A versatile platform for bioactive *Monascus* pigments: From waste-based biosynthesis to neuroprotective potential and food fortification

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Introduction

The growing preference for “clean-label” foods and concerns regarding synthetic additives have intensified the search for multifunctional natural colorants (Egea et al., 2023). *Monascus* pigments are prominent due to their diverse color range (yellow, orange, red), food matrix compatibility, and biological activities, including antioxidant and anti-inflammatory effects (Chaudhary et al., 2021). Utilizing agro-food side streams as fermentation substrates is a key circular bioeconomy strategy that addresses waste management while producing sustainable ingredients (Khatami et al., 2026). Although *Monascus* fungi effectively exploit nutrient-rich by-products in various fermentation systems: submerged (SmF) and liquid surface (LSF) fermentation, comparative studies evaluating their impact on specific pigment biosynthesis in a strain remain limited. Furthermore, there is a critical need for natural products targeting non-communicable diseases, like dementia and type 2 diabetes. While *Monascus* pigments are promising, their potential as enzyme inhibitors (AChE, BChE, MAO-A) or receptor agonists (PPAR-γ) is not fully characterized. This study provides an integrated assessment of *Monascus ruber* SRZ112-m22 pigments produced from wastes as multifunctional colorants with neuroprotective and antidiabetic potential, demonstrating their feasibility in bakery products.

Material and Methods

The tested fungus *Monascus ruber* SRZ112—m22 was selected among several gamma radiation mutant fungal strains based on their improved pigment productivity from our previous studies. WGS datasets were deposited in the SRA database under PRJNA1091857 for ONT and Illumina libraries. A total of six different by-products and wastes, namely linseed press cake (LPC), brewer’s spent grains (BSG), bread waste (BR), apple pomace (AP), sugar beet pulp (SBP), and acid cheese whey (CW) were used as solid substrates for testing the pigment profile production ability of *Monascus ruber* SRZ112—m22. To investigate the pigment biosynthesis capability of this strain using different agro-food side streams as substrates, pigment production was assessed under submerged (SmF) and liquid surface (LSF) fermentation systems. Pigments were quantified by HPLC analysis in comparison with *Monascus* pigments authentic standards (monascorubramin, rubropunctamine, monascorubrin, rubropunctatin, and monascin). Pigment were purified using puriFlash XS520Plus. The separated pigments were subjected to LC/ESI–MS/MS analysis to identify the chemical structure. The neuroprotective and anti-Alzheimer’s potential were evaluated by determining their AChE and BChE inhibitory activities. Antidepressant potential was evaluated by measuring MAO-A inhibitory activity. PPAR-γ agonist potential was used to assess the antidiabetic activity. Two types of sweet baked products were prepared: muffin-leavened by the addition of baking powder, and pastry rolls-leavened by yeast fermentation.

Results and Discussion

Our study evaluated a panel of food industry by-products and wastes as substrates for *Monascus* pigment production under submerged (SmF) and liquid surface fermentation (LSF). The side streams included bread residues (BR), brewer’s spent grain (BSG), apple pomace (AP), sugar beet pulp (SBP), linseed press cake (LPC) and acid cheese whey (CW). In the literature, all studies focused on the pigments collectively not on individual compounds. Therefore, our work focused on evaluating individual pigment molecules, such as monascorubramin, rubropunctamine, monascorubrin, rubropunctatin, and monascin (Figure 1). Regarding the effect of fermentation mode on the type of pigments, our results showed that the type of pigments was greatly affected by fermentation mode. In our study, LSF often led to distinct pigment spectra compared to SmF on the same substrate. These differences can be attributed to oxygen and nutrient gradients where LSF cultures are static, expose the fungal mat to air which may enhance oxidative steps in pigment biosynthesis. Our observation that SmF generally gave higher pigment titers is also consistent with *Monascus* often growing in pellet form in submerged culture. However, larger pellets can suffer oxygen/nutrient diffusion limits, lowering pigment yields, so carefully controlling morphology in SmF to favor smaller mycelial clumps could improve pigment production (Silbir and Goksungur 2019).

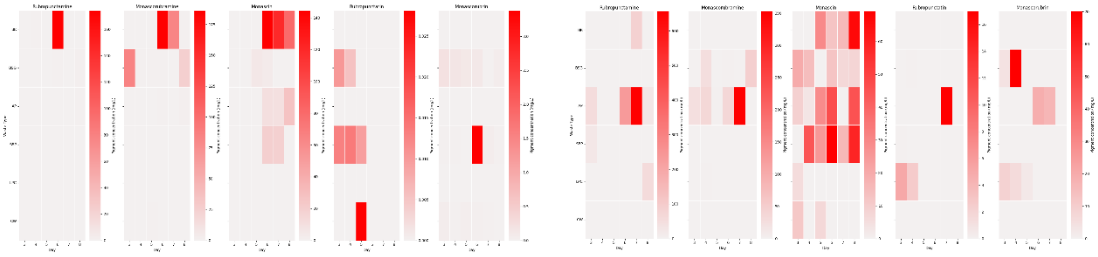

Figure 1. Pigment concentrations (mg/mL) of *Monascus* grown for different periods (day) on by-products and wastes (BR, AP, BSG, CW, LPC and SBP) under liquid surface (on left) and submerged (on right) fermentations.

Isothermal titration calorimetry of the red, yellow, and orange pigments revealed strong, micromolar-to-submicromolar binding of pigments to acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and monoamine oxidase A (MAO-A), as well as moderate affinity to PPAR-γ, indicating anti-Alzheimer's, antidepressant and antidiabetic potential. In our study the pigmentation of sweet baked goods was only slight which may suggest low concentration of the pigments in the post-cultivation liquid besides its strong red color (Figure 2). The aroma of the products was assessed by a panel five persons of which none reported any foreign or unpleasant smell of the product which suggests that the addition of red post-cultivation liquid does not contain significant levels of unwanted volatiles nor it do not yield high levels of unpleasant aroma during baking.

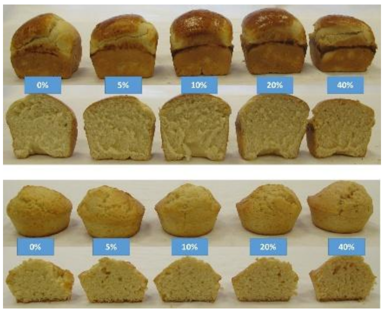

Figure 2. The prepared bakery products: pastry (upper) and muffin (lower) by different concentrations of *Monascus* post fermentation liquid.

Conclusions

In summary, the present study demonstrates that agro-industrial by-products, particularly brewer's spent grain, apple pomace and bread waste, are promising substrates for the targeted biosynthesis of structurally defined *Monascus* pigments under submerged and liquid fermentation modes. Our results concluded that fermentation mode and waste type can be used to steer pigment types towards red or yellow–orange compounds, enabling more flexible design of bio-colorant production processes. Selected pigments act as multi-target modulators of AChE, BChE, MAO-A and PPAR-γ, underscoring their potential as leads for neuroprotective, antidepressant and antidiabetic applications. Proof-of-concept incorporation of pigment-rich post-cultivation liquid into muffins and pastry rolls suggests that such process streams can be directly recycled into food formulations as natural color sources, although the relatively low pigment concentration, limited color impact. Together, these findings position *Monascus* fermentation on food wastes as a versatile platform for the production of natural colorants and bioactive metabolites, while emphasizing the need for further optimization of substrate pretreatment, process conditions, pigment recovery and food formulation strategies to fully exploit their functional and sustainability benefits.

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