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Proceedings

Inoculum production of *Monascus purpureus* with *Chenopodium quinoa* in submerged culture †

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Abstract: *Monascus purpureus* is widely used in different substrates for the production of secondary metabolites of interest; requiring an inoculum of easy handling. That is why this research provides information necessary to produce an inoculum of *M. purpureus* for future research. A maximum production of 34.83 mg of glucosamine/g dry weight was obtained under the conditions of NaCl 0.01%, pH 6.0 and agitation of 120 rpm ($p<0.05$), in addition to generating in the fungus an adaptation to the final medium which would give a rapid propagation in solid state fermentation with quinoa.

Keywords: *Monacus purpureus*; submerged culture; biomass; N-acetyl glucosamine; inoculum

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1. Introduction

Natural dyes reflect freshness and safety in food, as well as being indicators of aesthetic and sensory values, unlike synthetic dyes that have been linked to health problems, which is why the popularity of natural dyes is gaining strength among consumers [1]. One producer of natural pigments is *Monascus purpureus* that has been used in China for hundreds of years, which by solid fermentation is capable of producing food products through fermentation, which are used for food coloring, seasoning and preserving. The fungus also produces monacolin K that regulates the production of cholesterol in humans and animals, but also other undesirable metabolites such as citrinin that at high doses can become toxic [2].

On the other hand, quinoa has been cultivated since pre-Hispanic times in Peru, which has been considered by the FAO as a food of the future because of its good adaptability and its high protein content [3]. In addition to containing good levels of fatty acids, vitamins, minerals, dietary fibers, bioactive compounds and phenolic compounds, quinoa is a gluten-free pseudocereal, rich in protein for people with celiac sensitivity and disease [4].

Taking into consideration the natural components of *M. purpureus* and the nutritional source of quinoa, a new fermented food could be generated that has characteristics of both, but this requires a suitable inoculum and rapid propagation. Through the production of submerged culture, the mycelium production time is reduced and allows automation for industrial production. Many species of edible mushrooms produce their "seeds" by

submerged culture, such as *Flammulina velutipes*, *Hypsizigus marmoreus*, *Pleurotus eryngii* and *Lentinula edodes* [5]. Therefore, the objective of this research was to produce mycelial biomass of *M. purpureus* in submerged culture with quinoa flour as substrate at varying conditions of pH, agitation and NaCl concentration so that the best condition for inoculum preparation could be established for future research.

2. Materials and Methods

2.1. Strain of interest

M. purpureus 2955 strain was reactivated in potato dextrose broth, incubated for a period of 3 days. Later, the inoculum obtained was seeded in PDA medium and incubated for 5 days at 30°C. After the incubation time, cuts were made with a 5 mm diameter punch and seeded in the center of the medium formulated with quinoa, then incubated at 30°C until the fungus invaded the plate. These plates were refrigerated at 4°C to obtain the material necessary for the research.

2.2. Evaluation of the parameters rpm, pH and sodium chloride concentration.

In 250-mL flasks, 4 g of quinoa flour was added to 100 mL of distilled water with sodium chloride in different concentrations (0, 0.01, 0.05, 0.1 M), adjusted to different pH (5, 6, 7). Flasks were sterilized, and after cooled down at room temperature, 0.5 mL of inoculum was added and incubated with agitation of 100, 120, 140 rpm according to the treatment, at a constant temperature of 30°C for 5 days in darkness. After the time elapsed, the pellets formed were filtered and dried at 60 °C to a constant weight. The dried samples were ground and stored at 4°C until the respective analyses of N-acetylglucosamine concentration (mg) and pigments. The flasks were prepared in triplicate.

2.3. Quantification of N-acetylglucosamine.

Dry sample (0.1 g) was taken and submitted to acid hydrolysis (H_2SO_4 72%) for 4 hours, then diluted to 3% and kept at 100°C for 2 hours. It was cooled down and neutralized with NaOH 10 N, then centrifuged. A volume of 0.5 mL of the supernatant was taken and 0.25 ml of solution A (4% (v/v) acetylacetone in 1.25 N sodium carbonate) was added, left at 90 °C for 1 hour. It was cooled and 0.8 mL of 96% ethanol was taken, and added to 0.25 ml of Ehrlich's Reagent. The amount of N-acetylglucosamine was estimated from the calibration curve of the N-acetylglucosamine standard.

