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 μg/g) > IOR-20 (716.0 μg/g) > IOR-40 (690.5 μg/g) and those of betulin content were OR-30 (1273.7 μg/g) > IOR-40 (1247.81 μg/g) > IOR-20 (1209.82 μg/g). The highest content of total polyphenols (353.6 μg/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 μg/100 g), threonine (5.43 to 11.00 μg/100 g), serine (8.84 to 14.53 μg/100 g), glutamic acid (31.01 to 53.61 μg/100 g), glycine (4.77 to 10.26 μg/100 g), valine (2.93 to 6.57 μg/100 g), and lysine (5.20 to 14.41 μg/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±8.1 mg/kg), Mg(427.3±9.3 mg/kg), Ca(480.2±6.9 mg/kg), Na(296.1±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
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. xylglucans 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*, *Schizopyllum 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/v) 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: μBondapak C18 10 μm 125A, Mobile phase: 20.8% DMSO, Flow rate: 1.0 ml/min, UV detector: 260 nm, injection volume: 10 μl 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- μBondapak C18 10 μm 125A; mobile phase, 86% acetonitrile; Flow rate 1.0 ml/min; UV detector, 200 nm; injection volume, 10 μl 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-Ciocalteau method (Singleton et al., 1999). A 50 μl sample were added to 250 μl of undiluted Folin-Ciocalteau-reagent. After 1 min, 750 μl 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.
**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 acetic acid buffer pH 5.5 was prepared. An aliquot of 0.1 ml (at concentrations 0.5 to 1 ng/ml) of an antioxidant extract solution was added to 2 ml acetic acid buffer, 1.9 ml methanol and 1 ml DPPH' solution.

Blanks contained 2 ml acetic acid buffer, 1.9 ml methanol and 0.1 ml cherry wine, while the control contained 2 ml acetic acid 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:

\[
\text{Scavenging effect (\%) = } \left[\frac{(A_b - (A - A_b))}{A_b}\right] \times 100
\]

Where \(A_b\) 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,), b* (yellowness, + 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, Pharacia Biotech Co., Swiss).

**Determination of mineral content**

About 0.5 ml sample was put into a cup and 15 ml pure HNO₃ 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 ± 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 μg/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 μg/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 whiles 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*. Each data represents the mean±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 in 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 μg/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 μg/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>
<thead>
<tr>
<th>Sample</th>
<th>DPPH (% dry matter edible part)</th>
<th>Total polyphenol content (μg/g dry matter edible part)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polished rice</td>
<td>70.75±1.27²</td>
<td>114.7±10.2²</td>
</tr>
<tr>
<td>Brown rice</td>
<td>77.82±1.31c</td>
<td>310.5±12.3b</td>
</tr>
<tr>
<td>IOR-20</td>
<td>91.84±2.21a</td>
<td>353.6±23.1a</td>
</tr>
<tr>
<td>IOR-30</td>
<td>86.82±1.07b</td>
<td>102.9±19.1d</td>
</tr>
<tr>
<td>IOR-40</td>
<td>90.73±1.21a</td>
<td>319.4±16.2b</td>
</tr>
</tbody>
</table>

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).

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

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
20% *Inonotus 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 *Inonotus 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 *Inonotus 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 phenyl 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 methananol extracts of the mushroom produced significant scavenging for ABTS radical cations and DPPH radical, the mechanism for antioxidant activity of *Inonotus obliquus* remains unknown. However, Saltarelli et al. (2009) indicated that such antioxidant activity of *Inonotus 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 *Inonotus 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 *Inonotus 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) *Inonotus 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 *Inonotus obliquus* on production and antioxidant activity of extracellular (EPC) and intracellular phenolic compounds (IPC) by *Inonotus 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 *Inonotus 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 *Inonotus 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 *Inonotus 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 *Inonotus obliquus* mycelial

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Color value</th>
<th>L (Lightness)</th>
<th>a (Redness)</th>
<th>b (Yellowness)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polished rice</td>
<td>91.43±1.09</td>
<td>-0.82±0.09</td>
<td>6.61±0.89</td>
<td></td>
</tr>
<tr>
<td>IOR-20</td>
<td>79.17±2.21</td>
<td>1.74±0.31</td>
<td>16.19±0.72</td>
<td></td>
</tr>
<tr>
<td>IOR-30</td>
<td>62.80±1.17</td>
<td>3.21±0.21</td>
<td>13.57±1.09</td>
<td></td>
</tr>
<tr>
<td>IOR-40</td>
<td>56.96±1.07</td>
<td>2.83±0.16</td>
<td>8.51±1.72</td>
<td></td>
</tr>
</tbody>
</table>

1 IOR-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*. 2 L, lightness (100, white; 0, black), a, redness (-, green; +, red); b: yellowness (-, blue; +, yellow). 3 Quoted values are means of triplicate experiments. Different superscripts within the same column indicate significant differences (P < 0.05).
inoculated rice in which the 20% incubation with the fungi

(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 *Inonotus 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

### Table 3. Free amino acid composition(μg/100 g-dry weight) of
*I. obliquus* rice

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

1 IOR-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*: ND, not detected.

2 Quoted values are means of triplicate experiments.
with *Inonotus 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) showed that the highly nutritious product resulting from applying treatments such as pre-germination and inoculating rice with *Inonotus 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 *Inonotus obliquus* mycelia incubated rice showed significant differences across all treatments (Table 4). The 30% *Inonotus 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) concentration of the *Inonotus 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 *Inonotus obliquus* may not contribute significantly to mineral accumulation in rice. For best performance, however, treatment with 30% concentration of the *Inonotus obliquus* mycelial extract would produce the most significant effect in minerals content of rice. Among the three levels of *Inonotus 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 *Inonotus obliquus* mycelial extract enhances the quality of rice for human consumption.

### ACKNOWLEDGEMENTS

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### REFERENCES


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

<table>
<thead>
<tr>
<th>Sample ¹</th>
<th>K (mg/kg-dry weight)</th>
<th>Mg (mg/kg-dry weight)</th>
<th>Ca (mg/kg-dry weight)</th>
<th>Na (mg/kg-dry weight)</th>
<th>Fe (mg/kg-dry weight)</th>
<th>Zn (mg/kg-dry weight)</th>
<th>Mn (mg/kg-dry weight)</th>
</tr>
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<tbody>
<tr>
<td>IOR-20</td>
<td>746.1±7.1ᵃ</td>
<td>375.2±8.2ᵇ</td>
<td>450.3±5.7ᶜ</td>
<td>252.2±6.1ᵇ</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IOR-30</td>
<td>899.1±8.1ᵃ</td>
<td>427.3±9.3ᵇ</td>
<td>480.2±6.9ᵃ</td>
<td>296.1±9.2ᵃ</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IOR-40</td>
<td>812.1±10.1ᵇ</td>
<td>396.2±7.5ᵇ</td>
<td>467.4±7.8ᵇ</td>
<td>226.5±7.3ᶜ</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

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.


