



BITTERNESS REDUCTION IN LINGZHI (*GANODERMA LUCIDUM*) DRIED POWDER BY DRYING, EXTRACTION, AND MICROENCAPSULATE MATERIAL SPRAY DRYING

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Abstract

Ganoderma lucidum, commonly referred to as Lingzhi or Reishi, is a basidiomycete rot fungus which has been used for centuries. *Ganoderma lucidum* is a bitter fungus with a glossy exterior and a woody texture. Reishi mushroom (*Ganoderma lucidum*) is a type of mushroom believed to extend life and promote health. The main bioactive components of *G. lucidum* can be broadly grouped into polysaccharides and triterpenes. It is likely to have some benefits for cancer patients. However, oral consumption of *Ganoderma lucidum* products could be a problem due to the bitter taste. Bitter taste should be eliminated. β -cyclodextrin is one of the most common materials used in microencapsulation process. β -cyclodextrin comprises seven units of glucopyranose. An attempt studied the feasibility of bitterness reduction in *Ganoderma lucidum* dried powder. Fresh *Ganoderma lucidum* was fermented with *Lactobacillus plantarum* in 48h. After that, *Ganoderma lucidum* would be dried under different temperature and time (35°C in 18h; 40°C in 16h, 45°C in 14h, 50°C in 12h), extraction by hot water at different temperature and time (100°C in 60 min, 110°C in 50 min, 120°C in 40 min, 130°C in 30min), and spray drying by different microencapsulate materials (β -cyclodextrin, sodium alginate, carrageenan, pectin). Reduction of bitterness and enhancing palatability are obviously observed by fermentation with *Lactobacillus plantarum* in 48h, drying *Ganoderma lucidum* in 40°C in 16h, extraction by hot water at 120°C in 40 min and spray drying at inlet temperature 150°C, outlet temperature 80°C, feed rate 12 ml/min with β -cyclodextrin 0.4% as wall material.

Keywords: *Ganoderma lucidum*, dried powder, bitterness, drying, extraction, spray drying

Introduction

Ganoderma lucidum, also known as Reishi, Ling-zhi, mannentake or mushroom of immortality, is a type of mushroom that has been used in medicine (Ahmet Unlu *et al.*, 2016). *Ganoderma lucidum* extracts contain carbohydrates, glycosides, triterpenoids and phenolic compounds, alkaloids, proteins, coumarin, flavonoid, phenols, lignocellulose degrading enzymes and nucleosides (Pooja, *et al.*, 2014; Kirar *et al.*, 2015; Chien *et al.*, 2008; Rathor *et al.*, 2014). It contains active compounds known as beta glucans which are polysaccharides with immune-boosting effects (Nguyen Minh Thuy and Nguyen Thi My Tuyen, 2015). Various pharmacological activities have been reported such as, hepatoprotective, anti-diabetic, anti-hypertensive, cardioprotective, immune modulatory, antioxidant, anticancer (Mani Rupeshkumar *et al.*, 2016). *G. lucidum* extracts possess compounds with antifungal properties that can be used as antifungal agents in new drugs for the therapy of infectious diseases caused by pathogens (Naveen *et al.*, 2018). *G. Lucidum* products are sold in a variety of forms such as powder, nutritional supplements, tea, syrup, cream, hair tonic, and particularly capsule or tablet after being turned into powder (McMeekin D, 2005). These products can be produced from different parts of the mushroom, such as micella, spore, and stem. The polysaccharides fraction of *G. lucidum* has been manifested to activate immune effector cells and suppress the growth of several cancer cells in vivo (Gao-Qiang L *et al.*, 2011). *Ganoderma lucidum* extracts significantly also decrease the viability of both cancer cells in a time- and concentration-dependent manner, with abilities to reduce cell migration over time, which is correlated with a lower release of matrix metalloproteinases (Antonio *et al.*, 2017). The effect of *G. Lucidum* on cancer is based on glucan and triterpenes that it contains. Beta glucans are thought to activate the immune

system, and triterpenes are thought to have a cytotoxic effect against various cancer cells (Lin, 2005; Liao, 2013; Lin *et al.*, 2003). Triterpenes are also alleged to inhibit tumor invasion by reducing the expression of matrix metalloproteinases and inhibit tumor metastasis by limiting the binding to endothelium (Chen *et al.*, 2008; Li *et al.*, 2008). The structural similarity of the intensely bitter compounds was also elucidated, from which it was concluded that the spatial relationship of the hydrophobic methyl groups to the three functional oxygen atoms plays an important role in generating the bitterness (Tsuyoshi Nishitoba *et al.*, 1988).

