CORRELATION BETWEEN SOLUBLE DRY MATTER AND β-1.3 D-GLUCAN CONTENT IN *AGARICUS BLAZEI* MURRILL MUSHROOMS

CORELAŢIE ÎNTRE SUBSTANŢA USCATĂ SOLUBILĂ ŞI CONŢINUTUL DE β-1,3 D-GLUCAN LA CIUPERCILE *AGARICUS BLAZEI* MURRILL

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Abstract. *Experiments carried out by the researchers, about the extraction of the anti-tumour substances, like protein complex polysaccharides, which have demonstrated favorable effects of the immunostimulatory preparations and biologically active biomass obtained from certain species of fungi from the Basidiomicetae group. Thus, the antitumor activity of the β-1.3-D-glucan polysaccharide fractions extracted from mycelial biomass and fructification bodies of Agaricus blazei Murrill are well known. Mushrooms grown on synthetic substrate with wheat bran addition have a higher content of soluble dry substance (8.80%) and proteins (30.63% of the dm). The content of β-1.3-D-glucan, a specific substance in Agaricus blazei Murrill mushrooms, with a high biological value, is relatively high in the product obtained on the classic compost with the addition of corn flour and wheat bran (3.22-3.41 mg 100g*$^{-1}$*dm). Regression obtained from the correlation of these, shows strong link between the two components being very significant in both cases.*
Key words: mushrooms, *Agaricus blazei* Murrill, polysaccharides, β-1.3-D-glucan, protein addition

Rezumat. *Experimentele realizate de cercetători, privind extracţia de substanţe antitumorale de tipul unor polizaharide complexe cu proteine, au demonstrat efectele favorabile şi imunostimulatoare ale preparatelor biologic active obţinute din biomasa anumitor specii de ciuperci din grupul basidiomicetelor. Astfel, este bine cunoscută activitatea antitumorală a fracţiilor polizaharidice de tip β-1,3 D-glucan, extrase din biomasa micelianăşi corpurile de fructificaţie ale ciupercii Agaricus blazei Murrill. Ciupercile cultivate pe compost sintetic cu adaos de tărâţe de grâu, au un conţinut mai ridicat de substanţă uscată solubilă (8,80%) şi proteine (30,63% din s.u.). Conţinutul de β-1,3 D-glucan, substanţă specifică în ciupercile Agaricus blazei Murrill, cu valoare biologică ridicată, este relativ ridicat în produsul obţinut pe compost clasic cu adaos de făină de mălai şi cu tărâţe de grâu (3.22-3.41mg100g*$^{-1}$ *s.u.). Dreapta de regresie obţinută în urma corelaţiei dintre acestea, arată legătura strânsa între cele două componente, fiind foarte semnificativă în ambele cazuri.*
Cuvinte cheie: ciuperci, *Agaricusblazei* Murrill, polizaharide, β-1,3-D-glucan, adaos proteic

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INTRODUCTION

In addition to their food value, the mushrooms are also a cost-effective crop, which ensures a high yield, which is obtained on the used surface unit, in spaces arranged for this purpose. It is also worth noting that the cultivation of mushrooms does not use agricultural land. The advantages of a mushroom culture are many, both economic, occupational, medicinal, and reconversion of ligno-cellulosic waste (Stamets, 2005).

Worldwide, specialists are looking for new products from different plants as well as mushrooms, containing vitamins, minerals, enzymes to improve people's health. Viewed from this point of view, mushrooms are a food of high nutritional value, containing essential amino acids in the structure of complex proteins, and some species also have real therapeutic and medicinal virtues (Stamets, 2000).

Traditionally, mushrooms have also been given therapeutic, antitumor and immunological virtues that have begun to be scientifically tested, especially over the past three decades. The most important medical effects of the mushrooms are as follows: immunomodulators, antioxidant genoprotective, antiallergic, antitumoral, hepatoprotective, antidiabetic, antiatherogenic, hypocholesterolemic. Mushroom growers provide consumers with a fresh and valuable product (Stamets, 2010).

Nowadays, nutritional supplements and natural medicines are also provided by many mushroom-based, cultivated and medicinal basidiomycetes (Halpern, 2007).

Bioactive compounds in fungi can be isolated from fruit plants or extraction of pure mycelial culture (Chang and Miles, 2004). It has been reported that the *Agaricus blazei* Murrill mushroom produces various bioactive compounds that have the potential to treat several diseases (Firenzuoli *et al.*, 2008). This mushroom has been used as a drug for the prevention of cancer, diabetes, hyperlipidemia, arteriosclerosis and chronic hepatitis and is known as an immune system stimulator (Takaku *et al.*, 2001).

