

Conversion of Brewers' Spent Grain into Proteinaceous Animal Feed using Solid State Fermentation

Christos Iliopoulos

Institute of Technology of Agricultural Products: Institouto Technologias Agrotikon Proionton & Agricultural University of Athens

DIMITRIOS ARAPOGLOU (✉ dimarap@yahoo.com)

Institute of Technology of Agricultural Products: Institouto Technologias Agrotikon Proionton
<https://orcid.org/0000-0002-6526-5945>

Nikos Chorianopoulos

Institute of Technology of Agricultural Products: Institouto Technologias Agrotikon Proionton

Giorgos Markou

Institute of Technology of Agricultural Products: Institouto Technologias Agrotikon Proionton

Serkos Haroutounian

Agricultural University of Athens: Geoponiko Panepistemio Athenon

Research Article

Keywords: Brewers' Spent Grain, Pleurotus ostreatus, Solid-State Fermentation, Protein enrichment, Waste valorization

Posted Date: April 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-375812/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on July 23rd, 2021. See the published version at <https://doi.org/10.1007/s11356-021-15495-w>.

Abstract

Brewers' Spent Grain (BSG) represents the 85% of the total residue produced during the beer brewing process, with a global annual production volume exceeding the 30 Mtons. Study herein concerns the application of solid state fermentation (SSF) process for the efficient transformation of BSG into high nutritional value animal feed. The investigated SSF procedure was initiated by *Pleurotus ostreatus*, which constitutes a natural source of β -glucans and metabolites (vitamins, nutrients). Thus, it is possible to reduce the environmental impacts caused by BSG production and simultaneously contribute to the tackling of proteinaceous animal feed shortage observed during the last decade. The method developed resulted in a significant increase of protein content by 49.49%, a 10-fold increase of their 1,3 – 1,6 β -glucans content and a respective reduction of cellulose content by 11.42%. The application of this method is expected to provide a solid background for the utilization of BSG as substrate for fungi initiated SSF, a bioprocess allowing the significant reduction of the environmental impact caused by the beer brewing industry and simultaneously produce animal feed with high protein content and nutritional characteristics suitable for fulfilling animals' nutritional needs and improving their welfare.

Introduction

The adoption of the European Strategy and Action Plan for the Circular Economy in 2015 initiated a vigorous search towards the exploitation of more efficient utilization means for natural resources and anthropogenic products in the terms of an environmentally oriented economy (https://ec.europa.eu/info/research-and-innovation/research-area/environment/bioeconomy_en). In this respect, there is a recent global trend towards the reduction of environmental pollution caused by the remaining and/or byproducts of industrial activities. Among the broad variety of diverse industrial residues, those produced from the agro-industrial activities and exemplified by lignocellulosic materials present an intriguing case. They are produced in large quantities and are recyclable or reusable through the application of appropriate techniques and procedures of circular economy. In addition, their nutritional composition justifies their characterization as raw materials and not as wastes or by-products, since the lignocellulosic residues are mainly composed of Cellulose, Hemicellulose and Lignin. These constituents are also indicative of their suitability for serving as substrates of SSF, a procedure that can be initiated by various fungi (Mussato et al. 2006; Ritota and Manzi 2019), exemplified by white rot fungi *Pleurotus ostreatus* that is well known as potent means for the degradation of various lignocellulosic materials and their transformation into useful substrates. In this context, *Pleurotus ostreatus* is an edible basidiomycete, displaying the capability of colonizing and degrading lignocellulosic materials by secreting lignocellulolytic enzymes, which are classified as hydrolytic (Cellobiohydrolases, Endoglucanases, β -glucosidases) and oxidative enzymes (Laccases, Lignin Peroxidases, Manganese peroxidases) (Ankifermi et al 2020; Han et al. 2020). Their ability of initiating bioconversion via a SSF process has been previously utilized for the production of high added value products, such as antibiotics, vitamins amino acids, enzymes, biofuels, organic acids, and biopolymers (Cooray and Chenc 2018; Liguori et al. 2015).

Beer is considered as the fifth most consumed beverage worldwide, following tea, coffee, soda carbonates and milk. For 2018 global beer's annual production reached almost 182 Mtons whereas Europe's production was almost 52 Mtons (FAOSTAT 2019).

The brewing procedure produces large amounts of wastes, with prevailing by-product the Brewers' Spent Grain (BSG), which represents almost the 85% of the total brewing industry residues. BSG is derived from malted grain, the main raw material of brewing industry (Khidzir et al. 2010). It must be noted that the annual production of BSG exceeds the 30 Mtons, since every hectoliter of beer results in the production of 15–20 kg of BSG (Radosavljevic et al. 2017). The annual European production of BSG is estimated to 3.4 Mtons, from which 2 Mtons are produced in Germany and 288,000 in Italy (Bianco et al. 2020).

BSG disposal is a high-cost procedure. After the mashing process, all the exploitable ingredients are extracted. Although, the remaining barley is still consisted of digestible fibers and protein justifying the utilization of spent grains in agricultural applications (Westendorf and Wohlt 2002). On the other hand, the BSG content of exploitable compounds indicates potentials for its utilization as an appealing source for the production of high added value commodities, such as novel proteinaceous animal feed, enriched with bioactive compounds (Cooray and Chenc 2018).

