



Effect of Fish Hydrolysate and Sodium Chloride on the Colour of Quinoa Flour Fermented by *Monascus purpureus*[†]

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Abstract: *Monascus purpureus* is a red pigment-producing fungus. In the present study, the effect of fish hydrolysate and sodium chloride on the colour of quinoa flour fermented with *M. purpureus* was analysed. The colour of each sample was evaluated in CIELAB space (L^* , a^* , b^*), in addition to the C:N ratio. The minimum fermentation time was eight days and the values of L^* (44.66 ± 0.532), a^* (20.27 ± 0.323), b^* (17.89 ± 1.342) and a C:N ratio of 11.05 ± 0.240 were obtained. Therefore, there is an effect of supplementation on increasing the red colour of the meal. This work opened up the possibility that red quinoa flour could be used as a raw material in the production of other food products.

Keywords: nitrogen source; salts; novel foods



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1. Introduction

In the food industry, pigments are used to restore the colour lost by processing or to improve the appearance of a product; however, there is a trend to replace artificial dyes with natural ones and a good alternative is the red pigments from the filamentous fungus *Monascus*, which have been used as food dyes for many years in Asia and the annual demand is progressively increasing [1].

In addition, these pigments exhibit biological activities such as anti-inflammatory, antimicrobial, anticancer and anti-obesity properties, as well as advantages for harvesting and their readiness for large-scale production [1].

Fish hydrolysate is an excellent and effective nitrogen source to be part of a culture medium as it allows the growth and pigment production of *M. purpureus* [2]. As well as sodium chloride, another supplement, it generates an osmotic stress that aids the pigment production of *M. purpureus*, inducing conidial formation at high concentrations [3].

The evaluation of the C:N ratio plays an important role in pigment production, and it is different for each product to be obtained and varies for each fungal strain [4].

In this sense, the research team focused on obtaining red-coloured quinoa seed flour from solid-state fermentation by *M. purpureus* in order to evaluate the effect of fish hydrolysate and sodium chloride on the colour of quinoa flour fermented by *Monascus purpureus*.

2. Materials and Methods

2.1. Preparation of Inoculum

The present inoculum preparation methodology was previously performed by our research group [5], where the filamentous fungus *M. purpureus* CECT 2955 from the Spanish Type Culture Collection (CECT) was used. The strain was resuspended and seeded on PDA (potato dextrose agar) in a Petri dish at 30 °C for 7 days, then seeded on QFH (quinoa flour agar), adjusted to pH 6 and incubated at 30 °C for 7 days. The spore suspension was obtained by adding 10 mL of Tween-80 (0.01%) per Petri dish and swabbing to detach the mycelium from the medium. Then, by filtration, the hyphae were removed, allowing only the collection of spores, shaken for 5 min, and spores were counted in a Neubauer chamber and adjusted to 1.0×10^6 spores/mL.

2.2. Solid-State Fermentation of Quinoa Seeds

Solid-state fermentation was carried out in 250 mL flasks containing quinoa grains (30 g) at four sodium chloride factor levels (0.05, 0.10, 0.20 and 0.40%) and with 1.0% fish hydrolysate. The pH was adjusted to 6, then sterilised at 121 °C for 15 min. 1 mL spore concentrate (1.0×10^6 spores/mL) was added to the flasks after cooling to room temperature. Each flask was covered with sterile absorbent cotton wool and shaken vigorously for good mixing, then placed in an incubator (ILW, Pol Eko, Poland) at 30 °C for 0, 2, 4, 6, 8, 10, 12 and 14 days.

2.3. Production of Fermented Quinoa Flour

Four flasks were randomly selected from the incubator according to fermentation time (0, 2, 4, 6, 8, 10, 12 and 14 days), and each treatment was dried at 65 °C for 24 h [6]. Pigmented quinoa flour samples were ground using a mill (CS-1000, SHANG-JUN, China).

2.4. Colourimetric Analysis of Flour Samples

The colour of fermented and red pigmented quinoa flours was analysed at 25 °C with a CM-5 colourimeter (Minolta Camera Co., Osaka, Japan), with a D65 light source and an observation angle of 10°. It was calibrated with a standard white plate before taking the measurements according to the manufacturer's instructions. One reading (shot) was taken for each sample placed inside the Petri dish, being four samples per treatment. The determinations were made in CIELAB colour space (L^* , a^* , b^*) and the results were expressed as L^* (lightness) from 0 (black) to 100 (white); a^* from $-a^*$ (green) to $+a^*$ (red) and b^* from $-b^*$ (blue) to $+b^*$ (yellow) [7].

2.5. Carbon:Nitrogen Ratio

The Walkley–Black method was used to analyse the samples of pigmented quinoa flour to obtain the amount of carbon, while the Kjeldahl method was used to determine the amount of nitrogen. Finally, the quotient of both results was used to obtain the C/N ratio.

2.6. Experimental Design and Statistical Analysis

An 8×4 factorial design was used, where the independent variables were fermentation days 0, 2, 4, 6, 8, 10, 12, 14 and sodium chloride concentrations 0.05, 0.10, 0.20, 0.40% with 4 replicates for each. Statistical analysis was performed with R software (version 4.1.0, R Foundation for Statistical Computing, Vienna, Austria).

