Potential for manipulating the polysaccharide content of shiitake mushrooms

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Shiitake mushroom growers may be able to use the presence of health promoting constituents as a marketing tool to promote sales of their products for premium prices. There are few reports on the effects of management protocols for log-grown shiitakes on the concentrations of constituents to guide growers. In this chapter, a summary is given of the relationship of carbohydrate content as affected by shiitake strains, mushroom cap development, and length of saprophytic association. Shiitake carbohydrates include starch and lentinan, a compound identified as promoting human health. Results suggest that growers through the use of different fungal strains and growing conditions can manipulate carbohydrate constituents of shiitake mushrooms. Starch concentrations tended to be several fold greater than that of the lentinan fraction. In many instances, the concentrations of starch and the fraction containing lentinan were positively related.

Keywords: Lentinan, fungal strain, environment, mushroom maturation, saprophytic association, polysaccharide.

1. Introduction

Consumers worldwide are seeking healthy food and products. Shiitake (*Lentinula edodes* (Berk.) Pegler) mushrooms have had a reputation as being a health promoting food [1] and products derived from shiitakes are suitable for the health foods industry. As such, shiitake mushroom production in the United States has expanded greatly since its start about 40 years ago [2]. Worldwide sales of medicinal mushroom products were estimated to exceed $ 10 billion (US) in 2000 [3] and have probably increased since 2003, when skin care products made from shiitakes were introduced into the marketplace [4].

Fungal polysaccharides have been identified as promoting human health [5]. Specifically, lentinan has been identified as a health promoting, water-soluble β-glucan from shiitakes [6, 7]. Lentinan consists of a β-(1-3) linked glucan backbone with two β-(1-6) linked glucose side chains for every five β-(1-3) linked glucose residues and has a molecular weight of about 400 kilodaltons [8]. Lentinan is readily soluble in water but insoluble in 50% (v/v) ethanol [9, 10]. Shiitakes may contain other health promoting glucans, but specific polysaccharides have not been identified.

Several methods for lentinan and β-glucans quantitation from mushrooms have been reported. Minato et al. [9] and Mizono et al. [10] reported the development of an enzyme-linked immunosorbent assay to detect levels of lentinan in mushrooms. Widespread adoption of the procedure of Mizono et al. [10] has not occurred, possibly because of the limited availability of the lentinan reactive antibody. Brauer et al. [11] reported a method to quantitate a fraction that included lentinan, which is labeled as a high molecular weight polysaccharide (HMWP). In this method, aqueous extracts of shiitake mushrooms were fractionation by ethanol precipitation and size exclusion chromatography. Lentinan recovery during these two steps was quantitative [11]. Brauer et al. [12] reported that over 80% of the carbohydrates in the HMWP had the molecular weight reported for lentinan. The HMWP fraction had only trace amounts of starch and/or glycogen [12].

HMWP concentrations in shiitake mushrooms were higher when produced as log grown compared to substrate grown [11]. More recently, Kimmons et al. [13] demonstrated that the concentrations of HMWP in log-grown shiitake mushrooms varied with fungal strain, stage of development of the cap, and the length of the saprophytic association between fungi and tree log.

Manzi and Pizzoferrato [14] have reported on the β-glucan content of shiitakes. These scientists adapted the protocol of McCleary and Glennie-Holmes [15], by which the β-glucans from cereal grains are first digested to glucose by a highly purified β-glucanase (lichenase) from *Bracillus subtilis* and β-D-glucosidase. Glucose is quantified by changes in absorbance after oxidization by glucose oxidase and peroxidase. Manzi and Pizzoferrato [14] reported that shiitake mushrooms contained between 1 and 3 mg β-glucan g^{-1} dry weight. Brauer et al. [12] demonstrated that β-glucanase from *Bracillus subtilis* and β-D-glucosidase did not degrade purified lentinan; therefore, the polysaccharide quantified by the Manzi and Pizzoferrato [14] method is unique from lentinan and HMWP fraction.

