



## Note

# Heat treatment of wheat straw by immersion in hot water decreases mushroom yield in *Pleurotus ostreatus*

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## ABSTRACT

**Background:** The oyster mushroom, *Pleurotus ostreatus*, is cultivated worldwide. It is one of the most appreciated mushrooms due to its high nutritional value. Immersion of the substrate in hot water is one of the most popular and worldwide treatment used for mushroom farmers. It is cheap and easy to implement.

**Aims:** To compare the yields obtained during mushroom production of *P. ostreatus* using different pre-treatments (immersion in hot water, sterilization by steam and the use of fungicide) to determine if they influence mushroom crop.

**Methods:** Four different treatments of substrate (wheat straw) were carried out: (i) immersion in hot water (IHW); (ii) steam sterilization; (iii) chemical; and (iv) untreated. The residual water from the IHW treatment was used to evaluate the mycelium growth and the production of *P. ostreatus*.

**Results:** Carbendazim treatment produced highest yields (BE: 106.93%) while IHW produced the lowest BE with 75.83%. Sugars, N, P, K and Ca were found in residual water of IHW treatment. The residual water increased the mycelium growth but did not increase yields.

**Conclusions:** We have proved that IHW treatment of substrate reduced yields at least 20% when compared with other straw treatments such as steam, chemical or untreated wheat straw. Nutrients like sugars, proteins and minerals were found in the residual water extract which is the resultant water where the immersion treatment is carried out. The loss of these nutrients would be the cause of yield decrease. Alternative methods to the use of IHW as treatment of the substrate should be considered to reduce economical loss.

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## La inmersión en agua caliente de la paja del trigo como sustrato para la producción de *Pleurotus ostreatus* disminuye la producción de esta seta

## RESUMEN

### Palabras clave:

*Pleurotus ostreatus*

Inmersión en agua caliente

Pasteurización con vapor

Carbendazima

**Fundamento:** La seta de ostra, *Pleurotus ostreatus*, se cultiva en todo el mundo y es una de las especies más apreciadas debido a su alto valor nutricional. El método de inmersión del sustrato en agua caliente es uno de los más empleados en todo el mundo por los cultivadores. Su implementación es conveniente y sus costes son bajos. Es importante someter el sustrato a un tratamiento térmico que destruya semillas, insectos parásitos y hongos que podrían aparecer en el cultivo.

**Objetivos:** Comparar la producción obtenida de *P. ostreatus* durante el cultivo usando diferentes métodos de tratamiento del sustrato: inmersión en agua caliente, esterilización con vapor y uso de un fungicida para determinar si influyen en la cosecha de la seta.

**Métodos:** Se llevaron a cabo 4 tratamientos diferentes del sustrato (paja de trigo): a) inmersión en agua caliente; b) esterilización con vapor; c) químico, y d) sin tratamiento. El agua residual del tratamiento de inmersión en agua caliente se usó para evaluar el crecimiento del micelio y la producción de *P. ostreatus*.

**Resultados:** El tratamiento con carbendazima dio lugar a la mayor producción (eficiencia biológica [EB]: 106,93%), mientras que, mediante inmersión en agua caliente, se obtuvo la más baja (EB: 75,83%). En el agua residual del método de inmersión en agua caliente se detectaron azúcares, N, P, K y Ca. El agua residual aumentó el crecimiento del micelio, pero no incrementó el rendimiento.

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**Conclusiones:** Hemos demostrado que la inmersión en agua caliente del sustrato reduce la producción en hasta un 20% cuando se compara con otros tratamientos como el vapor, químicos, o la paja de trigo sin tratar. En el extracto de agua residual se detectaron nutrientes, como azúcares, proteínas y minerales. La pérdida de esos nutrientes sería la causante de la disminución de la producción. Para reducir las pérdidas económicas es preciso considerar el uso de otros métodos alternativos al de la inmersión en agua caliente del sustrato.

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The oyster mushroom, *Pleurotus ostreatus* is cultivated worldwide.<sup>6</sup> It is one of the most appreciated mushrooms due to its high nutritional value and its flavor. This species can be cultivated on a wide range of cellulosic materials.<sup>5,22</sup>

Before spawning, vegetative forms of competing microorganisms present in substrate have to be killed or diminished. Zadrazil and Kurtzman<sup>24</sup> pointed out five different substrate treatments for *Pleurotus* cultivation: (i) sterile technique, (ii) pasteurization technique, (iii) hot water treatment, (iv) fermented substrate and (v) chemical treatment.

