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# The potential, strategies, and challenges of *Monascus* pigment for food application

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The demand for dyes from natural sources to substitute synthetic dyes for application in the food industry has been continuously increasing due to some synthetic dyes being associated with several problems, including hypersensitivity, carcinogenesis, and negative environmental impacts. Furthermore, dyes from natural sources (like pigments) are generally regarded by the consumer as safer or with fewer side effects—a fact that requires in-depth investigation—which increases the commercial interest in such products. In this sense, great focus has been given to the biotechnological potential of *Monascus* sp. to produce red, orange, and yellow pigments using different types of the fermentation process (submerged or in solid-state fermentation), substrates, and process parameters (temperature, pH, agitation, aeration, etc.), aiming at optimizing and reducing costs in pigment production. In general, *Monascus* pigment has shown stability at neutral and basic pH, at elevated temperatures for a few hours, and to some metallic ions while not showing stability at acidic pH, elevated temperatures for many hours, and in the presence of light. Applications of *Monascus* pigment with colorant function in foods (candies, bread, yogurt, cheese, beer, and meat products) reported improvement in the color aspect by sensory analysis. The application of *Monascus* pigment still seems promising and incipient, demonstrating that it needs to be further studied, mainly concerning the stability of the pigment *in vivo* systems (inside the food) where adverse conditions are combined. Regulatory issues are heterogeneous around the world, which creates difficulties to expand production and commercialization but also demonstrates the need for studies to confirm its safety. In this sense, this mini-review presents the potential, strategies, and challenges of *Monascus* pigment for food application.

## KEYWORDS

bioprocesses, monascorubramine, natural pigment, color, biological properties

## 1. Introduction

The global demand for natural pigments is constantly growing, a fact that place pressure on the sector to offer more products to meet this demand. In this sense, was estimated that the global pigment market—considering the demand from textiles, food and beverages, paints and coatings, and cosmetic industries— was USD 2.0 billion in 2021, reaching USD 2.8 billion by 2028. And this growth is also in line with the demand from the consumer for sustainable raw materials ([MarketWatch](https://www.marketwatch.com), 2022).

The use of pigments is part of human culture. This pigment application aims to (i) make the products where they are applied more attractive for consumption and (ii) afford, standardize, or intensify the color, providing identity to certain products, among others (Manan et al., 2017; Muthusamy et al., 2020). In the food manufacturing process and during storage, the original color can often be lost or degraded. Faced with this obstacle, the food industry resorts to the use of synthetic (synthesized/produced in a laboratory or industrial setting) or natural dyes (substances derived from natural sources) to correct or provide original color, improving the attractiveness of products for consumption and maintaining their visual identity (Dikshit and Tallapragada, 2018).

In this context, pigments from natural sources such as plants (flowers, fruits, and leaves), animals (insects), and microorganisms (fungi, bacteria, yeasts, and algae) stand out because they act as dyes (Sudhakar et al., 2016; Muthusamy et al., 2020; Aman Mohammadi et al., 2022). The increase in demand for pigments can be associated with the consumer market's perception that this type of product is safer and more environmentally friendly (Kobylewski and Jacobson, 2012). It is commonly reported that pigments are considered safe, non-toxic, non-carcinogenic, biodegradable, and of low risk to the environment (Wrolstad and Culver, 2012; Dikshit and Tallapragada, 2018) as they do not interfere with the aquatic biota, not showing an undesirable/harmful tendency toward allergic reactions, intolerances, in addition to other effects such as mutagenicity and potential carcinogenic effect (Dikshit and Tallapragada, 2018; Aman Mohammadi et al., 2022). However, like any other product available for use, natural pigments need to be extensively studied for toxicity and safety (Kobylewski and Jacobson, 2012), as several other widely used components have been banned due to proven adverse effects, including synthetic dyes such as ponceau, tartrazine, and sunset yellow, for example (Kobylewski and Jacobson, 2012; Poorniammal et al., 2021). Additionally, it is important to emphasize that legal aspects related to doses and approval of natural pigments use are important for feasibility and safety of use (Commission Regulation, 2014).

Thus, due to its high demand, the evaluation of toxicology, economical, and technologically viable new sources of natural pigments has increased. Depending on the type of pigment and the color that is necessary to assign to the food, the extraction of pigments of plant origin in high quantities may not be feasible, in addition to their limited application due to low thermal stability or at different pHs. Pigments of plant origin may not be available throughout the year and their products depend on environmental conditions, demand a large area of cultivation, and compete with vegetables that would be destined for human consumption (Sudhakar et al., 2016; Fernández-López et al., 2020; Poorniammal et al., 2021). In addition, the pigment extraction process often generates a large amount of waste material for the extraction of a small amount of pigments (Sharma et al., 2021).