Polysaccharides from *Agaricus blazei* Murrill include several immunologically active low molecular weight fractions: α-1.6 and α-1.4 complex glucan, several polysaccharide-protein complexes and a heteropolysaccharide composed mainly of glucose, arabinose and mannose, all presenting anti-tumour properties (Kawagishi *et al.*, 1989; Fujimiya *et al.*, 1999; Ebina and Fujimya, 1998; Fujimyama *et al.*, 2000; Ohno *et al.*, 2004; Gonzaga *et al.*, 2005; Dong *et al.,* 2007; Johnson *et al.*, 2009; Niu *et al.*, 2009, Forland *et al.*, 2010; Zhang *et al.*, 2010). Importantly, there is an increase in the structural diversity of polysaccharides with fruit maturation (Camelini *et al.*, 2005).

MATERIAL AND METHOD

To study the influence of compost recipes on the quality of mushrooms, a bifactorial experience has been organized. Factor A was the 4-gradient culture substrate: C1 classic substrate (composed of horse manure and wheat straw70-75%, calcium sulphate, superphosphate, ammonium sulphate); C2 syntheticsubstrate (composed of wheat straw, poultry manure, calcium sulphate, urea); C3 Mixed substrate (horse manure, poultry manure, wheat straw, calcium sulphate, urea); C4 reed substrate (shredded reed, horse manure, poultry manure, calcium sulphate and urea).Factor B was the protein addition with 3 graduations: A1 without addition; A2 wheat bran 3%; A3 corn flour 3%.

The physical and chemical determinations in each experimental compost variant were made from an aqueous extract using 100 grams of compost in one litter of distilled water, and after filtration the obtained solution was used to perform the determinations.

The amount of 100 grams of compost was composed of 5 random harvests of 20 grams of compost from different sites of each experimental variation.

Sample preparation required the use of chromatographic purity extraction solvents (96% ethanol - FlukaChemie AG, bidistilled water) for the interpretation of the results for the study of the growth conditions of mycelium mycelium*Agaricusblazei* Murrill. The observations were made using the current technique of making them based on specific descriptors and bonuses (CIULCA, 2002).

Experimental data on the soluble dry substance and the amount of β-glucan of the *Agaricusblazei* Murrill mushrooms, the calculation of the regression line was made using the data processing program "Statistica 10".

RESULTS AND DISCUSSION

Analysing the results of two years of experience on total and soluble dry matter content in mushrooms (tab. 1), it is noted that in 2015 the total dry substance variation range was 7.60% in the mushrooms harvested from the canecompost (C4) with no additional protein supplement (A1) and 9.77% for mushrooms harvested on the mixed compost (C3) with wheat bran (A2) supplementary protein addition.

In 2016, lower dry matter content was recorded for all experimental variants compared to 2015.

Experimental factors had the same effect in 2015 as well as in 2016. Mushrooms harvested from the mixed compost (C3) with the addition of wheat bran (A2) recorded the best values for the soluble dry matter of 8.83% in 2015 and 8.17% in 2016. The recorded average of the dry substance in the two years was 8.50%.

With regard to the insoluble dry matter (i.d.m.), it can be noticed that in the year 2015 the mushrooms harvested from the cane compost (C4) with the addition of wheat barn (A2) have the lowest content ofi.d.m.(0.13%), and for the year 2016 the lowest values were obtained in the mushrooms harvested from the synthetic compost (C2) with added corn flour (A3), (0.04% i.d.m.).

Table 1

Effect ofcompost x protein addition (C x A) interaction on the mushrooms dry matter content (g/100g d.m./f.m.)

Experimental variant		Content of:								
		Total drymatter (d.m.) %			Solubled.m. %			Insolubled.m. %		
		2015	2016	Avg.	2015	2016	Avg.	2015	2016	Avg.
V1	C1 A1	9.03	8.23	**8.63**	7.73	7.07	**7.40**	1.30	1.16	**1.23**
V2	C1 A2	9.43	8.70	**9.07**	8.80	8.13	**8.47**	0.63	0.57	**0.60**
V3	C1 A3	9.73	8.87	**9.30**	8.07	7.40	**7.73**	1.66	1.47	**1.57**
V4	C2 A1	8.20	7.57	**7.87**	8.17	7.30	**7.75**	0.13	0.27	**0.12**
V5	C2 A2	9.10	8.50	**8.80**	8.50	7.77	**8.13**	0.60	0.73	**0.67**
V6	C2 A3	8.70	7.77	**8.23**	8.33	7.73	**8.03**	0.37	0.04	**0.20**
V7	C3 A1	9.40	8.60	**9.00**	7.93	7.27	**7.60**	1.47	1.33	**1.40**
V8	C3 A2	9.77	9.10	**9.43**	8.83	8.17	**8.50**	0.94	0.93	**0.93**
V9	C3 A3	8.47	7.80	**8.13**	7.43	6.77	**7.10**	1.04	1.03	**1.03**
V10	C4 A1	7.60	6.83	**7.13**	7.37	6.67	**7.10**	0.23	0.16	**0.03**
V11	C4 A2	8.37	7.83	**8.10**	7.63	6.87	**7.25**	0.74	0.96	**0.85**
V12	C4 A3	7.70	7.17	**7.43**	7.57	6.57	**7.07**	0.13	0.60	**0.36**