*P. ostreatus* has been commercially cultivated in Argentina for about 35 years. The majority of farmers (95%) use wheat straw as substrate, which is esterilized by steam or hot water.<sup>2</sup> The highest biological efficiency obtained varies between 80 and 120%; annual production is, at present, estimated in 100 ton per year, and most of it (95%) is sold fresh.<sup>15</sup>

Hot water treatment is also widely used in many other countries in Latin America,<sup>16,17</sup> India,<sup>4</sup> Asia, China, Taiwan, Indonesia and Africa.<sup>25</sup> This simple method has been described by Kurtzman.<sup>13,14</sup> Wheat, ragi, rice or other cereal straw is treated with hot water (60–70 °C) from 10 min to 1 h. Water is heated in tanks by gas, electricity or fire using wood or charcoal; the excess water is drained off and discarded. After cooling, substrate is spawned and bagged.<sup>1,21</sup> This method has small differences which are temperature of water and time of immersion. For example, Guzmán et al.<sup>11</sup> proposed 85 °C during 40 min while Diana et al.<sup>8</sup> proposed 100 °C during 1 h.

The aim of this work is to compare the yields obtained during mushroom production of *P. ostreatus* using 3 different pre-treatments: immersion in hot water, sterilization by steam and the use of fungicide in order to determine if they influence mushroom crop.

## Material and methods

### Strain used

*P. ostreatus*, ARGENTINA, Chascomús, Leg. E. Albertó, commercial strain, ICFC 153/99 deposited in the IIB-INTECH collection of fungal cultures (WFCC 826).

### Spawn production

750 ml glass jars filled with boiled wheat grains and 1% (w/w) CaCO<sub>3</sub> were sterilized for 1.5 h at 121 °C, cooled and inoculated with an agar plug (1 cm diameter) cut from the advancing margin of a 5-day-old colony of ICFS 153/99 grown on PDA medium. Bottles were incubated in the dark, at 25 °C, with periodical shaking.

### Substrate treatments

Chopped wheat straw (3–5 cm long) was used as substrate. Four different treatments were carried out: (i) *immersion in hot water*, straw was immersed in hot water (80 °C) for 90 min, then it was drained for 12 h to a final moisture of 70%; (ii) *steam sterilization*, straw was bagged, tap water was added up to 70% of final humidity

and then sterilized during 2 h at 120 °C and 1.2 psi of pressure; (iii) *chemical*: straw was bagged, a solution of carbendazim (0.5% of commercial formula, CARBENDAZIM 50 KAYKUN<sup>TM</sup>) was added up to a final humidity of 70%; (iv) *untreated*: straw was hydrated up to 70% of final humidity. After cooling, bags were inoculated with 5% of spawn and then incubated in a room in the dark at a constant temperature of 25 °C for 21 days. Each treatment had 10 replicates.

### Hydration of wheat straw using residual water extract

Chopped wheat straw was hydrated with residual water (REW), which is the water obtained after the immersion in hot water treatment, up to 70% of final humidity and then sterilized during 2 h at 120 °C and 1.2 psi of pressure. Straw was bagged and spawned as was explained above to evaluate mushroom crop.

### Fruiting conditions

After spawning-run, six small cuts (20 mm long) were regularly made on the bags surface. Bags were transferred to a culture room (2.5 m × 4.5 m) for basidiomata induction. Room conditions were controlled: temperature was kept at 18–20 °C with 9 h light/15 h dark photoperiod (20 W fluorescent light), humidity levels were kept between 75% and 85%; watering by spray (fog type) was automatically provided (5 min every 4 h).

### Cropping period and crop yield assessment

Two to three flushes were collected during the cropping period (68 days), defined as the time elapsed between the induction day and the last harvest day. Mature basidiomata were collected and the following production traits were registered: (A) *first harvest day*: time (in days) from the start of fruiting conditions until harvest of the first fruiting bodies; (B) *yield*: harvested basidiomata; (C) *biological efficiency* (BE): the ratio of kg of fresh mushrooms harvested per kg of dry substrate and expressed as a percentage; (D) *percentage of yield distribution* (first, second and third flush yield); and (E) *production rate* (PR): BE/days including incubation time.

