is mainly consumed as white grain, but in the last decade dozens of products containing rice as an ingredient have been appeared on the food market. Several treatments have been applied to improve rice quality, but

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no major study on use of the medical mushroom, *Inonotus obliquus*, on rice have been cited (Rosell and Marco, 2008). The most important new pharmaceutical products from medicinal mushrooms include polysaccharides, antioxidants, and lectins (Ng, 2004). The functional role of medical mushrooms include a number of processes from the hydrolysis of macromolecular substrates under extremely low nitrogen content to initiation and maintenance of pathogenesis and also widely used as a source of nutrients and medicines (Kudryavtseva et al., 2008). Polysaccharides are one of the main bioactive constituents of *I. obliquus* with health functions (Myung-ja et al., 2008; Ma et al., 2013). Although, this mushroom has been known to exhibit potent antioxidant activity, the mechanisms responsible for this activity remain unknown (Lee et al., 2007). These medical mushrooms are known in China, Russia, Japan, Korea, as well as the U.S.A. and Canada. There are about 200 species of mushrooms that have been found to markedly inhibit the growth of different kinds of tumors. Searching for new antitumor and other medicinal substances from mushrooms and to study the medicinal value of these mushrooms have become a matter of great significance.

However, most of the origin of mushroom antitumor substances has not been clearly defined. Several antitumor polysaccharides such as mixed-beta-glucans and their protein complexes (e.g. xyloglucans and acidic beta-glucan-containing uronic acid) as well as dietary fibers, lectins and terpenoids have been isolated from medicinal mushrooms. In Japan, Russia, China and the U.S.A., several different polysaccharide antitumor agents have been developed from the fruiting body, mycelia and culture medium of various medicinal mushrooms (*Lentinus edodes*, *Ganoderma lucidum*, *Schizophyllum commune*, *Trametes versicolor*, *I. obliquus*, and *Flammulina velutipes*). Both cellular components and secondary metabolites of a large number of mushrooms have been shown to affect the immune system of the host and therefore could be used to treat a variety of disease states (Song et al., 2003).

In view of the substantial health and functional benefits of medical mushrooms, this study was set out to evaluate the biochemical properties that *I. obliquus* mycelia inoculum impacts on rice in terms of antioxidant properties and other bioactive compounds.

MATERIALS AND METHODS

Microorganism and culture

The strain of *I. obliquus* (KCTC 256152) used in the present experiment was provided by the Korea Research Institute of

Bioscience and Biotechnology (Daejeon, Korea). The stock culture was maintained on potato dextrose agar (PDA) slants. Slants were incubated at 25°C for 20 days and then stored at 4°C for use as subcultures every month.

Inoculum preparation and culture conditions

I. obliquus (KCTC 256152) was initially grown on PDA medium (2.4% potato dextrose broth and 2% agar) in a Petri dish and then transferred to the seed culture medium by punching out 5 mm of the agar plate culture with a sterilized house-developed cutter. The liquid seed cultures were 500 ml Erlenmeyer flask containing 300 ml of PDA medium at 25°C on a rotary shaker incubator at 130 rpm for 10 days.

For the preparation of *I. obliquus* mycelia rice, sterilized rice was incubated with 20, 30 and 40%(v/w) of the seed culture and the fermentation culture was carried out in a 5 L jar fermenter (working volume: 3 L, PH: 5, temperature: 22°C agitation speed: 130 rpm and aeration rate: 1 vvm) for 20 days.

Determination of β -1,3-glucan content

A 10 g of sample was extracted with 20 ml of methanol at room temperature for overnight. 5 ml of the supernatant obtained by centrifugation and then concentrated under reduced pressure and dissolved in methanol 3 ml was used to measure concentration. Conditions for the HPLC were detector : waters, US/M996, 717 plus photodiode array detector, column : μ Bondapak C₁₈ 10 μ m 125A, Mobile phase : 20.8% DMSO, Flow rate : 1.0 ml/min, UV detector : 260 nm, injection volume : 10 μ l β -1,3-glucan (Sigma Co.) was used as a standard (Harada et al., 1968).

Determination of betulin contents

A 10 g of sample was extracted overnight at room temperature with 20 ml of methanol. 5 ml of the supernatant obtained by centrifugation and then concentrated under reduced pressure and dissolved in 3 ml methanol was used to measure concentration. Conditions for the HPLC were: detector- waters, US/M996, 717 plus photodiode array detector; column- μ Bondapak C₁₈ 10 μ m 125A; mobile phase, 86% acetonitrile; Flow rate 1.0 ml/min; UV detector, 200 nm; injection volume, 10 μ l Betulin (Sigma Co.) was used as a standard (Oliveira et al., 2002).

Determination of total phenols contents

The total phenols of the samples were estimated according to the Folin-Ciocalteu method (Singleton et al., 1999). A 50 μ l sample were added to 250 μ l of undiluted Folin-Ciocalteu-reagent. After 1 min, 750 μ l of 20% (w/v) aqueous Na₂CO₃ were added, and the volume was made up to 5.0 ml with H₂O.