Todate, 70% of BSG is used as animal feed, 10% for the production of biogas and 20% is disposed as landfills (Bianco et al. 2020). Its precise chemical composition depends on the variety of barley, the harvest period and the applied malting and mashing processes (Liguori et al. 2010). Furthermore, BSG is rich in fibers (cellulose, hemicellulose and lignin), proteins, essential amino acids, minerals and antioxidants (polyphenols, flavonoids), vitamins and lipids (Bianco et al. 2020; Cooray and Chenc 2018). Currently, brewery waste is mainly used as a low nutritional value animal feed of low economic cost, since BSG constitutes a source of lower protein quality content compared to other proteinaceous supplements such as soybean meal, fishmeal and milk. On the other hand, its unique nutritional profile consisted of Ca, Na, and K justifies its utilization as predominant material that should be combined with other cereal grains, forages, and protein supplements for the creation of feedstuff with high impact on animals' growth (Westendorf and Wohlt 2002). Thus, there is an emerge for the upgrade of its nutritional content, in order to obtain higher commercial value and concurrently provide animals with nutrients and bioactive compounds. In this respect, the increased protein content, improved amino acid profile and enhanced β -glucans content which contribute in animals' good health and welfare are being considered as the most crucial factors. These constituents also contribute on the improvement of the quality value of produced meat. As we have already pointed out, the polysaccharide content and abundancy highlights the BSG as a suitable substrate for SSF, which proceeds through its colonization initiated by fungi, bacteria or microorganisms (Ritota and Manzi 2019; Tan et al. 2019). This constitutes an environmentally friendly process that is capable of upgrading raw materials to overcome the problem of low protein content and high lignin concentration. So far in the literature there are only few studies on the upgrading of BSG by SSF focusing mainly on the fungus *Rhizopus*. Aim of the present study is to investigate and demonstrate an exploitation pathway of BSG by its conversion into proteinaceous animal feed by SSF with the fungus *Pleurotus ostreatus*. The study herein, highlights the proteins increase, as well as the

presence of β -glucans, indicating BSG potential as a novel animal feed with high nutritional value and content.

Materials And Methods

Materials and Microorganisms

BSG substrate for the growth of *Pleurotus ostreatus* was kindly provided by the Athenian Brewery, one of the biggest beer industries of Greece. To achieve the optimum moisture content (approximately 70%) for an unhindered SSF process, tap water was added and renewed every day. All samples were weighed to a final weight of 200g (fresh matter). The substrate was placed into test vessels of 750 ml volume and sterilized at 121°C for 15min. *Pleurotus ostreatus* White 2000 P67 LOTTO 1551 MN 01827 strain was used for the inoculation of substrates. Throughout all experiments the fungi stock cultures were stored at 4°C.

Inoculation and Solid-State Fermentation

Inoculation was performed under aseptic conditions. Inoculum of *Pleurotus ostreatus* strain (5% w/w) was added at substrate's surface and transferred into a bioclimatic chamber with stable temperatures of 25°C and 60% humidity. The total incubation time was 12 days, while samples of Day 0, Day 2, Day 4, Day 6, Day 9 and Day 12 were taken for analyses purposes. Every sample was prepared and studied in triplicate.

Analytical methods

Moisture, proteins content and ashes were determined according to AOAC methods (AOAC 1995). Total soluble sugars were determined according to method developed by Dubois et al. (1951) and reducing sugars were determined according to Miller (1959). The determinations of sugars were performed on the aqueous extracts of the respective samples. Crude fiber substances content was determined in accordance with AOAC method (AOAC 1995). Cellulose and lignin presence were evaluated according to the acid-detergent fiber (ADF) method (AOAC 1995). Finally, β -glucans were assessed using the Megazyme enzymatic assay kits (β -Glucan Assay Kit (Mixed Linkage), MEGAZYME Product code: K-BGLU and β -Glucan Assay Kit Yeast & Mushroom, MEGAZYME Product code: K-YBGL).

Statistical analysis

All analyses were performed in triplicates and the results were expressed in means \pm standard deviation (\pm S.D.). Data normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk test. The differences between the groups were analyzed by paired t-test ($p \leq 0.05$ was considered significant). To all proportions of both substrates, a statistical analysis was performed between Day 0 and Day 12.

Results

Figure 1 illustrates the gradual mycelium growth during Days 2 to 12 while Table 1 tabulates the physicochemical parameters (moisture content, Total and Reducing Soluble Sugars concentration and Ash content) of the examined substrates, which were analyzed throughout all stages of mycelium growth, from the beginning (Day 0) until the end (Day 12) of the fermentation process. As shown, the moisture content although fluctuated during incubation, remained practically unaffected since only a slight increase (no significant, $p \geq 0.05$) was determined between Days 0 and 12. The concentration of the Total Soluble Sugars underwent a statistically significant decrease ($p \leq 0.05$), reduced from 10.34–7.66% from Day 0 to Day 12. A similar pattern was observed for the Reducing Soluble Sugars content, since a statistically significant decrease ($p \leq 0.05$) by 41.91% was observed between Days 0 and 12. Finally, the Ash content displayed a 2-fold significant increase ($p \leq 0.05$), as the ash content ranged from 1.13–2.27% between Days 0 and 12.

Table 1
Assessment of Physicochemical Parameters

	<i>Day 0</i>	<i>Day 2</i>	<i>Day 4</i>	<i>Day 6</i>	<i>Day 9</i>	<i>Day 12</i>
Moisture (%)	76.10 ± 2.08 ^a	73.17 ± 0.45	73.61 ± 0.23	73.97 ± 0.49	75.52 ± 0.17	76.86 ± 0.30 ^a
TSS (%)	10.34 ± 0.16 ^a	9.99 ± 0.39	10.11 ± 1.29	10.81 ± 0.88	7.14 ± 0.53	7.66 ± 1.00 ^b
RSS (%)	8.78 ± 0.80 ^a	11.04 ± 0.44	9.72 ± 1.76	9.28 ± 0.47	5.77 ± 0.13	5.10 ± 0.12 ^b
Ash (%)	1.13 ± 0.26 ^a	1.66 ± 0.26	2.21 ± 0.59	1.74 ± 0.29	1.35 ± 0.13	2.27 ± 0.19 ^a
TSS: Total Soluble Sugars, RSS: Reducing Soluble Sugars						
Differences between same symbols represent significance > 5%.						
Statistical analysis was performed between Day 0 and Day 12						

As shown in Fig. 2 protein content (% w.d.) increased gradually during the incubation period. In specific, a statistically significant increase ($p \leq 0.05$) by 49.49% was observed, since the proteins content was ranged from 19.73–25.01% between Days 0 and 12.

