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Proceeding Paper

Colorimetric Evaluation of Quinoa Flour Fermented by *Monascus purpureus* Enriched with Monosodium Glutamate and Sodium Chloride †

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Abstract: In the present study, the colour and C:N ratio of quinoa flours pigmented with *M. purpureus* supplemented with monosodium glutamate and sodium chloride were evaluated during 14 days of fermentation. The best values of L*, a* and b*corresponded to the eighth day. This research showed that the pigmented flour produced by solid-state fermentation of quinoa by *M. purpureus* showed variations in red colour along with the C:N ratio during the fermentation time, resulting in a product with good visual sensory attribute that can be used to develop new naturally pigmented products with potential functional characteristics.

Keywords: *Chenopodium quinoa*; CIELAB; pseudocereal; carbon:nitrogen ratio

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1. Introduction

The source of natural pigments from microorganisms such as bacteria, algae and filamentous fungi are good alternatives because their manipulation is more controllable and they have a tendency for large-scale production. Among the producing microorganisms, fungi are considered to be the best source due to their ability to synthesise more soluble and stable pigments [1].

Monascus is a fungus widely used in Asian countries because it produces secondary metabolites of interest in the food industry, the most important of which are the red pigments that serve to colour foods and improve their appearance [2,3]. They also have good antioxidant, anti-inflammatory and anticarcinogenic properties, which is why they have received widespread attention in solid fermentation studies [3].

Nitrogen source such as monosodium glutamate is exploited by *Monascus*, which is used to evaluate the pigment synthesis kinetics and mycelial development of the fungus, where its addition in solid state fermentation enhances the production of water-soluble pigments [4,5]. Additionally, sodium chloride, a common salt compound, produces significant changes in red pigment production and growth of *Monascus* [6]. Therefore, it was considered important in this research to evaluate the colour of quinoa flour fermented by *M. purpureus* supplemented with monosodium glutamate and sodium chloride.

2. Materials and Methods

2.1. Strain Monascus purpureus CECT 2955

The *M. purpureus* strain was acquired from the Spanish Type Culture Collection (CECT). It was previously resuspended and seeded in PDA (Potato Dextrose Agar) at 30 °C for 7 days, then seeded in QFA (Quinoa Flour Agar) at pH 6, then incubated at 30 °C for 7 days. A stock inoculum containing a suspension of 1.0×10^6 spores/mL was prepared and used for solid state fermentation [7].

2.2. Fermentation of quinoa grains by M. purpureus

Quinoa was used as substrate where 30 g of quinoa grains, NaCl at 0.05, 0.10, 0.20 and 0.40% (*w*/*w*) and monosodium glutamate at 1.0% (w/w) were added with 25 mL of distilled water per flask; it was sterilised in an autoclave (PRESOCLAVE III 80, J.P. SELECTA, s.a., Spain) at 121 °C for 15 min. Fermentation of the quinoa grains was carried out by adding 1 mL of *M. purpureus* spore suspension to the sterile substrate previously cooled to room temperature. It was placed in an incubator (ILW, Pol Eko, Poland) at 30 °C for 0, 2, 4, 6, 8, 10, 12 and 14 days. At the end of the fermentation time, each treatment was dried at 65 °C until a constant weight was reached. It was then milled to obtain pigmented quinoa flour.

2.3. Colorimetric analysis of CIELAB

The evaluations were carried out in CIELAB (L*, a*, b*) colour space [8] where the colour of red fermented quinoa flours was analysed at room temperature with a CM-5 colorimeter (Minolta Camera Co., Osaka, Japan), with a D65 light source and an observation angle of 10°. A standard white plate was used for calibration before measurements were taken. One reading was taken per sample placed inside the plate for four replicates per treatment.

2.4. Determination of the C:N Ratio

The carbon and nitrogen content were determined by the Walkley-Black and Kjeldahl methods respectively, where the samples were the fermented quinoa flours previously dried for analysis. The results were divided to find the C:N ratio. The analysis was done for days 0, 2, 4, 6, 8, 10, 12 and 14 at four levels of sodium chloride.

2.5. Statistical Analysis

For the statistical analysis, the software R (version 4.1.0, R Foundation for Statistical Computing, Vienna, Austria) was used. An 8 × 4 factorial arrangement was used with the independent variables being the days of fermentation (0, 2, 4, 6, 8, 10, 12 and 14) and the concentration of sodium chloride (0.05, 0.01, 0.20 and 0.40%).

3. Results and Discussions

The determination of L*, a* and b* values for flour samples fermented by *M. purpureus* using sodium chloride and monosodium glutamate during a 14-day incubation period are shown in Table 1. The values obtained for the lightness (L*) ranged from 45.95 to 64.24, showing that on days 8, 10, 12 and 14 the lowest values were obtained as 45.95 ± 2.334, 46.33 ± 1.950, 46.13 ± 2.819 and 46.97 ± 2.335 respectively, the above results showed no significant differences. The lightness of the flour samples decreases due to the fermentative process of the fungus where the production of secondary metabolites such as pigments increases. This production of pigments, mainly red, also caused a* values to vary between 6.52 and 19.79 belonging to the red colour range (+a*), where the highest values were obtained on days 6, 8 and 10 with a mean of 19.33 ± 1.026, 19.79 ± 1.064 and 19.17 ± 0.985 respectively with no significant differences between them. These values are favoured by the nitrogen source (monosodium glutamate) which is related to a higher production

of red pigments [9], solid-state fermentation is also considered to improve the production of red pigments [4,10]. On the other hand, the values obtained for b* were located in the yellow colour zone (+b*) between 18.95 and 27.78, with the lowest values on days 12 and 14, with a mean of 18.75 ± 0.770 and 18.95 ± 0.999 respectively, showing no significant differences, where the initial yellow colour of the quinoa began to decrease due to the production of pigments in the *Monascus* fermentation.