The controls contained all the reaction reagents except the extract. After 2 h of incubation at 25°C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. Total phenols were determined as gallic acid equivalents (mg gallic acid /g extract), and the values are presented as means of triplicate analyses.

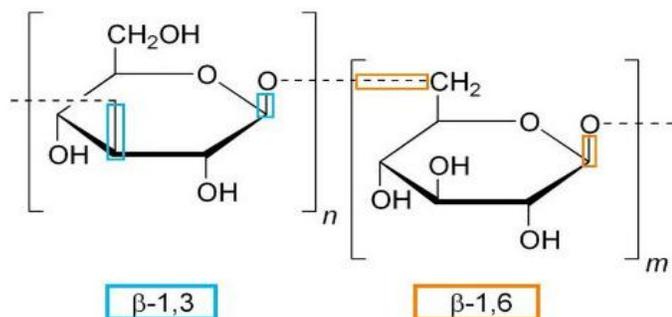


Figure 1. Chemical structures of the two glucans.

Determination of antioxidant activity using free radical scavenging activity (DPPH')

The DPPH' (1,1-diphenyl-2-picrylhydrazol) radical scavenging activity of sample was measured as follows: A 0.5 mM solution of DPPH' in methanol and 0.05 M acetate buffer pH 5.5 was prepared. An aliquot of 0.1 ml (at concentrations 0.5 to 1 mg/ml) of an antioxidant extract solution was added to 2 ml acetate buffer, 1.9 ml methanol and 1 ml DPPH' solution.

Blanks contained 2 ml acetate buffer, 1.9 ml methanol and 0.1 ml cherry wine, while the control contained 2 ml acetate buffer, 1 ml DPPH' and 2 ml methanol. The mixture was shaken immediately after adding DPPH' and allowed to stand at room temperature in the dark, and the decrease in absorbance at 517 nm using a Shimadzu UV-1700UV spectrophotometer was measured after 30 min until they reached a plateau. All determinations were performed in duplicate. The inhibitory percentage of the DPPH' radical by the samples was calculated according to Shyu and Hwang (2002) as follows:

$$\text{Scavenging effect (\%)} = [(A_0 - (A - A_b)) / A_0] \times 100$$

Where A_0 is the A_{517} of DPPH' without sample (control), A is the A_{517} of sample and DPPH', and A_b is the A_{517} of sample without DPPH' (blank).

Colour measurement

L^* (lightness), a^* (redness, + or greenness, - or blueness, + or blueness, -) values of sample powder were measured using a Minolta Chroma meter CR-300 (Minolta Corp., Japan). A Minolta calibration plate (YCIE = 94.5, XCIE = 0.3160, YCIE = 0.330) and a Hunter Lab standard plate ($L^* = 97.51$, $a^* = -0.18$, $b^* = +1.67$) were used to standardize the instrument with D65 illuminant. Colour was measured directly on three zones of the sample and the average was calculated.

Determination of free amino acid content

A 1 g of sample was hydrolyzed with 6 N HCl (10 ml) in a sealed-vacuum ampoule at 110°C for 24 h for amino acid composition analysis. The HCl was removed from the hydrolyzed sample on a

rotary evaporator, brought to a known volume (5 ml) with 0.2 M sodium citrate buffer (pH 2.2). The sample was passed through a C-18 Sep Pak (Waters Co. Milford, USA) cartridge and filtered through a 0.22 μm membrane filter (Millipore, USA). Amino acids were determined on an automatic amino acid analyzer (Biochrom-20, Pharcia Biotech Co., Swiss).

Determination of mineral content

About 0.5 ml sample was put into a cup and 15 ml pure HNO_3 was added. A solution was diluted to certain volume with distilled water. Mineral concentrations were determined by inductively coupled plasma atomic emission spectrometer (ICP AES: Varian Vista) (Skujins, 1998). The instrument was calibrated using known standards for each mineral. Average values of 2 replicate samples are reported.

Statistical analysis

All values for each group are given as means \pm standard deviation. The data were analysed using one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test (DMRT) using the SAS system (version 8.2). P -values less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

β -1,3-Glucan content

β -1,3-Glucan content varied significantly among the rice incubated with three different levels of *I. obliquus* mycelia inoculum. Incubating rice with 20% concentration of the *I. obliquus* mycelial culture (IOR - 20) reduced β -1,3-glucan content by 6.58% over what pertained (740 $\mu\text{g/g}$) with those inoculated with 30% *I. obliquus* mycelial culture (IOR - 30). Increasing the concentration of the Inoculum applied to rice to 40% (IOR - 40) produced significantly low amounts of β -1,3-glucan (680 $\mu\text{g/g}$) compared to the 30% level (IOR - 30).