There are only few reports regarding the starch and/or glycogen content of shiitake mushrooms. Dikeman et al. [16] reported that cooked mature and immature shiitake mushrooms contained slightly more than 20% of dry weight as starch as determined by amyloglucosidase digestion. More recently, Brauer et al. [18] reported that starch content of log-grown shiitake mushrooms varied from 1% to 15% of the dry weight using an amyloglucosidase / α-amylase

method [19]. In addition, the starch content was affected by the fungal strain, species of the tree log inoculated, developmental stage of the mushroom cap and the length of saprophytic association [18]. In general, starch concentrations were higher than reported concentrations for β-glucans and HMWP.

Results from these studies indicate that the water soluble constituents contain several different types of polysaccharides. Marketers of mushroom products have started to use the polysaccharide content as a possible indicator of the products' functional food activity [19]. However, such polysaccharide measurements may not be indicators of functional food activity if shiitake mushrooms or resulting products are high in polysaccharides such as starch that have little functional food activity [20]. The objectives of this study were to: 1) compare the content of starch and HMWP in shiitake grown under a variety conditions; and 2) assess the possibility that polysaccharide content may or may not represent the functional food activity of shiitake mushrooms if they contain significant concentrations of starch.

2. Materials and Methods

2.1 Log-grown shiitake mushroom production method

Log-grown mushrooms were grown in Shirley, Arkansas, USA (approximately N 35.655 W 92.318) using fungal inoculums obtained from Field and Forest Products* (Peshtigo, WI, USA) as described previously [11-13]. Many of these inoculums are not commerically available at this time. Briefly, logs were cut green from white oak (*Quercus alba* L.) trees after leaf drop in the fall but before bud break in the spring (i.e., mid-November to late March). Inoculated logs were incubated outside. Fruiting usually occurred immediately after a heavy rainfall event at suitable ambient temperatures. Mushroom caps were collected at one of three development stages: bud, veil break and open. Bud stage refers to the initial mushroom cap appearance, when the cap is swollen and distinct from its stalk. Buds are usually dome-shaped between 1 and 2 cm in diameter. Veil break refers to the stage of mushroom cap development where the veil begins to open and separate from the stalk, exposing the gills or lamellae. The mushroom cap is flat and the outside edges are slightly curled with the gills clearly exposed at the fully open stage. Over 3,000 logs were inoculated for the studies described below. All experiments had three replications with each replication representing numerous logs.

Three studies were conducted. In the first study (fungal strain comparison), mushrooms produced by 12 shiitake strains growing on logs were compared (Table 1). Mushrooms were harvested only in the veil break stage in the first study. In the second study (fungal strain and cap development experiment), mushrooms in the bud, veil break and open developmental stages were collected from the logs inoculated with one of five fungal strains. Mushrooms from logs' second and third harvests were included. In the third study (length of saprophytic association), mushrooms were from logs inoculated from one of two strains (NN-430 and 569-430) either two, three or four years prior. Mushrooms were collected only in the bud stage. More details on the design and execution of these experiments can be found elsewhere [11, 13].

2.2 Sample processing and polysacharride analyses

Collected mushroom caps were sliced (approximately 5 mm thick) and then dried at room temperature (20 to 22^{o} C) with circulating air in a industrial type food drier immediately after harvesting. Dried samples were ground to a 20-mesh powder using a grinding mill and stored at – 20^{o} C until analyses were performed. All samples from a harvest period were collected and prepared prior to analyses. The HMWP content was determined as described previously [11-13]. Starch concentrations were determined by amyloglucosidase / α-amylase method [18] using the ethanol/water extractant. Further details on the starch analyses procedure can be found elsewhere [12, 17]. Duplicate determinations were routinely performed on each sample. Additional replicates of analyses were performed until a coefficient of variation among analyses for a sample's starch content was less than 5%. Procedures for conducting analysis of varaince were described previously [11, 13]. Regression analyses were performed using PROC REG of SAS [21].

* Mentioning of a brand or company does not constitute endorsement by U.S. Department of Agriculture.

Table 1 Summary of the shiitake strains, and time of inoculations and harvests for the three experiments reported herein. Time is represented by 3 letter abbreviations for months, and two digits for years between 2002 and 2008. Logs incoluated with fungal strain 603 did not produce enough mushrooms for analyses.