According to Fig. 3, crude Fiber Substances content was increased without statistical significance ($p \leq 0.05$). In particular, crude Fiber Substances content presented a slight increase of 9.24% ranging from 13.63–14.89% between Days 0 and 12.

The Cellulose content presented a fluctuation during incubation. In particular, cellulose content was found to be reduced by 11.42% without statistical significance ($p \leq 0.05$), ranging from 21.10–18.69% between Days 0 and 12.

According to Fig. 5, Lignin concentration resulted to a slight increase by 27.59% at the end of fermentation without statistical significance ($p \leq 0.05$). In specific, lignin content ranged from 5.58–

7.12% between Days 0 and 12 by presenting the highest value.

Table 2
Assessment of β -glucans content

B-glucans content (g/g)	Day 0	Day 12
1,3 – 1,4 β -glucans	3.24 \pm 0.07 ^a	0.78 \pm 0.09 ^b
1,3 – 1,6 β -glucans	0.1 \pm 0.01 ^a	11.68 \pm 0.84 ^b
Differences between same symbols represent significance > 5%.		
Statistical analysis was performed between Day 0 and Day 12		

1,3 – 1,4 β -glucans content was reduced between Days 0 and 12, whereas the presence of 1,3 – 1,6 β -glucans was increased. In particular, the 1,3 – 1,4 β -glucans content from 3.24g to 0.78g between Days 0 and 12 presenting a statistically significance reduction ($p \leq 0.05$) of 75.92%, while on the contrary 1,3 – 1,6 β -glucans content resulted to a statistically significant 10-fold increase ($p \leq 0.05$) on the respective days increasing from 0.1g to 11.68g.

Discussion

Study herein aimed the utilization of BSG as substrate for the solid-state fermentation process initiated by *Pleurotus ostreatus*. Final goal of the study was the evaluation of fermentation products as proteinaceous animal feed. In this respect, BSG's moisture content constitutes a crucial factor for fungi growth, since the increased moisture content inhibits the oxygen transfer, generating concurrently a suitable environment for contamination. On the contrary, low moisture levels prevents the fungi/microbial growth, enzyme production and confines the nutrition availability (He et al. 2019). In our experiments the moisture content always maintained around 76%, a value that is in line with previous literature reports (Khidzir et al. 2020; Musstato et al. 2006; Wang et al. 2001; Xiros and Christakopoulos 2012). On the other hand, the most crucial variable for BSG upgrade is the protein content of the fermentation outcome. Previous studies have demonstrated the ability of fungi initiated SSF procedure to increase the protein content. In this respect, Xiros and Christakopoulos (2012) have reported that the initial protein content of unfermented BSG which varied between 10–30%, was significantly increase upon SSF initiated by *Pleurotus ostreatus*, *Trichoderma pseudokoningii* and *Rhizopus* sp respectively (Wang et al. 2001, Bayitse et al. 2015 and Ibarruri et al. 2019). These findings are in accordance with our results indicating that the protein content was gradually increased during incubation to conclusively result a statistically significant increase at the end of fermentation (Day 12). In similar studies, according to Akinfemi et al. (2010) proteins' content was increased probably because of the excretion of certain extra cellular enzymes which are proteinaceous in nature into the waste during their breakdown and its subsequent metabolism. Additionally, proteins' increase could probably be explained by the intake of nitrogen excess via aerobic fermentation. Darwish et al. (2012) and Terassan and Carmona (2015) have reported that proteins' content increase is probably due the fungal biomass accumulation.

BSG is considered that constitutes a source of lower quality protein content as compared to other proteinaceous supplements such as soybean meal, fishmeal and milk (Westendorf and Wohlt 2002). This upgrade through SSF could probably improve its amino acid profile by upgrading the protein content, thus recommending the treated BSG as suitable and enriched with proteins substrate for livestock. On the other hand, the content of crude fiber substances varied between Days 0 and 12, recording a slight increase at the end of fermentation (Day 12), possibly as a result of a parallel Lignin increase.

Ritota and Manzi (2019) and Darwish et al. (2012) noted that *Pleurotus* spp. excrete hydrolytic enzymes (lignin peroxidase and manganese peroxidase) displaying the potential of degrading lignocellulosic raw materials, resulting to their improved digestibility (Akinfemi et al. 2010). Herein, the cellulose content was diminished without significance, whereas the lignin content showed a slight increase at the end of fermentation. It is notable that Fibers' concentration affects the feed conversion rate. According to Lao et al. (2020) growing/finishing pigs that were fed with BSG up to 23% didn't result to a significant gain reduction or a decline in quality's carcass.

The augmented BSG's ratio, over more than 6%, initially increased fibers content but concurrently caused a decreased conversion rate of feed in pigs. Thus, a decreased performance was observed, with the authors concluding that in order to have an essential management, BSG could be absorbed at a rate up to 50%, fulfilling the additional protein needs without a decrease in performance. (Westendorf and Wohlt 2002). Thus, the present study highlights the importance of BSG's biotransformation by SSF where the entire BSG's proportion can be used since a reduction of cellulose is observed.

Both, Total and Reducing Soluble Sugars concentrations varied until Day 6 and then they were gradually diminished until the end of fermentation. This may be rationalized considering that *Pleurotus ostreatus* consumed fermentable sugars as an energy source for its growth.