This therefore established the 30% *I. obliquus* mycelial culture (IOR - 30) as the optimum level of *I. obliquus* extract to derive the highest functional properties when used on rice. The chemical structure of the two types of glucans obtained, β -1,3-glucan and β -1,6-glucan, are shown in Figure 1 while the trend of results is presented in Figure 2. Rhee et al. (2008) indicated that the extraction method does not affect β -glucan content in *Inonotus obliquus* as indicated by no significant difference between extraction method in comparative study of analytical methods for alkali-soluble β -glucan in *Inonotus obliquus*. Harikrishnan et al. (2012) suggested that supplementation diet with the mushroom positively enhances the immune system and confers diseases resistance which may be potentially used as an immunoprophylactic.

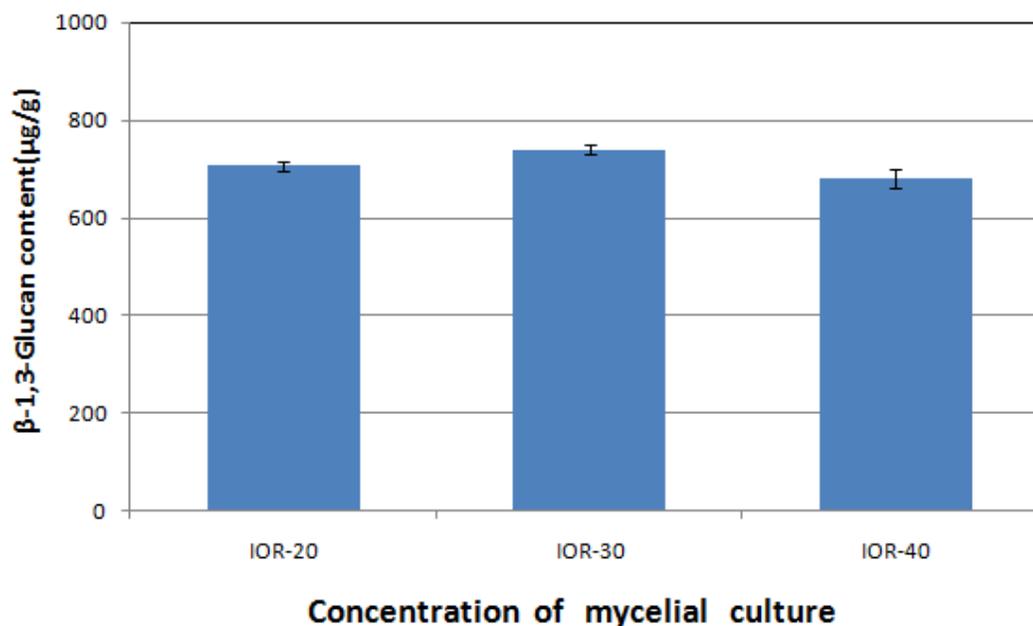


Figure 2. The content of β -1,3-Glucan in rice incubated with mycelial culture of *Inonotus obliquus*. ¹IOR-20, Rice incubated with 20%(v/w) of mycelial culture of *Inonotus obliquus*. IOR-30, Rice incubated with 30%(v/w) of mycelial culture of *Inonotus obliquus*. IOR-40, Rice incubated with 40%(v/w) of mycelial culture of *Inonotus obliquus*.²Each data represents the mean \pm SD of three independent experiments.

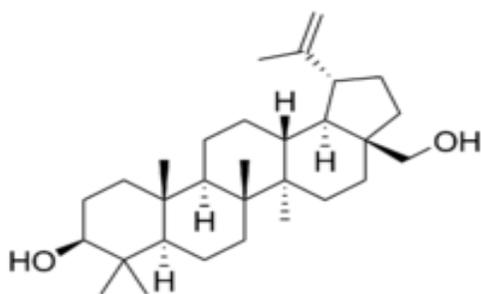


Figure 3. Chemical structure of betulin.

Smiderle et al. (2008) reported that animals previously treated with the glucan showed a reduction of writhes and also caused significant inhibition of both the early (neurogenic pain) and the late phases. These data showed that the glucan had potent anti-inflammatory and analgesic (antinociceptive) activities, possibly by the inhibition of pro-inflammatory cytokines. In a study of mice, Mizuno et al. (1999) observed that the mushroom (*Inonotus obliquus*) produced an enzyme inhibitory activity and hypoglycemic effects in normal mice with both water-soluble and water-insoluble polysaccharides. The active principles were β -glucan, heteroglucan, and

their protein complexes. No clear antienzymic traits were recognized by polysaccharides prepared from mycelia.

Betulin content

Betulin content, just as β -1,3-glucan, was highest (1284.3 $\mu\text{g/g}$) in the rice inoculated with 30% *I. obliquus* mycelial culture (IOR - 30) compared to those incubated with 20% (IOR - 20) and 40% (IOR - 40) of the inoculum which produced 1186.3 and 1235.7 $\mu\text{g/g}$ β -1,3-glucan contents, respectively. This represents an 8.3% higher content of the compound in rice with 30% of the inoculum (IOR - 30) over those receiving 20% of the mycelial culture (IOR - 20) and an increase of 6.6% over rice incubated to 40% of *I. obliquus* (IOR - 40). This showed that inoculating rice with 30% concentration of the inoculum (IOR - 30) was the most appropriate concentration for obtaining maximum biochemical activity from *I. obliquus* application to rice. The chemical structure of Betulin is shown in Figure 3 and Figure 4 shows the trend of results. In a study concerned with optimization of submerged culture conditions for the mycelial growth and botulin production by *I. obliquus*, Bai et al. (2012) proved that mycelial growth and pellet morphology (that is, compactness, mean diameter and roughness) may be the critical para-