Bitter taste is a major problem in the food and pharmaceutical industries due to its negative hedonic impact on ingestion (Drewnoswki 2001; Drewnoswki and Gomez Carneros 2000). Encapsulation is a process in which flavors, nutrients, oils, proteins, or probiotic bacteria are enveloped into a starch, protein, or lipid carrier matrix for preservation, masking, or delivery of the encapsulated agent. Taste masking is defined as a perceived reduction of an undesirable taste that would otherwise exist. The ideal solution to reduce or inhibit bitterness is the discovery of a universal inhibitor of all bitter tasting substances that does not affect the other taste modalities such as sweetness or saltiness (Pandey *et al.*, 2010; Sapkal *et al.*, 2007). One of the major problems of masking of off-taste is the complex mixture of sensations. The ingestible is not only perceived as bitter, but is also astringent and/or sour. Each modality is transduced by different molecular sensing systems in the mouth, and the sensation consciously recognized is again a difficult mixture to separate into individual taste qualities (Jakob, 2008).

Cyclodextrins (CDs) are naturally occurring molecules composed of glucose units linked by β (1-4) linkages, arranged in bucked shape with a central cavity. These oligosaccharides, produced from starch, are composed of six,

seven and eight glucose units, α , β , and γ cyclodextrins (CDs), respectively. Native cyclodextrins are available in large volumes and are food quality. It is the three-dimensional structure of these molecules that makes them so important. These starch derivatives are non-toxic ingredients; they are not absorbed in the upper gastrointestinal tract, however they are completely metabolized by the colon microflora. They meet the requirements for neutrality in terms of odour and taste since, In fact, although cyclodextrin molecules are composed of glucose units, α - and β -cyclodextrin do not taste sweet at all, whilst γ -cyclodextrin has only a slightly sweet taste. In addition, the fact that cyclodextrins occur in the form of a colourless powder makes them easy to process.

Several researches mentioned to application of bitterness reduction. A research determined the effect of limonin core to coating ratio on limonin microencapsulation efficiency and its stability in pH 4 and 7 solution. Encapsulant used was β cyclodextrin with core to coating ratio 1:10 and 1:20. Microencapsulation process was conducted using freeze dryer. Results revealed that limonin microencapsulation with core to coating ratio of 1:10 had efficiency of 68.14 %, while that with ratio of 1:20 had efficiency of 80.52% (Dewi Cakrawati et al., 2018). The use of insoluble β -cyclodextrin polymer for the reduction of limonin in Thai tangerine juice by batch, column and fluidized bed processes were studied and compared. Direct correlation between complexation of limonin and amount of β -cyclodextrin polymer was observed. Using 3g% β -CD polymer at room temperature, limonin reduction by the batch and column processes were 70 and 94% respectively. In a fluidized bed column (50x3cm.i.d.) under the condition of 15g β -cyclodextrin polymer with a feed flow rate of 100mL/min at room temperature, the initial efficiency of debittering was about 90% and gradually decreased (Mongkolkul, 2006). β -Cyclodextrin at 0.4% is able to reduce the bitterness of a 0.05% caffeine solution by about 90%. The α - and the γ -cyclodextrins are much less active and higher concentrations of β -cyclodextrin taste sweet. In the same study, the authors demonstrated that the bitterness of various plant extracts such as artichoke or gentian can be selectively reduced by β -cyclodextrin (Binello et al., 2004). A polymer-supported cyclodextrin using chitin as base was also successfully tested as a bittermasking agent (Binello et al., 2004). β -Cyclodextrin polymer and XAD-4 and XAD-16 resins were used in a pilot-scale fluidized bed process to reduce bitterness from naringin and limonin in grapefruit juice and limonin in California navel orange juice. For β -cyclodextrin polymers cross-linked with epichlorohydrin, naringin reduction in grapefruit juice ranged from 18 to 61% and limonin reduction ranged from 28 to 67%. Limonin reduction in navel orange juice ranged from 29 to 55%. Bitterness reduction in grapefruit juice from XAD-16 resin was 55-58% for naringin and 90-97% for limonin; with navel orange juice limonin was reduced 93%. For grapefruit juice debittered with XAD-4 naringin reduction was 32-38%, and for both grapefruit and navel orange juice limonin reduction was 58% (Wilson, 1989). Use of β -cyclodextrin polymer at 1 g of polymer/50 mL of juice in a continuous flow fluid-bed or a batch process lowered the major bitter components limonin, nomilin, and naringin in grapefruit juice and limonin and nomilin in navel orange juice by about 50% (Shaw et al., 1984). Effect of debittering techniques on the chemical characteristics of stored kinnow juice was examined. Five