The pigments produced by microorganisms have shown low production costs compared with plant pigments—especially when agro-industrial waste and by-products are used as a substrate for their production (Lemes et al., 2021, 2022). It is important to highlight that the question of costs for the use of by-products is still a challenge—but dependent on the type of by-product, seasonality, and quantity generated, as well as the process used, geographic location, among other factors—and need to be

overcome for complete viability of the process (Carvalho et al., 2023).

On the other hand, microbial pigments present higher yields, as well as simpler and cleaner extraction and purification processes compared with plant pigments. Pigments from microorganisms show no seasonal variation (as with pigments extracted from plant sources, for example), and demonstrate the possibility of improvements in the process to increase production yield and ease of scalability (Galaffu et al., 2015; Panesar et al., 2015; Aman Mohammadi et al., 2022; Bakhshi et al., 2022).

In addition to benefiting the coloring of products—and overcoming toxicological and regulatory issues—pigments of microorganisms can provide health benefits when consumed, as their composition contains bioactive compounds from the secondary metabolism of the producing organism itself, including alkaloids, phenols, flavonoids, polysaccharides, terpenes, and several other widely studied compounds. These bioactivities enable a reduction of the incidence of many diseases of cardiovascular origin, in addition to chronic degenerative diseases and cancer, in addition to acting as antioxidant and antimicrobial components (Lavecchia et al., 2013).

In this sense, among several microorganisms producing pigments, the natural pigment produced by *Monascus* sp.—which is used in products and processes of several countries specially in Asia—has gained huge prominence for food application (the advantages are presented in item 2), demonstrating the potential for the production of yellow, orange, and red pigments by use of different types of the fermentation (submerged or in solid-state fermentation), substrates (synthetic, natural and also by-products), and process parameters (temperature, pH, agitation, aeration, etc.). Furthermore, *Monascus* pigment has been associated with antioxidant, antiosteoporosis, anti-inflammatory, and antimicrobial, properties, as well as anti-diabetic, antidepressant, anticancer, antiobesity, neurocytoprotective, antihypertensive, and hepatoprotective effects (Feng et al., 2016; Agboyibor et al., 2018; Chaudhary et al., 2022). In this way, this mini-review presents the trend of production and application *Monascus* pigment in foods, as well as its strategies and challenges.

## 2. *Monascus* sp. and its pigment production potential

*Monascus* is a homothallic fungus related to the *Ascomycetes* class and *Monascaceae* family and are the famous producers of several important secondary metabolites, for example, pigments (Mahmoud et al., 2021). The genus *Monascus* is described as a filamentous, aerobic, saprophytic, prototrophic, mesophilic (optimum temperature of 30–35°C), xerophilic fungus, with respiration-fermentative metabolism (Feng et al., 2016; Agboyibor et al., 2018), and reproduction both sexually (asci) and asexual (conidia). Over glucose in the culture medium, microorganisms of this genus can form ethanol under aerobic conditions, and therefore can be classified as Crabtree negative with limited respiration (Crabtree, 1929).

*Monascus* fungus has 7 species belonging to the genus, but the most relevant species in the food industry are *Monascus purpureus*, *Monascus ruber*, and *Monascus pilosus* (Carvalho et al., 2005;

Agboyibor et al., 2018). The advantage of this genus over other microorganisms is due to the easy production of the pigment mainly on non-expensive substrates and this pigment presents characteristics such as bioactive properties as antioxidant and antimicrobial activities, good solubility in water and ethanol that facilitates the extraction step, and also its safety for consumption (Vendruscolo et al., 2016).

*Monascus* are filamentous fungi and can grow on simple substrates and produce different types of metabolites, including alcohols, vitamins, enzymes, polysaccharides, fatty acids, flavor compounds, flocculants, ketones, organic acids, and antioxidant, antibiotic, and antihypertensive agents (Vendruscolo et al., 2016). The growth and secondary metabolism of these microorganisms are directly influenced by several cofactors, such as the sources of carbon and nitrogen and conditions used to obtain the compound including pH, and temperature, among others (Chen and Johns, 1994; Huawei et al., 2019).

