

Effect of Some Technological Factors of Extraction on Total Lentinan Content in Sapa Shiitake Mushroom Extract

Nguyen Duc Tien¹, Nguyen Dinh Khoa², Nguyen Trong Duy³ & Nguyen Thi Huyen¹

¹ Vietnam Institute of Agricultural Engineering and Postharvest Technology, Hanoi, Vietnam

² Faculty of Food Science and Technology, Vietnam National University of Agriculture, Hanoi, Vietnam

³ Department of Chemistry, VNU University of Science, Hanoi, Vietnam

Correspondence: Nguyen Duc Tien, Vietnam Institute of Agricultural Engineering and Postharvest Technology, No. 60 Trung Kinh Road, Cau Giay District, Hanoi, Vietnam. Tel: 091-702-2777. E-mail: nguyenductienviaep@gmail.com

Received: April 9, 2024

Accepted: May 24, 2024

Online Published: June 15, 2024

doi:10.5539/jas.v16n7p131

URL: <https://doi.org/10.5539/jas.v16n7p131>

Abstract

In Vietnam, the source of shiitake mushroom is very abundant and grown in many provinces/cities, the total yield reaches hundreds of thousands of tons/per year, mainly serving the demand of domestic food processing, and did not develop into medicinal mushrooms yet. Among them, Sapa shiitake mushroom is being widely cultivated in Sapa-Vietnam, and is a raw material with great potential for lentinan exploitation. Until now, researches on extracting and obtaining lentinan in Vietnam are still limited. Those are reasons to carry out this research. The effects of solvent in combination with assistance of ultrasound wave in lentinan extracting capability in Sapa shiitake mushroom were studied. Before carrying out extracting of lentinan; The dried fruit bodies of Sapa shiitake mushroom was crushed in to 1mm. Five extracting parameters include concentration of NaOH solvent (%), proportion of NaOH solvent and raw material (v:w), extracting temperature, ultrasound time and ultrasound intensity were carried out. An extraction without the use of ultrasound for 180 min was control sample. Total lentinan content was obtained during the extracting process. The results indicated that shiitake mushrooms were extracted by using 0.35% NaOH solvent, the rate of NaOH solvent and mushrooms was 12:1 (v:w), intensity of ultrasound was 58 W/cm², frequency was 20 kHz, extracting temperature was 65 °C, time of extraction was 6 min gave total lentinan content 1.63 times higher than the control.

Keywords: Shiitake mushroom, *Lentinula edodes*, lentinan, ultrasound, extraction

1. Introduction

The shiitake fungus, *Lentinula edodes*, is nutritional and has a specific aroma which makes shiitake the second most popular edible medicinal herb fungus in the world (Miles & Chang, 2004). Lentinan is a β -glucan from shiitake mushroom, a biologically active polysaccharide. Lentinan extracts in Shiitake fungus having many biologically active ingredients, such as antitumor, antioxidant, immunomodulatory, antiviral, and anti-inflammatory effects (Yin et al., 2018; Ahn et al., 2017; Ziaja-Sołtys et al., 2020; Wang et al., 2019; Ren et al., 2018). Therefore, many studies have focused on lentinan, including its extraction, purification (Morales et al., 2020; Wang et al., 2018; Li et al., 2016). Therefore, different extraction methods can result in different yields of polysaccharides (Chen et al., 2018). Meanwhile, in Vietnam, there are abundant sources of shiitake mushrooms and grown a lot in provinces/cities but mainly used for food and not yet developed for medicinal mushrooms.. There were a lot of methods to help the polysaccharides extracting in plant materials such as enzymes, microwaves, etc. On the base of the use of ultrasonic energy in the extracting process increasing the rate of mass transfer of the extract, helping extract diffuses easily from the inside of the material to the outside (Liu et al., 2010).

The purpose of this research was to evaluate the effects of some technological factors of extraction such as: (concentration of NaOH solvent, proportion of solvent/material, extracting temperature, ultrasonic intensity, ultrasonic time) on the total content of lentinan in Sapa shiitake mushrooms extract during the extraction with the assisting of ultrasound.