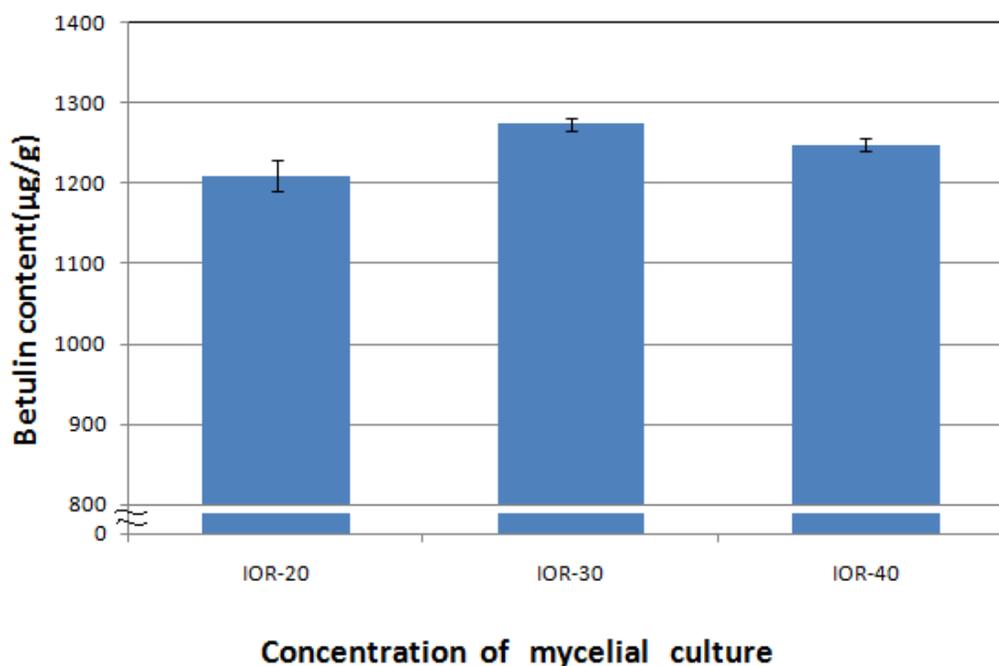


Figure 4. The content of betulin in rice incubated with mycelial culture of *Inonotus obliquus*. ¹IOR-20, Rice incubated with 20%(v/w) of mycelial culture of *Inonotus obliquus*. IOR-30, Rice incubated with 30%(v/w) of mycelial culture of *Inonotus obliquus*. IOR-40, Rice incubated with 40%(v/w) of mycelial culture of *Inonotus obliquus*.²Each data represents the mean±SD of three independent experiments.

Table 1. Antioxidant capacity and total polyphenol content of *Inonotus obliquus* rice.

Sample ¹	DPPH (% dry matter edible part)	Total polyphenol content (µg/g dry matter edible part)
Polished rice	70.75±1.27 ^{d2}	114.7±10.2 ^c
Brown rice	77.82±1.31 ^c	310.5±12.3 ^b
IOR-20	91.84±2.21 ^a	353.6±23.1 ^a
IOR-30	86.82±1.07 ^b	102.9±19.1 ^d
IOR-40	90.73±1.21 ^a	319.4±16.2 ^b

¹IOR-20, Rice incubated with 20%(v/w) of mycelial culture of *Inonotus obliquus*. IOR-30, Rice incubated with 30%(v/w) of mycelial culture of *Inonotus obliquus*. IOR-40, Rice incubated with 40%(v/w) of mycelial culture of *Inonotus obliquus*. ²Quoted values are means of triplicate experiments. Different superscripts within the same column indicate significant differences (P < 0.05).

meters affecting betulin production which helps to scavenge DPPH radical and hydroxyl radical. Won et al. (2011) indicated that this potential allows for regulation of immune responses through suppression of *in vivo* growth of melanoma tumor in tumor bearing mice receiving oral administration of polysaccharides isolated from fruiting body of *I. obliquus*.

Total phenolic content and DPPH radical scavenging ability

The rice inoculated with *I. obliquus* mycelial culture exhibited higher DPPH antioxidant activity compared to the polished and brown rice which were of significantly less antioxidant activity (Table 1). Incubating rice with

Table 2. Hunter's color values of *Inonotus obliquus* rice.