The secondary metabolites, like occur with natural pigments, produced by *M. purpureus* and *M. ruber* are extremely important and have an economic impact (Agboyibor et al., 2018; Chaudhary et al., 2022). Among the pigments produced by *Monascus* sp. are ankaflavin and monascin (yellow), monascorubrin and rubropunctatin (orange), and monascorubramine and rubropunctamine (purple-red) (Figure 1) (Kim and Ku, 2018). The pigments produced by *Monascus* sp. have a stereotype of azaphilones, which are a family of natural cyclic compounds that have at least one chiral center. These azaphilones have a great interest in demonstrating biological activities, which include adipogenesis and lipolysis (antiobesity effect), anticancer, and anti-inflammatory activities, as well as antidepressant, anti-osteoporosis, and anti-diabetic effects, and others (Gao et al., 2013; Agboyibor et al., 2018).

Obtaining pigments from fermentation using *Monascus* can be carried out by (i) solid-state fermentation and (ii) submerged fermentations. The (i) solid-state fermentation consists of cultivation with a low amount of water (De Carvalho et al., 2006; Zhang et al., 2013; Almeida et al., 2019) using a wide variety of agricultural substrates such as fruit pulp, grains, straw, and bagasse (Lemes et al., 2021). In addition, solid-state fermentation has low water content in the medium reducing contamination problems, and offers high volumetric productivity, concentrated target compounds, tolerance of high substrate concentration, and less waste and water generation (Lemes et al., 2021). On the other hand, solid-state fermentation presents difficulty in scale-up and control of process parameters such as pH, heat, moisture, and nutrient homogenization, among other, problems with heat build-up and higher impurity product, increasing the complexity of the recovery protocol due to the heterogeneity of the medium and complexity of the matrix used, impacting recovery and purification costs (Couto and Sanromán, 2006).

The (ii) submerged fermentation that are the microorganisms growing in a liquid medium with nutrients, which demonstrates the ease of homogenization and process control such as oxygenation, pH, and temperature (Agboyibor et al., 2019; Chai et al., 2020; Abdollahi et al., 2021; Abuthahir et al., 2021; Chen et al., 2021; de Almeida et al., 2021). In addition, the pigments are secreted into the fermentation medium and then recovered in a separation

step, such as centrifugation or using simple solvent extraction (Lemes et al., 2021). Furthermore, submerged fermentation allows the proper mixing of nutrients due to the high amount of free water and is a method of easy handling and scaling up. Nevertheless, the target products tend to be diluted at the end of fermentation (Lemes et al., 2021), but they can be easily solved using membrane concentration processes or a suitable technique for pigment processing (Vandanjon et al., 1999).

Regardless of the method chosen for fermentation, the availability of nutrients in the chosen medium and the fermentation conditions directly impact the synthesis of pigments by the species of fungus used, making it necessary to investigate and provide a broad interpretation of primary and secondary metabolism (Carvalho et al., 2005; Agboyibor et al., 2018).

Pigments obtained from the secondary metabolism of *Monascus* sp. are originates from medium-chain fatty acids, such as octanoic acid, which in turn are synthesized by the fatty acid metabolic pathway and bind to the chromophore structure through a transesterification reaction, resulting in the orange pigment (monascorubrin— $C_{23}H_{26}O_5$  or rubropunctatin— $C_{21}H_{22}O_5$  in trans-esterification with octanoic acid). The reduction of the orange pigment, monascorubramine, gives rise to the yellow pigment (ankaflavin— $C_{23}H_{30}O_5$  or monascin— $C_{21}H_{26}O_5$ ). The red pigments (monascorubramine— $C_{23}H_{27}NO_4$  and rubropunctamine— $C_{21}H_{23}NO_4$ ) are produced by the reaction of the orange pigment with compounds that contain  $NH_3$  and  $NH_2$  in the molecule (Feng et al., 2016; Chen et al., 2017).