Barley as well as mushrooms, are composed by many bioactive compounds such as β -glucans, which constitute polysaccharides composed by D-glucose monomers linked through β -glycosidic bonds. β -glucans from barley consist of 1,3 - 1,4 bonds, while β -glucans originated from mushrooms consist of 1,3 - 1,6 linkages (Jin et al. 2004; Zhu et al. 2015). Previous studies have displayed the β -glucans beneficial impacts on human and animal health, which vary due to the differences between their linkage bonds. Barley's β -glucans have been displayed on the list of the European Food Safety Authority (EFSA) by claiming that they can exhibit positive health effects under certain circumstances. Furthermore, the consumption of (1,3 - 1,4) β -glucans is associated with a decrease of blood cholesterol (Steiner et al. 2015; Zhu et al. 2015). Additionally, β -glucans derived from mushrooms are known for their antitumor and immune-stimulating properties that are responsible for health promotion and welfare. β -glucans derived from barley, are the predominant constituent (70%) of barley endosperm cell walls. According to previous brewing literature, barley's β -glucans content ranged from 2-6% (w/w) (Jin et al. 2004), which is also in line with the results of the present work. Additionally, the content of 1,3 - 1,4 β -glucans derived herein from BSG were metabolized and therefore resulted in a significant reduction between Days 0 and 12, while the content of 1.3-1.6 β -glucans was significantly increased. This notable increase of 1,3 - 1,6

β -glucans content was due to the fungal growth. Finally, the fungal growth was achieved by breaking the cell walls, utilizing enzymes such as cellulases and glucosidases which resulted to reducing sugars release. *Pleurotus ostreatus* consumed these fermentable sugars as an energy source as well as structural components. Overall, according to Ibaruri et al. 2019, BSG proteins increased by 54% ranging from 20.5–31.7% via SSF with *Rhizopus*. Notwithstanding BSG used in this study, had lower initial protein concentration probably due to its variety or cultivation practices, a similar pattern was observed, via SSF initiated by *Pleurotus ostreatus* demonstrated a notable increase of 50% ranging from 16.73–25.01%. Also, BSG is consisted of 1,3 – 1,4 β -glucans which contribute to the cholesterol and blood glucose regulation. SSF initiated by *Pleurotus ostreatus* enriched BSG with 1,3 – 1,6 β -glucans, which impact to the immunostimulation and the animals' good health and welfare. This procedure generates a novel fermentation outcome as a proteinaceous animal feed consisted of 1,3 – 1,4 and 1,3 – 1,6 β -glucans contributing to the economic and environmental impact in the context of the circular economy.

Conclusion

In conclusion, study herein aimed the valorization of Brewers' Spent Grain (BSG) bioconversion to a novel proteinaceous animal feed. According to the results of the current study, BSG bioconversion into a high added value product through SSF with *P. ostreatus*, enhanced its nutritional value displaying, thus, its potential for use for animal nutrition. SSF initiated by *Pleurotus ostreatus* using exclusively BSG as substrate, increased protein content by 49.49%, reduced cellulose concentration of 11.42% and enriched BSG with a significant amount of 1,3 – 1,6 β -glucans of 11.68g indicating its potential as animal feed, since so far is used for different commercial purposes. Furthermore, from an economic point of view, the current study also highlights BSG's potential for augmented commercial price which is in the line/related with the protein's content increase achieved by SSF bioprocess. Overall, BSG is strongly recommended for exploitation in the field of animal nutrition for its physicochemical properties as well as by reducing its environmental impact in the context of the circular economy.

Declarations

Acknowledgements

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH—CREATE—INNOVATE (project code: T1EDK-04331).

Author's contribution

PhD candidate Christos Iliopoulos: conducted the experiments, analyzed the data, discussed the results and manuscript preparation.

Dr Dimitrios Arapoglou: had the idea for the article, designed the experiments, discussed the results and manuscript preparation.

Dr Nikos Chorianopoulos: literature survey, data analysis, discussed the results and manuscript preparation.

Dr Giorgos Markou: literature survey, data analysis, discussed the results and manuscript preparation.

Professor Serkos A. Haroutounian: had the general supervision and revised the manuscript.

All authors read and approved the final manuscript.

Data availability

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable

References

1. Akinfemi A, Adu OA, Doherty F (2010) Conversion of sorghum stover into animal feed with white-rot fungi: *Pleurotus ostreatus* and *Pleurotus pulmonarius*. African Journal of Biotechnology 9(11): 1706-1712
2. Aliyu S, Bala M (2011) Brewer's spent grain: A review of its potentials and applications. African Journal of Biotechnology 10(3):324-331. <https://doi.org/10.5897/AJBx10.006>

3. AOAC 1995 Official Methods of Analysis, 14th ed. Association of Official Analytical Chemists, Washigton, DC
4. Bayitse R, Hou X, Laryea G, Bjerre AB (2015) Protein enrichment of cassava residue using *Trichoderma pseudokoningii* (ATCC 26801). *AMB Express* 5:80. [http://doi.org/ 10.1186/s13568-015-0166-8](http://doi.org/10.1186/s13568-015-0166-8)
5. Bianco A, Budroni M, Zara S, Mannazzu I, Fancello F, Zara G (2020) The role of microorganisms on biotransformation of brewers' spent grain. *Applied Microbiology and Biotechnology*. <https://doi.org/10.1007/s00253-020-10843-1>
6. Cooray TS, Chenc NW (2018) Valorization of brewer's spent grain using fungi solid-state fermentation to enhance nutritional value. *Journal of Functional Foods* 42:85–94. <https://doi.org/10.1016/j.jff.2017.12.027>
7. Darwish G, Bakr AA, Abdallah MMF (2012) Nutritional value upgrading of maize stalk by using *Pleurotus ostreatus* and *Saccharomyces cerevisiae* in solid state fermentation. *Annals of Agricultural Sciences* 57(1): 47–51. <http://dx.doi.org/10.1016/j.aoas.2012.03.005>
8. Dubois M, Gilles K, Hamilton KJ, Rebers AP (1951) A Colorimetric Method for the Determination of Sugars. *Nature* 168:167. <https://doi.org/10.1038/168167a0>
9. European Commission.
10. https://ec.europa.eu/info/research-and-innovation/research-area/environment/bioeconomy_en Accessed 9 March 2021
11. Faostat 2019.
12. <http://www.fao.org/faostat/en/#data/FBS>. Accessed 12 February 2021
13. Han LM, An Q, He FS, Zhang LX, Zhang HM, Gao HX, Wu Q, Bian SL (2020) Solid State Fermentation on Popular Sawdust and Corncob Wastes for Lignocellulolytic Enzymes by Different *Pleurotus ostreatus* strains. *Bioresources* 15(3) 4982-4995
14. He Q, Peng H, Sheng M, Hu S, Qiu J, Gu J (2019) Humidity Control Strategies for Solid-State Fermentation: Capillary Water Supply by Water-Retention Materials and Negative-Pressure Auto-controlled Irrigation. *Frontiers in Bioengineering and Biotechnology* 7:263. <https://doi.org/10.3389/fbioe.2019.00263>
15. Ibarriuri J, Cebrian M, Hernadez I (2019) Solid State Fermentation of Brewer's Spent Grain Using *Rhizopus* sp. to Enhance Nutritional Value. *Waste and Biomass Valorization*. <https://doi.org/10.1007/s12649-019-00654-5>
16. Jin Y, Speers AR, Paulson TA, Stewart JR (2004) Barley b-Glucans and Their Degradation During Malting and Brewing. *MBAA TQ* 41(3): 231–240
17. Khidzir KM, Abdullah N, Agamuthu P (2010) Brewery Spent Grain: Chemical Characteristics and utilization as an Enzyme Substrate. *Malaysian Journal of Science* 29(1):41-51
18. Lao JE, Dimoso N, Raymond J, Mbega RE (2020) The prebiotic potential of brewers' spent grain on livestock's health: a review. *Tropical Animal Health and Production* 52:461–472.