2. Materials and Methods

2.1 Materials

Fresh shiitake mushrooms (fruiting body) were harvested in a farm around February 2022 in Sapa district, Lao Cai province, Vietnam. After 3-5 days from the appearance of fruiting body sprouts, mushrooms can be harvested, fruit diameter was 3-4 cm. Samples were collected according to the method of Codex Methods of Sampling (Codex Methods of Sampling, 2004), Fresh shiitake mushrooms were firstly removed foreign bodies, cleaned, dried by vacuum drying at 45 °C/-0.8 atm, then ground into a powder of 1 mm particle in size with 12% moisture, mix well to homogenize the sample, shiitake powder namely L09 and then were stored at a temperature of 5 °C, protected from light and moisture to be used for experiments.

All chemicals were employed in this research meeting the standard qualities of chemie analyzing at laboratorial level.

Ultrasonic device TJS-3000 intelligent Ultrasonic Generator V6.0 (Hangzhou Success Ultrasonic Equipment Co., Ltd), capacity 20 l, frequency 20 kHz.

2.2 Methods

2.2.1 Effects of NaOH Solvent Concentration

03 kg of L09 were extracted by NaOH solvent at concentrations of 0.0 (deionized water), 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50% NaOH, proportion between NaOH solvent and L09 was 12:1 (v:w), intensity of ultrasonic wave was 40 W/cm² at frequency of 20 kHz, temperature of extraction was 65±2 °C and time of extraction was 8 min. Collected extract was filtered and then determined the lentinan (β-D-Glucan) content.

2.2.2 Effects of the Ratio Between NaOH Solvent and L09

03 kg of L09 were extracted by NaOH solvent at concentrations of 0.35% NaOH to ratio among NaOH solvent and L09 was 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1 and 14:1 (v:w), intensity of of ultrasonic wave was 40 W/cm² at frequency of 20 kHz, temperature of extraction was 65±2 °C and time of extraction was 8 min. Collected extract was filtered and then determined the lentinan content.

2.2.3 Effects of Extracting Temperature

03 kg of L09 were extracted with temperatures of 35, 45, 55, 65 and 75±2 °C by 0.35% NaOH solvent, the rate among solvent and L09 was 12:1 (v:w), intensity of ultrasonic wave was 58 W/cm² at frequency of 20 kHz, and time of extraction was 8 min. Collected extract was filtered and then determined the lentinan content.

2.2.4 Effects of Time and Intensity of Ultrasound

03 kg of L09 were extracted by intensity of ultrasound 0, 30, 40 and 58 W/cm² for 0, 3, 4, 5, 6, 7 and 8 min, at frequency of 20 kHz, the rate among 0.35% NaOH solvent and L09 was 12:1 (v:w) and time of extraction was 180 min in non-ultrasonic extraction method. Collected extract was filtered and then determined the lentinan content.

2.2.5 Analysis of Lentinan

(1) Extraction Method of Lentinan

The extract was obtained after centrifuge (at 4000 × g for 10 min), the supernatant was deproteinated with 3% trichloroacetic acid (v/v = 1:1) overnight at 4 °C. The supernatant was obtained by centrifugation (at 7000× g for 10 min) and then precipitated with the same volume of ethanol overnight at 4 °C. After centrifugation (at 7000× g for 10 min), the precipitate was vacuum freeze-dried for 12 h to obtain the lentinan samples. The extraction yield (%) was calculated using the following equation (Equation 1).

$$\text{Yield (\%)} = \text{Weight of dried crudes polysaccharides (g)} / \text{Weight of dry materials (g)} \times 100\% \quad (1)$$

(2) Measurement of Lentinan (β-D-Glucan) Content

The lentinan (β-D-Glucan) was measured according to the method of Dubios et al. (1956) by the use of phenol-sulfuric acid. A standard curve of glucose content was prepared by using glucose solution as a standard to obtain a linear regression equation. The sample solution was measured according to the linear regression equation.

2.2.6 Analysis of Data

The data were analyzed by variance (ANOVA) to define different among mean values, significance with confidence $p < 0.05$, using Statgraphics Plus software, version 5.1.