different debittering methods viz., lye, florosil, naringinase, lye and florosil, florosil and naringinase were used for the debittering of kinnow juice. It was concluded that naringinase enzyme was best among all treatments given for removal of bitterness and also had not much effect on characteristics of stored juice (Piyush Kashyap, Shailza Anand, 2017). Florosil (activated magnesium silicate) has been shown to reduce limonin without adversely affecting its nutritive quality (Barmore et al., 1986).

Healthy or functional food often contains phytochemical that has bitter taste. Thus, it is important to reduce bitterness due to consumer sensory perception (Coupland and Hayes, 2016). Oral consumption or addition of limonin to food system could be a problem due to the bitter taste. Bitter taste can be masked by the application of encapsulation using polymer, cyclodextrin, lipid, or surfactant (Coupland and Hayes, 2016). Microencapsulation does not only help masking bitterness of bioactive compound but also help protect them from damages caused by oxygen, heat, or light (Dias et al., 2015). The effectiveness of phytochemical compound antioxidant activity depends on bioactivity, stability, and bioavailability of these compounds, which could be overcome with encapsulation technology (Fang and Bhandari, 2010). Microencapsulation process commonly uses spray dryer or freeze dryer. Bioavailability of bioactive compound and organoleptic characteristics in freeze dried microencapsulated product were better since the minimum use of heating compared with spray dried microencapsulated product (Chranioti et al., 2015). *Ganoderma lucidum* contains bitter chemicals like charantin, vicine, glycosides and karavilosides. The aim of this work was to study the feasibility of bitterness reduction in *Ganoderma lucidum* dried powder by treatment of drying, enzyme, and wall material.

Material and Method

Material

Ganoderma lucidum was collected from Soc Trang province, Vietnam. *Ganoderma lucidum* was stored at 8 °C and transferred quickly to laboratory for experiment.



Fig. 1 : *Ganoderma lucidum*

Researching Method

- **Effect of drying temperature and time to bitterness of *Ganoderma lucidum* extract :** Fresh *Ganoderma lucidum* was fermented with *Lactobacillus plantarum* in 48h. After that, *Ganoderma lucidum* would be dried under different temperature and time (35°C in 18h; 40°C in 16h, 45°C in 14h, 50°C in 12h), extraction by hot water at 100°C in 60 min. Sensory evaluation would be conducted to estimate the bitterness reduction.
- **Effect of extraction temperature and time to bitterness of *Ganoderma lucidum* extract :** Fresh *Ganoderma lucidum* was fermented with *Lactobacillus plantarum* in 48h. After that, *Ganoderma lucidum*

would be dried under temperature and time 40°C in 16h. The extraction was conducted by hot water at different temperature and time (100°C in 60 min, 110°C in 50 min, 120°C in 40 min, 130°C in 30min). Sensory evaluation would be conducted to estimate the bitterness reduction.

- **Effect of wall material in spray drying to bitterness of *Ganoderma lucidum* dried powder :** Fresh *Ganoderma lucidum* was fermented with *Lactobacillus plantarum* in 48h. After that, *Ganoderma lucidum* would be dried under temperature and time (40°C in 16h), extraction by hot water at temperature and time (120°C in 40 min), and spray drying by different wall materials (β -cyclodextrin, sodium alginate, carrageenan, pectin) in the same concentration 0.4% at inlet temperature 150°C, outlet temperature 80°C, feed rate 12 ml/min. Sensory evaluation will be conducted to estimate the bitterness reduction.

