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Review

Monascus spp. and citrinin: Identification, selection of *Monascus* spp. isolates, occurrence, detection and reduction of citrinin during the fermentation of red fermented rice

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ABSTRACT

Red fermented rice (RFR) is rice fermented using *Monascus* spp. This product contains monacolin K, providing health benefits including mitigation of diarrhoea and improving blood circulation. RFR can produce pigments that can act as natural colour and flavouring agents. However, *Monascus* spp. (a fungal starter to ferment RFR) can also produce the mycotoxin, citrinin (CIT) which is believed to have adverse effects on human health. CIT in RFR has been reported worldwide by using different methods of detection. This review focuses on the production of RFR by solid-state fermentation (SSF) and submerged fermentation (SmF), the occurrence of CIT in RFR, CIT quantification, the factors affecting the growth of *Monascus* spp., pigments and CIT production in RFR, and possible methods to reduce CIT in RFR. This review will help the food industries, researchers, and consumers understand the risk of consuming RFR, and the possibility of controlling CIT in RFR.

1. Introduction

Red fermented rice (RFR) is a fermented product consumed in East Asia for centuries and is particularly popular in Chinese dishes. RFR is also known as red rice, red leaven, zhitai, hong qu, angkak, and hung-chu among the Chinese while the Japanese call the product beni-koji (Erdoğrul and Azirak, 2004). Other names for RFR are rotscimmelreid (Europe), Anka, Ang-Khan, Anka-Koji, red mould rice and red yeast rice (Chiu et al., 2006; Patcharee et al., 2007; Ristiarini et al., 2017). RFR is widely used as a therapy for hyperlipemia. RFR is available in the market in dried and powder forms (Abdul-Manan et al., 2017). RFR has been used to give colour and flavour, and act as a preservative in East Asian foods and cuisine, especially in China, Korea, and Japan. RFR is also consumed as a traditional Chinese medicine (Nguyen et al., 2017) and dietary supplement in Western countries (Zhu et al., 2019). Table 1 summarizes the benefits of RFR to improve the quality of human health.

During fermentation of the RFR, secondary metabolites such as pigments, lovastatin/monacolin K, polysaccharide, γ-aminobutyric acid (GABA), ergosterol, and CIT are produced (Srianta et al., 2014). Monacolin K, also known as lovastatin in lactone form (Younes et al., 2018), is a natural statin, acting as an inhibitor of the enzyme 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoA reductase) that prevents the formation of mevalonate from HMG-CoA during cholesterol biosynthesis (Suraiya et al., 2018). The United States Food and Drug Administration (USFDA) has approved the use of statins to treat hyperlipidaemia (abnormally high level of fats/lipids in the blood including cholesterol and triglycerides) (Gregory et al., 2012). Statins can be used either as a single-ingredient such as Lipitor (atorvastatin), Lescol (fluvastatin), Mevacor (lovastatin), Altoprev (lovastatin extended-release), Livalo (pitavastatin), Pravachol (pravastatin), Crestor (rosuvastatin) and Zocor (simvastatin); or in combination with other products including Advicor (lovastatin/niacin extended-release), Simcor (simvastatin/niacin extended-release), and Vytorin (simvastatin/ezetimibe) (USFDA, 2016). The average amount of prescription lovastatin (Monacolin K) is 10–80 mg/day (Gregory et al., 2012). Consumption of RFR containing RFR could lead to adverse effects on the liver, and musculoskeletal system including a breakdown of muscle tissue. This muscle tissue breakdown releases a damaging protein (myoglobin) into the blood and can damage the kidneys (Younes et al., 2018). However, the European Food Safety Authority (EFSA) Panel is unable to recommend a safe dietary intake of monacolins from RFR due to several uncertainties (Younes et al., 2018). Another metabolite such as citrinin (CIT), a mycotoxin of food safety concern, can be produced by some of *Monascus* spp. such as *M. purpureus* during fermentation of RFR

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Table 1
The benefits of RFR.

Benefits	References
Improve the quality of eggs for human consumption:	(Pengnoi et al., 2018; Sun et al., 2015; Zhu et al., 2019)
(a) Decreasing the level of serum and egg yolk cholesterol (b) Enhance the egg quality in laying hens without disablement of laying production	
Anticarcinogenic compounds against liver, colon, breast, and prostate cancer	(Chiu et al., 2013; Klingelhöfer and Morlock, 2019)
Inhibitors of cholesterol biosynthesis	(Man et al., 2002)
Lipid and cholesterol-lowering properties	(Becker et al., 2009; Bogsrud et al., 2010; Heber et al., 1999; Ma et al., 2009; Wei et al., 2003; Yang et al., 2021; Zhou et al., 2019)
Prevent the build-up of fats, cholesterol, and other substances in and on the artery walls	(Lin et al., 2008; Lin et al., 2011; Liu et al., 2017; Shen et al., 2017; Wu et al., 2017)
Neurocytoprotective activity	(Lee et al., 2009; Lee et al., 2007b; Lee et al., 2010; Lin et al., 2015)
Preventing osteoporosis	(Cho et al., 2010; Gutierrez et al., 2006; Wang et al., 2015; Wong and Rabie, 2008)
Anti-obesity activity	(Chen et al., 2008)
Anti-fatigue effects	(Wang et al., 2006; Xue et al., 2017)
Anti-diabetic benefits	(Chen and Liu, 2006; Chen et al., 2008)
Regulatory effects on the immune system	(Patakova, 2013)
Anti-microbial activities	(Ferdeş et al., 2009; Milanda et al., 2021)
Anti-inflammatory effects	(Ding et al., 2014; Hsu et al., 2010)
Anti-hypertensive benefits	(Wang et al., 2010)
Anti-cancer activity	(Hong et al., 2011; Hsu et al., 2010; Lee et al., 2013; Lin et al., 2007; Xue et al., 2017)
Prevent damage to the liver	(Cheng and Pan, 2011; Hong et al., 2007; Lee et al., 2012)

(Silva et al., 2021).

This review will focus on *Monascus* spp. as the starter fungi for RFR, secondary metabolites produced during the fermentation of RFR including beneficial compounds such as pigments and monacolin K (Table 1), methods used including different ways to ferment RFR, factors affecting the growth, pigments production by *Monascus* spp. and CIT production during fermentation of RFR, and the occurrence, detection and reduction of CIT in RFR. This review will help the food industries, researchers, and consumers to understand food safety risks associated with the consumption of RFR, and the possibility of producing RFR with low or no CIT.

2. Methods used to ferment RFR

There are variations in the methods used for the fermentation of RFR, but the general processes are the same. All rice types can be used as a substrate for the RFR, but non-glutinous rice is preferable to avoid the agglomeration of rice during the fermentation process (Wen et al., 2020). A variety of substrates and nutritional broths are used in solid-state fermentation (SSF) or liquid/submerged fermentation (SmF) respectively, for improving RFR quality in terms of yield, pigments, and monacolin K. Most of the SSF processes require several weeks to produce RFR (Chairote et al., 2009; Chen and Hu, 2005; Dogra and Kumar, 2017; Li et al., 2003; Patcharee et al., 2007). The type of inoculum, inoculum concentration and size (the ratio of the inoculum to the substrate, v/w) are important to produce RFR. Selection of *Monascus* spp. isolates, medium formulation and optimization of fermentation conditions help to improve RFR (and other *Monascus* fermented products), enhancing the pigments and monacolin K production, and possibly novel unknown compounds that might be produced during the fermentation of RFR. Comprehensive research and development are needed to identify additional bioactive compounds. Table 2 shows the methods that have been used in the literature to ferment RFR.