3. Results and Discussion

3.1 The Effect of the Concentration of Extracted NaOH Solvent on Total Lentinan Content of Extract

The impact of the concentration of NaOH solvent on the ability to extract lentinan in the Sapa shiitake mushrooms (L09) by ultrasonic wave assistance is shown in Figure 1. In general, different NaOH concentration gave different lentinan ability extraction.

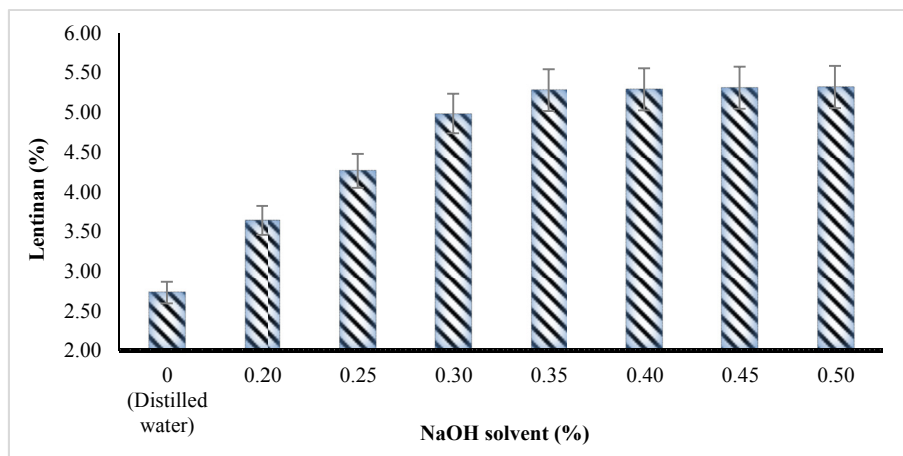


Figure 1. The effect of the concentration of extracted NaOH solvent on total Lentinan content of extract

Effect of solvent in combination with NaOH at concentration of 0.00 (distilled water), 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50% NaOH obtaining the lentinan content in the extract respectively were 2.74; 3.63; 4.26; 4.99; 5.28; 5.29; 5.31 and 5.32% lentinan. These results demonstrated that the concentration of NaOH solvent impacts the lentinan extracting ability from Sapa shiitake mushrooms. Hot water extractions have been shown to be inefficient, producing low yields of lentinan (2.73%). This could be clarified that at each given concentration of solvent not only has the capability to dissolve lentinan, but also could dissolve impurities, decreasing the capability to dissolve lentinan into the solvent. The highest yield of extract was obtained in the NaOH concentration of 0.35% (5.33% lentinan). Thus, the NaOH concentration of 0.35% was selected as the optimal concentration for extracting lentinan in the L09 by ultrasonic wave. Surenjav et al. (2006) used NaOH/NaBH₄ mixture to separate lentinan with a yield of 3.5-10% (dry weight). However, Rincao et al. (2012) found that the hot water extract mainly contained polysaccharides with β -1,6 and α -1,4 glucosidic linkages without the β -1,3 linkage of lentinan.

3.2 The Impact of the Proportion Among NaOH Solvent and L09 on Total Lentinan Content of Extract

The use of a lot of solvents to extract, the higher capability to diffuse lentinan from mushroom to the solvent, however if the use of so much solvent the capability to collect lentinan active ingredients is insignificant, it will not be practical because of costing solvent, duration and energy to obtain solvent. Therefore, defining the rate among NaOH solvent and mushroom for lentinan extracting to highly obtain efficiency is necessary. Figure 2 indicated that the use of solvent to different rates, the extracting ability of lentinan was different, when solvent increases the concentration of lentinan also increases.