### Residual water extract analysis

Water obtained after the immersion of wheat straw in *hot water treatment* was analyzed to determine the composition. (A) Total sugar was determined by anthrone method,<sup>20</sup> (B) protein content was determined by Lowry,<sup>7</sup> (C) nitrogen content was determined using the Kjeldahl method.<sup>3</sup> Phosphorus was determined by ascorbic acid<sup>3</sup>; the concentration of K<sup>+</sup>, Na<sup>+</sup> and Ca<sup>2+</sup> in the extracts were estimated by Atomic Absorption Spectrometry through the use of a Perkin Elmer AAnalyst 100 spectrometer (emission mode for K<sup>+</sup> and Na<sup>+</sup>, absorption mode for Ca<sup>2+</sup>).<sup>18</sup>

### Mycelium growth

Cultures were inoculated with 5 mm diameter cylinder of strain IFC 153/99 in 90 mm Petri dishes containing 3 different mediums:

**Table 1**

First harvest days, number of flushes, yield, biological efficiency, yield distribution, production rate and basidiome number of *P. ostreatus* using 4 different substrate pretreatments.

Pretreatment	First harvest day	No. of flushes	Yield (g)	EB%	Yield distribution (%)			PR	Basidiome number
					Flush 1	Flush 2	Flush 3		
Sterilization	8 <sup>a</sup>	3	282.63 <sup>a</sup>	94.91 <sup>a</sup>	80.82 <sup>ac</sup>	10.92 <sup>ac</sup>	8.26 <sup>a</sup>	1.39 <sup>a</sup>	49.63 <sup>a</sup>
IHWater	8 <sup>a</sup>	2	228.00 <sup>b</sup>	75.83 <sup>b</sup>	85.10 <sup>ab</sup>	14.90 <sup>ab</sup>	0	1.1 <sup>b</sup>	32.7 <sup>b</sup>
Chemical	12 <sup>b</sup>	3	322.06 <sup>a</sup>	106.93 <sup>a</sup>	86.37 <sup>b</sup>	5.40 <sup>c</sup>	8.23 <sup>a</sup>	1.6 <sup>a</sup>	50.1 <sup>a</sup>
Untreated	8 <sup>a</sup>	3	312.24 <sup>a</sup>	104.00 <sup>a</sup>	76.7 <sup>c</sup>	15.5 <sup>b</sup>	7.72 <sup>a</sup>	1.5 <sup>a</sup>	44.4 <sup>a</sup>

IHWater: immersion in hot water; EB: biological efficiency; PR: production ratio. Values (means of ten replicates) not sharing common letters are significantly different at  $P=0.05$  compared by Tukey's test.

(i) water agar (WA) (20 g/L), (ii) water agar with D-glucose (GA) (1 g glucose/L; 20 g agar/L) and (iii) agar residual water extract (REA) (20 g agar/L) and incubated in the dark at 25 °C. Growth of mycelium was measured as radio of the colony in triplicate during 7 days with a ruler (0.5 mm scale). Each treatment had 5 replicates.

#### Experimental design and statistical treatments

The data presented are the average of the results of at least two replicates with a standard error of less than 5%. Analysis of variance (ANOVA) and repeated measures analysis were tested by the Software SigmaPlot 11.0. The significant differences between treatments were compared by Tukey's test at 5% level of probability.

#### Results and discussion

Fruiting bodies were obtained in all treatments assayed including the untreated treatment. We considered the latter treatment as a "positive control". We expected that the straw would have been rapidly colonized with other contaminant fungi, such as green molds as *Trichoderma* or *Aspergillus* but this did not occur. Bags were completely colonized by the white mycelium of *Pleurotus*; only two bags presented some small spots of black and green color, which were fungal contaminants. Anyway, this situation did not reduce mean yields (Table 1). The low contamination observed could be due to good quality of the wheat straw. It had low water content at the moment of the wheat cropping period; bales were made and were immediately kept in a barn in dry conditions. This situation defines a substrate of excellent quality. When contaminants are scarce in the substrate they do not offer a competence for the mycelium of *Pleurotus* which quickly colonized it. The proportion of inoculum of *Pleurotus* against contaminants is much higher.

Mushrooms were collected in all treatments at day 8 after the start of fruiting conditions with the exception of chemical treatment in which the first collect was obtained at day 12. The use of carbendazim extended the time of harvest in 4 days compared to the other treatments. Anyway, yields were not affected resulting in the highest yield obtained in this experience (BE: 106.93%) although without significant differences among untreated (104%) and sterilized substrates (94.91%). BE obtained were similar to those reported by Omarini et al.,<sup>19</sup> Lechner and Albertó,<sup>15</sup> for the cultivation of *P. ostreatus* using wheat straw without supplements, and remarkably higher than those obtained by Shah et al.<sup>22</sup>

The lower yield obtained with the immersion in hot water treatment was also accompanied by a decrease in the number of

basidiomes (Table 1). The PR also significantly decreased with this treatment.