3. Production of RFR

RFR can be produced by traditional and industrial methods. In the traditional process (Chiu et al., 2006), cooked rice is inoculated with *Monascus* spp., and put in a round shallow bamboo tray (about 5–6 cm in depth) to control aeration and temperature. The trays are stored on shelves in a fermentation room at 33–35 °C. Hands are used to turn over the rice koji (rice inoculated with *Monascus* spp. culture) (the frequency was not mentioned) to remove the heat of fermentation. To maintain the appropriate moisture content of the koji, each tray is taken out at least three times from the room and soaked in water during the fermentation process. Some of the traditional methods to ferment RFR involve hand stirring and water soaking for several days. Another method that does not involve hand stirring and water soaking, adjusts the moisture content and temperature of incubation of RFR in a temperature-controlled (the temperature was not mentioned) chamber with regularly stirring and moistening (Chen et al., 2015).

During fermentation, the rice may be contaminated by the environment and hand turning. High labour costs are involved in stirring and water soaking. Fermentation in a closed environment (anaerobic) produces an unsatisfactory product (Chiu et al., 2006) with a very low monacolin K level (9 μg/g) compared to aerobic fermentation (157 μg/g) under the same fermentation conditions (Tsukahara et al., 2009).

There are two different ways of producing RFR, either by SSF or SmF. SSF involves the addition of starter fungi into the solid medium (rice) whereas SmF provides nutrients for fungal growth in a liquid medium. For both fermentations, the temperature is very important as it affects microbial growth, spore formation and germination, and pigments and monacolin K production (Darwesh et al., 2020; Tsukahara et al., 2009). A low moisture content (20–70 %) is needed for fungal growth, whereas bacteria need a moisture content higher than 70 % (Babitha et al., 2007). At high initial moisture content, the rice will agglomerate and this limits the supply of oxygen for fungal growth, resulting in low pigments production (Gautam, 2002). A low moisture level will reduce the risk of contamination with bacteria and yeast, resulting in high productivity during SSF (Kraboun et al., 2019).

SSF also provides ideal conditions for fungal hyphae to grow on the surface of the rice and penetrate the substrate such as rice generating high pigments production. One of the advantages of SSF is the low cost of production because a variety of carbon sources such as jackfruit seed powder, sesame oil cake, coconut oil cake, wheat bran, palm kernel cake, grape waste, rice bran, cassava powder, spent brewing grain, and tamarind seed powder can be used as substrates (Babitha et al., 2007). Zhang et al. (2013) showed that the type of fermentation (SSF or SmF) influenced the production of CIT and pigments, with SSF producing 1000 times lower CIT than SmF (0.018 μg/g and 19.02 μg/g respectively).

Fungi in SmF culture are often grown in pellets, which are the form of compact spherical masses of mycelium (Pirt, 1966). There are two types of fungal pellets formations, which are coagulative and non-coagulative (Veiter et al., 2018). In the coagulative agglomeration type, spores agglomerate fast and subsequently germinate involving hyphal tip growth (Zhang and Zhang, 2016). Finally, a great number of spores of the coagulative type form pellets. Meanwhile, for the non-coagulative agglomeration type, spores germinate to hyphae. Branched hyphal elements subsequently agglomerate to form a pellet. In theory, one spore can form a pellet (Veiter et al., 2018). Fungi in SmF can also grow in the filamentous form, featuring homogeneously dispersed hyphae (Veiter et al., 2018).

The drawback of SmF is that the growth of fungi during the fermentation results in an increase in the viscosity of the broth. After 8–9 days of fermentation, pigments productivity decreases due to the

Table 2
Methods used to ferment RFR.

Country	Fermentation type	Type of rice	Culture media	Inoculum	Inoculum ratio (*v/w*)	RFR process										Reference
						Soaking time	Steaming time	Cooling time	Weight of steamed rice	Sterilization condition	Cooling temperature	Incubation temperature	Incubation time	Drying information	Final product	
India	SSF	Basmathi white rice	Rose Bengal Agar (30 °C, 7–8 days)	Pre-culture *M. purpureus* (1 week old)	5 %	2 h	20 min	Yes (NM)	20 g	15 psi, 121 °C, 15 min	NA	30 °C	2–3 weeks	65 °C, 2 h (Oven)	Dried RFR	(Dogra and Kumar, 2017)
Thailand	SSF	Polished rice	PDA (30 °C, 10 days)	*M. purpureus*/ *M. ruber*	NA	NA	NA	Yes (RT)	NA	121 °C, 15 min	RT	RT	14 days	55 °C, 3 days (NM)	Powder RFR	(Patcharee et al., 2007)
Thailand	SSF	Non-glutinous rice and glutinous rice	NA	*M. purpureus* (1 week old)	NA	6 h	20 min	Yes (NM)	50 g	15 psi, 121 °C, 15 min	NA	30°C	2–3 weeks	65 °C, 6 h (Oven)	–Dried RFR –Dried RFR + [a]1 mL of 0.25 g/mL soybean milk solution	(Chairote et al., 2009)
China	SSF	NM	[b]GBP medium slant	*Monascus*	5 mL spore solution (1 × 10^6/mL)	2 h (Soaked in water at 30°C)	100 g rice, 1 g wheat, 70 mL water/ 50 mL water + 20 mL [c]nutritional broth were mixed well			121 °C, 30 min	NA	30°C	Several days	50 °C (until constant weight)	Dried RFR	(Chen and Hu, 2005)
China	SSF (industrial, using Modified Nagata type koji maker)	Indica rice	NA	*M. purpureus*	0.7–4 %	0–25 min	Yes (100 °C, 30 min)	From 100 °C to 30 °C (5 min)	NA	NA	NA	SSF: 37–38 °C 86th hour until the end of fermentation: 26, 30, 34 °C		NA	Dried RFR	(Chiu et al., 2006)
China	SSF (Traditional)	NM	NA	*Monascus* koji	NA	6–8 h	Yes (NM)	Yes (40 °C)	NA	1st day: Inoculated with *Monascus* koji (33–35 °C) 2nd day: Stirring and mixing of koji (34 °C) 3rd day: 1st water soaking of koji for 30 min (moisture: 50 %) 4th day: 2nd water soaking of koji (moisture: 47 %) 5th day: last time water soaking of koji (moisture: 48 %) 6th day: Post maturing, stirring every 10 h (30 °C)				45 °C, 22 h	Dried RFR	(Chiu et al., 2006)
China	SSF (traditional)	Long-shaped non-glutinous rice	NA	*Monascus* starter culture	NA	1 day	Yes (steamed in [d]ZENG with vapour)	NA	NA	NA	NA	25–28 °C	2–4 weeks	NA	Powder RFR	(Chen et al., 2015)
China	SSF	Long-grained non-glutinous rice	NA	SJS1–SJS35	NA	24 h (Soaked in acidified water (pH 4.5)	60 g of soaked rice were assigned to a 500 mL flask. After autoclaved at 121 °C for 20 min, 6 mL seed cultures[e] were inoculated and incubated. Triplicate flasks were inoculated with each strain.			121 °C, 20 min	NA	30 °C, 3 weeks ([f]RH 70 %) 1–3 days: all flasks were left standing and hereafter shaken daily to reduce clumping. To maintain MC, autoclave water (pH 4.5) was added during the later stage of incubation		NA	Dried RFR	(Li et al., 2003)
China	SmF	NA	NA	*Monascus* single-spore culture (10-day old)	NA	300 mL flasks containing 50 mL of submerged culture (g/L): Glucose (20), monosodium glutamate (5), KH_2PO_4 (5), K_2HPO_4 (5), $MgSO_4{\cdot}7H_2O$ (0.5), $CaCl_2$ (0.1), $FeSO_4{\cdot}7H_2O$ (0.01), $ZnSO_4{\cdot}7H_2O$ (0.01) and $MnSO_4{\cdot}H_2O$ (0.03). Triplicate flasks were inoculated with each isolate				121 °C, 20 min	NA	30 °C	3 weeks	NA	SmF broth	(Li et al., 2003)

NM: not mentioned. NA: not applicable. RT: room temperature. MC: moisture content.