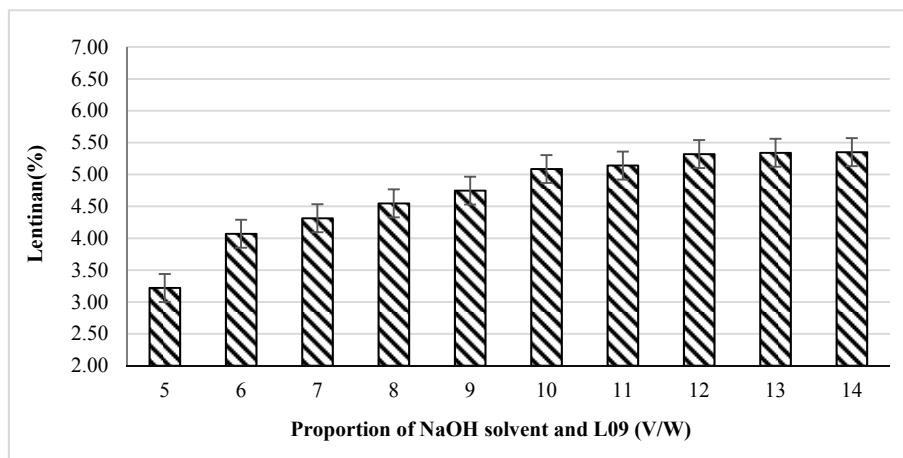


Figure 2. The impact of the proportion among NaOH solvent and L09 on total lentinan content of extract

Lentinan content strongly grew up at the rate of solvent/mushroom was 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, and 11:1 (v/w) (3.22, 4.07, 4.31, 4.55, 4.75, 5.09, and 5.14% lentinan respectively). The result indicated that at proportion of 5:1, the amount of using solvent is small, not enough to osmotic the entire extracting raw material, the diffusion of solute into the solvent, and a certain amount of solvent will be kept in the mushroom is the cause of the decreasing in extracting efficiency. proportion of solvent increase leading to promoting lentinans dissolves into solven at the first stage. The solutes dissolve good in solvent due to a big amount of solvent will help increasing of capacity contacted of cell and solvent, making concentration of enviromental inside and outside of the celldifferently increasing there will solutes be diffused into the solvent, lead to there is an creasing in the content of lentinan in extract. At firstly, the concentration of lentinan in the raw material is high, lentinan fastly diffuses out of the cell. At high rate of solvent of 12:1, 13:1 and 14:1 (v/w), the lentinan extraction capacity did not increase significantly, respectively 5.32, 5.34 and 5.35% lentinan when compared to a ratio of 14:1 (v/w). This result indicated that the rate among solvent and mushroom was 4:1 to be the best proportion to extract lentinan in the L09 not only saving solvent but also energy.

3.3 The Impact of Temperature of Extraction on Total Lentinan Content of Extract

At higher temperature of extraction, raw material is swelled leading to there will be increasing of porosity of the material, the active ingredient to dissolve easy into the solvent due to the decreasing of viscosity. The number of air formed bubbles increased when extract by temperature with the assistance of ultrasonic waves. However, air bubble' intensity breaking will be decreased when increasing of temperature because of effect of increasing of vapour pressure and playing a role as a buffer layer, prevention of the collision of surrounding molecules when the air bubble is broken. The high increase in temperature of extraction can increase solubility of some impurities, and it results in the promotion of chemical changes then making the quality of the extract changed unfavourably. In contrast, when temperature decrease air bubbles will be difficultly ruptured due to the increasing of environmental viscosity. So, it is necessary to determine suitable temperature of extracting to obtain not only pure extracts but also high productivity. The Figure 3 indicated that extracting capability with amount of lentinan high increasing (3.20, 3.84 and 4.24%) with the increasing of extracting temperature (35, 45, and 55 °C respectively).

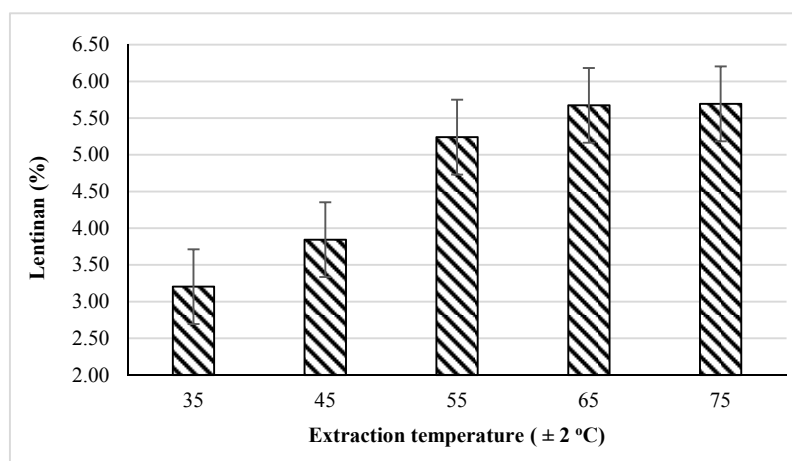


Figure 3. The impact of temperature of extraction on total lentinan content of extract