Once produced, pigments need to be properly obtained, separated, and identified, since their value and application are directly related to purity degree (Mukherjee and Singh, 2011) and stability, which can be affected by factors such as light, oxygen, and metal ions presence, pH levels, as well as high temperatures (Vendruscolo et al., 2016; Abdollahi et al., 2021). Separation methods vary according to the location of the pigment (intracellular or extracellular) production and the need for use for each application. The ratio of intracellular and extracellular pigments produced by *Monascus* is dependent on fungus culture conditions, such as carbon, nitrogen, and pH sources. Therefore, conditions can be used to convert intracellular into extracellular pigments such as cultivation at pH 8.5 which reduces from 75 to 17% intracellular pigments (Orozco and Kilikian, 2008). As the aim of the industry is not normally to add steps in the production process, pigment extraction has been directed toward extracellular produced pigments. *Monascus* extracellular pigments can be obtained using appropriate organic solvents (Carvalho et al., 2005) and simple, low-cost, and easy physical methods considered environmentally friendly such as separation by centrifugation or filtration (Almeida et al., 2019; de Almeida et al., 2021). On the other hand, intracellular pigments—less frequently used due to their difficulty of extraction and low yield—need to be “released” using some method that promotes cell lysis (rupture of the cell wall) for subsequent separation, which can involve multiple steps as the use of specific organic solvents (Carvalho et al., 2005), ultrasound-assisted extraction (Kraboun et al., 2017), extractive fermentation with surfactant (Chen et al., 2018), ethanol/ammonium sulfate aqueous two-phase system (Dong et al., 2020), among several other procedures. Each of the operations used can significantly affect the

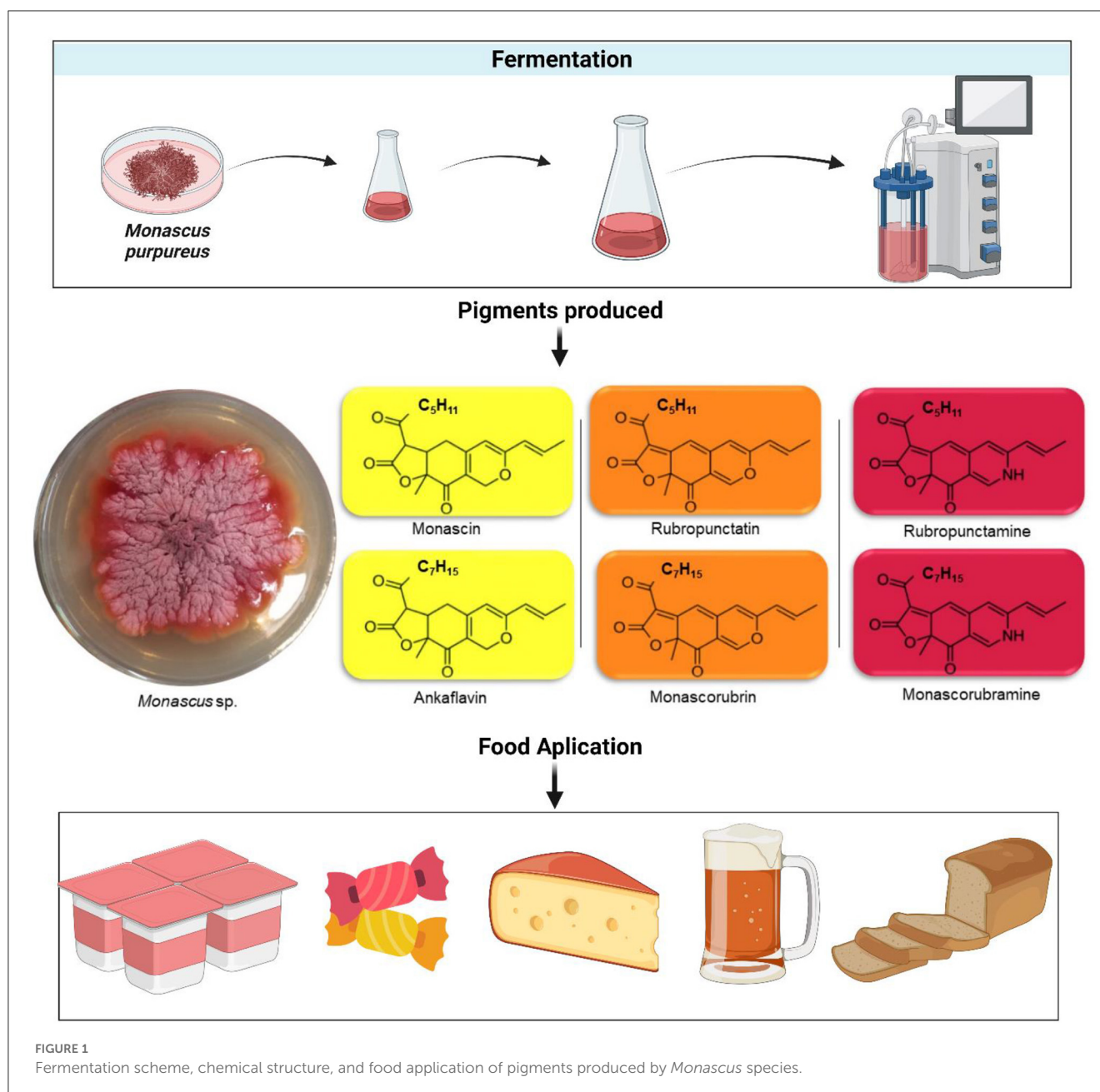


FIGURE 1  
Fermentation scheme, chemical structure, and food application of pigments produced by *Monascus* species.

yield, purity, and functional properties of the target compound (Gautério et al., 2022).