The content of β-1.3-D-glucan (mg $100g^{-1}$d.m.) in *Agaricus blazei* Murrill mushroom, ranged between 2875 and 3605 mg $100g^{-1}$d.m. in 2015 and 2640 and 3381 mg $100g^{-1}$d.m. in 2016 (tab. 2).

Both in the 2015 and 2016 experimental years, the maximum value was recorded in the mushroom harvested from classical compost (C1) with corn flour protein admixture (A3), with a maximum of 3414 mg $100g^{-1}$d.m. in 2015, and 3411 mg $100g^{-1}$d.m. in 2016.

Table 2

β-1.3 D-glucan content of *Agaricus blazei* Murrill mushrooms (mg $100g^{-1}$d.m.)

Experimental factors		β-1.3 D-glucan content (mg $100g^{-1}$d.m.)	
Variant	Combination	2015	2016
V1	C1 A1	3143	3143
V2	C1 A2	3208	3244
V3	C1 A3	3414	3411
V4	C2 A1	2917	2917
V5	C2 A2	3076	3078
V6	C2 A3	3155	3158
V7	C3 A1	3109	3110
V8	C3 A2	3133	3133
V9	C3 A3	3167	3167
V10	C4 A1	2581	2651
V11	C4 A2	2766	2766
V12	C4 A3	2990	2990

Regarding the content of β-1.3 D-glucan in *Agaricus blazei* Murrill mushroom, Zied *et al.* (2010) found the amount of 5000 mg $100g^{-1}$d.m., Cohen *et al.* (2014) 2550 mg $100g^{-1}$d.m., so that the values determined by us are found within these ranges.

The therapeutic quality is given in particular by the amount of β-1.3 D-glucan, which is correlated with the soluble dry matter and depends on the experimental factors.

As a result of the calculations, the correlation coefficient between the amount of soluble dry substance and the mushrooms β-1,3 D-glucan content, in the variant without additional protein supplement yielded r = 0.45652, being significant (fig. 1).

Comparing this value with the correlation coefficient with the probability of 5% and 1%, respectively, r = 0.95> 0.50 and 0.80 on the basis of these comparisons, it can be stated that between the amount of soluble dry matter and the content in β-1.3 D-glucan from mushrooms, the correlation coefficient is significantly positive, indicating a relationship between the dry matter content and the β-1.3 D-glucan content of *Agaricus blazei* Murrill mushroom. A higher amount of dry matter in mushrooms, induced a higher β-1.3 D-glucan content.

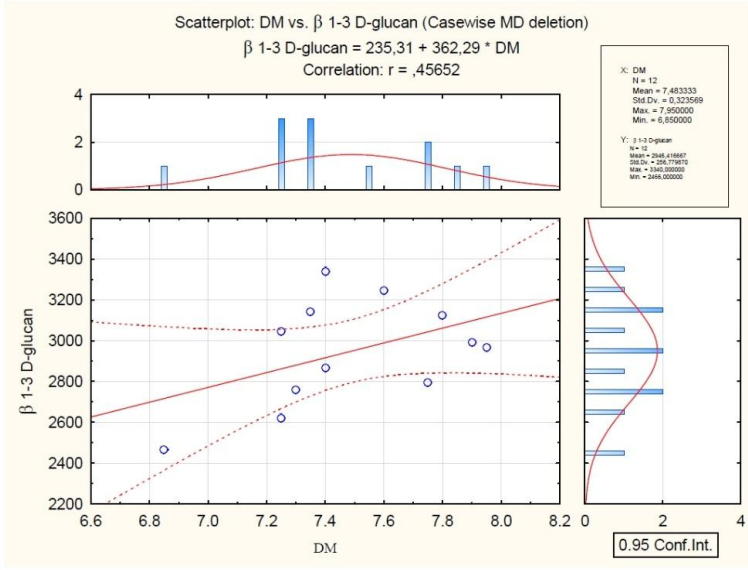

Fig. 1 Correlation between dry substance and β-1.3 D-glucan content of *Agaricus blazei* Murrill mushrooms, harvested from composts without added protein supplement

The additional protein supplement with 3% wheat bran indicates that with increasing quantity of dry matter the content of β-1.3 D-glucan increases (fig. 2).