Sample ¹	Color value ²		
	L (Lightness)	a (Redness)	b (Yellowness)
Polished rice	91.43±1.09 ^a	-0.82±0.09 ^d	6.61±0.89 ²
IOR-20	79.17±2.21 ^{b3}	1.74±0.31 ^c	16.19±0.72 ^a
IOR-30	62.80±1.17 ^c	3.21±0.21 ^a	13.57±1.09 ^b
IOR-40	56.96±1.07 ^d	2.83±0.16 ^b	8.51±1.72 ^c

¹IOR-20, Rice incubated with 20%(v/w) of mycelial culture of *Inonotus obliquus*. IOR-30, rice incubated with 30%(v/w) of mycelial culture of *Inonotus obliquus*. IOR-40, Rice incubated with 40%(v/w) of mycelial culture of *Inonotus obliquus*. ²L, lightness (100, white; 0, black), a, redness (-, green; +, red); b: yellowness (-, blue; +, yellow). ³Quoted values are means of triplicate experiments. Different superscripts within the same column indicate significant differences (P < 0.05).

20% *I. obliquus* mycelial culture (IOR - 20) produced the highest (91.84%) antioxidant activity followed by incubation with 40% of the mycelial culture (IOR - 40) which had a similar antioxidant capacity (90.73%) as incubating with 20% of the inoculum (IOR - 20). Among the *I. obliquus* inoculations, antioxidant capacity was lowest (86.84% DPPH and 102.9 µg/g total phenol content) when the rice was incubated with 30% of the mycelia (IOR - 30). The ideal *I. obliquus* incubation condition for maximum antioxidant activity in rice was obtained when the rice was treated to 20%(v/w) mycelial culture (IOR - 20) as this produced the highest DPPH percentage in the edible part and a commensurate high total phenol content of 353.6 µg/g. incubating rice with a high dose of more than 30% of *Inonotus obliquus* mycelial culture as occurred in the 40% (IOR - 40) level did not promote increased antioxidant capacity and total phenol content of rice.

Lee et al. (2007) indicated that though methanolic extracts of the mushroom produced significant scavenging for ABTS radical cations and DPPH radical, the mechanism for antioxidant activity of *I. obliquus* remains unknown. However, Saltarelli et al. (2009) indicated that such antioxidant activity of *I. obliquus* may be influenced by the low molecular weight compound which has been found to produce significant antioxidative properties with a correlation between total phenol content and DPPH scavenging activity. Ma et al. (2012a) explained that *I. obliquus* polysaccharides has a hyperbranched conformation with triple helix, higher antioxidant abilities on DPPH radical scavenging, ferric-reducing power and lipid peroxidation inhibition activity which could have transferred antioxidative properties to the inoculated rice especially when the rice was inoculated with 30% (IOR - 30) of the mycelial culture. Freeze drying was found as a good choice for the preparation of polysaccharides from *I. obliquus* and could

be used to produce antioxidants for food industry.

Ma et al. (2012b) concluded that chemical modification of polysaccharides exerted potent biological property which was related to the physicochemical properties that may have been in detection with inoculation of rice. The 30% (IOR - 30) *I. obliquus* mycelial culture could have produced higher amounts of free phenolics accounting for the significantly high antioxidant capacity at that level of inoculum concentration. Ju et al. (2010) found that free phenolics produced during inoculation of the rice with the mycelia culture could have significantly enhanced the radical scavenging activity of treated rice. Xu and Zhu (2011) found that in dose-dependent experiments, antioxidant activity of *I. obliquus* on production and antioxidant activity of extracellular (EPC) and intracellular phenolic compounds (IPC) by *I. obliquus* from corn stover medium demonstrated a significantly stronger free radical scavenger activity against DPPH and hydroxyl radicals similar to the effect exhibited by different doses of *I. obliquus* in rice in this study. Hu et al. (2009) explained that the different biological activities among the untreated rice and those incubated with the *I. obliquus* mycelial extracts may be attributed to the chemical composition of the inoculum, partially supported by polysaccharide, protein and phenolic content.

Colour values

Polishing increased the lightness of rice to 91.43 with low yellowness of 6.61 and a negative redness (-0.82) which varied significantly from rice incubated with the *I. obliquus* mycelia culture (Table 2). Following a similar trend as antioxidant and total phenol content, rice incubated with 30% of the inoculum (IOR - 30), was of greater lightness (62.80) and redness (3.21) and moderate yellowness (16.91) compared to the other *I. obliquus* mycelial

Table 3. Free amino acid composition($\mu\text{g}/100$ g-dry weight) of *Inonotus obliquus* rice¹.