The temperature of extraction grew from 65 to 75 °C gave lentinan content to increase insignificantly by 5.67 and 5.69%, respectively. The result indicated that the temperature of extraction increases to 75 °C gave negligible increasing of extracting lentinan ability when compare to at temperature of 65 °C. The result above could justify that 65 °C is a suitable temperature for lentinan extracting in Sapa shiitake mushrooms. An increasing of temperature could enhance the permeability of lentinan molecules inside the cell. In the range of temperature from 35 to 65 °C, the total yield of lentinan increasing as the increasing of temperature. On the contrary, Onyebuchi and Kavaz (2020) concluded that chemical and bioactive properties of all extracts indicated significantly dependence on the temperature of extraction and type of solvent. The increasing of contacting surface among solvents and raw material and the permeability of cell walls is due to the affect of mechanic of acoustic cavitation from the ultrasonic wave. Physical and chemical properties of the materials subjected to ultrasound are altered and disrupt the plant cell wall, facilitating the release of compounds and enhancing mass transport of the solvents into the plant cells (Dhanani et al., 2017).

3.4 The Impact of Ultrasonic Time and Intensity of Ultrasonic Wave on Total Lentinan Content of Extract

Impact of ultrasonic duration and ultrasonic intensity on extracting capability of lentinan in the Sapa shiitake mushrooms is indicated in the Figure 4, the control was mushroom did not extract by ultrasonic wave for 0, 3, 4, 5, 6, 7 and 8 min obtained lentinans content were 0.44, 0.46, 0.49, 0.53, 0.58, 0.61 and 0.67% lentinan, respectively.

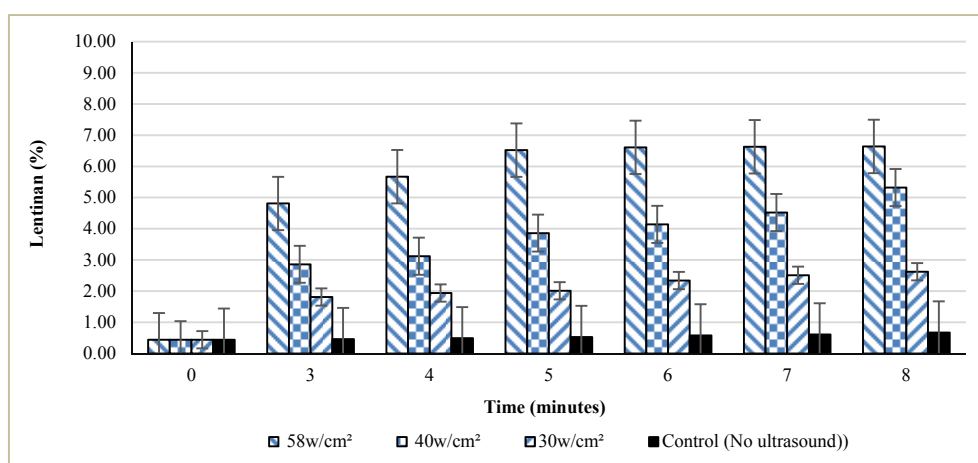


Figure 4. The impact of ultrasonic time and intensity of ultrasonic wave on total lentinan content of extract