[a] To study the effect of adding nitrogen-containing nutrients.

[b] GBP medium slant: 10 g glucose, 5 g beef extract, 10 g protease peptone, 5 g NaCl, 15 g agar, 1000 mL distilled water, pH 5.0, sterilized for 30 min at 121 °C.

[c] Nutritional broth: 40 g glucose, 4 g sodium glutamate 3 g protease peptone, 3 g NH_4NO_3, 1000 mL distilled water.

[d] ZENG: a kind of wooden rice steamer.

[e] Seed cultures: Transferring a small piece of 10-day-old *Monascus* single-spore culture from an MEA slant into a 500 mL flask containing 50 mL autoclaved yeast extract sucrose medium (by mixing 10 g yeast extract and 100 g sucrose in 1 L distilled water). Cultures were incubated in the shaker at 30 °C for 3 days.

[f] RH: Relative humidity.

lack of oxygen (Agboyibor et al., 2018). The formation of pellets might be disturbed due to the high shear force at high agitation speed (Wang et al., 2014), leading to low pigments concentration and high CIT. Non-coagulative pellet formation is connected with agitation and aeration (Pazouki and Panda, 2000).

Yang et al. (2015) studied the effect of oxygen supply on the pigments and CIT production by *M. ruber* HS.4000 in a shake-flask and fermenter. High agitation speeds for the extraction leads to a high oxygen supply. The results showed that an agitation speed of 300 rpm produced lower CIT compared to 600 rpm. In contrast to earlier findings by Pereira et al. (2008), the optimum agitation speed was 600 rpm with a dissolved oxygen concentration of 60 %. Yang et al. (2015) suggested ending the fermentation once the optimum pigments concentration was produced before most of the CIT is produced.

The high viscosity of the broth was observed when *Monascus* sp. J101 was fermented at 30 °C, resulting in poor oxygen transfer and low pigments yield. Lower viscosity was achieved when the culture was incubated at 25 °C due to a reduced fungal growth rate, resulting in 10 times higher pigments yield than when incubated at 30 °C (Ahn et al., 2006). Low RFR yields occur when *Monascus* mycelia are easily attached to the stirring paddle and fermentation tank wall (Srianta et al., 2014). Another problem with SmF is low pigments production. Due to the presence of free water in SmF, the pigments are not just associated with the fungal cells (intracellular) but are also in the surrounding medium (Hamano et al., 2005). Intracellular pigments are insoluble in water, while extracellular pigments are water-soluble and can be affected by the nitrogen source and pH of the liquid medium. The intracellular pigments accumulate mainly in the mycelium and are retained within the fungal cells resulting in a lower pigments yield (Hamano et al., 2005). In contrast, Mukherjee and Singh (2011) stated that a high pigments yield can be achieved by SmF, and the pigments yield is affected by fermentation conditions such as pH, medium composition and agitation (Hamdi et al., 1996; Mukherjee and Singh, 2011). In the food industry, the production of RFR by SmF remains confidential to many companies. Therefore, there is limited technical information published in the literature (Srianta et al., 2014).

SmF has many advantages over SSF such as a smaller surface area, less labour, and a short fermentation time. Less labour also reduces the chance of contamination (Agboyibor et al., 2018; Srianta et al., 2014). Therefore, the purification of RFR products of SmF is easier compared to SSF (Subramaniyam and Vimala, 2012). It is also easier to control the production of secondary metabolites of RFR such as pigments, CIT, and monacolin K (Patakova, 2013).

4. Fungal growth identified in RFR

RFR is usually produced by traditional methods in most Asia countries. However, there is usually no monitoring procedure during production, storage and transportation to ensure the safety of RFR (Samsudin and Abdullah, 2013). Fungal contamination and growth by genera such as *Aspergillus*, *Fusarium* and *Penicillium* may result in mycotoxins such as CIT, aflatoxins (AFs) and ochratoxin A (OTA) (Samsudin and Abdullah, 2013). Most of the fungal growth in RFR is from *Monascus* spp. (as a fungal starter), but RFR can be contaminated with other species such as *Aspergillus* spp. and *Penicillium* spp. Table 3 shows the fungi that have been isolated from RFR.

5. Occurrence of mycotoxins in RFR and RFR products

Table 4 shows the occurrence of mycotoxins in RFR in different countries, especially in Asia. Samsudin and Abdullah (2013) found that RFR is often contaminated with CIT and other mycotoxins, such as AFs and OTA. Meanwhile, other researchers only found CIT in RFR. This may be because RFR was stored in different storage conditions: (1) in wooden drawers without any packaging and stored together with other herbs, (2) in packaging, (3) in the refrigerator, (4) at room temperature, (5) in an air-conditioning system, and (6) in open-air (Samsudin and Abdullah, 2013). The study found that RFR was contaminated with *A. flavus* and *A. niger*, which are AFs and OTA producers, respectively (Samsudin and Abdullah, 2013).

Table 3
Fungal isolates from RFR.

Fungal isolates	References
M. purpureus	(Abdul-Manan et al., 2017; Chen et al., 2015; Samsudin and Abdullah, 2014)
M. pilosus	(Abdul-Manan et al., 2017; Chen et al., 2015; Dai et al., 2021; Samsudin and Abdullah, 2014)
M. ruber	(Abdul-Manan et al., 2017; Chen et al., 2015; Samsudin and Abdullah, 2014)
M. floridanus	(Barnard and Cannon, 1987; Chen et al., 2015)
M. pallens	(Abdul-Manan et al., 2017; Chen et al., 2015; Shao et al., 2014)
M. lunisporas	(Abdul-Manan et al., 2017; Chen et al., 2015; Shao et al., 2014)
M. argentinensis	(Abdul-Manan et al., 2017; Chen et al., 2015; Shao et al., 2014)
M. sanguineus	(Abdul-Manan et al., 2017; Chen et al., 2015; Shao et al., 2014)
M. eremophilus	(Abdul-Manan et al., 2017; Chen et al., 2015; Shao et al., 2014)
P. chrysogenum	(Samsudin and Abdullah, 2013)
A. niger	(Samsudin and Abdullah, 2013)
A. flavus	(Samsudin and Abdullah, 2013)

Avula et al. (2014) found that there was no detectable CIT in authentic RFR samples (obtained from Beijing Peking University WBL Biotech Co., Ltd., China) and dietary supplements (labelled as 600 or 1200 mg of RFR were purchased online from supplement retailers in the USA), but 19 % of commercial RFR were contaminated with CIT (10,000–80,000 μg/kg). This was thought to be due to variations in storage conditions in the RFR shops effects the fungal contamination of RFR, leading to CIT production (Samsudin and Abdullah, 2013).