The methods of pigments identification and/or purification that may or may not be used for *Monascus* pigment are including ultraviolet (UV) spectrometer, luminescence, spectrophotodensitometer, nuclear magnetic resonance spectroscopy, enzyme immunoassay (ELISA), immunochromatographic tests, capillary zone electrophoresis, thin layer chromatography (TLC), mass spectrometry, capillary chromatography micellar electrokinetics, mass spectrometry, high-performance liquid chromatography (HPLC) with UV light, fluorescence (FLD), liquid chromatography-mass spectrometry (LC-MS), LC-MS/MS, LC-FLD, ultra-performance liquid chromatography-quadrupole/time-of-flight mass spectrometry

(UHPLC-DADQToF-MS), and gas chromatography-mass spectrometry (GC-MS). Among these, HPLC is the most used technique for the determination of 80% of organic compounds worldwide due to its precision and is considered the most effective in the detection of natural pigments (Liu and Chen, 2019; Singh and Mehta, 2020).

In some cases, a sequence or combination of the mentioned techniques can be used for separation and better identification of the pigments, allowing the complete characterization of the compound, as well as the verification of its purity and even its stability. These determinations are important to ensure standardization, comply with regulatory aspects in different countries, avoid adulterations, and even check for the presence of undesirable components, such as toxic components, among others,

allowing for proper and safe application in products. However, the application of *Monascus* pigment has also been carried out directly, that is, the fermented extract itself where the pigment was produced is added to the food product without the need for isolation and/or purification (as will be discussed in subitem 3).

### 3. *Monascus* pigment for food applications: Trends and challenges

The addition of natural pigments to food has several advantages, with the main purpose of stimulating and improving sensory perception, in addition to reducing the use of synthetic components, due to health and safety concerns (Egea and Marcionilio, 2022). In this sense, due to its production capacity, the *Monascus* pigment is often evaluated for its application in food and beverages, including dairy products, candies, bakery products, cereals, meat products, and beverages, among others (Table 1) (Mal'a et al., 2010; Gomah et al., 2017; Darwesh et al., 2020; Monteiro et al., 2021; Abdel-Raheam et al., 2022; Srianta et al., 2022).

With the advent of the plant-based market, dyes for application in meat analogs, especially red dyes, have been studied more to meet the needs of the industry. To our surprise, only two of the studies that we found tested *Monascus* pigment in meat products (Mal'a et al., 2010; Zheng et al., 2023). In meat analogs currently, the main colorings used are annatto extracts (E 160b), lycopene, beet juice extract, or leghemoglobin to imitate meat red color, and titanium dioxide to imitate chicken color. However, when using these dyes, ingredients to promote thermal stability must be added (Boukid, 2021; Ishaq et al., 2022).

As with any component applied to food, pigments are subject to changes triggered by the physical-chemical characteristics of the matrices, as well as by operating conditions (pH, acidity, temperature, salts, among others) during processing and shelf life, and may impact on pigment degradation, structural alteration, and even the loss of bioactive, technological, and sensorial capacity, mainly about its colorific capacity (Egea and Marcionilio, 2022; Oliveira Filho et al., 2023).

In this sense, the *Monascus* pigment has already had its stability evaluated under different conditions (Abdollahi et al., 2021; de Almeida et al., 2021), to allow its application and/or improvement of its stability to avoid changes in its properties (Vendruscolo et al., 2013; Xu et al., 2020a). Among the main limitations, related to its degradation, factors such as light, oxygen, metal ions, pH levels, and high temperatures are always highlighted (Agboyibor et al., 2018; Abdollahi et al., 2021), which vary according to the producing microorganism, way of obtaining, and method of extraction, among others.

Regarding pigment stability, it has also been reported that the stability is affected by different types of solvents during the extraction process, with an influence on yield, stability, and pigmentation capacity (Srianta et al., 2022). Furthermore, *Monascus* pigment does not completely lose pigmentation at temperatures up to 97.8°C (Abdollahi et al., 2021; da Silva et al., 2022), pH in the range of 3 to 8 (da Silva et al., 2022), presence of several components, including salts (2, 5%) (Abdollahi et al., 2021)

and solvents (Srianta et al., 2022), which corroborates and justifies its efficient application in different food matrices.