The extraction using ultrasonic waves at different intensities from 30 to 58 W/cm² for 3, 4, 5, 6, 7 and 8 min obtained lentinan content ranging from 1.81 to 6.64%. A significantly different in extracted lentinan content between control and samples were extracted by ultrasonic wave at different intensities ($P \leq 0.05$). At a different

extracting times with different powers also obtained additional lentinan content (Figure 4). As seen in the Figure 4, the extracted lentinan content sharply increased after 3, 4, 5 and 6 min of extraction; after that, at 7 and 8 min of extraction, the extracted lentinan content in the extract did not increase significantly. At extracting conditions with ultrasonic intensity of 58 W/cm² gave higher lentinan content when compared to the other extracting conditions and the control. At the ultrasonic intensity of 58 W/cm² with an ultrasonic extraction times of 3, 4 and 5 min, lentinan content increased by 4.81, 5.67, 6.52 and 6.61% lentinan, respectively; after 6 and 7 minutes of ultrasonic extraction, the lentinan content still increased at 6.63 and 6.64%, respectively, at the time level of 5, 6 and 7 min, the lentinan content in the extract did not increase significantly. These results justified that at an ultrasonic intensity of 58 W/cm² with an ultrasonic extraction time of 6 min were the best condition to extract lentinan in Sapa shiitake mushrooms. The time and intensity of ultrasound was investigated as when the ultrasonic intensity and time increased, the ability to extract lentinan highly increased and much higher than that of the control (without using ultrasound, for lentinan content in the extract is the lowest). The higher the ultrasonic intensity, the higher the effervescence, the high breaking force, which increases the mass transfer rate of the extract, and the foam breaking also creates agitation to help diffuse the extract inside the material to escape more easily (Jian-bing et al., 2005). The increase in extraction capacity is due to the higher the ultrasonic intensity, the higher the effervescent effect of high breaking force, which increases the mass transfer rate of the extract. In addition, the foam bubbling also creates an agitation that allows the diffusion of the extracts inside the material to escape more easily (Jian-bing et al., 2005). The longer the ultrasonic time, the greater the intensity, the more the particles of the extracted material are destroyed, the more the viscosity and solubility increase respectively. Viscosity increases with increasing ultrasonic intensity and time, the number of short-chain molecules more increases, the viscosity also increases, but when the intensity reaches a certain value, the viscosity decreases. The higher the ultrasonic intensity, which is agent to contribute increasing the efficiency of the extraction process, the rapid expansion of bubbles during the negative pressure cycle makes the bubbles no chance to shrink during the positive pressure cycle. Conversely, when the ultrasonic intensity is smaller, the number of times the bubbles expand and compress will increase, the extraction time will be longer (Hecceg et al., 2010). The intensity of ultrasonic wave is all the stronger, the extracting capability of the active ingredient is all the higher and time is all the shorter. Therefore, for saving time, the time and intensity of extracting ultrasound is 6 min and 58 W/cm² respectively to be the most effectively to extract lentinan in Sapa shiitake mushrooms

3.5. Lentinan Extraction by Method of Ultrasonic Wave Compared to Without Ultrasonic Wave for Sapa Shiitake Mushrooms (L09)

Lentinan extracting capability by ultrasonic waves (intensity was 58 W/cm², frequency was 20 kHz, temperature was 65±2 °C, the rate of 0.35% NaOH solvent/mushroom was 12:1 (v:w), time was 6 minutes is showed in the Table 1. According to the Table1, for above extracting condition gave the highest extracting capability of lentinan of 6.63% and soluble dry matter of 16.14%.

Table 1. Lentinan extraction by method of ultrasonic wave compared to without ultrasonic wave for Sapa shiitake mushrooms (L09)

Norm	Unit	Lentinan extract		
		Intensity ultrasonic wave was 58 W/cm ² , frequency was 20 kHz	Without ultrasonic	
Material size	mm	1.0	1.0	30.0
The solvent		0.35% NaOH	0.35% NaOH	Distilled water
Proportion of solvent/mushroom	v:w	12	12	12
Temperature of extraction	°C	65±2.0	65±2.0	98±2.0
Time of extraction	min	6	6	180
Lentinan (β-D-Glucan)	%	6.63±0.04	0.58±0.03	4.08±0.12
Dry matter	%	16.14±0.05	1.89±0.04	14.74±0.07