Most of the studies on RFR focus on the occurrence of CIT in the final products. However, other studies have determined CIT, pigments or monacolin K during fermentation of RFR (Table 5). Li et al. (2003) compared the CIT levels and pigments of RFR produced from SSF and SmF. The results found that SSF produced RFR with higher CIT and pigments value compared to SmF. The researchers also mentioned that long-grained non-glutinous rice was the preferable medium for CIT production.

6. Factors affecting growth and pigments production of *Monascus* spp.

Red mould species such as *Monascus* spp. are xerophilic fungi (Silbir and Goksungur, 2019) and are commonly used as fungal starters in RFR (Dogra and Kumar, 2017). RFR is produced by inoculating rice (preferably white rice) with a fungal starter consisting of one or more *Monascus* spp. There are twenty-nine species of *Monascus* spp. identified globally (Chiu et al., 2006) including *M. purpureus*, *M. pilosus*, *M. ruber*, *M. pallens*, *M. lunisporas*, *M. argentinensis*, *M. sanguineus*, *M. floridanus*, and *M. eremophilus* (Chen et al., 2015; Shao et al., 2014). However, the main fungal starters for RFR production are *M. purpureus*, *M. pilosus*, and *M. ruber* (Chen et al., 2015; Samsudin and Abdullah, 2014). The taxonomy of *Monascus* is kingdom *Fungi*, phylum *Ascomycota*, class *Ascomycetes*, order *Eurotiales*, family *Monascaceae*, *and* genus *Monascus* (Pan and Hsu, 2014). These fungal isolates can secrete various secondary metabolites of polyketide structure. Polyketides are rich sources of pharmaceuticals, including antibiotics, anticancer drugs, cholesterol-lowering drugs and immune suppressant. (Chakravarti and Sahai, 2004; Jůzlová et al., 1996).

The growth of *M. purpureus* starts with whitish-coloured mycelium, followed by rich orange and then a clear rich red colour. The colour change happens because of the increase in acidity of the medium resulting in the production of red-orange hyphae. As the culture ages, *M. purpureus* will change to a deep crimson (Abdul-Manan et al., 2017; Blanc et al., 1994). There are several factors affecting the growth and colour production (pigments) of *Monascus* spp. These include *Monascus*

Table 4
Occurrence of CIT, AFs, OTA, and monacolin K in RFR and RFR products.

Samples	Country	Methods of detection	Total of tested samples	Percentage of positive and mycotoxins level (μg/kg)			Monacolin K (mg/g)	References
				CIT	AFs	OTA		
RFR[a]	China	[c]LC-FLD	9	<3–3200	NA	NA	NA	(Marley et al., 2016)
RFR	Malaysia	[d]ELISA	50	100 % (230–20,650)	92 % (0.61–77.33)	100 % (0.23–2.48)	NA	(Samsudin and Abdullah, 2013)
RFR (SSF)	China	[e]HPLC, [f]LC-MS	35	100 % (280–2,460,000)	NA	NA	NA	(Li et al., 2003)
RFR (SmF)	China	[e]HPLC, [f]LC-MS	30	86 % (90–56,000)	NA	NA	NA	(Li et al., 2003)
RFR (raw material)	Taiwan	[g]HPLC-FLD	84	69 % (400–93,500)	NA	NA	NA	(Liao et al., 2014)
RFR (supplements)	Taiwan	[g]HPLC-FLD	77	35 % (100–15,200)	NA	NA	NA	(Liao et al., 2014)
RFR (processed products)[b]	Taiwan	[g]HPLC-FLD	141	6 % (100−1300)	NA	NA	NA	(Liao et al., 2014)
RFR (raw material)	Taiwan	[h]LC-MS/MS, HPLC-FLD	33	64 % (1450–63,400)	NA	NA	NA	(Chen et al., 2016b)
RFR (dietary supplements)	Taiwan	[h]LC-MS/MS, HPLC-FLD	58	24 % (70–4900)	NA	NA	NA	(Chen et al., 2016b)
RFR (processed products)	Taiwan	[h]LC-MS/MS, HPLC-FLD	115	18 % (70–1290)	NA	NA	NA	(Chen et al., 2016b)
RFR	China	[e]HPLC	10	50 % (2.13–11.97)	NA	NA	NA	(Xu et al., 2003)
RFR food additives	China	[g]HPLC-FLD	11	73 % (127-4960)	NA	NA	NA	(Li et al., 2012)
RFR medicinal materials	China	[g]HPLC-FLD	19	100 % (18.2–5253)	NA	NA	NA	(Li et al., 2012)
RFR functional food and medicine products	China	[g]HPLC-FLD	29	14 % (16.6–62.5)	NA	NA	NA	(Li et al., 2012)
Authentic RFR samples	China	[i]UHPLC-DAD-QTOF-MS	3	0 %	NA	NA	100 % (1.97–2.33)	(Avula et al., 2014)
Commercial RFR materials	China	[i]UHPLC-DAD-QTOF-MS	31	19 % (10000–80,000)	NA	NA	71 % (0.54–24.27)	(Avula et al., 2014)
RFR dietary supplements (600 or 1200 mg)	USA	[i]UHPLC-DAD-QTOF-MS	14	0 %	NA	NA	100 % (0.03–2.62)	(Avula et al., 2014)

NA: not applicable.
[a] Granules – sold loose, powder – sold loose and packet, capsules, tablets.
[b] Including RFR sauce, crackers, oatmeal, soy sauce and wine.
[c] LC-FLD: liquid-chromatography with fluorescence detector.
[d] ELISA: enzyme-linked immunosorbent assay.
[e] HPLC: high-performance liquid chromatography.
[f] LC-MS: high-performance liquid chromatography coupled with mass spectrometry.
[g] HPLC-FLD: High-performance liquid chromatography with fluorescence detector.
[h] LC-MS/MS: High-performance liquid chromatography and tandem mass spectrometry.
[i] UHPLC-DAD-QTOF-MS: ultra-high performance liquid chromatography-diode array detector-mass spectrometry.

spp. isolates, cultivation conditions, the substrate, inorganic nitrogen, organic nitrogen, carbon source, pH, temperature, and moisture content (Kraboun et al., 2019; Liu and Chen, 2019).

Monascus spp. can produce three types of pigments, which are yellow (monascin and ankaflavin), orange (rubropunctatin and monascorubrin), and red (rubropunctamine and monascorubramine) pigments. The maximum absorbance wavelengths for the yellow, orange and red pigments are 330–450 nm, 460–480 nm, and 490–530 nm, respectively (Liu and Chen, 2019). The maximum wavelengths of pigments produced by *Monascus* vary among species. For example, 370 nm for *M. kaoliang*, 420 nm for *M. anka*, and 500 nm for *M. purpureus* and *M. barkeri* (Kraboun et al., 2019). These pigments have benefited human health.

Yellow *Monascus* pigments has anti-obesity activity and obesity-related diseases such as hyperlipidemia, steatohepatitis, and hyperglycemia (Hsu and Pan, 2014). ANKASCIN 568-R, is a patented RFR, free of monacolin K, high level of yellow pigments, and have been verified by animal and clinical studies. The product has been accepted by USFDA as a new dietary ingredient (NDI) (Liu et al., 2018). Rubropunctatin, an orange *Monascus* pigments, has an anti-proliferative effect on BGG-823 (human gastric adenocarcinoma) cells and has the potential to be developed as a new natural anti-cancer agent (Zheng et al., 2010). The red pigments produced by *Monascus* spp. is important for food additives, food colourants and condiments (He et al., 2020; Lagashetti et al., 2019). Red pigments can also induce cellular senescence and reduce viability in the hepatocarcinoma cell line (HepG2). Wei and Popovich (2013) suggested that this capability may halt the progression of carcinomatous cells to invasive malignancy and provide an alternative strategy for cancer prevention.