Strain Name	Month of Inoculation	Fruiting for strain comparison	Fruiting for cap development	Fruiting for time after inoculation
Night Velvet	Jan-03	Oct-05		
Mori 290	Mar-03	Oct-05		
Biyang Flower	Apr-03	Sep-05		
Sefi 30	May-03	Oct-05		
K-6	Jan-04	Sep-05		
603	Jan-04			
762	Jan-04	Sep-05	Sep-05; Oct-06	
MY-602	Jun-04	Sep-05	Sep-05; Jun-06	
29-430	Jun-04	Oct-05	Oct-05; Oct-06	
CW-25	Jan-02	Jan-06		
569-430	Jun-04	Sep-05	Sep-05; Jun-06	Mar-08
569-430	Jan-06			Mar-08
569-430	Dec-06			Mar-08
NN-430	Jun-04	Oct-05	Oct-05; Oct-06	Apr-08
NN-430	Jan-06			Apr-08
NN-430	Dec-06			Apr-08

3. Results

3.1 Effects of Shiitake Strains

Fungal strain had a strong effect on both HMWP and starch concentrations in mushrooms harvested in the veil break stage during the fall of 2005 (Table 2). Logs inoculated with fungal strain 603 did not produce enough mushrooms for analyses and thus, were omitted from the study. HMWP concentrations varied over five-fold from 1.9 mg g^{-1} in mushrooms from the strain Night Velvet to 10.4 mg g^{-1} in mushrooms from the strain NN-430. The variations in starch concentrations were at least four-fold from 21.4 mg g^{-1} in MY-602 to 92.0 mg g^{-1} in strain 29-430.

In general, mushrooms from strains with high starch content tended to have high concentrations of HMWP. There was a strong association between the concentrations of starch and HMWP among the fungal strains (Figure 1). The F-value and R-square for the model of predicting HMWP from starch were 30.01 ($P < 0.001$) and 0.769, respectively. The regression equation was: HMWP concentration, mg g^{-1} = 1.209 + 0.0855*[starch concentration, mg g^{-1}]. The t-values for the Y-intercept and slope were 1.4 and 5.48, respectively, indicating that the Y-intercept was not significantly different from zero and the slope was significantly different ($P < 0.001$).

Table 2 Effects of shiitake strains on concentrations of HMWP and starch in mushrooms harvested during the fall of 2005 (second harvest). Mushrooms were collected in the veil break stage.

Fungal Strain	HMWP (mg g^{-1})	Starch (mg g^{-1})
Night Velvet	1.9	25.3
MY-602	2.7	21.3
Sefi 30	6.8	76.0
K-6	4.5	36.0
762	3.8	27.7
569-430	7.5	49.0
Mori 290	4.3	26.3
Biyang Flower	4.4	42.0
CW-25	5.7	65.7
29-430	8.0	92.0
NN-430	10.4	85.0
Least Square Standard Error	1.3	5.0