Sensory evaluation

The sensory attributes were carried out by selected panel of judges (9 members) rated on a nine point hedonic scale.

Statistical analysis

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan’s multiple range test (DMRT). Statistical analysis was performed by the Statgraphics Centurion XVI.

Result and Discussion

Effect of drying temperature and time to bitterness of *Ganoderma lucidum* extract

G. lucidum spore powder revealed high antioxidant activity, in some cases even higher than antioxidant property of ascorbic acid. Antioxidant activity was enhanced during high temperature drying due to increasing antioxidant power of polyphenols at an intermediate state of oxidation, increase in reducing sugar and formation of Maillard reaction products, which have a great antioxidant activity. The product of spore powder (density of 1.38×10⁹ CFU/g) remained light brown color and flavor characteristics of *G. lucidum* and could be soluble in hot water with light bitter taste. (Madrau *et al.*, 2009). Fresh *Ganoderma lucidum* was fermented with *Lactobacillus plantarum* in 48h. After that, *Ganoderma lucidum* would be dried under different temperature and time (35°C in 18h; 40°C in 16h, 45°C in 14h, 50°C in 12h), extraction by hot water at 100°C in 60 min. Sensory evaluation would be conducted to estimate the bitterness reduction. Results revealed in table 1. From table 1, the bitterness in *Ganoderma lucidum* could be effectively eliminated by drying at 40°C in 16h so this value was selected for further experiments.

Table 1 : Effect of drying temperature and time to bitterness of *Ganoderma lucidum* extract

Drying	35°C in 18h	40°C in 16h	45°C in 14h	50°C in 12h
Sensory score	5.21±0.03 ^b	6.87±0.02 ^a	4.12±0.03 ^c	3.04±0.00 ^d

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ($\alpha = 5\%$).

Four different drying methods were investigated for drying of *G. lucidum*, namely convective hot air drying, vacuum drying, freeze drying and heat pump drying. The results show that heat pump dried *Ganoderma* retained most of the active ingredients with the shortest total drying time required as compared to other drying methods. It could retain 94% of crude ganoderic acids and 88.5% of water soluble polysaccharides (Siew Kian Chin *et al.*, 2011). The effect of drying temperature to the antioxidant activity of spore powder was made. Spore powder was dried at temperatures ranging from 95 to 105°C until 2 to 3% moisture content (Nguyen Minh Thuy and Nguyen Thi My Tuyen, 2015).

Effect of extraction temperature and time to bitterness of *Ganoderma lucidum* extract

Fresh *Ganoderma lucidum* was fermented with *Lactobacillus plantarum* in 48h. After that, *Ganoderma lucidum* would be dried under temperature and time 40°C in 16h. The extraction was conducted by hot water at different temperature and time (100°C in 60 min, 110°C in 50 min, 120°C in 40 min, 130°C in 30 min). Sensory evaluation would be conducted to estimate the bitterness reduction. Results revealed in table 2. From table 2, the bitterness in *Ganoderma lucidum* could be effectively eliminated by extraction with hot water at 120°C in 40 min so this value was selected for further experiments.

Table 2 : Effect of hot water extraction temperature and time to bitterness of *Ganoderma lucidum* extract

Hot water extraction	100°C in 60 min	110°C in 50 min	120°C in 40 min	130°C in 30 min
Sensory score	6.87±0.02 ^c	7.21±0.00 ^{bc}	7.95±0.03 ^a	7.32±0.01 ^b

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ($\alpha = 5\%$).

Optimization of extraction and characterization of polysaccharides from medicinal mushroom *Ganoderma lucidum* was conducted. The optimum conditions were an extraction temperature of 100°C, an extraction time of 3 h, NaOH concentration of 6% and ratio of liquid to solid of 20 ml (Gunjan Sood *et al.*, 2013). One research was conducted by Nguyen Minh Thuy and Nguyen Thi My Tuyen (2015). The content of this paper including (i) optimization extracting process based on the experimental design of time (15-45 minutes) and temperature (70-130°C) and (ii) fermentation of *G. lucidum* spore with *Lactobacillus plantarum* for breaking spore wall from 24 to 72 hours. The optimum extraction conditions were achieved at temperature of 130°C for 40-45 minutes.