The substrate is important for the growth of *Monascus* spp. and the production of RFR. Chairote et al. (2008) found that RFR produced from non-glutinous rice with the addition of soybean milk and fermented for 3 weeks had a softer texture, a pleasant sweet odour and a dark red colour compared with RFR from non-glutinous rice without the soybean milk.

Inorganic nitrogen, such as ammonium chloride (NH_4Cl), produces higher *Monascus* pigments yield during the stationary phase even though the development of conidial germination and the sexual cycle of *Monascus* is suppressed (Chen and Johns, 1994). Sodium nitrate ($NaNO_3$) stimulates sporulation and high pigments yield, but the growth of *Monascus* is restricted (Chen and Johns, 1994).

To increase the pigments production of *Monascus* spp., organic nitrogen such as peptone, yeast extract and MSG can be added as supplements (Dufossé et al., 2005; Lin and Demain, 1991; Silveira et al., 2008; Subsaendee et al., 2014; Vidyalakshmi et al., 2009). The carbon

Table 5
Citrinin, pigment or monacolin K production during the fermentation of RFR by traditional and industrial methods.

Samples/ media	Country	Type of fermentation	Fermentation approach	Methods of detection	CIT	Pigments/ Monacolin K	References
1. Yes medium (SmF) 2. Wet rice medium (SSF) 3. Synthetic medium	France	SSF and SmF	Laboratory scale	[a]UV spectrometer, spectrophotodensitometer, [b]NMR spectroscopy, [c]TLC, HPLC, and mass spectrometer	1. [d]YES medium – *M. ruber*: 370 mg/L – *M. purpureus*: 240 mg/L 2. [e]Wet rice medium – *M. ruber*: 300 mg/kg dried fermented rice powder – *M. purpureus*: 100 mg/kg dried fermented rice powder 3. [f]Synthetic medium: no CIT produced	No pigments are produced in synthetic medium	(Blanc et al., 1995)
RFR	Taiwan	SSF	Industrial (modified Nagata type koji maker)	NM	331–617 ppb	– Red pigment: 0.75–0.91 [g]OD_{500nm} – Monacolin K: 47–54 ppm	(Chiu et al., 2006)
Rice waste	Taiwan	SmF	Laboratory scale	HPLC	From 30.36 ppb (control) reduced to 0.057 ppb (forecast optimal conditions) and 0.055 ppb (verification experiment).	The best yield of pigment: – Yellow: 4.132 ppm – Red: 8.480 ppm – Orange: 4.573 ppm	(Chung et al., 2009)
RFR	China	SSF and SmF	Laboratory scale (autoclaved at 121 °C for 20 min)	HPLC	SSF: 0.28–2460 mg/kg SmF: 0.09–56 mg/L	Pigment: SSF: 26–1134 U/g SmF: 0.11–35.33 U/mL	(Li et al., 2003)
RFR	Korea, USA, Taiwan, China	SSF	Traditional (culturing *M. ruber* on steamed rice)	HPLC	0–11.97 μg/g (Samples were cultured in the lab)	NA	(Xu et al., 2003)

NM: not mention. NA: not applicable.
[a] UV spectrometer: ultraviolet spectrometer.
[b] NMR spectroscopy: nuclear magnetic resonance spectroscopy.
[c] TLC: thin layer chromatography.
[d] Yeast extract sucrose (YES) medium: composed of yeast extract (40 g) and sucrose 160 g/L of deionized tap water. This medium was incubated at 27 °C without agitation for 2 weeks.
[e] Wet rice medium (50 % of water w/w): incubated at 27 °C for 2 weeks.
[f] Synthetic medium: composed of monosodium glutamate (MSG) (5 g), K_2HPO_4 (5 g), KH_2PO_4 (5 g), MgSO4.7H_2O (0.5 g), CaCl2 (0.5), $FeSO_4{\cdot}7H_2O$ (0.5 g), $ZnSO_4{\cdot}7H_2O$ (0.01 g) and $MnSO_4{\cdot}H_2O$ (0.03 g), ethanol 20 g/L deionized tap water. The initial pH of the medium was adjusted to 6.5 with ammonium hydroxide. This medium was incubated with or without agitation at 27 °C until exhaustion of ethanol.
[g] OD: optical density.

source also affects the production of *Monascus* spp. pigments. Ghada and Walid (2017) studied the factors affecting pigments production by *M. purpureus*. The results showed that the optimal growth and pigments production was achieved when corn starch was used as a carbon source, yeast extract was used as a nitrogen source, initial pH was adjusted to pH 6, and incubated at 30 °C for 12 days with shaking speed at 150 rpm.

The use of glucose as a carbon source increased *M. purpureus* growth but suppressed the sporulation rate. In contrast, sucrose increased sporulation rates, but cell mass was inhibited (Ajdari et al., 2011). Li et al. (2017) studied the effect of different carbon sources on CIT production by *P. citrinum* and used transcriptome analysis to study the mechanism at the molecular level. CIT produced by glucose-cultured *P. citrinum* was 49 % higher than sucrose-cultured *P. citrinum*. The glucose-cultured *P. citrinum* changed its primary metabolic pathways, with more carbon passing through acetyl-CoA and malonyl-CoA, resulting in increased levels of precursors for polyketide synthesis. The polyketide synthase involved in secondary metabolism and CIT biosynthesis was increased (up-regulated) in the glucose-cultured *P. citrinum*, resulting in higher CIT production. As a carbon source, glucose suppresses *P. citrinum* to produce energy, activates the electron transport chain (ETC) process, forms reactive oxygen species (ROS), and produces higher hydrogen peroxide (H_2O_2) content due to the up-regulation of glucose oxidase (GOX). In response to oxidative stress, *P. citrinum* might produce higher CIT concentrations by altering expression levels of signalling pathway genes, antioxidant enzymes and others via transcriptional regulation. Ajdari et al. (2011) suggested that glucose and sucrose are a good combination of carbon sources to enhance sporulation and cell mass of *Monascus* spp. The effects of these combinations on the CIT produced by *Monascus* spp. and understanding the mechanism for CIT biosynthesis at the molecular level are interesting areas to be discovered.

According to Wang et al. (2004), the pH required for *Monascus* pigments production is between pH 2.5 to 10.0. The production of yellow and orange pigments is higher at pH 2.5 than more alkaline pH (Babitha et al., 2006). Another main factor involved in the hydrolysis of *Monascus* spp. is temperature. Temperatures between 35 °C and 37 °C encourage growth and glucoamylase production, while temperatures in the range of 30–40 °C produce pigments (Babitha et al., 2007; Kraboun et al., 2019). The highest pigments intensity occurs when the initial moisture content is between 30 % and 50 % (Kongbangkerd et al., 2014). For high pigments intensity, Kraboun et al. (2019) suggested the moisture content of the rice must be <30 %. Moisture content higher than 50 % reduces oxygen transfer, heat exchange and ventilation, leading to carbon dioxide accumulation. This is unsuitable for the formation of secondary

metabolites, resulting in lower pigments intensity (Kongbangkerd et al., 2014).