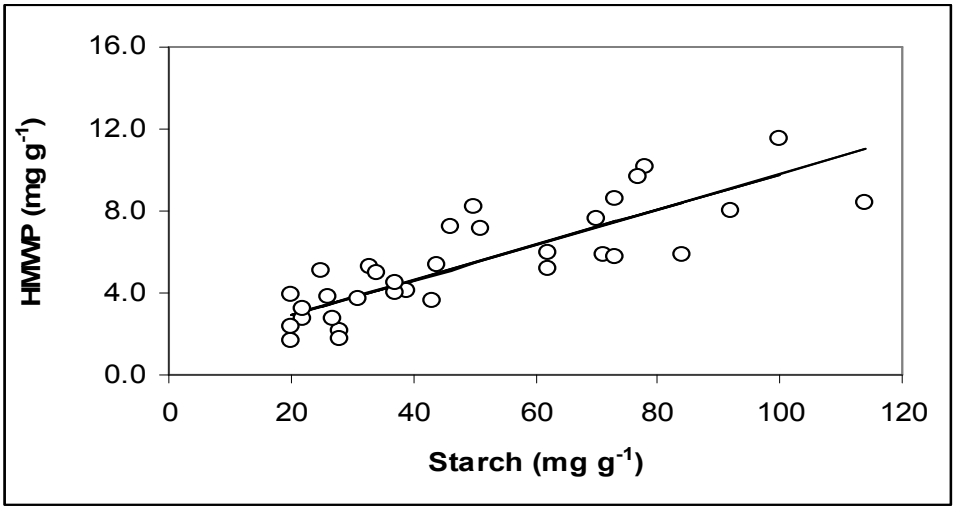

Figure 1 Association between starch and HMWP concentrations from mushrooms harvested in the veil break stage in the fall of 2005. Data are mean values for each replication and there were three replications per fungal strain entry (11 strains). The regression equation (HMWP concentration = 1.209 + 0.0855*[starch concentration]; R-square = 0.769) is depicted by a solid line.

3.2 Effects of Fungal Strain and Mushroom Development

In the second experiment, the starch and HMWP concentrations were compared in mushrooms harvested from the second and third harvests in three cap developmental stages (bud, veil break and fully open) from logs inoculated with one of five fungal strains (Table 1). Previous analyses indicated that both cap development and fungal strains affected starch and HMWP concentrations [11, 13]. In general, HMWP content was greatest in bud break mushrooms. HMWP concentrations averaged approximately 6 mg g^{-1} across the five fungal strains in the bud stage and declined by approximately 50% by the fully open stage. Starch concentrations increased as mushrooms matured beyond the bud stage. Starch concentrations in the bud, veil break and fully open stages averaged 32.5, 43.5 and 41.5 mg g^{-1}, respectively (least square standard error = 1.7 mg g^{-1}) across the five fungal strains. Across developmental stages and fungal strains, HMWP and starch concentrations were less for mushrooms in the third harvest than in the second harvest [11,13].

There was a strong positive association between HMWP and starch concentrations for the data from this study (Fig. 2). The F-value and R-square for the regression equation predicting HMWP concentrations from starch concentrations were 100.28 ($P < 0.001$) and 0.486, respectively. Therefore, the predictive power of the regression equation from the second experiment was not as strong as in the first experiment. The regression equation was HMWP (mg g^{-1}) = 1.73 + 0.0660*[starch, mg g^{-1}]. The t-values for the intercept and slope were 4.31 and 10.01, respectively; both being significant at $P < 0.001$. Thus, a major difference between the results from the first and second experiment was that the Y-intercept of the regression equation between starch and HMWP concentrations was significantly different from zero in the second experiment.

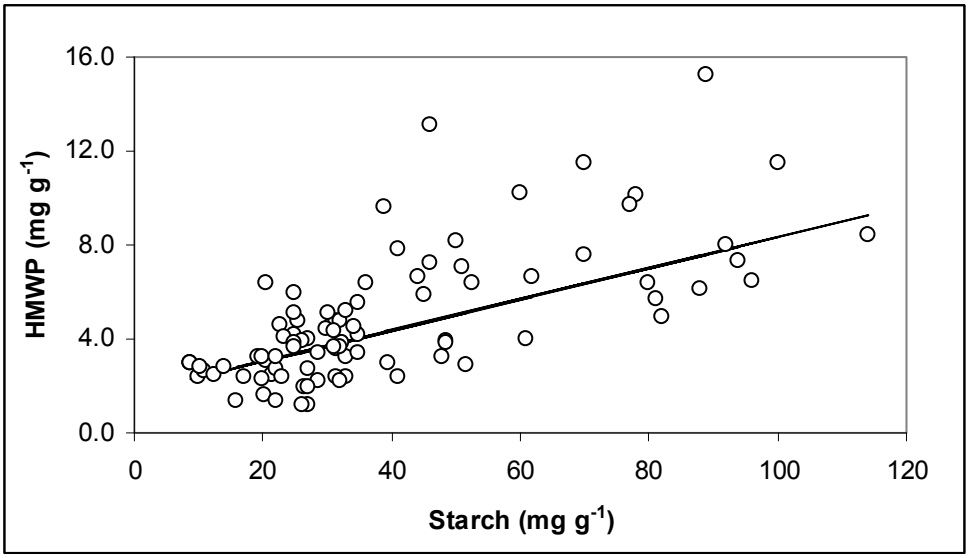

Figure 2 Relationship between starch and HMWP concentrations for mushrooms collected in a two successive harvests and at three different developmental stages from logs inoculated with one of five fungal strains. Data represent the mean from each replication. The regression equation, (HMWP = 1.73 + 0.0660*[Starch]; R-square = 0.486), is depicted as a solid line.

