INTERACTION BETWEEN GROWING SUBSTRATE NITROGEN CONTENT AND *AGARICUS BLAZEI* MURRILL MUSHROOMS PROTEIN CONTENT

INTERACȚIUNEA DINTRE CONȚINUTUL DE AZOT AL SUBSTRATULUI DE CULTURĂ ŞI CONȚINUTUL DE PROTEINĂ AL CIUPERCILOR *AGARICUS BLAZEI* MURRILL

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Abstract. *Mushrooms provide important sources of protein extracted from materials of very low economic value, such as manure, agricultural waste, forestry, wood industry.Cultivated mushrooms having a saprophyte diet are forced to feed their enzymes from decomposing organic substances.During the composting and pasteurization of the substrate, the protein nitrogen is transformed into peptides and amino acids that are absorbed by mycelial hippocampal cells.Research has shown that there is a direct correlation between the total nitrogen content of the nutrient substrate (up to 2.7%), the production of mushrooms and the protein content of the mushrooms.In this study, four types of compost and three types of additional protein additions were investigated.The highest values of correlation coefficient (r = 0.88187) were obtained with the addition of 3% wheat bran, additional protein supplement in the substrate.*

Key words: mushrooms, *Agaricus blazei* Murrill, protein, substrate, protein addition

Rezumat. *Ciupercile asigură importante surse de proteine extrase din materiale cu valoare economică foarte scăzută, cum ar fi gunoiul de grajd, deşeurile din agricultură, din silvicultură, industria lemnului.Ciupercile de cultură, având un regim de nutriție heterotrof saprofit sunt obligate să-i ia hrana din substanțele organice în descompunere, cu ajutorul enzimelor.În timpul compostării şi pasteurizării substratului, azotul proteic se transformă în peptide şi acizi aminici, care sunt absorbiți de celulele hifelor miceliene. În urma unor cercetărilor, s-a constatat că între conținutul de azot total al substratului nutritiv (de până la 2,7%), producția de ciuperci şi conținutul în proteină al ciupercilor, există o corelație directă.În studiul de față s-au efectuat cercetări pe patru tipuri de compost şi trei tipuri de adaosuri proteice suplimentare.Valorile cele mai ridicate ale coeficientului de corelație (r=0,88187) au fost obținute în cazul utilizării ca şi adaos proteic suplimentar în substrat a tărâțelor de grâu 3%.*

Cuvinte cheie: ciuperci, *Agaricus blazei* Murrill, proteină, substrat, adaos proteic

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INTRODUCTION

The chemical composition of fungi differs from one species to another, their stage of development, the nutritive substrate used to produce them, the part of the mushroom from which the samples are harvested, the duration of growth and development of the mushrooms, as well as the microclimate in the spaces of culture (Manzi *et al.*, 1999). Compared with spontaneous flora, mushrooms are much appreciated due to the increase in protein content and higher commercial quality (Abah and Abah, 2010).

The use of mushrooms with therapeutic effects is one of the most exciting areas of natural medicine, offering a significant therapeutic benefit, backed by a long history of traditional use and more and more scientific evidence (Wasser, 2010). The *Agaricus blazei* Murrill species (fig.1) is called popular the royal fungus, God's mushroom, the goddess's mushroom, the fungus of the sun.

Fig.1 *Agaricus blazei* Murrill mushrooms (original)

semi-globular in the beginning, then convex, smooth on the edges and in the centre, white, yellow-creamy like an almond, or light brown to dark brown, brown. On the edge of the hat remain veils. It has almond flavour.The lamellas are free, dense, 8-10 mm wide in white, pale pink when young, and later chocolate brown.Basidiospores are dark brown up to chocolate,6-8 x 4-5 microns,wide-elliptical to oval, without epispore.The leg is short and loud, like a column, filled in, cylindrical, white, attached to a mycelian base. Touched with hand is coloured in yellow. On the leg remains a ring after the velvet breaks. The length is 6-13 cm, and the diameter is 1-3 cm (Stamets, 2000).

Agaricus blazei Murrill mushrooms are secondary saprophytes, which develop on a partially processed substrate in which microorganisms reduce complex ligno-cellulosic compounds (Chen, 2003).

Increasing the nutritional quality of mushroom compost is a prime factor in increasing yield. Schisler and Sinden (1966) have shown that when the compost was supplemented with various seeds ground together with refined and crude seed oils applied to the compost before applying the coating, the production of mushrooms increased.The addition of compost with various protein additions was also attempted by Sinden and Sehialer (1962). Vijay *et al.* (2002) have found that supplements in compost and coating have increased the yield of *Agaricus bisporus*.