7. Factors affecting CIT production in RFR

It is critical to design a safe process for RFR production, as it can be contaminated with mycotoxins such as CIT, AFs, and OTA (Samsudin and Abdullah, 2013). The most commonly reported mycotoxin in RFR is CIT (Avula et al., 2014; Chen et al., 2016a; Chen et al., 2016b; Li et al., 2003; Li et al., 2012; Liao et al., 2014; Marley et al., 2016; Xu et al., 2003). However other mycotoxins such as AFs and OTA are reported (Samsudin and Abdullah, 2013) but appear to be associated with *P. chrysogenum*, *A. niger* and *A. flavus* contamination in RFR from contaminated air. This review will focus on CIT contamination as the starter fungi, *Monascus* spp. that can produce CIT during fermentation (Blanc et al., 1995).

Some aspects that influence the level of CIT produced include the *Monascus* species and isolates, amino acids, trace elements, carbon and nitrogen sources, nutritional factors, the ratio of nitrogen to carbon concentration, pH, moisture content, light, oxygen, temperature and environmental factors (Blanc et al., 1995; Comerio et al., 1998; Marič et al., 2019; Ostry et al., 2013; Patakova, 2013; Wong and Koehler, 1981; Yang et al., 2019; Yang et al., 2015).

Several *Monasus* species and isolates produce different levels of CIT. Blanc et al. (1995) studied the CIT concentration produced by *M. ruber* and *M. purpureus* in YES medium (SmF), wet rice (SSF) and synthetic media (Table 5). YES medium is used to produce fungal toxins, wet rice is traditionally used to produce RFR, and synthetic medium is used to produce red pigments. The results showed that *M. ruber* produced higher CIT levels than *M. purpureus* on the YES and wet rice medium. In contrast to later findings, five of the *M. ruber* isolates produced no CIT, four of the *M. ruber* isolates produced CIT in the range of 59–137 ng/g, and two of the *M. purpureus* produced higher CIT concentrations (8470–11,064 ng/g) (Li et al., 2020). Selecting a *Monascus* spp. that does not produce CIT or produced minimal CIT would help in ensuring a safe product of RFR.

The optimum temperature for *Penicillium viridicatum* and *P. citrinum* to produce CIT is 30 °C, but CIT can be produced over a wide temperature range between 15 °C to 37 °C (Montani et al., 1988; Silva et al., 2021; Wu et al., 1974). The optimum temperatures for *M. purpureus* to produce CIT in SSF and SmF are 35 °C and 32 °C, respectively (Zhang et al., 2013). In contrast, the study by Camardo Leggieri et al. (2016) found that the highest amount of CIT produced by *P. citrinum* occurs when incubated at 35 °C and 0.99 a_w. Another important finding was CIT production decreased rapidly when a_w decreased from 0.99 to 0.96 ($T = 20$ °C) and stopped production at 0.93 a_w. This result differs from Comerio et al. (1998) who reported that the minimum a_w for *P. citrinum* growth on MEA (30 °C) and CIT accumulation in the substrate was 0.775 and 0.810, respectively.

High red pigments and low CIT are achieved when RFR is fermented at pH 5.5, followed by the addition of an alkaline medium (the medium added was not mentioned) to adjust the pH to 8.5 (Orozco and Kilikian, 2008).

8. Detection methods of CIT

There are several methods used to detect CIT in RFR: (1) colorimetric techniques such as fluorometer, UV spectrometer and luminescence material (eg: carbon dot) as a fluorescence probe for CIT detection in the picomole range; (2) spectrophotodensitometer; (3) NMR spectroscopy; (4) enzyme immunoassays (EIA) such as ELISA and indirect competitive enzyme-linked immunosorbent assays (ic-ELISA); (5) immunochromatographic assay (ICA); (6) immunochromatographic strip (ICS); (7) capillary zone electrophoresis (CZE), including CZE along with an ultraviolet detector (CZE-UV); (8) micellar electrokinetic capillary chromatography; (9) TLC; (10) mass spectrometer; (11) HPLC with ultraviolet (UV) light, fluorescence (FLD) or photodiode-array (PDA) detector; (12) LC-MS; (13) LC-MS/MS; (14) LC-FLD; (15) UHPLC-DAD-QToF-MS; and (16) gas chromatography–mass spectrometry (GC–MS) (Avula et al., 2014; Blanc et al., 1995; Chen et al., 2016b; Cheng et al., 2018; Kamle et al., 2022; Marley et al., 2016; Nigović et al., 2013; Xu et al., 2006; Zhang et al., 2021). Some of them were listed in Tables 4 and 5.

HPLC is used to determine 80 % of the world's organic compounds due to providing accurate results and is the most frequently used method to detect CIT in RFR (Ji et al., 2015; Singh and Mehta, 2020). The limitations of using HPLC are the practical issues on the choice of calibration, sample preparation, sample type, and matrix effects (Singh and Mehta, 2020). Even though GC–MS provides high specificity and sensitivity, this method has drawbacks such as requiring samples volatilization, the nonlinearity of calibration curves, reminiscence properties from earlier samples, weak fluorescent and absorption groups, drifting responses, column blockage, and the risk of contamination compared to HPLC (Perez et al., 2016; Pettersson and Langseth, 2002).

Some of the mycotoxins such as CIT and AFs have natural fluorescence properties, and HPLC-FLD is used to identify analytes based on the occurrence of the chromophore in the particles (Singh and Mehta, 2020; Vazquez et al., 1997). Ji et al. (2015) compared the use of HPLC-FLD and LC-MS/MS for the quantification of CIT in RFR. The results showed that LC-MS/MS offers better sensitivity, accuracy, and reproducibility than HPLC-FLD. However, these instrument-based methods have disadvantages such as the instruments need maintenance, the use of large amounts of organic solvents, cost, time, complex pre-treatments, and requires a good technique and training for troubleshooting, method development, system testing, analyzing chromatograms and data analysis (Haidar Ahmad, 2017; Kamle et al., 2022; Singh and Mehta, 2020). Meanwhile, the drawbacks of biological methods such as TLC and ELISA are lack of sensitivity, difficult reproducibility, and the possibility of false-positive results (Kamle et al., 2022; Shekhar et al., 2017). Therefore, it is important to find a simple and rapid method for screening fungi that can produce CIT.

Coconut Cream Agar (CCA) has been used as a screening method for the detection of other mycotoxins such as AFs (Dyer and McCammon, 1994) and OTA (Heenan et al., 1998). Studies from Mohamed et al. (2013) found that CCA can be used to detect CIT from *P. citrinum* isolated from Maldive fish. This method has been verified by Farawahida et al. (2022) to screen CIT-producing *Monascus* spp. isolates isolated from RFR. This method can be adopted to select *Monascus* spp. isolates without or with low CIT to produce RFR.

9. Toxicity of CIT

AFs, CIT, and OTA can be produced during the fermentation of RFR (Blanc et al., 1995; Dogra and Kumar, 2017; Samsudin and Abdullah, 2013). *Monascus* pyridines are toxic metabolites that can also be present in RFR (Blanc et al., 1995). CIT can be produced by *Aspergillus*, *Fusarium* and *Penicillium* species across the temperature range of 15–37 °C, but the optimum temperature is 30 °C (Silva et al., 2021). Zhang et al. (2013) reported the optimum temperatures for CIT production by *M. purpureus* in SSF and SmF are 35 °C and 32 °C, respectively. CIT production could be minimised by incubation of *M. purpureus* for SSF and SmF at 28 °C. CIT has antibacterial properties against Gram-positive bacteria. However, due to its high mammalian nephrotoxicity, it is not allowed to be used as a drug (Flajs and Peraica, 2009).