3.3 Effects of fungal strain and length of saprophytic association

The concentrations of starch and HMWP in mushrooms collected at the bud break developmental stage in the spring of 2008 were significantly affected by length of the saprophytic association and the shiitake strain used to inoculate the logs [11, 13]. When averaged across the two fungal strains NN-430 and 569-430, starch and HMWP concentrations decreased with increasing age of the saprophytic association. However, the trends with time after inoculation were not the same for the two fungal strains (Table 3). Starch concentrations in NN-430 mushrooms declined by 25% as the time from inoculation was increased from 2 to 4 years, while HWMP concentrations declined by approximately 30%.

Table 3 Variations in HMWP and starch concentrations among logs inoculated with two fungal strains and different lengths of time after inoculation.

Years after Inoculation	Fungal Strain	HMWP (mg g^{-1})	Starch (mg g^{-1})
2	NN-430	12.1	50.9
3	NN-430	8.3	43.8
4	NN-430	8.2	38.2
2	569-430	17.6	24.6
3	569-430	17.0	30.3
4	569-430	16.4	25.2
Least Square Standard Error		0.4	1.1

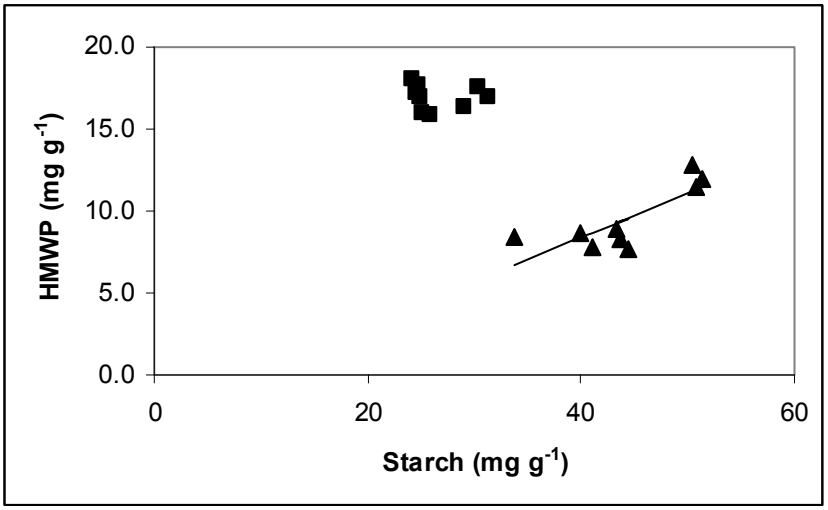

Figure 3 Relationship between starch and HMWP concentrations in mushrooms harvested in 2008 in the bud stage from logs inoculated with one of two fungal strains (triangles, NN-430 and squares, 569-430). Mushrooms were harvested from logs inoculated two to four years previously. Data are means from three replications. The regression equation for data from fungal strain NN-430 (HMWP, mg g^{-1} = 0.0268*[starch, mg g^{-1}] -2.33; R-square of 0.635) is depicted by a solid line.