Amino acid	IOR-20	IOR-30	IOR-40
O-Phospho-L-serine	ND ²	ND	ND
Taurine	ND	ND	ND
O-PhosphoEthanolAmine	ND	ND	ND
Urea	ND	ND	ND
L-Aspartic acid	29.44 ³	11.03	10.28
Hydroxy-L-proline	ND	ND	ND
L-Threonine	11.00	7.10	5.43
L-Serine	14.53	11.33	8.84
L-Asparagine	ND	14.74	ND
L-Glutamic acid	53.61	32.84	31.01
L-Sarcosine	ND	ND	ND
L-a-Aminoadipic acid	ND	ND	ND
L-Proline	ND	ND	ND
Glycine	10.26	5.41	4.77
L-Alanine	20.42	16.32	25.93
L-Citrulline	ND	ND	ND
L-a-Amino-n-butylric acid	ND	ND	ND
L-Valine	6.57	3.16	2.93
L-Cystine	ND	ND	ND
L_Methionine	ND	ND	ND
Cystathionine	ND	11.58	10.28
L-Isoleucine	10.61	ND	ND
L-Leucine	19.54	7.97	ND
L-Tyrosine	6.77	6.95	9.15
B-Alanine	ND	ND	ND
L-Phenylalanine	13.27	9.72	12.22
D,L-B-Aminoisobutyric acid	ND	ND	ND
L-Homocystine	ND	ND	ND
r-Amino-n-butylric acid	ND	ND	ND
Ethanolamin	ND	ND	ND
Ammonium Chloride	52.72	78.59	107.21
Hydroxylysine	ND	ND	ND
L-Ornithine	11.41	9.93	19.20
L-Lysine	14.41	9.30	5.20
1-Methyl-L-histidine	ND	ND	ND
L-Histidine	ND	8.53	ND
L-Tryptophan	ND	ND	ND
3-Methyl-L-histidine	ND	ND	ND
L-Anserine	ND	ND	ND
L-Carnosine	ND	ND	ND
L-Arginine	29.24	27.41	27.65

¹IOR-20, Rice incubated with 20%(v/w) of mycelial culture of *Inonotus obliquus*. IOR-30; Rice incubated with 30%(v/w) of mycelial culture of *Inonotus obliquus*. IOR-40, Rice incubated with 40%(v/w) of mycelial culture of *Inonotus obliquus*.²ND, not detected.

³Quoted values are means of triplicate experiments.

(IOR - 20) was of high level of lightness (79.17) and the 40% incubation with the inoculum (IOR - 40) showed very low yellowness level (8.51). The color expression of inoculating rice with *I. obliquus* mycelia culture seemed similar to parboiling where the color of rice increased with severity of parboiling conditions. Redness increased more than yellowness when parboiling brown rice. It changed the levels of glucose, fructose, sucrose and maltose (Lamberts et al., 2008). The results show that treatment of rice with extract of the medical mushroom impacts significant improvement in rice quality by enhancing the redness and yellowness of rice while reducing lightness.

Free amino acid content

Free amino acid content showed varied degrees of detection in the various *I. obliquus* mycelia treated samples (Table 3). Where the amino acids were detected, slightly higher levels of the amino acids occurred when incubated under 20% (IOR - 20) and 40% (IOR - 40) concentration of the inoculum, respectively, than was observed when 30% concentration of *I. obliquus* mycelia culture (IOR - 30) was applied to the rice. Asparagine was detected in the 30% treatment level (IOR - 30), but not in the 20% (IOR - 20) and the 40% incubations (IOR - 40). Isoleucine occurred only at low concentrations at 20% incubation of *I. obliquus* mycelia but not at higher levels of concentration of the inoculum. Total amino acid content was highest at the 40% incubation of rice with the mushroom (IOR - 40) and least at the 20% incubation level (IOR - 20) level of *I. obliquus* mycelial treatment of rice.

Borah and Johari (1987) showed high amino acid content in grains, indicated that higher levels of reduced nitrogen, efficient translocation of vegetative nitrogen into developing grains, higher level of free amino acids and higher rate of incorporation of amino acids into grain proteins were some of the important factors for higher protein content rice grains. Pineda-Hidalgo et al. (2011) indicated that the increased levels of free amino acids in rice are mainly due to the reduction of storage proteins and the failure to incorporate their amino acids into other proteins, as well as the alteration of carbohydrate metabolism that may favor amino acid biosynthesis. Das et al. (2008) suggested that treatment of rice with cellulase enzymes of microbial sources increases water uptake ratio, volume expansion ratio with reduced cooking time and better cooking and nutritional attributes over untreated brown rice. The color expressions obtained in this study are comparative to the findings of Frank et al. (2012) who showed that with treatment of rice

inoculated rice in which the 20% incubation with the fungi

Table 4. Mineral contents of *Inonotus obliquus* rice.

Sample ¹	Element (mg/kg-dry weight)						
	K	Mg	Ca	Na	Fe	Zn	Mn
IOR-20	746.1±7.1 ^{c2}	375.2±8.2 ^c	450.3±5.7 ^c	252.2±6.1 ^b	ND ³	ND	ND
IOR-30	899.1±8.1 ^a	427.3±9.3 ^a	480.2±6.9 ^a	296.1±9.2 ^a	ND	ND	ND
IOR-40	812.1±10.1 ^b	396.2±7.5 ^b	467.4±7.8 ^b	226.5±7.3 ^c	ND	ND	ND

¹IOR-20, Rice incubated with 20%(v/w) of mycelial culture of *Inonotus obliquus*. IOR-30, Rice incubated with 30%(v/w) of mycelial culture of *Inonotus obliquus*. IOR-40, Rice incubated with 40%(v/w) of mycelial culture of *Inonotus obliquus*.²Quoted values are means of triplicate experiments. Different superscripts within the same column indicate significant differences (P < 0.05). ³ND, not detected.

with *I. obliquus* mycelial culture; the treatment exhibited higher levels of fatty acid methyl esters, free fatty acids, organic acids and amino acids. Ohtsubo et al. (2005) shown that the highly nutritious product resulting from applying treatments such as pre-germination and inoculating rice with *I. obliquus* mycelial culture would be acceptable to consumers or the food industry as a promising foodstuff containing more nutritional and bio-functional components than ordinary rice products.