The lowest BE was obtained with immersion in hot water treatment (75.83%). The latter treatment was the only one that produced 2 flushes (Table 1). Probably, the third flush was delayed because of the loss of nutrients in IHW treatment. When the distribution of the crop along flushes was observed we found that for the strain and the substrate used, the first flush was the most important with 76.7–80.82%. The second flush varied from 5.4 to 14.9%.

The treatment of immersion in hot water produced a reduction of at least 20% in yields. We supposed that some nutrients, which were important for *P. ostreatus* production were lost during immersion in hot water treatment. It produced a dark brown water extract, which was enriched with some soluble compounds presented in the straw. When the residual water extract was analyzed we found the presence of sugars (which are expressed as it equivalent to g of glucose), proteins and minerals (Table 2). The content of N and P was 2.99 mg/L and 16.98 mg/L, respectively. Values of K found in the extract were 36 times higher than those we found in TAP water. Nitrogen is very important in the growth of all organisms. In mushrooms, it is necessary for nucleic acids, protein, and for the chitin of cell walls. The values here found are low if we compare them with the concentration normally employed in culture media. It is also very low if we compare it with values found in wheat straw which has 0.62%.<sup>23</sup> Anyway, Ginterová and Maxianova<sup>9</sup> stated that *P. ostreatus* has the ability to fix atmospheric N. If we consider this fact, probably deficient N content may not be so important. Glucose is important for mycelium growth; biomass production of *P. ostreatus* increases when glucose concentration is increased in the culture media.<sup>10</sup>

Houdeau et al.<sup>12</sup> considered that the immersion of substrate in water can have different consequences according to the type of raw material. They pointed out that there is a "nutrients washing" effect that can be negative when old raw material is used, but useful in new raw material because there is a decrease of soluble sugars that can prevent the development of antagonistic microorganisms. The raw material herein used can be considered "new" type. The method probably helped to decrease contamination as Hodeau et al.<sup>12</sup> pointed out but as a negative effect, has significantly decreased yield.

To evaluate if the water residual extract was important for mycelium growth we made a medium of culture using it and we compared it with 2 other culture media: water agar and glucose agar (0.1%). Results showed that the extract improved the mycelia growth showing that the compounds which were extracted from wheat straw were important and useful for fungal metabolism (Fig. 1). This medium with glucose produced a low

**Table 2**

Glucose, protein, nitrogen, phosphor, potassium, sodium and calcium content presented in residual water immersion (RW) and water before treatment (WBT).

	Glucose (g/L)	Protein (mg/mL)	N (mg/L)	P (mg/L)	K (ppm)	Na (ppm)	Ca (ppm)
RW	0.99	3.75	2.99	16.98	912.97	546.4	22
WBT	0	0	0	0	24.8	488.3	15.5

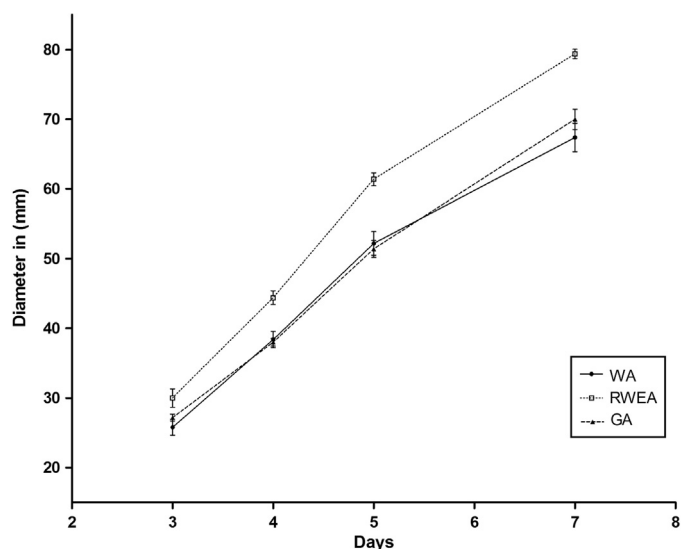


Fig. 1. Mycelium growth of *P. ostreatus* in 3 different agar media. WA: water agar; RWEA: residual water extract agar; GA: glucose agar.