3. Results and Discussions

The analysis of the flour samples fermented by *M. purpureus* is presented in Table 1 (L^* , a^* and b^*), obtained over 14 days. The analysis of lightness (L^*) ranges from 44.43 to 62.89, where the lowest values obtained were on days 8, 10 and 14 with 44.43 ± 2.933 , 46.07 ± 3.295 and 45.97 ± 2.867 , respectively, which did not show significant differences because the longer the fermentation time, the higher the pigment production, changing the initial L^* value (62.89 ± 0.987). The a^* values are found within the red colour range from

6.81 to 19.62, showing an increase as the fermentation time elapsed due to the increase in red pigments, being 19.03 ± 1.486 , 19.62 ± 1.179 and 19.51 ± 0.991 for days 6, 8 and 10, respectively, which presented the highest values without significant differences. The b^* values tend to decrease as time passes without showing significant differences on days 10, 12 and 14 with a value of 18.85 ± 1.136 , 18.32 ± 1.020 and 18.48 ± 1.109 , respectively, due to the decrease in the slightly yellow colour of the quinoa grains.

Table 1. CIELAB colourimetric analysis of pigments produced by *M. purpureus* grown on different fermentation days.

Fermentation Time ¹ (Days)	CIELAB Colour System ²		
	L*	a*	b*
0	62.89 ± 0.987^e	6.81 ± 0.137^a	28.13 ± 0.397^e
2	62.39 ± 1.528^e	8.11 ± 0.393^b	27.87 ± 0.802^e
4	57.70 ± 2.099^d	12.71 ± 2.013^c	25.74 ± 1.984^d
6	49.04 ± 2.551^c	19.03 ± 1.486^{ef}	21.27 ± 1.879^c
8	44.43 ± 2.933^a	19.62 ± 1.179^f	19.59 ± 1.765^b
10	46.07 ± 3.295^{ab}	19.51 ± 0.991^f	18.85 ± 1.136^{ab}
12	47.25 ± 3.501^{bc}	17.84 ± 0.875^d	18.32 ± 1.020^a
14	45.97 ± 2.867^{ab}	18.32 ± 1.294^{de}	18.48 ± 1.109^{ab}

¹ Fermentation day with four levels of sodium chloride concentrations. ² Mean \pm standard deviation (SD). Black letters (a–e) represent statistically significant differences ($p < 0.05$).

The samples analysed are influenced by the supplementation of fish hydrolysate, which is a source of nitrogen widely used in the preparation of different culture media in microbiology [8], which in turn generates a higher pigment production of *M. purpureus* [2]. Among the values shown for L^* , a^* and b^* , the most important is a^* , as it is related to the red colour in the production of pigmented meal in the present investigation. Therefore, taking into account the values that did not show significant differences and the time–cost relationship of fermentation, it was considered to stop the process on the eighth day.

Table 2 shows the values obtained on day 8 by varying the sodium chloride concentrations, where the L^* measurements with the concentrations of 0.20 and 0.05% gave the lowest values of 40.84 ± 2.469 and 44.66 ± 0.532 , respectively. For the determination of a^* , the concentrations of 0.05 and 0.2% gave the highest values linked to the red colour of 20.27 ± 0.323 and 20.88 ± 0.112 , respectively. In the determination of b^* , the concentrations of 0.05 and 0.2% presented the lowest values with 17.89 ± 1.342 and 18.49 ± 1.343 , respectively. The values obtained for L^* , a^* and b^* on the eighth day show that the minimum concentration in common is 0.05%, making it important to decrease the use of this supplement in the fermentation cost. In different investigations, it has been shown that salt concentration decreases or increases pigment production in *M. purpureus*, taking into account that a low salt concentration favours pigment production, while a high concentration generates osmotic stress in the fungus, decreasing its production [9].

Table 2 also shows that at a concentration of 0.05% sodium chloride, day 8 had the lowest C:N ratio with a value of 11.05 ± 0.240 . It is known that the C:N ratio is of utmost importance since in culture media, it is an indispensable factor to be taken into account for the production and yield of secondary metabolites, including pigments [10].

Table 2. CIELAB system colourimetric analysis of pigments produced by *M. purpureus* at different sodium chloride concentrations on the eighth day of fermentation.

Sodium Chloride (%)	CIELAB Colour System ¹			C/N Ratio
	L*	a*	b*	
0.05	44.66 ± 0.532 ^{ab}	20.27 ± 0.323 ^{bc}	17.89 ± 1.342 ^a	11.05 ± 0.240 ^a
0.1	45.46 ± 2.403 ^b	18.82 ± 1.268 ^{ab}	21.37 ± 0.645 ^c	11.04 ± 0.176 ^a
0.2	40.84 ± 2.469 ^a	20.88 ± 0.112 ^c	18.49 ± 1.343 ^{ab}	13.32 ± 0.329 ^b
0.4	46.77 ± 2.209 ^b	18.53 ± 0.312 ^a	20.62 ± 0.689 ^{bc}	12.99 ± 1.124 ^b

¹ Mean ± standard deviation (SD). Black letters (a–e) represent statistically significant differences ($p < 0.05$).

4. Conclusions

Fish hydrolysate and sodium chloride have an effect on the increase in the red colour of quinoa flour fermented by *M. purpureus* at a concentration of 0.05% sodium chloride and 1.00% fish hydrolysate. The best results were obtained on the eighth day, with the highest values for a* (20.27 ± 0.323) and the lowest values for L* (44.66 ± 0.532) and b* (17.89 ± 1.342) with a C:N ratio of 11.05 ± 0.240, which showed that the parameters varied over the fermentation time. This work opened up the possibility that quinoa flour could be used as a raw material pigmented by this fungus in the production of other food products.

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