Following Siqueira *et al*. (2011), if an adequate amount of nitrogen is added to a carbon-rich substrate, the mycelium growth and the quality of the fructification bodies are considerably improved. According to Andrade *et al.* (2007) and Siqueira *et al*. (2011), the optimal initially nitrogen content of the substrate should be 1-1.5%. Selection of the nitrogen source is essential because the *Basidiomycetes* mushrooms do not produce nitrate reduction enzymes (Gerrits, 1998). For *Agaricus blazei* Murrill mushrooms, the best source of nitrogen is urea and the most useful C: N ratio ranges from 10:1 to 50:1 (Mantovani *et al.*, 2007). The nutrient substrate must exhibit degradation or microbial synthesis products in assimilable form as quickly as possible by the fungus, having a biochemical specificity corresponding to the metabolic requirements of the fungus (Zicari *et al.*, 2012).

MATERIAL AND METHOD

To study the influence of compost recipes on substrate quality and mushroom production, a bifactorial experience has been organized. Factor A was the culture substrate with 4 graduations: a_1-classical compost, a_2-synthetic compost, a_3-mixed compost and a_4-groove + horse manure compost. Factor B was the protein addition with 3 graduations: b_1 - without protein addition, b_2 - 3% wheat bran protein addition and b_3 - 3% corn flour protein addition. The combination of experimental factors resulted in 12 variants.

RESULTS AND DISCUSSION

At the beginning of the anaerobic composting phase, the water content of the mixtures for each experimental variant ranged from 74-78%, organic matter determined at 63-68%, nitrogen 1.7-1.9%, calcium 82-95 mg/l, magnesium 4-5 mg/l, ammonia (NH_3) 0.5-0.6%, pH 8.87-8.98, and electrical conductivity (EC) 3.45-3.78 dS/cm. The data obtained are also found in the literature (Andrade *et al.*, 2007, Chatterjee *et al.*, 2013, Xiang *et al.*, 2014).

At the end of the aerobic composting phase, the water content of the mixtures was in the range of 67-71%, the organic matter determined at DM 53-56%, nitrogen between 1.7-1.8%, calcium between 79-90 mg/L, magnesium between 3.7-4.85 mg/L, ammonia (NH_3) between 0.20-0.29%, pH between 7.98-8.24, and the electrical conductivity (EC) determined in this phase was in the range 2.83-2.98 dS/cm.

By comparing the data presented above, it can be said that during aerobic composting, water content in compost decreases from 74-75% to 67-71%, the content of organic matter drops from 63-68% to 53-56% nitrogen content drops by about 0.1%, calcium content decreases by 5mg/l, magnesium decreases by 0.1-0.3mg/L, ammonia (NH_3) decreases by 0.3% and pH decreases at 8.95 to 8.24, the best values being obtained on the mixed compost version.

Following the dynamics of physicochemical transformations during pasteurization for each type of compost, it was observed that after the pasteurization, the relative humidity of the compost decreased and stabilized in the range of 63-65% for each type of compost, the data being also found in the literature Garcia *et al.*, (1992) mentions 60-0%. After pasteurization, the pH of the compost decreased from 7.98-8.24 to 7.21-7.5, being in the range set by Gonzales Matute *et al.* (2011), 7.1-7.9. After pasteurization, total nitrogen in compost increased by 85%, ranging from 2.1-2.6%. Colak (2004) mentions 2.8% total nitrogen after pasteurization.

From the presented data, it can be noticed that during the pasteurization of the compost there are losses of up to 36.3% of the fresh matter and up to 30.1% of the dry matter of the compost, the biggest losses being recorded in the cane compost for the fresh substance and for the dry matter in the mixed compost. Gerrits *et al.* (1997) reported losses during compost pasteurization of up to 40% of the fresh and 35% of the dry matter of the compost, values higher than those in our experience.

At the end of the pasteurization phase, the compost water content for each experimental variation ranged from 63-65%, Horm and Ohga (2008) mentioning 60-65%, Chatterjee *et al.*, (2013) 60-70%.

Organic matter determined at DM at this stage, it was in the range of 49-52%, falling within the studied values by other authors Stanek (2010) 45-50% and Siqueira *et al.* (2011) 50%.

Nitrogen determined at DM at this stage, it was in the range of 2.1-2.6%, below the value obtained by Gerben *et al.*, (1998) 2.8%.

The interaction of experimental factors on the correlation between the amount of nitrogen in the culture substrate and the amount of protein in the harvested mushrooms highlights the close link between them.

From the results obtained it was found that the amount of protein in the mushrooms increase with the amount of nitrogen in the culture substrate. Regardless of the culture substrate, linear equations calculated for each protein addition were emphasized.

In the first case, on the supplementary proteinfree substrate (fig. 2) it can observe the value of the correlation coefficient r = 0.76603 is distinctly significant.