mycelium growth, comparable to water agar, showing that sugar was not the only compound responsible for the improvement in the mycelium growth and that the rest of compounds, many of them unknown for us, also helped mycelium development. If we compared the mycelium growth in GA with WA we found that there were no significant differences in the diameter of the colony (Fig. 1). Guillén-Navarro et al.<sup>10</sup> compared the production of biomass of *P. ostreatus* at different concentration of glucose. They found that biomass production was increased with the increase of glucose concentration. Although the concentration of 1 g/L was one of the lower concentrations assayed they found that the fungal biomass increased at low glucose concentration with the higher efficiency (measured as g of biomass by g of glucose). When we compared the aspect of the colony of mycelium obtained (data not shown) we observed that it was very different for each medium. The aspect of the colony of mycelium obtained in REA was dense and cottony showing a good mycelium biomass development. The mycelium obtained in WA was lax and weak. The aspect of the mycelium of GA was intermediate between REA and WA.

To evaluate if the water residual extract was important for mushroom production, a third experiment was carried out. We used the immersion residual water to hydrate the wheat straw and we compared it with straw hydrated with tap water (TW). Results showed that the latter treatment produced significantly higher yields (BE: 94.54%) that REW treatment (70.02%) after 68 days of crop (Fig. 2). We expected to obtain higher yields with REW treatment but it did not happen. Probably, it could be due to the low concentration of nutrients present in the residual water used to hydrate the wheat. In fact, the volume of water needed for the immersion treatment was major (twice) that the volume needed to obtain 70% of final humidity on the substrate. As a consequence, we used only part of nutrients which were extracted during the immersion process in relation to the mass of wheat straw used. There could probably have been an effect of transformation or loss of available nutrients in the water or effect of toxicity due to heating (first time at 80 °C during 90 min, and second time autoclaved 2 h at 120 °C.), causing polymerization/ionization of nutrients extracted from the substrate. López et al.<sup>16</sup> determined the quality of water from pasteurizing substrate (coffee wastes), for *P. ostreatus* cultivation. They found out that residual water could become highly polluted. As a consequence, this factor may have affected mushroom yield.

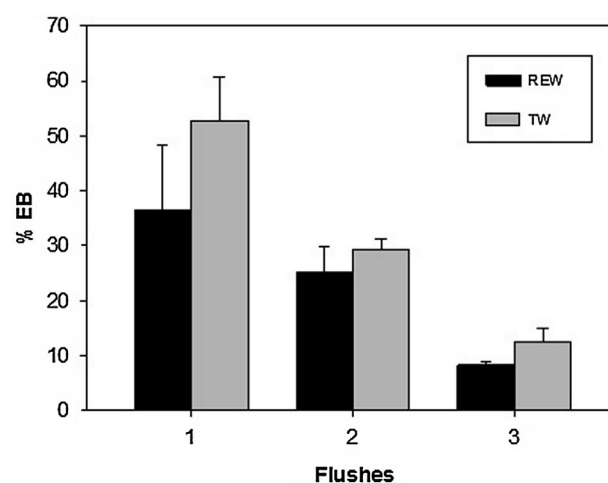


Fig. 2. Production of *P. ostreatus* in wheat straw. REW: using residual water for hydration; TW: using tap water for hydration.

## Conclusions

We have proved that immersion in hot water treatment of substrate reduces yields at least 20% when compared to other straw treatments, as steam, chemical or untreated wheat straw. Compounds which are hydro-soluble are lost during wheat straw immersion in hot water. The loss of these nutrients would be the cause of yield decrease.

Although this method is inexpensive and easy to implement, the reduction of crop is very important causing significant loss, especially when the majority of *Pleurotus* farmers in Latin America, India or Africa use this methodology to treat the substrate. Additionally, other import factor to take into account is that this method uses a high amount of water, which could be a negative factor due to scarcity of this resource in some areas.

In this experience, the chemical treatment produced higher yields. Anyway the use of this treatment has to be discussed since its success depends on the microbiology population of the substrate. It is a selective fungicide which does not control all possible contaminants as some fungi or bacteria. The use of pesticides is unpopular and mushrooms are considered as an “organic product”, environmentally and pesticide free.

Alternative methods to the use of immersion in hot water as treatment of the substrate should be considered to reduce economical loss especially in mushroom farms with large production.

## Conflict of interest

The authors declare that they have no conflict of interest.

## Acknowledgments

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