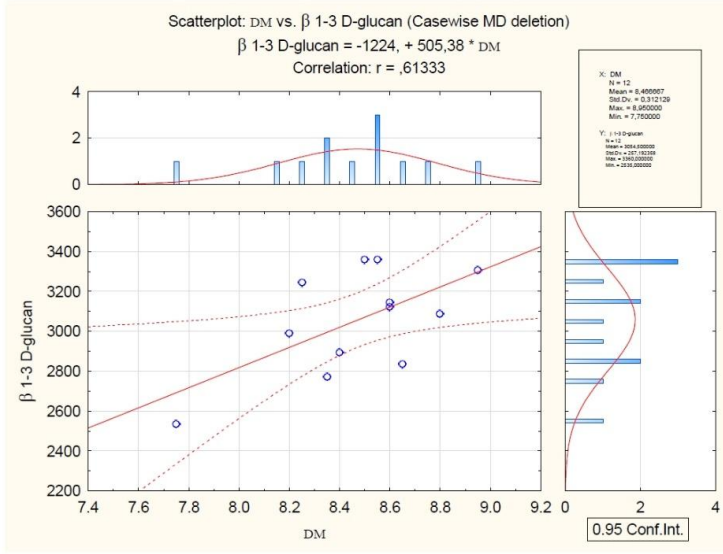

Fig. 2 Correlation between dry substance and β-1.3 D-glucan content of *Agaricus blazei* Murrill mushrooms, harvested from composts with 3% wheat barn protein supplement

It is noted that the experimental data faithfully follows the right regression. The relation between the two characters estimated by simple linear regression is illustrated by the following equation of degree I: y = 1224 + 505.38 * d.m. and the value of the coefficient of determination r = 0.61333 is distinctly significant.

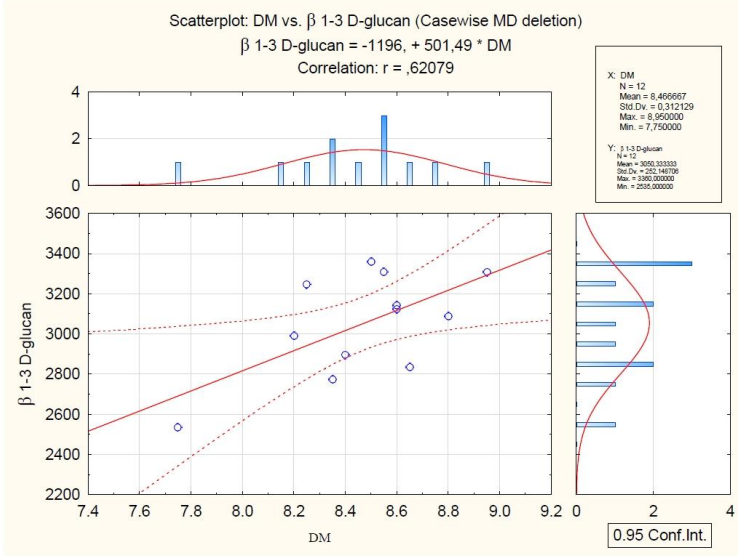

Fig. 3 Correlation between dry substance and β-1.3 D-glucan content of *Agaricus blazei* Murrill mushrooms, harvested from composts with 3% corn flour protein supplement

The experimental results in the case of 3% corn flour protein addition, accurately follow the right regression (fig. 3). The relation between the two characters estimated by linear regression is illustrated by the following equation of degree I: y = 1196 + 501.49 * d.m. and the value of the coefficient of determination r = 0.62079. On the basis of these comparisons it can be stated that between the quantity of dry substance and β-1.3 D-glucan the correlation coefficient is distinctly significant.

CONCLUSIONS

1. From the results obtained, the content of β-1.3 D-glucan was increased with the increase in the amount of soluble dry substance.

2. Regardless of the culture substrate, linear equations were calculated for each protein addition.

3. It can be argued that the two additional protein additions lead to an increase in the correlation coefficient, so it is beneficial for the cultivation of *Agaricus blazei* Murrill mushrooms using additional protein additions.

4. Based on the results obtained and the data processing through correlations, the regression has highlighted the link between the studied factors.

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