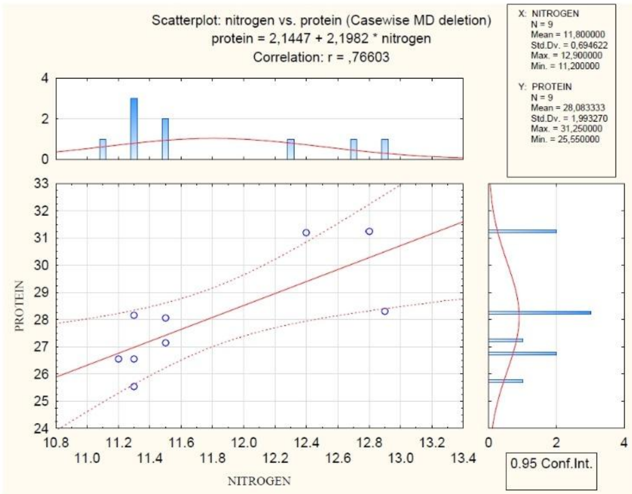

Fig. 2 Correlation between the amount of nitrogen in the substrate without added protein and the amount of mushroomsprotein content

In the case of the use of additional proteinaceous wheat bran 3% in the culture substrate, the coefficient of correlation increases (r = 0.88187) being very significant (fig. 3).

Higher correlation coefficients show a much closer direct link between the nitrogen content of the culture substrate and the protein content of the mushrooms. The addition of 3% wheat bran has positively influenced the amount of mushrooms protein.

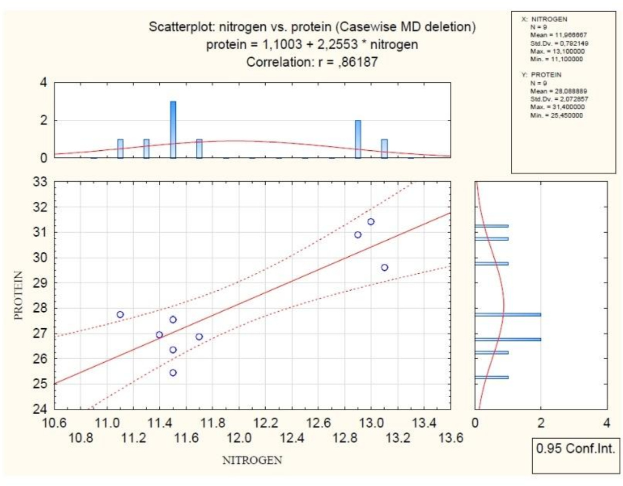

Fig. 3 Correlation between the amount of nitrogen in the 3% wheat bran addition substrate and the amount of mushroomsprotein content

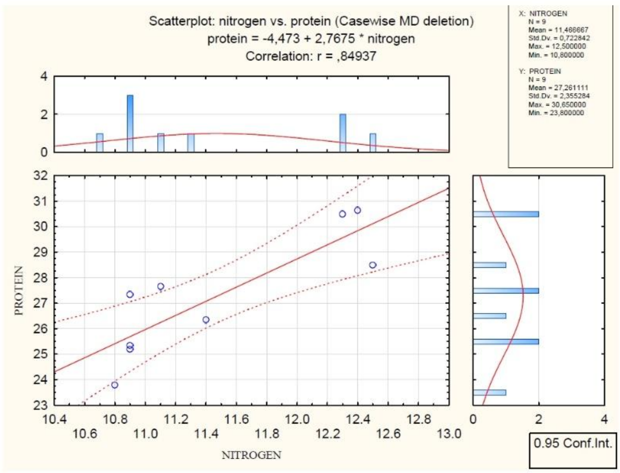

Fig.4 Correlation between the amount of nitrogen from the 3% corn flour addition substrateand the amount of mushroomsprotein content

In case 3% corn flour protein added as a protein additive, correlating the nitrogen content of the substrate with the amount of protein of the mushrooms, very significant linear correlations (r = 0.84937) were obtained (fig. 4). However, these values were lower than in the case of additional protein supplement with 3% wheat bran.

Regarding the protein content of *Agaricus blazei* Murrill mushroom, Tsai et al. (2008) mentions 26.7% protein from DM, Carneiro *et al.* (2013) 31.3%, Cohen *et al.* (2014) 28% and Stojkovic *et al.* (2014) 13.4%.

Compared with *Agaricus bisporus* mushroom, the protein content of *Agaricus blazei* Murrill is similar. Thus, Matilla *et al.* (2002) reported 26.5-27.1%, Akyuz and Kirbag (2010) 36.3%, Ulziijargal and Mau (2011) 26.5%, Reis *et al.* (2012) 14.1-15.4%.

CONCLUSIONS

1. From the obtainedresults, it was found that the as the amount of nitrogen in the substrate increase, will increase also the amount of mushrooms protein.

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

3. The closest connection between the amount of nitrogen in the culture substrate and the protein content of the mushrooms is found in the case of compost with 3%wheat bran addition, the correlation coefficient being very significant (r = 0.88187).

4. It can be argued that the two additional protein additions lead to an increase in the correlation coefficient, being beneficial to the culture of *Agaricus blazei* Murrill mushrooms.

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