<https://doi.org/10.1007/s11250-019-021209>

19. Liguori R, Soccol RC, Vandenberghe PL, Woiciechowski LA, Ionata E, Marcolongo L, Faraco V (2015) Selection of the Strain *Lactobacillus acidophilus* ATCC 43121 and Its Application to Brewers' Spent Grain Conversion into Lactic Acid. *BioMed Research International*.
<http://dx.doi.org/10.1155/2015/240231>
20. Miller GL (1959) Use of Dinitrosalicylic Acid Reagent for determination of reducing sugar. *Analytical Chemistry* 31(3):426-428. <https://doi.org/10.1021/ac60147a030>
21. Mussatto IS, Dragone G, Roberto CI (2006) Brewers' spent grain: generation, characteristics and potential applications. *Journal of Cereal Science* 43:1-14. <https://doi.org/10.1016/j.jcs2005.06.001>
22. Rachwal K, Wasko A, Gustaw K, Polak – Berecka M (2020) Utilization of brewery wastes in food industry. *PeerJ* 8: e9427. <http://doi.org/10.7717/peerj.9427>
23. Radosavljevic M, Pejin J, Kocic-Tanackov S, Mladenovic D, Djukić-Vuković A, Mojovic L (2017) Brewers' spent grain and thin stillage as raw materials in L- (+)-lactic acid fermentation. *J. Inst. Brew.* <https://doi.org/10.1002/jib.462>
24. Ritota M, Manzi P (2019) *Pleurotus* spp. Cultivation on Different Agri-Food By-Products: Example of Biotechnological Application. *Sustainability* 11:5049. <https://doi.org/10.3390/su11185049>
25. Steiner J, Procopio S, Becker T (2015) Brewer's spent grain: source of value-added polysaccharides for the food industry in reference to the health claims. *Eur Food Res Technol.* <https://doi.org/10.1007/s00217-015-2461-7>
26. Tan XY, Mok KW, Lee J, Kim J, Chen NW (2019) Solid State Fermentation of Brewers' Spent Grains for Improved Nutritional Profile Using *Bacillus subtilis* WX-17. *Fermentation* 5:52.
<https://doi.org/10.3390/fermentation5030052>
27. Terrasan FRC, Carmona CE (2015) Solid – State Fermentation of Brewer's Spent Grain for xylanolytic enzymes production by *Penicillium janczewskii* and analyses of the fermented. *Bioscience Journal* 31(6):1826-1836
28. Wang D, Sakoda A, Suzuki M (2001) Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent grain. *Bioresource Technology* 78:293-300
29. Westendorf LM, Wohlt EJ (2002) Brewing by-products: their use as animal feeds. *The Veterinary Clinics Food Animal Practise* 18 233-252
30. Xiros C, Christakopoulos P (2012) Biotechnological Potential of Brewers Spent Grain and its Recent Applications. *Waste Biomass Valorization* 3:213–232. <http://doi.org/10.1007/s12649-012-9108-8>
31. Zhu F, Du B, Bian XZ, Xu B (2015) β -Glucans from edible and medicinal mushrooms: Characteristics, physicochemical and biological activities. *Journal of Food Composition and Analysis* 41: 165-173.
<http://dx.doi.org/10.1016/j.jfca.2015.01.019>