Meanwhile the extracting without ultrasound at temperature of 65±2 °C, the proportion of 0.35% NaOH solvent/mushroom was 12:1 (v:w), time was 6 min gave less lentinan content (0.58%) and soluble dry matter of 1.89%. Extracting without ultrasound at lentinan extraction in Sapa shiitake mushrooms by 0.35% NaOH solvent to the proportion among solvent and raw material was 12:1 in combination with helping temperature of 98±2 °C (traditional technology), a proportion of water/raw material (d = 30 mm) was 12:1 (v:w), time was 180 min (3 h)

obtained possible extraction of lentinan content only was 4.08% and 14.74% of soluble dry matter. Extracting did not use ultrasound obtained lower lentinan content than extracting by ultrasound. At intensity of ultrasound was 58 W/cm², frequency was 20 kHz, time was 12 min, lentinan content obtained 1.63 times higher than nonultrasound for 3h at 98±2 °C and obtained total lentinan content was about 11.43 times higher than nonultrasound for 12 min at 65±2 °C. Temperature of extraction significantly decreased from 98±2 °C in normal extracting process to 65±2 °C during the use of ultrasound. Finding result demonstrated that energy of ultrasound affecting on lentinan extraction in Sapa shiitake mushrooms.

4. Conclusions

Lentinan extraction in Sapa shiitake mushrooms by 0.35% NaOH solvent to the proportion among solvent and raw material was 12:1 in combination with helping of ultrasound at intensity of 58 W/cm², frequency of 20 kHz for 6 min at temperature of 65±2 °C instead of normal extracting method supplies an attractive alternative technology to obtain the total lentinan content 1.63 times higher than the traditional technology. This research indicates the potential of applying ultrasound technology is more effectiveness than traditional technology that did not use wave of ultrasound. Applying ultrasonic wave technology not only shorten time of extraction but also getting more lentinans in Sapa shiitake mushrooms..

References

- Ahn, H., Jeon, E., Kim, J.-C., Kang, S. G., Yoon, S., Ko, H.-J., ... Lee, G.-S. (2017). Lentinan from Shiitake Selectively Attenuates AIM2 and Non-Canonical Inflammasome Activation while Inducing pro-Inflammatory Cytokine Production. *Sci. Rep.*, 7, 1314. <https://doi.org/10.2147/DDDT.S243311>
- Chen, G., Chen, K., Zhang, R., Chen, X., Hu, P., & Kan, J. (2018). Polysaccharides from Bamboo Shoots Processing By-Products: New Insight into Extraction and Characterization. *Food Chem.*, 245, 1113-1123. <https://doi.org/10.1016/j.foodchem.2017.11.059>
- Codex Methods of Sampling. (2004). *General Guidelines on Sampling (CAC/GL 50)*.
- Dhanani, T., Shah, S., Gajbhiye, N. A., & Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry*, 10, S1193-S1199. <https://doi.org/10.1016/j.arabjc.2013.02.015>
- Dubios, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric Method for Determination of Sugar and Related Substances. *Anal. Chem.*, 28, 250-256. <https://doi.org/10.1021/ac60111a017>
- Hereceg, I. L., Jambrak, A. R., Šubarić, D., Brnčić, M., Brnčić, S. R., Badanjak, M., ... Ježek, D. (2010). Novotni and Z. Hereceg, Texture and pasting properties of ultrasonically treated corn starch. *Czech J. of Food Sci.*, 28(2), 83-93. <https://doi.org/10.1016/j.carbpol.2009.07.051>
- Jian-bing, J., Xiang-hong, L., Mei-qiang, C., & Zhi-chao, X. (2005). Improvement of leaching process of Geniposide with ultrasound. *Ultra. Sonoche.*, 13(5), 455-462. <https://doi.org/10.1016/j.ultsonch.2005.08.003>
- Li, Z., Nie, K., Wang, Z., & Luo, D. (2016). Quantitative Structure-Activity Relationship Models for the Antioxidant Activity of Polysaccharides. *PLoS ONE*, 11, e0163536. <https://doi.org/10.1371/journal.pone.0163536>
- Liu, J., Li, J. W., & Tang, J. (2010). Ultrasonically assisted extraction of total carbohydrates from *Stevia rebaudiana* Bertoni and identification of extracts. *Food and Biopro. Process.*, 88, 215-221. <https://doi.org/10.1016/j.fbp.2009.12.005>
- Miles, P. G., & Chang, S.-T. (2004). *Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact*. CRC Press: Boca Raton, FL, USA. <https://doi.org/10.1201/9780203492086>
- Morales, D., Rutckeviski, R., Villalva, M., Abreu, H., Soler-Rivas, C., Santoyo, S., ... Smiderle, F. R. (2020). Isolation and Comparison of α - and β -D-Glucans from Shiitake Mushrooms (*Lentinula edodes*) with Different Biological Activities. *Carbohydr. Polym.*, 229, 115521. <https://doi.org/10.1016/j.carbpol.2019.115521>
- Onyebuchi, C., & Kavaz, D. (2020). Effect of extraction temperature and solvent type on the bioactive potential of *Ocimum gratissimum* L. extracts. *Scientific Reports*, 10(1), 21760. <https://doi.org/10.1038/s41598-020-78847-5>

