Gossypol's degradation via Solid-State Fermentation initiated by *Pleurotus ostreatus* in the mixture of Cotton Seed Cake with *Lathyrus clymenum* pericarp

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Keywords: Gossypol, *Pleurotus ostreatus*, Protein enrichment, Solid-State Fermentation, Feedstuff.

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Introduction

Cotton *(Gossypium hirsutum L.)* is widely famous for its valuable fiber content. It is an annual plant which is cultivated in more than 75 countries worldwide, covering an area that exceeds 32.4 million hectares. Cottonseed Cake (CSC) is considered as one of the major by-product of cotton's cultivation which is derived during its process. The main aggravating factor for CSC's exploitation is the presence of a toxic compound in its chemical content, which is called gossypol. CSC could be utilized as a feed supplement in ruminants' diets; however, its adoption by non-ruminants is limited due to the high content of gossypol. Gossypol is a polyphenolic compound, divided into Free and Bound form, which both constitute Total Gossypol and play a key role in cotton's self-defense mechanism since latter is able to protect the crop from insects, pests, and potentially from some diseases (Shaikh et al., 2014).

Legumes that include *Lathyrus* species are categorized into the Fabaceae family and are well known for their rich nutritional content since their seeds are highlighted by the high presence of proteins and minerals. *Lathyrus clymenum* forms an annual plant that is mainly located in the Aegean islands (Santorini, Karpathos, and Anafi). The pericarp (LCP) forms the major by-product that is generated during its cultivation. However, its nutritional value is characterized as poor due to the low protein content, digestibility, and poor palatability (Eliopoulos et al., 2022).

The application of Solid-State fermentation (SSF) seems to be an efficient and eco-friendly strategy for the reduction of gossypol levels and the nutritional upgrade of the respective substrate. Therefore, the employment of suitable fungi such as *Pleurotus ostreatus,* which is characterized as a lignocellulosic degrader, is crucial since it can be used as a potent means in order to deal with the aforementioned nutritional and toxicity issues.

The main objective of the present study concerns the development of a substrate by mixing CSC and LCP in a ratio of 80-20% w/w in order to apply SSF procedure initiated by *P. ostreatus*, targeting its nutritional upgrade and gossypol's reduction for the production of a novel proteinaceous animal feed supplement in the context of circular economy.

Materials and Methods

The raw materials were hydrated by adding tap water to obtain the optimum moisture content. Subsequently, the investigated substrate was prepared in triplicates by mixing CSC with LCP in a ratio of 80%-20% w/w in a final weight of 300g. The developed substrate was placed into glass test vessels and subjected to sterilization at 121°C for 15 minutes. Then, the sample was inoculated with *P. ostreatus* at a ratio of 5% w/w and incubated at 25°C in the dark for 10 days. The prepared substrate was examined concerning its moisture, protein, ash content, total and reducing water-soluble sugars, crude fiber substances, cellulose's presence, and lignin at the beginning (Day 0) and at the end of the process (Day 11). Furthermore, 1,3-1,6 β-glucans were evaluated with the Megazyme commercial kit enzymatic assay kit. Finally, Total and Free gossypol content were determined by performing HPLC analysis according to Hron et al. (1990). A statistical analysis was performed for the evaluation of the results, which were expressed as means ± standard deviation (±S.D.). Kolmogorov-Smirnov and Shapiro-Wilk tests were applied in order to determine data normality. The differences between the groups were analyzed by independent and paired *t*-test ($p \leq 0.05$ was considered significant).