Figures

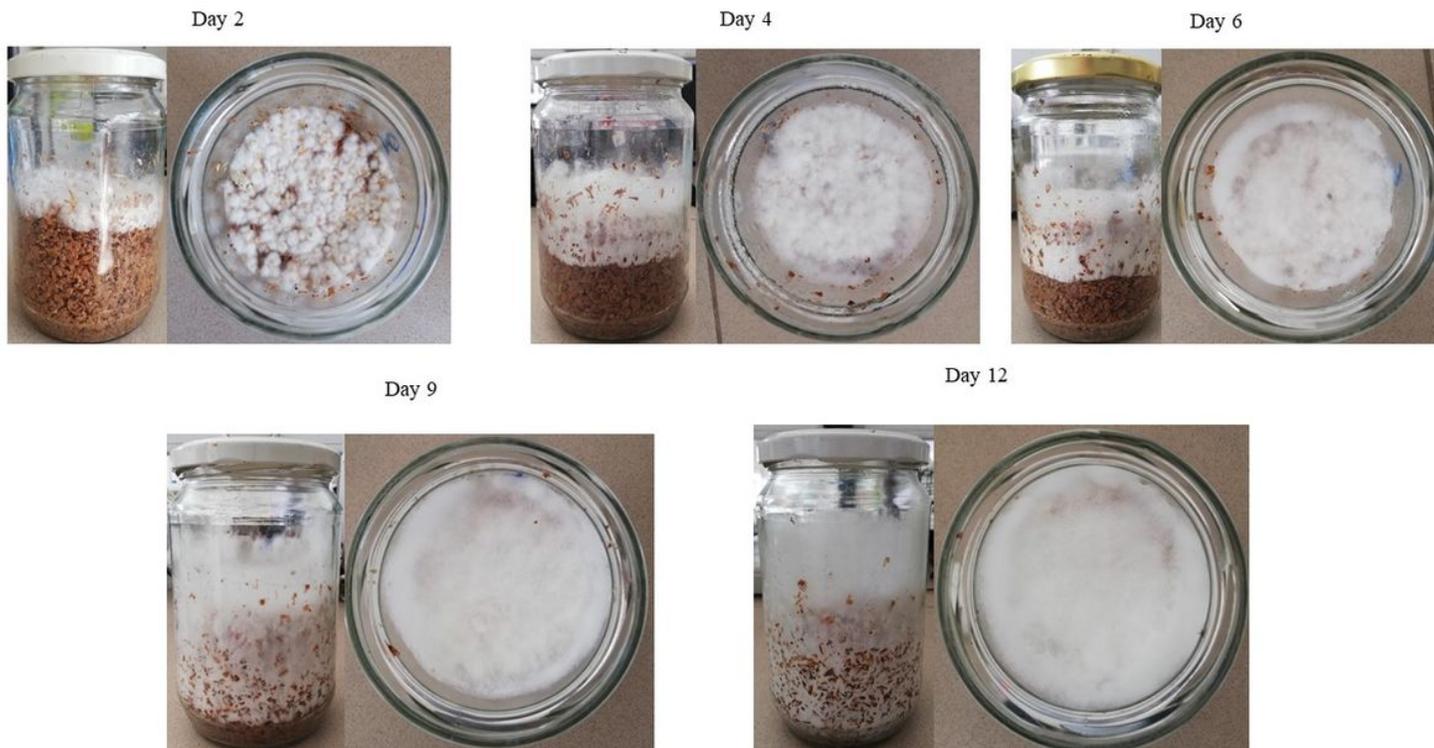


Figure 1

Representative images of stages of mycelial growth during Days 2 to 12 of fermentation.

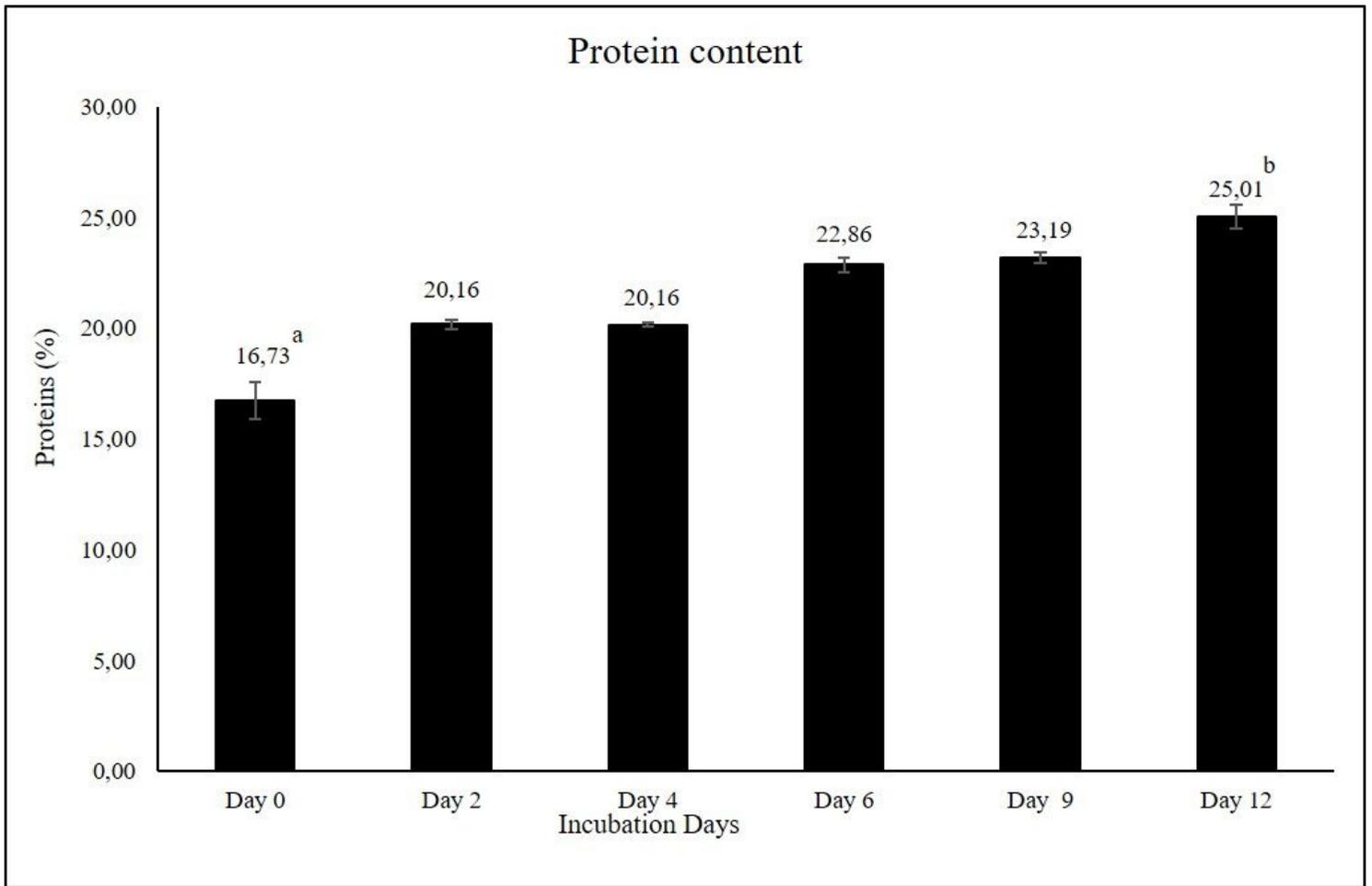


Figure 2

Assessment of proteins content Differences between same symbols represent significance >5%. Statistical analysis was performed between Day 0 and Day 12