Mineral content

The mineral contents (K, Mg, Ca, Na, Fe, Zn and Mn) of *I. obliquus* mycelia incubated rice showed significant differences across all treatments (Table 4). The 30% *I. obliquus* incubated rice (IOR - 30) produced the optimum level (899.1 mg/kg dry weight) of K, but a 20% (IOR - 20) and 40% (IOR - 40), incubations of rice with the inoculum reduced K content of rice. Mg also produced similar effect as the expression of K where 40% inoculation (IOR - 40) stimulated the highest (427.3 mg/kg dry weight) Mg accumulation. Incubation of the rice with 20% (IOR - 20) and 40% (IOR - 40) concentration of the *I. obliquus* gave significantly low amounts of Ca and Na as were observed with all the other minerals. This showed that treatment of the rice with 20 and 40% concentrations of *I. obliquus* may not contribute significantly to mineral accumulation in rice. For best performance, however, treatment with 30% concentration of the *I. obliquus* mycelial extract would produce the most significant effect in minerals content of rice. Among the three levels of *I. obliquus* treatment, Fe, Zn and Mn were not detected just as heavy metals AS, Pd, Cd and Hg (date not shown), indicating the hygienic condition under which experiment was conducted.

Rivero-Huguet (2007) indicated that in terms of mineral composition, potassium (K) is the most abundant mineral found in rice (brown, parboiled brown, milled and parboiled milled rice) followed by magnesium (Mg) and

calcium (Ca). Among microelements, the presence of Cu, Fe, molybdenum (Mo), manganese (Mn), sodium (Na) and Zn in rice is significant. It is generally accepted that as greater amounts of rice bran are removed from the grain during milling and polishing, more vitamins and minerals are lost. Milled rice shows a significantly lower content of K, Mg, Mn, Na and Zn than brown rice, therefore, the higher levels of minerals obtained with treatment of rice with the *I. obliquus* mycelial extract enhances the quality of rice for human consumption.

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REFERENCES

- Bai YH, Feng YQ, Mao DB, Xu CP (2012). Optimization for betulin production from mycelial culture of *Inonotus obliquus* by orthogonal design and evaluation of its antioxidant activity. J. Taiwan Inst. Chem. Eng. <http://dx.doi.org/10.1016/j.jtice.2012.03.004>
- Borah RC, Johari RP (1987). Uptake and Assimilation of Nitrogen and its Relationship to Grain Protein Accumulation in Field-Grown Rice (*Oryza sativa* L.) II. Protease Activity, Reduce Nitrogen, FreeAmino Acid Content in Different Organs, Incorporation of [U-14C]-Leucine and Accumulation of Grain Protein. Biochem. Physiolog. Pflanzen. 182(4):333-341.
- Das M, Banerjee R, Bal S (2008). Evaluation of physicochemical properties of enzyme treated brown rice (Part B). LWT. Food Sci. Tech. 41(10):2092-2096.
- Frank T, Reichardt B, Shu Q, Engel KH (2012). Metabolite profiling of colored rice (*Oryza sativa* L.) grains. J. Cereal Sci. 55(2):112-119.
- Harada T, Misaki J, Saito H (1968). Curdlan: A Bacterial Gel-Forming B-1,3-Glucan. Arch. Biochem. Biophysics. 124:292-2
- Harikrishnan R, Balasundaram C, Heo MS (2012). *Inonotus obliquus* containing diet enhances the innate immune mechanism and disease resistance in olive flounder *Paralichthys olivaceus* against *Uronema marinum*. Fish Shellfish Imm. 32(6):1148-1154.
- Hu H, Zhang Z, Lei Z, Yang Y, Sugiura N (2009). Comparative study of antioxidant activity and antiproliferative effect of hot water and ethanol extracts from the mushroom *Inonotus obliquus*. J. Biosci. Bioeng. 107(1):42-48.