The values indicated did not show significant differences between the results of L*, a* and b*, a* being the most important within the results as it is related to the red colour. It is necessary to consider the fermentation time in the production of this sensory attribute, so day 8 was considered as the minimum time to stop the fermentation of the quinoa grains, obtaining a substrate with good colour.

Table 1. Colorimetric characteristics of *M. purpureus* pigments on different fermentation days.

Fermentation Time [1]	CIELAB Colour System [2]		
(Day)	L*	a*	b*
0	64.24 ± 1.457 ^{e}	6.52 ± 0.290 ^{a}	27.72 ± 0.205 ^{e}
2	61.34 ± 1.650 ^{d}	7.80 ± 0.408 ^{b}	27.78 ± 0.632 ^{e}
4	55.93 ± 2.200 ^{c}	14.25 ± 0.868 ^{c}	25.65 ± 0.984 ^{d}
6	49.14 ± 2.725 ^{b}	19.33 ± 1.026 ^{ef}	21.99 ± 1.442 ^{c}
8	45.95 ± 2.334 ^{a}	19.79 ± 1.064 ^{f}	20.28 ± 0.857 ^{b}
10	46.33 ± 1.950 ^{a}	19.17 ± 0.985 ^{ef}	19.99 ± 1.079 ^{b}
12	46.13 ± 2.819 ^{a}	18.50 ± 1.211 ^{de}	18.75 ± 0.770 ^{a}
14	46.97 ± 2.335 ^{a}	17.93 ± 0.865 ^{d}	18.95 ± 0.999 ^{a}

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

The data obtained in the fermentation at day 8 with sodium chloride at different percentages were analyzed and are shown in Table 2. The data for lightness (L*) were found in a range from 44.09 to 48.48, being the concentrations of 0.10, 0.20, 0.40% with a value of 44.09 ± 3.146, 45.70 ± 0.890 and 45.53 ± 1.615 the ones that presented a lower value without presenting significant differences. The results obtained for a* were positive, that is to say that they are oriented to the red color, and showed no significant differences for any of the concentrations used such as 0.05, 0.10, 0.20 and 0.40% of sodium chloride, obtaining 19.85 ± 1.174, 19.51 ± 0.198, 20.47 ± 0.289 and 19.35 ± 1.780, respectively. The results obtained for b* show that at 0.05, 0.20 and 0.40% sodium chloride were the lowest values with a mean of 19.90 ± 0.775 ± 1.174, 19.56 ± 0.349 and 20.39 ± 0.638 respectively with no significant differences.

The values shown in Table 2 indicate that sodium chloride concentration did not affect red color (+a*), but did affect lightness (L*) and yellow color (+b*). The variation of L* and b* were the result of the effect of osmotic stress on pigment production of *M. purpureus* [6]. Considering that the research is focused on the production of a red flour with fermentation supplements, the lowest concentration of sodium chloride (0.05%) was considered due to the cost-performance.

At a concentration of 0.05% sodium chloride on day 8 the lowest C:N ratio was produced with a mean value of 11.31 ± 0.258, the C:N ratio is of great importance for pigment production considering that an amount higher than 20 could give a greater intensity to the red colouring, although the production of the dyes will also depend on the strain and other factors [11].

Table 2. Colour characteristics of *M. purpureus* pigments 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	48.48 ± 0.713 [b]	19.85 ± 1.174 [a]	19.90 ± 0.775 [a]	11.31 ± 0.258 [a]
0.1	44.09 ± 3.146 [a]	19.51 ± 0.198 [a]	21.26 ± 0.592 [b]	11.95 ± 0.313 [b]
0.2	45.70 ± 0.890 [ab]	20.47 ± 0.289 [a]	19.56 ± 0.349 [a]	12.56 ± 0.199 [c]
0.4	45.53 ± 1.615 [ab]	19.35 ± 1.780 [a]	20.39 ± 0.638 [ab]	13.14 ± 0.248 [d]

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

4. Conclusions

This research showed that the pigmented flour produced by solid-state fermentation of quinoa by *M. purpureus* supplemented with monosodium glutamate and sodium chloride showed variation in the days of fermentation with respect to the red colour, with the eighth day being the appropriate time to stop fermentation, obtaining the values of L* (48.48 ± 0.713), a* (19.85 ± 1.174), b* (19.90 ± 0.775) and C:N (11.31 ± 0.258); resulting in a product with a good visual sensory attribute that can be used to develop new naturally pigmented products with possible functional characteristics.

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