CIT exposure has been tested on human cells and animals such as guinea pigs, mice, rats, rabbits, bovines, hamsters, and zebrafishes (de Oliveira Filho et al., 2017). CIT has detrimental health consequences for humans and animals including nephrotoxicity and hepatoxicity, but the level of acute toxicity varies in different species (Kumar et al., 2010). CIT is embryocidal and fetotoxic in mice, meanwhile, rats exposed to high doses of CIT cause renal tumours, and teratogenic effects and induce the enlargement of tubular necrosis of the kidney (Mayura et al., 1984). The major target organ of CIT is the kidney (Kamle et al., 2022). In cereals

and grains, CIT and OTA are the two mycotoxins frequently occurring together as co-contaminants and cause toxicity in the kidneys and reduce RNA synthesis in murine kidneys (Knecht et al., 2005; Sansing et al., 1976). Consumption of foods contaminated with these mycotoxins increased the toxicity due to the addition or combination effect of these mycotoxins, leading to kidney diseases in humans and animals (Bouslimi et al., 2008; Vrabcheva et al., 2000). Exposure of CIT to male Fisher 344 rats for 60 and 80 days weeks leads to tumour formation in their kidneys (Arai and Hibino, 1983). Vero cells (kidney cell culture) exposed to CIT for 24 h produce DNA damage, and a combination of OTA and CIT exposure simultaneously causes renal diseases due to enhanced oxidative stress (Bouslimi et al., 2008).

CIT also targets other organs such as the liver, heart, immune and reproductive system. The toxic effects of CIT have been associated with CIT-mediated oxidative stress and mitochondrial dysfunction in biological systems (de Oliveira Filho et al., 2017). Kumar et al. (2010) studied the immunotoxicity of New Zealand White rabbits. The combination of CIT and OTA caused several humoral and immunodepression in the rabbits.

In 1979, monacolin K was isolated from *M. ruber* and *A. terreus* (Endo, 2004). Then, Merck, Sharp, and Domen commercialized monacolin K as a drug (lovastatin) in 1987 after obtaining validation from Food and Drug Administration (FDA) (Endo, 2004; Le Bloc'h et al., 2015). As RFR produces a drug (monacolin K) and mycotoxin (CIT), the United States Food and Drug Administration (USFDA) does not approve the RFR as a dietary supplement (Gordon et al., 2010). However, some researchers have shown that RFR or *Monascus* fermented products pose no threat to human or animal health, and this may be due to the low levels of CIT in RFR or other *Monascus* fermented products (Lee et al., 2006; Mohan Kumari et al., 2009; Venero et al., 2010). Due to inadequate exposure data, risk assessment of CIT in food was estimated based on the CIT concentrations in grains and grain-based products, resulting in an exposure equal to the level of no concern for nephrotoxicity (0.2 μg/kg body weight per day) (EFSA, 2012). However, most researchers consider that some action should be taken to control CIT concentration in RFR (Chen and Hu, 2005).

The European Commission (EC) has established the maximum permissible level of CIT in food supplements based on rice fermented with *M. purpureus* is 100 μg/kg (EU, 2019). Meanwhile, the maximum permitted level of CIT in RFR products in other countries is 50 μg/kg in China (Srianta et al., 2014), 200 μg/kg in Japan (Srianta et al., 2014), and 2000 μg/kg in Taiwan (Taiwan-FDA, 2020). The regulations vary among the countries due to different dietary patterns, the risk analysis of toxicological data available, sampling and analytical capabilities, information on susceptible commodities, the effect on the availability of an adequate food supply, environmental conditions and national practices (FSANZ, 2019; Stoloff et al., 1991).

10. Control and reduction of CIT

CIT problems can be minimised by: (1) preventing contamination, (2) removing contaminated material from the RFR, (3) reducing CIT in RFR, and (4) treating exposed individuals (Karlovsky et al., 2016). The CIT production during the fermentation of RFR is difficult to avoid since *Monascus* spp. produce CIT. However, it may be possible to select isolates that can produce high levels of desirable pigments and monacolin K but no or minimal CIT (Blanc et al., 1995; Li et al., 2020). Another approach is to reduce the amount of CIT produced by manipulating the fermentation conditions (Yang et al., 2015). Three other approaches to prevent CIT production are (1) generating mutant *Monascus* spp. isolates free of the gene *pksCT* to obtain an isolate that does not produce CIT (Dikshit and Tallapragada, 2018; Jia et al., 2010; Li et al., 2020), (2) optimizing the culture media for SmF (Chen et al., 2016a), and (3) genetic engineering such as disrupting the *pksCT* gene (Jia et al., 2010).

Treatments to reduce CIT concentration in CIT standard, RFR and other samples have been reported (Table 6). These treatments can be categorized as physical, chemical, natural substances, and microbiological.

Physical methods include heating at 140 °C–160 °C which reduced 20 % of CIT and converted it to CIT H2 (non-cytotoxic to human cervical cancer, HeLa cells) (Chen et al., 2013; Hirota et al., 2002). Surface Active Maghemite Nanoparticles (SAMNs) removed 70 % CIT by adsorption and binding iron (III) in solution (Magro et al., 2016). This study suggested that the addition of SAMNs can also reduce other

Table 6
Methods to control and reduce CIT.

Category	Control of CIT during fermentation	Fungal species	Media/substrates	Treatment	Reduction of CIT	References
Physical	Initial $a_w = 0.800$	*P. citrinum*	Wheat	NA	NA	(Comerio et al., 1998)
Physical	pH = 5.5, then adjust the pH to 8.5	*M. purpureus*	[a]Bioreactor cultures	NA	NA	(Orozco and Kilikian, 2008)
Physical	NA	*P. citrinum*	[b]YES broth	Heating at 140 °C–160 °C	20 %	(Hirota et al., 2002; Trivedi et al., 1993)
	NA	*M. purpureus*, *M. ruber*	[c]Rice medium	SAMNs	70 %	(Magro et al., 2016)
Chemical	NA	*M. purpureus*	RFR	Phosphate-ethanol extraction	92 % CIT was reduced, and 80 % monacolin K has remained	(Lee et al., 2007a)
Natural substance	NA	*M. aurantiacus*	[d]SmF	Genistein (Flavonoid)	80 %	(Wang et al., 2020)
Microbiology	NA	NA	Nutrient yeast dextrose broth (NYDB)	*C. podzolicus* Y3	94 %	(Zhang et al., 2017)
	NA	*M. purpureus*	[e]YES medium	*S. cerevisiae*	98 %	(Davoudi Moghadam et al., 2019)
	NA	NA	[f]Mineral broth	*K. pneumoniae*	100 %	(Chen et al., 2011)

NA: not applicable.

[a] Bioreactor cultures (g/L): [KH_2PO_4 (1.5 g), K_2HPO_4 (1.5 g); $ZnSO_4{\cdot}7H_2O$ (0.01 g), MSG (7.6 g), NaCl (0.4 g), $FeSO_4$ (0.01 g), yeast extract (1.0 g) (pH 5.5)] with addition of glucose solution (g/L) [glucose (20.0 g); $MgSO_4{\cdot}7H_2O$ (4.8 g)].

[b] YES broth: 2 % yeast extract and 15 % sucrose.

[c] Rice medium: (20 g/L, 5 g/L glycine and 20 g/L agar-agar).

[d] SmF: (2.0 % rice powder, 0.2 % $NaNO_3$, 0.05 % KH_2PO_4, 0.1 % K_2HPO_4, 0.1 % $MgSO_4{\cdot}7H_2O$), with addition of rice powder inorganic salt medium (20.0 g/L) and 2.0 g/L of genistein.