As reported in Table 1, the different *Monascus* pigments have already been produced in different presentations (liquid and powder) and applied in different products such as cheese, yogurt, lollipops, jellybeans, meat products, bread, and beers demonstrating an impact positive in color, attractiveness, uniformity of color, pigment stability, in addition to high sensory scores for all aspects evaluated (taste, color, odor, texture, and total acceptance). In addition, it was verified that the addition of pigment confers the presence of bioactive substances, mainly phenolic compounds, with special antioxidant and antimicrobial activities. In all the cases cited, the added pigment seemed to withstand the process conditions, not being degraded to the point of making its application unfeasible, much less influencing the attractiveness and acceptance by the consumers (Mal'a et al., 2010; Gomah et al., 2017; Darwesh et al., 2020; Monteiro et al., 2021; Abdel-Raheam et al., 2022; Srianta et al., 2022).

Several strategies can be used to guarantee the stability of the pigment, such as pigment encapsulation using precipitation, spray or freeze drying, or liposome membranes methods with a different encapsulating agent (dextrin, maltodextrin, sodium caseinate, gum Arabic, among others) and carrier agents (propylene glycol, mannitol, among others), which can result in products with different structures and stability profiles (Priatni, 2015; Jian et al., 2017; Xu et al., 2020b; Ali et al., 2022; Long et al., 2023).

In the case of the protection offered by the sodium caseinate microcapsule, for example, the thermal degradation (100°C) of the encapsulated *Monascus* pigment demonstrated to follow the degradation of the pigment without encapsulation when the heating time is increased at pH 3.0 (Ali et al., 2022). On the other hand, the microcapsule can contribute by increasing the solubility and decreasing the precipitation of the *Monascus* pigment in an acid medium (Jian et al., 2017; Ali et al., 2022).

Spray or freeze-dried encapsulation methods require efforts to verify encapsulating agents and carriers that demonstrate better performance in preserving the *Monascus* pigment color after the process has been carried out. For example, Xu et al. (2018) verified that the combination of maltodextrin as an encapsulating agent and mannitol as a carrier agent increases the hydrophilicity and solubility of the encapsulated *Monascus* pigment, resulting in a greater red color.

Another way to increase the stability of *Monascus* pigment using the spray drying/freeze drying encapsulation process is to associate this process with combined treatment, for example, the emulsification/internal gelation method. Zhang et al. (2023) demonstrated that thermal degradation (50–90°C) was lower when spray-dried followed by freeze-dried combined with emulsification/internal gelation were applied compared to *Monascus* pigment extract without application of emulsification technique /internal gelation. Furthermore, even encapsulated, *Monascus* pigment showed low stability to illumination treatment (500 lx), pH below 5 and above 9, and the presence of Ca<sup>2+</sup>, Cu<sup>2+</sup> and Fe<sup>2+</sup>. This occurs because the emulsification/internal gelation technique combined with drying methods results in a controlled release of *Monascus* pigment under specific conditions, improving the stability profile of the pigment.

TABLE 1 Applications of *Monascus purpureus* and *Monascus ruber* pigments in food products (2000–2022 years) (+) positive and (–) negative aspects.