- Ren, G., Xu, L., Lu, T., & Yin, J. (2018). Structural Characterization and Antiviral Activity of Lentinan from *Lentinus edodes* Mycelia against Infectious Hematopoietic Necrosis Virus. *Int. J. Biol. Macromol.*, *115*, 1202-1210. <https://doi.org/10.1016/j.ijbiomac.2018.04.132>
- Rincao, P. V., Yamamoto, K. A., Ricardo, N. M. P. S., Soares, S. A., Meirelles, L. D. P., Nozawa, C., & Linhares R. E. C. (2012). Polysaccharide and extracts from *Lentinula edodes*: structural features and antiviral activity. *Virology Journal*, *9*, 2-6. <https://doi.org/10.1186/1743-422X-9-37>
- Surenjav, U., Zhang, L., Xu, X., Zhang, X., & Zeng, F. (2006). Effects of molecular structure on antitumor activities of (1/3)- β -D-glucans from different *Lentinus edodes*. *Carbohydr. Polym.*, *63*, 97-104. <https://doi.org/10.1016/j.carbpol.2005.08.011>
- Wang, T., He, H., Liu, X., Liu, C., Liang, Y., & Mei, Y. (2019). Mycelial Polysaccharides of *Lentinus edodes* (Shiitake Mushroom) in Submerged Culture Exert Immuno-enhancing Effect on Macrophage Cells via MAPK Pathway. *Int. J. Biol. Macromol.*, *130*, 745-754. <https://doi.org/10.1016/j.ijbiomac.2019.03.023>
- Wang, Y., Wei, X., Wang, F., Xu, J., Tang, X., & Li, N. (2018). Structural Characterization and Antioxidant Activity of Polysaccharide from Ginger. *Int. J. Biol. Macromol.*, *111*, 862-869. <https://doi.org/10.1016/j.ijbiomac.2018.01.087>
- Yin, C., Fan, X., Fan, Z., Shi, D., & Gao, H. (2018). Optimization of Enzymes-Microwave-Ultrasound Assisted Extraction of *Lentinus edodes* Polysaccharides and Determination of Its Antioxidant Activity. *Int. J. Biol. Macromol.*, *111*, 446-454. <https://doi.org/10.1016/j.ijbiomac.2018.01.007>
- Ziaja-Sołtys, M., Radzki, W., Nowak, J., Topolska, J., Jabłńska-Rys, E., Sławinska, A., ... Bogucka-Kocka, A. (2020). Processed Fruiting Bodies of *Lentinus edodes* as a Source of Biologically Active Polysaccharides. *Appl. Sci.*, *10*, 470. <https://doi.org/10.3390/app10020470>

Acknowledgments

Not applicable

Authors Contributions

Not applicable

Funding

This research was funded by the Vietnam National Science and Technology task, code: DADL.CN-02/18.

Competing Interests

Not applicable

Informed Consent

Obtained.

Ethics Approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and Peer Review

Not commissioned; externally double-blind peer reviewed.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data Sharing Statement

No additional data are available.

Open Access

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).

Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.