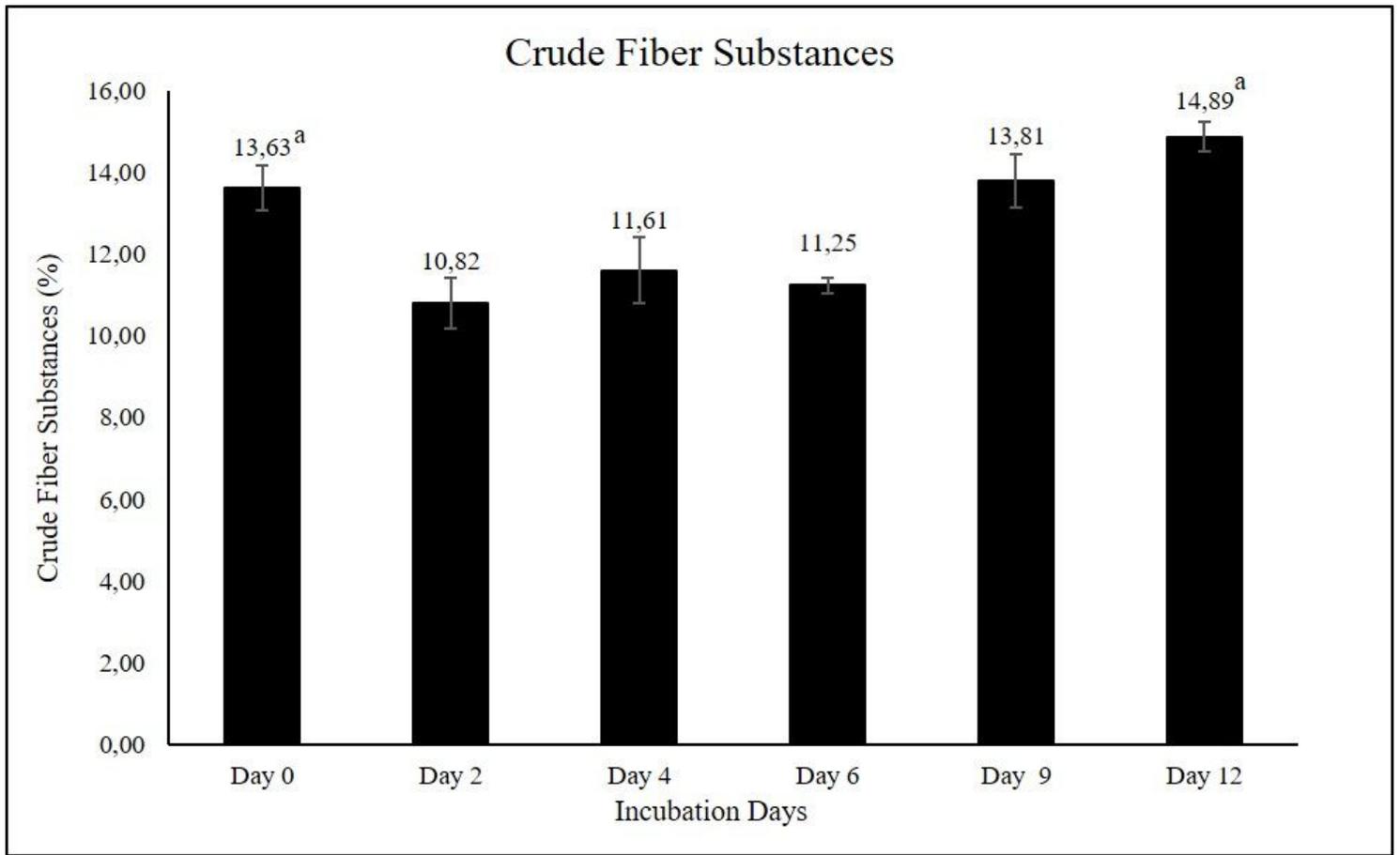


Figure 3

Assessment of crude Fiber Substances content Differences between same symbols represent significance >5%. Statistical analysis was performed between Day 0 and Day 12