There seemed to be a strong positive association between starch and HMWP concentrations for the data from fungal strain NN-430 (Figure 3). The F-value and R-square for the regression equation were 12.19 ($P < 0.01$) and 0.635, respectively. The regression equation was: HMWP (mg g^{-1}) = 0.268*[starch, mg g^{-1}] – 2.33. The t-value (-0.68) was insignificant for the Y-intercept while the t-value (3.49) for the slope was significant at $P < 0.01$. The results from fungal strain 569-430 were much different: time after inoculation did not significantly affect either polysaccharide concentration. There were no significant differences with time after inoculation in both polysaccharide concentrations; HMWP varied from 16.4 to 17.6 mg g^{-1} while starch varied from 30.3 to 24.6 mg g^{-1}. There was no significant association between starch and HMWP concentrations with data from fungal strain 569-430. When the polysaccharide data from both fungal strains were analyzed by regression analyses, a significant negative linear relationship between starch and HMWP was found; F-value and R-square were 18.94 ($P < 0.001$) and 0.542, respectively. The regression equation was: HMWP, mg g^{-1} = 23.82-0.297*[starch, mg g^{-1}].

4. Discussion

The results from this study provide a direct comparison between the concentrations of starch and HMWP in shiitake mushrooms produced by several fungal strains/phenotypes under various management conditions or environments. In general, the concentrations of starch in these mushrooms were several-fold greater than that of HMWP (Table 2). These results suggest that reports of the total water soluble polysaccharide content of either shiitake mushrooms or products derived from shiitakes will be an estimate of the starch concentration, but not likely that of the lentinan-containing fraction, HMWP, because of the differences in the amounts of these two carbohydrate fractions. .

Another generalization that can be derived from this study is that mushrooms with higher concentrations of starch tended to have higher concentrations of HMWP. Very strong positive relationships between starch and HMW content were found in the studies comparing effects of twelve different fungal strains (Figure 1) and three different developmental stages of the mushrooms among five different fungal strains (Figure 2). A strong positive relationship between starch and HMWP may indicate that environmental and phenotypic factors affect either the production or build-up of glucose, the common precursor to these two polymers.

There was an exemption to this trend with fungal strain 569-430 from bud break mushrooms harvested in 2008 from logs 2 to 4 years after inoculating (Table 3 and Figure 3). Data from fungal strain 569-430 were included in other analyses of the relationships between starch and HMWP concentrations that appear in Figure 1 and 2. In both of these cases the data from fungal strain 569-430 contributed to the generalized trends, i.e. increases in starch concentrations were associated with increasing HMWP concentrations.

The difference from the third study in 2008 involving fungal strain 569-430 may have resulted from a change in the mushroom production system [11, 13]. Fruiting from logs inoculated with fungal strain NN-430 occurred under the standard management conditions between February and March, 2008. Weather conditions during the spring of 2008 were unusually cold and wet, and thus were not conducive to fruiting of fungal strain 569-430. To meet the project's deadlines, a forced fruiting of logs inoculated with strain 569-430 was necessary to obtain samples. A subset of logs was randomly selected from each inoculation of the 569-430 strain. These logs were submerged in water for 48 h. Fruiting started three to five days after soaking during incubation at 18 and 21 ^{o}C at 90% relative humidity during the

first week in April 2008. The conditions of the forced fruiting may have changed the metabolism of these mushrooms, yielding a more narrow range between contents of starch and HMWP.

The general trend of increasing starch concentrations with increasing HMWP concentration may be helpful in developing management practices to produce mushrooms and/or their products high in functional food value from a high lentinan concentration, even if exceptions to the trend between starch and HMWP concentrations exist. Starch concentrations are much easier to measure than the employed HMWP assay. The starch assay employed in this study could be streamlined to produce results faster and easier. Screening mushrooms for high starch concentrations may be able to identify fungal strains and/or management practices that favour HMWP production because of the general trend between the concentrations of these two polysaccharides. Then, those favourable fungal phenotype/environmental conditions for high starch production could be tested by assaying for HMWP, instead of testing all possible combinations for HMWP.

One primary purpose for conducting this research project was to aid shiitake farmers in their knowledge of the best management practices needed to maximize potential health benefits from shiitake mushrooms. This knowledge includes selecting shiitake strains, establishing harvesting times and monitoring log or substrate maturity for optimal medicinal content in the Shiitake cap. Since the point of maximization of HMWP in maturing shiitake caps has not been precisely established, we have taken the average of HMWP content from our analysis of bud, veil break and open caps and have demonstrated that this average in all warm weather, all wide-range and most cold weather strains is always higher in the bud-stage (up to veil break) than in open, mature mushroom caps.

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