Food products	Microorganism, production, and pigment extraction	Pigments produced	Maximum or optimum concentration	General characteristics	References
Ice lolly	<i>M. purpureus</i> Went NRRL 1992; submerged cultivation (potato waste 7.81%; peptone 2.87%; 30°C, pH 6.5; 12.82 days); and extraction: solvent (ethyl acetate).	Red, orange, and yellow	29.86 absorbance unit/mL	Pigment concentration: 0.05%;	Monteiro et al. (2021)
				A sensory rating higher than 9 in all sensory aspects (taste, color, texture, odor, and overall acceptability);	
				Color uniformity (+);	
				The low concentration required for coloring (+)	
Yogurt and milk beverage from buffalo milk	<i>M. ruber</i> Went AUMC 5,705; solid-state fermentation (broken rice and monosodium glutamate (2%); pH 6.5; 30°C; 39% moisture content; inoculum rate of $126 \times 10^4$ spores/10 g of a fermented dried substrate; particle size larger than 2.0 mm; 15 days); and extraction: ethanol and water.	Red (strawberry-flavored milk beverage) and yellow (banana-flavored milk beverage)	(1,424.8 and 1,442.4 absorbance unit (AU)/g dried substrate, respectively).	Pigment concentration: Not determined—pigment added until standardization with a commercial product;	Abdel-Raheem et al. (2022)
				Uniform and attractive color, appearance, and distribution (+);	
				Total score > 87.8% (100%) for strawberry flavored yogurt and milk beverage;	
				Total score higher than 83.88% (100%) for banana flavored milk beverage.	
Yogurt	<i>M. purpureus</i> HM188425.1; 14 days at 30 °C; solid-state fermentation (durian seeds 50 g/batch; particle size 1 cm <sup>3</sup> ; inoculum 5% (v/w); and extraction: water and ethanol (1:50 p/v).	Red	N.d.	Pigment concentration: 0.15% for pigment powder and 7.5% for liquid pigment (water or ethanol);	Gomah et al. (2017)
				> red coloring (+);	
				Direct addition of pigment powder without loss of yield (+);	
				Increase in total phenolic content (+) (1.54 to 2.21 mg/GAE g);	
				Increase in antioxidant activity (+);	
Increase in color and flavor.					
Poultry meat products	<i>M. purpureus</i> (commercial extract) prepared as a powder of red yeast rice	Red	N.d.	Pigment concentration: ~0.6%;	Srianta et al. (2022)
				Increase color, taste, consistency, cutting view, and inner view parameters.	
Meat system as pork fat replacement	<i>Monascus sp.</i> (powder of red yeast rice)	Red	N.d.	<i>Monascus</i> pigment W/O/W emulsion meat system (10 to 30%);	Darwesh et al. (2020)

(Continued)

TABLE 1 (Continued)

Food products	Microorganism, production, and pigment extraction	Pigments produced	Maximum or optimum concentration	General characteristics	References
				<p>&lt;45.37% of lipid content (+);</p> <p>&gt;7.34% of protein content (+);</p> <p>&gt;de 36.27% polyunsaturated fatty acids composition (+);</p> <p>&lt;19.2% saturated fatty acids;</p> <p>&gt;water holding capacity (+);</p> <p>Oxidative stability (+);</p> <p>&lt;hardness without affecting the meat system (+);</p> <p>&gt;lightness and redness (+).</p>	
Lollipops and jellybeans	<i>M. ruber</i> OMNRC45; submerged fermentation; 30°C; pH 6.5; with modified rice broth medium; Peptone (100 mg/L); (pH 4.5–8.5); and extraction: ethanol 95% and centrifugation.	Red	1.4 absorbance unit (AU)/g dried	<p>Pigment concentration: 0.1%;</p> <p>Intense and persistent red color (+);</p> <p>Increased the appeal, color, appearance, and overall acceptability (+).</p>	Mal'a et al. (2010)
Loaf bread	<i>M. ruber</i> CCT 3802; solid-state fermentation; 30°C; 14 days; wheat grains as substrate; 5mL of spore solution for each 100 g of dry wheat; moisture of 43% (m/m); and extracted	Red	2.88 absorbance unit corresponding to 16.64 mg/g of dry fermented wheat	<p>Pigment concentration: up to 15% replacement of flour with pigment;</p> <p>Increased red color (+);</p> <p>Increased ash content (+) and water holding capacity;</p> <p>Reduction of dough development time (+);</p> <p>Less resistance to extension (-);</p> <p>Decrease in mass volume (-);</p> <p>Higher texture values (-).</p>	Egea and Marcionilio (2022)
Processed cheeses	<i>M. purpureus</i> (type Moguntia, International AG, Europa) powder of red yeast rice	Red	–	<p>Sensory characteristics when 0.015% pigment was added (+);</p> <p>Sensory characteristics (+) and color stability (+) when 0.02 and 0.05% pigment was added influenced sensory.</p>	Lin and Xu (2022)
Red beer (Pilsen)	Addition of 10% of rice fermented with <i>M. ruber</i> to barley malt	Red	N.d.	<p>pH and titratable acidity indicating high acidity (-);</p> <p>23.64 EBC staining (dark brown) (-);</p> <p>Presence of lovastative (+).</p>	Souza (2018)

N.d., Not determined; EAG, gallic acid equivalent; W/O/W, water/oil/water; EBC, European Brewery Convention.