Results and Discussion

Table1. Impact of SSF on the physicochemical composition of the examined substrate

Parameters (%)	Day 0	Day 11
Moisture	58.86 ± 0.89^{a}	68.18 ± 1.05^{b}
Total Soluble Sugars (TSS)	2.53 ± 0.19^{c}	4.39 ± 0.02^{d}
Reducing Soluble Sugars (RSS)	0.68 ± 0.10^{e}	1.57 ± 0.04^{f}
Ash	4.72 ± 0.06^{g}	8.03 ± 0.65^{h}

Proteins	17.67±0.91[i]	23.84±0.42[j]
1,3-1,6 β-glucans	0.38±0.12[k]	2.16±0.25[l]
Crude Fiber Substances	35.13±1.23	37.79±1.04
Cellulose	30.64±0.96	31.95±0.93
Lignin	19.20±0.86[a]	14.07±0.46[b]

Different superscripts indicate statistical significance $p \leq 0.05$.

According to Table 1, moisture and ash content were statistically significant increased ($p \leq 0.05$) during the procedure by 15.83% and 70.12%, respectively. Moisture is a crucial factor for the successful implementation of SSF, since latter's optimum levels facilitate nutrients' solubility and accessibility by the microorganisms, securing the ability for a proper fungal growth. Ash's increased presence at Day 11 can be rationalized by the organic matter's reduction and can be used as an indicator of minerals content. TSS were found to be statistically significant increased ($p \leq 0.05$) by 73.51%, whereas RSS presented an increment exceeding two-folds. The increased presence of TSS and RSS at the end of SSF could possibly be explained by the excessive secretion of the enzymes that participate in the degradation process. The presence of 1,3-1,6 β-glucans revealed a statistically significant increment ($p \leq 0.05$) with their content ranging from 0.38% to 2.16% at Day 11. The enhanced concentration of 1,3-1,6 β-glucans at Day 11 indicates *P. ostreatus'* biochemical ability to produce new organic compounds by reaping the benefits of the substrate's nutrients. Proteins were found to be significantly increased ($p \leq 0.05$) at the end of SSF by 34.91%. The observed increase in protein levels could be a result of the fungal biomass accumulation as well as could be rationalized by the uptake of nitrogen excess that can be modified into proteins via aerobic fermentation. Crude Fiber Substances and cellulose content recorded a slight increase at the end of SSF by 7.57% and 4.27%, respectively. On the other hand, lignin displayed a statistically significant reduction ($p \leq 0.05$) ranging from 19.20% to 14.07% at Day 11. Crude Fiber Substances afforded an increment at the end of the procedure presumably due to the parallel increase cellulose content, whereas the elevated cellulose presence at Day 11 could be justified by the different fiber fractions of the employed materials. On the other hand, lignin revealed a different pattern since it was found to be decreased, probably due to *P. ostreatus*' hydrolytic activity, which indicates its efficacy to act as a degradation agent (Ritota and Manzi, 2019).

Table 2. Assessment of Gossypol on the studied substrate

Gossypol (ppm)	Day 0	Day 11
Total (TG)	628.72±11.09[a]	66.50±4.63[b]
Free (FG)	2.73±0.23	2.39±0.34

Different superscripts indicate statistical significance $p \leq 0.05$.

Table 2 presents the results that concern Total and Free gossypol levels. In specific, Total Gossypol afforded a statistically significant 9-fold reduction ($p \leq 0.05$) while Free Gossypol was slightly decreased by 12.45% at the end of SFF process. The reduced content for both TG and FG can be rationalized by the fact that the participating microorganism in SSF procedure, namely *P. ostreatus* secrete some gossypol-modifying enzymes that release Bound Gossypol facilitating FG's and TG's degradation (Shaikh et al., 2014)

Conclusions

This study highlighted SSF's ability to act as a degradation agent concerning the antinutritional factors of the examined mixture. Considering our results, SSF upgraded the nutritional profile of CSC-LCP (80-20% w/w) mixture with a parallel reduction of gossypol, transforming it into a proteinaceous animal feed. Overall, SSF is an environmentally acceptable method that contributes to the bioconversion of CSC-LCP into a feed supplement, evading the constraints of gossypol's toxicity with a parallel reduction of the environmental impact in the context of the circular economy.

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