2.4. Statistical analysis

The results for N-Acetylglucosamine (mg/g) were analysed by ANOVA using the software Statgraphics 19 (Statpoint Technologies Inc, EE. UU.). The means were analysed by the Tukey's test, considering a significance level of $P<0.05$ throughout the study.

3. Results and Discussions

Table 1 presents the results from the analysis of variance (ANOVA) and Tukey's test, showing significant differences in mean effects and interactions.

All F-ratios are based on the residual mean square error. Since seven p-values are less than 0.05, these factors have a statistically significant effect on N-acetylglucosamine (N-AcG) at the 95.0% confidence level.

Table 1. Analysis of Variance for N-AcG (mg/g) - Type III Sums of Squares.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN EFFECTS					
A:rpm	100.82	2	50.41	855.15	0.0000
B:pH	5.11	2	2.56	43.42	0.0000
C:NaCl (M)	164.28	3	54.76	928.97	0.0000

INTERACTIONS					
AB	19.52	4	4.88	82.82	0.0000
AC	1.12	6	0.186	3.17	0.0081
BC	7.45	6	1.240	21.05	0.0000
ABC	12.03	12	1.003	17.02	0.0000
RESIDUAL	4.24	72	0.059		
TOTAL (CORRECTED)	314.60	107			

Table 2. Multiple Range Tests for N-AcG (mg/g) by agitation rpm.

RPM	Count	LS Mean	LS Sigma	Homogeneous Groups
100	36	29.9755	0.0404651	X
140	36	31.964	0.0404651	X
120	36	32.081	0.0404651	X

Method: 95.0 percent Tukey HSD.

In Table 2, two homogeneous groups are identified by columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Tukey's honestly significant difference (HSD) procedure. With this method, there is a 5.0% risk of calling one or more pairs significantly different when their actual difference equals 0. There are no significant differences between the rpm of 120 and 140; therefore, the use of 120 is considered to be favorable in energy saving and to avoid fungus stress. Furthermore, there is no significant difference between pH 5 and 6 (Table 3); therefore pH 6 was chosen as it is the closest value to the initial pH (~5.86) of the formulated culture medium, which would imply less time and cost for future scale-up.

Table 3. Multiple Range Tests for N-AcG (mg/g) by pH.

pH	Count	LS Mean	LS Sigma	Homogeneous Groups
7	36	31.0329	0.0404651	X
5	36	31.4769	0.0404651	X
6	36	31.5107	0.0404651	X

Method: 95.0 percent Tukey HSD.

Table 4. Multiple Range Tests for N-AcG (mg/g) by NaCl (M).

NaCl (M)	Count	LS Mean	LS Sigma	Homogeneous Groups
0.10	27	29.7666	0.046725	X
0.05	27	30.5383	0.046725	X
0.0?	27	32.2596	0.046725	X
0.01	27	32.7961	0.046725	X

Method: 95.0 percent Tukey HSD.