Effect of microencapsulate material in spray drying to bitterness of *Ganoderma lucidum* dried powder

Fresh *Ganoderma lucidum* was fermented with *Lactobacillus plantarum* in 48h. After that, *Ganoderma lucidum* would be dried under temperature and time (40°C in 16h), extraction by hot water at temperature and time (120°C in 40 min), and spray drying by different wall materials (β -cyclodextrin, sodium alginate, carrageenan, pectin) in the same concentration 0.4% at inlet temperature 150°C, outlet temperature 80°C, feed rate 12 ml/min. Sensory evaluation will be conducted to estimate the bitterness reduction. Results revealed in table 3. From table 3, the bitterness in

Ganoderma lucidum dried powder could be effectively eliminated by β -cyclodextrin 0.4%.

Table 3 : Effect of wall materials to bitterness of *Ganoderma lucidum* dried powder

Wall material	β -cyclodextrin 0.4%	Sodium alginate 0.4%	Carrageenan 0.4%	Pectin 0.4%
Sensory score	8.60 $\pm 0.02^a$	8.21 $\pm 0.03^b$	8.15 $\pm 0.02^{bc}$	8.08 \pm 0.01 ^c

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ($\alpha = 5\%$).

Cyclodextrins are cyclic oligosaccharides produced by the degradation of starch resulting from intramolecular transglycosylation reactions caused by cyclodextrin glucanotransferase enzyme. There are several types— α -cyclodextrin which have six glucose molecules in the ring, β -cyclodextrin which have seven glucose molecules in the ring, and γ -cyclodextrin, which have eight or more glucose units (Del Valle, 2004). The height of the cyclodextrin cavity is the same for all three types but the diameter varies with the number of glucose units. Small molecules are included in α -cyclodextrin, whereas larger molecules are included in γ -cyclodextrin. γ -Cyclodextrins have greater internal cavities, are more water soluble, and allows for the inclusion to be more bioavailable (Li *et al.*, 2007). β -cyclodextrin form inclusion complex with other molecule by non-covalent bond and complex stability becomes better with the availability of electron-donor character of the substituents. β -cyclodextrin can make a stable complex from with the guest molecule that is less polar than water or less hydrophilic (Coupland and Hayes, 2016). The use of β -cyclodextrin was expected to give limonin stability against light, pH, and heat, as well as mask their bitterness when added to food system or consumed by consumer. Sodium alginates were suggested for reduction of unpleasant off-tastes caused by tea catechins (Jakob, 2008). The chitin derivative, chitosan, at a concentration of 0.4 to 1.2% in water, is also able to reduce bitterness of caffeine and various plant extracts but also exhibits a strong astringency. Sulfated polysaccharides, such as carrageenan, were used to reduce the undesirable taste of amino acids mixtures (e.g., L-histidine, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-tryptophan, and L-valine, each at 10%). In a ratio of 9:1 carrageenan/amino acid cocktail, e.g., in beverages, the bitterness of an aqueous solution of such a mixture was reduced to 1 (weak bitterness) compared to 9 (strong bitterness) for the neat amino acid cocktail (Calton and Wood, 2002). The astringency of various tea catechins at 100 ppm was reduced using pectin at concentrations <0.1% (Hayashi *et al.*, 2005).

Conclusion

Ganoderma lucidum has been used for several decades for both treatment strategy and health promotion. The extracts contain bioactive compounds such as carbohydrates, glycosides, triterpenoids and phenolic compounds. Several studies have shown that *Ganoderma lucidum* contains a wide range of bioactive compounds associated with the promotion of good health. Numerous pharmacological effects associated with lingzhi have been recorded, among which are immunomodulatory, anti-inflammatory, antiviral, antioxidative, antiaging and antitumor properties. As the consumer rejects bitter or astringent taste therefore bitterness should be reduced by giving appropriate treatments.

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