- Ju HK, Chung HW, Hong SS, Park JH, Lee J, Kwon SW (2010). Effect of steam treatment on soluble phenolic content and antioxidant activity of the Chaga mushroom (*Inonotus obliquus*). Food Chem. 119(2):619-625.
- Kudryavtseva OA, Dunaevsky YE, Kamzolkina OV, Belozersky MA (2008). Fungal proteolytic enzymes: Features of the extracellular proteases of xylophilic basidiomycetes. Microbiol. 77(6):643-653.
- Lamberts L, Rombouts I, Brijs K, Gebruers K, Delcour JA (2008). Impact of parboiling conditions on Maillard precursors and indicators in long-grain rice cultivars. Food Chem. 110(4):916-922.
- Lee IK, Kim YS, Jang YW, Jung JY, Yun BS (2007). New antioxidant polyphenols from the medicinal mushroom *Inonotus obliquus*. Bioorg. Medicinal Chem. Lett. 17(24):6678-6681.
- Ma L, Chen H, Zhang Y, Zhang N, Fu L (2012a). Chemical modification and antioxidant activities of polysaccharide from mushroom *Inonotus obliquus*. Carbohydr. Poly., 89(2):371-378.
- Ma L, Chen H, Zhang Y, Zhang N, Fu L (2012b). Chemical modification and antioxidant activities of polysaccharide from mushroom *Inonotus obliquus*. Carbohydrate Polymers, 89(2):371-378.
- Ma L, Chen H, Zhu W, Wang Z (2013). Effect of different drying methods on physicochemical properties and antioxidant activities of polysaccharides extracted from mushroom *I. obliquus*. Food Res. Inter. <http://dx.doi.org/10.1016/j.foodres.2011.05.005>
- Mizuno T, Zhuang C, Abe K, Okamoto H, Kiho T, Ukai S, Leclerc S, Meijer L (1999). Antitumor and Hypoglycemic Activities of Polysaccharides from the Sclerotia and Mycelia of *Inonotus obliquus* (Pers.: Fr.) Pil. (Aphyllophoromycetideae). Int. J. Medical Mushroom., 9:301-316.
- Myung-Ja Y, Kim JK, Park SY, Kim YH, Kim SJ, Lee JS, Chai KY, Kim HJ, Cui MX, Hong SS, Kim KY, Park RK (2008). Chaga mushroom (*Inonotus obliquus*) induces G0/G1 arrest and apoptosis in human hepatoma HepG2 cells. W. J. Gastroenterol. 14(4):511-517.
- Ng TB (2004). Peptides and proteins from fungi. Peptides 25:1055-1073.
- Ohtsubo K, Suzuki K, Yasui Y, Kasumi T (2005). Bio-functional components in the processed pre-germinated brown rice by a twin-screw extruder. J. Food Comp. Anal., 18(4):303-316.
- Oliveira BHD, Cid AM, Santos CAM, Espindola APDM (2002). Determination of the Triterpenoid, Betulinic acid, in *Doliocarpus schottianus* by HPLC. Phytochem. Anal. 13:95-98.
- Pineda-Hidalgo KV, Lavin-Aramburo M, Salazar-Salas NY, Chavez-Ontiveros J, Reyes-Moreno C, Muy-Rangel MD, Larkins BA, Lopez-Valenzuela JA (2011). Characterization of free amino acid QTLs in maize opaque2 recombinant inbred lines. J. Cereal Sci., 53(2):250-258.
- Rhee SJ, Cho SY, Kim KM, Cha DS, Park HJ (2008). A comparative study of analytical methods for alkali-soluble β -glucan in medicinal mushroom, Chaga (*Inonotus obliquus*). LWT. Food Sci. Techn. 41(3):545-549.
- Rivero-Huguet M, Huertas R, Francini L, Vila L, Darre E (2006). Concentrations of As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb and Zn in uruguayan rice determined by atomic absorption spectrometry. Atomic Spectroscopy. 27(2) : 48-56.
- Rosell CM, Marco C (2008) Rice. Gluten-Free Cereal Products and Beverages. pp. 81-100.
- Saltarelli R, Ceccaroli P, Iotti M, Zambonelli A, Buffalini M, Casadei L, Vallorani L, Stocchi V (2009). Biochemical characterisation and antioxidant activity of mycelium of *Ganoderma lucidum* from Central Italy. Food Chem., 116(1):143-151.
- Shyu YS, Hwang LS (2002). Antioxidant activity of the crude extract of crude extract of lignan glycosides from unroasted Burma black sesame meal. Food Res. Int. 35:357-365.
- Singleton V, Orthofer LR, Lamuela-Raventós RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299:152-178.
- Skujins S (1998). Handbook for ICP AES (Varian Vista). A short guide to Vista series. ICP AES Operation. Varian Int. AG, Zug, Version 1.0, Switzerland.
- Smiderle FR, Lorena MO, Carbonero ER, Baggio CH, Freitas CS, Marcon R, Santos ARS, Gorin PAJ, Lacomini M (2008). Eur. J. Pharmacol. 597(1-3):86-91.
- Song YS, Kim SH, Sa JH, Jin C, Lim CJ, Park EH (2003). Anti-angiogenic, antioxidant and xanthine oxidase inhibition activities of the mushroom *Phellinus linteus*. J. Ethnopharmacol. 88:113-116.
- Won DP, Lee JS, Kwon DS, Lee KE, Shin WC, Hong EK (2011). Immunostimulating activity by polysaccharides isolated from fruiting body of *Inonotus obliquus*. Molecules Cells. 31(2):165-173.
- Xu X, Zhu J (2011). Enhanced phenolic antioxidants production in submerged cultures of *Inonotus obliquus* in a ground corn stover medium. Biochem. Eng. J. 58-59:103-109.