[e] YES medium: yeast extract 40 g and sucrose 160 g/L in distilled water.

[f] Mineral broth (1 L of deionised water): [KCl (0.7 g), KH_2PO_4 (2 g); Na_2HPO_4 (3 g), $MgSO_4{\cdot}7H_2O$ (0.7 g); and $CaCl_2{\cdot}2H_2O$ (0.02 g)] containing 10 ppm of CIT, 1.2 % glucose, 0.3 % peptone and 100 ppm of vitamin C.

mycotoxins such as dihydrocitrinone and OTA due to the presence of a strong iron-chelating agent on the toxin molecule, namely the keto-enol group. However, the effects of SAMNs on other compounds such as pigment and monacolin K have not been reported. Cold plasma can reduce fungi and mycotoxin contamination in roasted coffee and may be able to be applied to RFR. *A. westerdijikiae*, *A. steynii*, *A. versicolor*, and *A. niger* were unable to produce CIT after the exposure to cold plasma for 6 min, while exposure for 30 min reduced 33–61 % of OTA produced by these fungi (Casas-Junco et al., 2019).

Treatment with chemicals such as phosphate during extraction (phosphate-ethanol extraction) of RFR was sufficient to reduce CIT and retain the monacolin K content. Up to 92 %, CIT was reduced and 80 % monacolin K was retained under optimized conditions (45 % ethanol, 1.5 % phosphate, and extraction for 70 min) (Lee et al., 2007a).

Wang et al. (2020) studied the effect of adding nine flavonoids (natural substances) to SmF medium, incubated in a shaking incubator (180 rpm) for 12 days at 30 °C. The mycelium was measured after obtaining a constant weight. The results showed that by the addition of 20 g/L rice powder and 2 g/L genistein, pigments and biomass were increased by 20 % and 80 % of CIT was reduced.

Cryptococcus podzolicus Y3 can be used to reduce CIT by intracellular enzymes (Zhang et al., 2017). CIT was reduced by 94 % using *C. podzolicus* Y3 at 1×10^8 cells/mL incubated in nutrient yeast dextrose broth (NYDB) at 28 °C for 42 h. CIT can be reduced by 98 % when 20 μg/mL of CIT is incubated at pH 4.0, CIT can be reduced by 98 % when 20 μg/mL of CIT is incubated at pH 4.0.

Davoudi Moghadam et al. (2019) studied the effects of adding heat-treated *Saccharomyces cerevisiae* at different temperatures and yeast concentrations on CIT and pigments produced during SmF using *Monascus* spp. When *S. cerevisiae* (10^5 cells/mL) was heated at 121 °C CIT was reduced by 98 % through binding to the yeast. However, this treatment also significantly reduced extracellular pigments (Davoudi Moghadam et al., 2019).

Chen et al. (2011) isolated CIT-degrading isolates in 24 soil samples (>50 g each). CIT (1 ppm) was added to suspensions of the soil samples and incubated in a shaking incubator at 150 rpm and 30 °C for 2–3 days. The microbial growth was monitored using optical density (OD) at 660 nm. When the OD unit of the suspension had an OD value >2, the CIT level in the suspension was measured by HPLC. If there was a reduction in CIT, the suspension was transferred to a new broth containing 2 ppm of CIT, and the same process was repeated at a higher CIT concentration (4 ppm). After the CIT was fully degraded, samples were diluted and inoculated onto mineral agar plates containing 10 ppm of CIT and incubated at 30 °C for 1 day. Over 300 colonies grown on the CIT-containing mineral agar plates were picked and re-suspended in the fresh mineral broth added with 10 ppm CIT and incubated in a shaking incubator at 30 °C for 2–3 days. From the screening process, 10 isolates were isolated and characterized as *Klebsiella pneumoniae* based on 16S rDNA and these bacteria degraded CIT, with the most effective isolate NPUST-B11. The addition of *K. pneumoniae*, resulted in a 2 % reduction in CIT when incubated at 37 °C at 200 rpm for 1 h. After 5 h, 91 % of CIT was degraded, and CIT was completely degraded after 10 h of incubation (Chen et al., 2011).

Lactic acid bacteria (LAB) can reduce other mycotoxins such as AFs, OTA, aflatoxin M_1, patulin, deoxynivalenol, zearalenone, fumonisin B1 and B2 (Muhialdin et al., 2020). There is a possibility that LAB may also be able to degrade CIT.

To control CIT production in RFR, recent studies focused on optimization of fermentation conditions, enrichment nutrients used in SmF, and post-harvest degradation by physical, chemical, natural substances, and microorganisms. Optimization of fermentation and enrichment nutrients can be used to control CIT production, but it is difficult to block CIT biosynthesis completely in *Monascus* spp. (Li et al., 2020).

Variation in the CIT production and concentration could be due to different extrinsic factors during fermentation. Comerio et al. (1998) studied the influence of a_w on *P. citrinum* growth and CIT production in wheat. The finding showed that CIT in wheat was not detected at a_w 0.800, even though there was the development of *P. citrinum* mycelium and sporulation after 6 days and 12 days of incubation, respectively. Ristiarini et al. (2017) reported that RFR in Indonesia has a_w between 0.75 and 0.80. However, there is no information available on the effect of a_w on CIT produced by *Monascus* spp. (Silva et al., 2021).

Different *Monascus* species and isolates also can affect CIT production. Li et al. (2020) showed that *M. purpureus* R9 produced higher CIT than *M. purpureus* R3. However, some *M. ruber* isolates (R4, R15, R16, R17) produce CIT while other *M. ruber* isolates (R1, R2, R19, R20, R21) do not produce any CIT. The results contradict the results obtained by Blanc et al. (1995), who reported *M. ruber* produced higher CIT than *M. purpureus* in SSF and SmF. Another study found that from eight isolates of *M. purpureus*, two of the isolates (isolates IFRPD 4044 and IFRPD 4046) do not produce any CIT after fermentation for 14 days at 30 °C (Saithong et al., 2019).

Therefore, it is important to select *Monascus* spp. isolates as the inoculum for RFR production. CCA has been successfully used to screen other mycotoxins such as AFs and OTA, and CIT from Maldive fish (Mohamed et al., 2013). This method has been used to screen *Monascus* spp. isolates from RFR (Farawahida et al., 2022).

11. Conclusion

RFR is consumed as a traditional Chinese medicine producing a variety of metabolites, in particular, monacolin K, beneficial to human health for cholesterol reduction, and red pigments to add flavour and colour to food. However, CIT is produced during the fermentation of RFR and this is a concern for human health. To produce safe RFR, CIT production must be reduced. Several methods can do this in the final product, but many of these methods are not fully understood or are impractical for routine use. The ideal approach is to prevent CIT production. One of the methods is to select *Monascus* spp. isolates that produce no CIT, while still producing the desirable bioactive properties valued in RFR. There is another scope to explore which is applying different fermentation conditions, such as a_w, oxygen levels and temperature to prevent CIT production. Rapid screening tests would be useful to help select *Monascus* spp. isolates that do not produce CIT. The kinetics of CIT production during RFR fermentation does not appear to have been studied and is important to optimise the fermentation to minimise CIT presence. Alternative methods for CIT reduction could be investigated to produce safe RFR.

CRediT authorship contribution statement

Abdul Halim Farawahida: Conceptualization, Resources, Writing – original draft, Visualization, Project administration. **Jon Palmer:** Conceptualization, Writing – review & editing, Supervision. **Steve Flint:** Conceptualization, Resources, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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