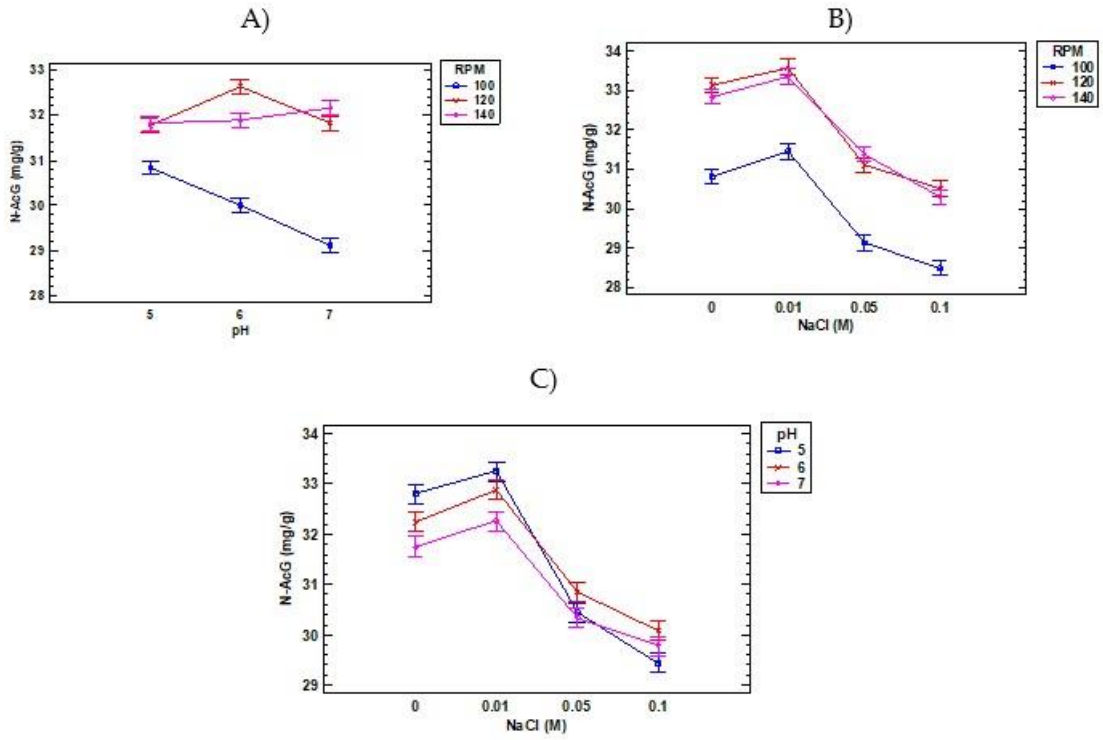

Figure 1. Interactions and 95.0 Percent Tukey HSD Intervals. The figure shows the interactions of pH and rpm A), NaCl and rpm B), NaCl and pH C) in relation to N-AcG production in mg/g dry matter.

In the interaction of pH and NaCl it can be seen that the highest production of N-AcG was at 120 rpm at pH 6 while the lowest production occurred at all pH (5, 6, 7) at 100 rpm (Figure 1A). In the interaction of NaCl and rpm, a decrease in the production of N-AcG can be seen from a concentration of NaCl higher than 0.5 M (Figure 1B). Similarly, in the interaction NaCl and pH, a concentration greater than 0. 05 M decreases the production of N-AcG at all pH levels (Figure 1C). This is because a concentration higher than 0.05 M is detrimental to the fungus causing a salt stress that alters its metabolism, thereby decreasing the production of N-AcG. On the other hand, at a concentration lower than or equal to 0.01 M the fungus can produce a greater amount of N-AcG being favorable at pH 6 and an rpm of 120.

4. Conclusions

These results demonstrate that a suitable inoculum with a considerable amount of mycelium can be generated in a reduced space and in a short time for cultures that have quinoa as food matrix. A maximum production of 34.83 mg of glucosamine/g dry weight ($p<0.05$) was produced under the conditions of NaCl 0.01%, pH 6 and at 120 rpm of agitation.

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References

1. Davoudi, H. M.; Shahidi, F.; Tabatabaei F. Y.; Sarabi, M. J; Eshaghi, Z. Biological detoxification of *Monascus purpureus* pigments by heat-treated Saccharomyces cerevisiae. *Journal of the Science of Food and Agriculture* **2019**, *99(9)*, 4439-4444.
2. Saithong, P.; Chitisankul, WT.; Nitipan, S. Comparative study of red yeast rice with high monacolin K, low citrinin concentration and pigments in white rice and brown rice. *Czech J. Food Sci.* **2019**, *37*, 75–80.
3. Arzapalo, DQ; Huamán, CK; Quispe SM; Espinoza SC. Extraction and characterization of the starch of three varieties of quinoa (*Chenopodium quinoa* Willd) black collana, pasakalla red and white junin. *Journal of the Chemical Society of Peru* **2015**, *81 (1)*, 44-54.
4. Pellegrini, M; Lucas, GR; Ricci, A; Fontecha, J; Fernández, LJ; Pérez, JA; Viuda, MM. **2018**. Chemical, fatty acid, polyphenolic profile, techno-functional and antioxidant properties of flours obtained from quinoa (*Chenopodium quinoa* Willd) seeds. *Industrial Crops and Products* **2018**, *111(1)*, 38-46.
5. Ma, L; Lin, YQ; Yang, C; Ying, ZH; Jiang, XL**.** Production of liquid seed of an edible fungus, Sparassis latifolia by submerged fermentation and mycelium growth in pine wood sawdust. *Scientia horticulturae* **2016**, *209(1)*, 22-30.