Encapsulation of *Monascus* pigment using liposome membranes using a thin-film ultrasonic method demonstrated stability at pH (2, 3, 4, 5, 6, 7, 8, 9, and 10), thermal (water bath at 60, 70, 80, and 90°C/5 h), light (500 lx), storage in refrigeration (4°C for 30 days in the dark), and *in vitro* simulated gastrointestinal digestion stability and stronger inhibitory effect on MKN-28 cells (gastric cancer cell line) by damaging the integrity of cells (Long et al., 2023). In addition, liposomes containing rubropunctatin (a *Monascus* pigment) demonstrated greater water solubility, stability to light (tungsten lamp (500 W, wavelength of 597–622 nm), also increasing anticancer activity and stimulating apoptosis-promoting mechanism (Xu et al., 2020b).

Although the encapsulation processes seem efficient in increasing the stability of *Monascus* pigment, the literature still lacks the application of these encapsulates, regardless of the method, directly in the food product where all adverse conditions are combined (acidic pH, high processing temperatures, exposure light during storage, among others).

However, for products that present adequate stability, the most common is to add them before the heat treatment to ensure the microbiological safety of the food, having already verified the maintenance of its properties in time/temperature binomials of 68°C/30 min, 90°C/5 min, 70°C/10 min, among others (Abdel-Raheem et al., 2022). In cases where the pigment has reduced stability, measures such as the addition after heat treatment can be considered, requiring additional care to avoid contamination that seems to have minor effects on the degradation of pigments such as sterilization procedures with filtering membranes and also the use of high pressure, ultrasound, for example (Sant'Ana, 2014; Singh et al., 2019). In addition, auxiliary stability-improving techniques, such as nanotechnology, can be successfully employed in this regard (Bhandari et al., 2022).

In addition to the concern with the stability of the pigment for application in food products, another concern is related to the regulatory and safety aspects of the *Monascus* pigment. Safety aspects for food products are still not very homogeneous across the world, since the use of *Monascus* pigment is restricted for application in products in the United States and the European Union countries, but allowed in Asian countries, where it is traditionally consumed in typical products (Manan et al., 2017; Liu et al., 2018). In countries where fermented products containing *Monascus* pigment are allowed, there is a position that the amount of pigment must be controlled, so for these countries, the safety of using *Monascus* pigment is related to the amount ingested (Commission Regulation, 2014). In the case of countries where the use of *Monascus* pigment is still not allowed, it is argued that the aspects of health hazards—the same ones related to synthetic pigments—are not yet fully elucidated and should be better analyzed before releasing for use. In addition, some countries raise the possibility of production and high doses of citrinin—a mycotoxin displaying hepatotoxic and nephrotoxic effects on humans—as one of the reasons for banning it, but which can be circumvented with the genetic manipulation of strains that do not produce the component, or with the control of the conditions of the fermentation process (Lin and Xu, 2022). In some cases, limits have been established for the presence of citrinin in food products containing *Monascus* pigment, such as in Japan, European countries, and the United States (Commission Regulation, 2014).

Thus, it is clear that the *Monascus* pigment is an important ingredient for the development of food products and can be used properly to improve the attractiveness, as well as the nutritional and bioactive aspects of food, provided that the limiting operational conditions are respected.

## 4. Conclusion and future directions

Several advantages related to the production and use of the *Monascus* pigment are presented when compared to synthetic and vegetable pigments, but it is still necessary to advance in points such as its economic viability, toxicity, mycotoxin production depending on the strain, safe consumption doses, among others, to make its application homogeneous, unrestricted and safe.

Regulatory aspects are still quite divergent around the world—as with any other product still in the implementation phase—and are mainly related to the citrinin content and the lack of studies on the effects of *Monascus* pigments and their doses on human health, which must be investigated and answered appropriately.

The main advantages associated with *Monascus* sp. for pigment production are related to its high pigment production capacity using non-expensive substrates in its cultivation and production of pigments with bioactive properties, good solubility (water and ethanol), and sensorial properties. The *Monascus* pigment can be efficiently applied in food with a color impact, conferring attractiveness, pleasant appearance, and color uniformity as well as promoting sensory aspects such as taste, color, odor, texture, and total acceptance.

Another aspect to be considered is the need to apply auxiliary stabilization techniques depending on the operational processing conditions, which may include the use of encapsulation in different systems, for example. Furthermore, economical and environmentally viable strategies must be studied, to offer a stable and low-cost pigment.

Additionally, due to its bioactive properties, with health effects, including anti-obesity activity as well as obesity-related-diseases, such as hyperlipidemia, steatohepatitis, and hyperglycemia the pigment stands out as an emerging compound in the pharmaceutical industry, which further contributes to the need for in-depth studies.

## Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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