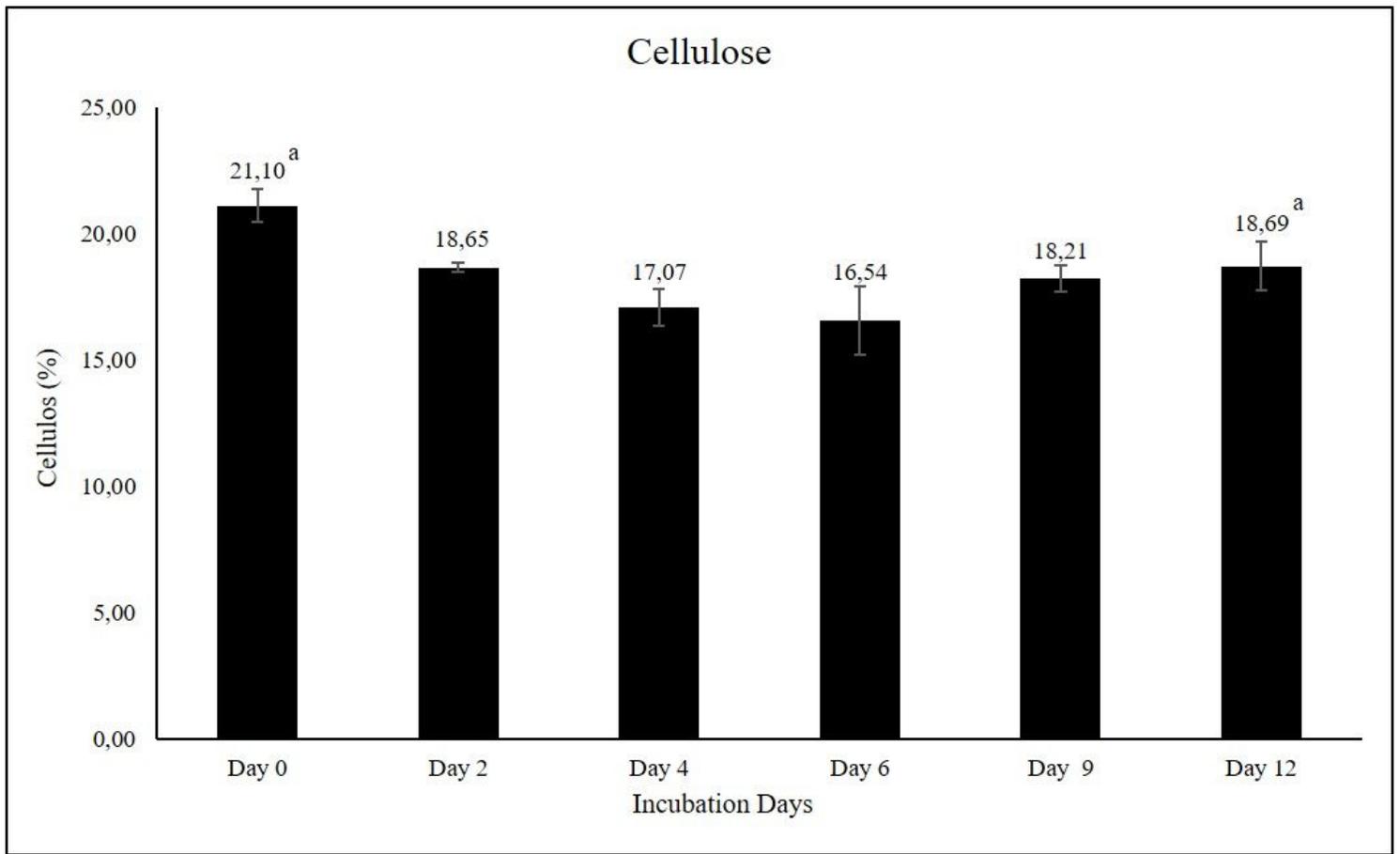


Figure 4

Evaluation of Cellulose content Differences between same symbols represent significance >5%. Statistical analysis was performed between Day 0 and Day 12

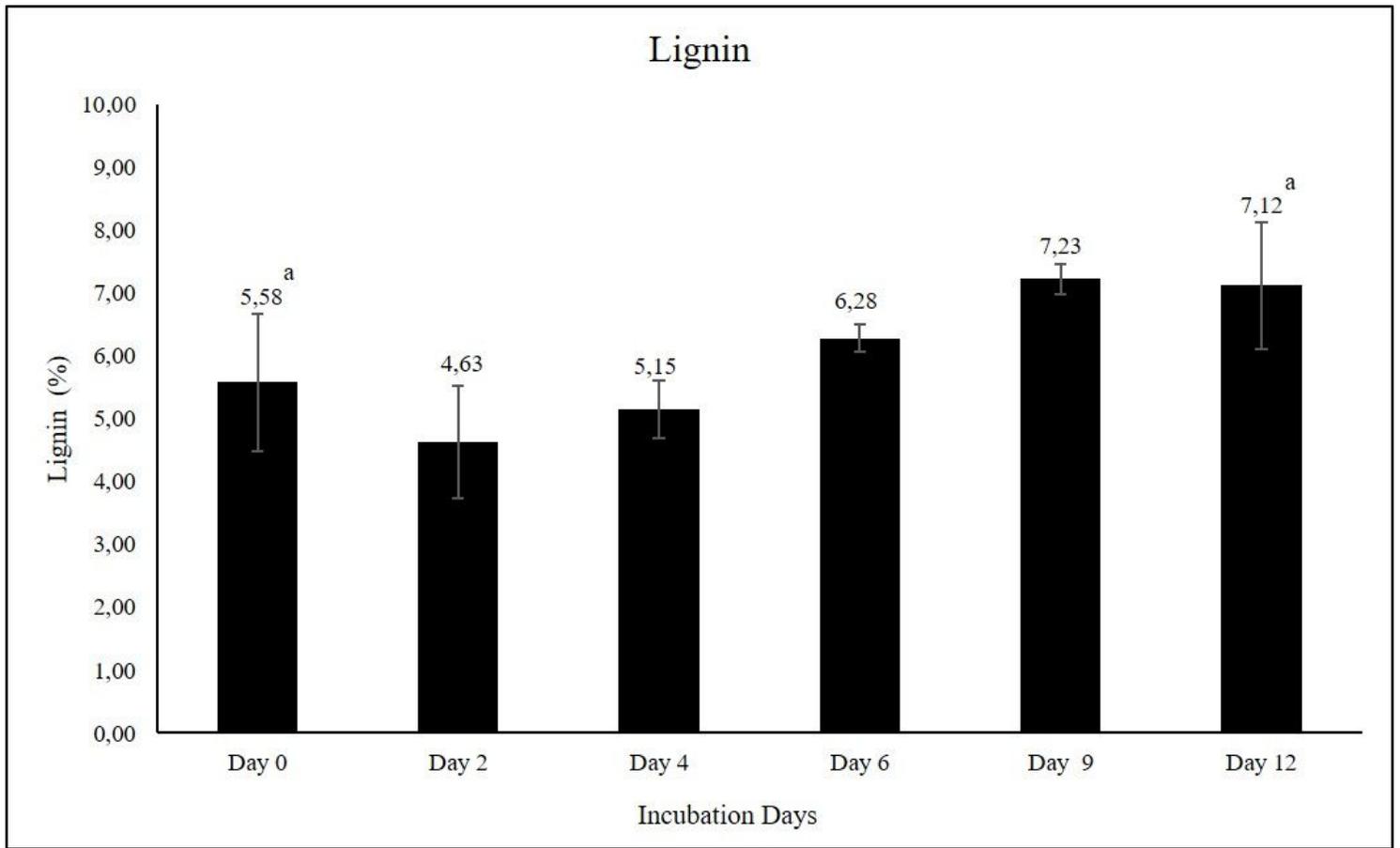


Figure 5

Evaluation of Lignin content Differences between same symbols represent significance >5%. Statistical analysis was performed between Day 0 and Day 12