

## Effects of pH on the Stability of *Monascus purpureus* Red Yeast IR-64 Rice (MpRYR) Extract

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### Abstract

*Monascus purpureus* red yeast rice (MpRYR), popularly known as angkak, is a Chinese fermented rice health product. The red pigments and monacolin K contained in MpRYR are sensitive to various factors, including acidity, temperature, oxidation, and light; thus, MpRYR is unstable and susceptible to damage during storage and processing. It was reported that monacolin K in red yeast rice solution was easily degraded under thermal treatment at 85–121°C, and the absorbance of MpRYR extract increased at an alkaline pH. This work aimed to study the effects of pH on the stability of pigment extract MpRYR. MPYR was purchased from Raja Godhong herbal supplier and extracted with 70% ethanol for 3 × 24 h. The resulting MpRYR extracts were subjected to various pH conditions, and the pH and absorbance were measured, and the degradation rate of MpRYR extracts was calculated. The initial pH of MpRYR extract is 5.75, which increased to 5.97 (+3.82%) at 3 h. The MpRYR extract was more stable in an acidic medium with a degradation rate of 3.80% at 3 h. Research results suggest that acidic conditions are preferable for storing and formulating MpRYR-based products to preserve their functional efficacy.

**Keywords:** angkak, *Monascus*, nutritional composition, proximate, yeast rice

## Pengaruh pH terhadap Stabilitas Ekstrak Beras Angkak IR-64 Fermentasi *Monascus purpureus* (MpRYR)

### Abstrak

*Monascus purpureus* red yeast rice (MpRYR), yang dikenal luas dengan nama angkak, merupakan produk kesehatan berupa beras hasil fermentasi dari Tiongkok. Pigmen merah dan monakolin K yang terkandung dalam MpRYR bersifat sensitif terhadap berbagai faktor, seperti keasaman, suhu, oksidasi, dan cahaya, sehingga MpRYR menjadi tidak stabil dan mudah rusak selama penyimpanan maupun proses pengolahan. Dilaporkan bahwa monakolin K dalam larutan angkak mudah terdegradasi ketika dipanaskan pada suhu 85–121°C dan daya serap ekstrak MpRYR meningkat pada pH basa. Penelitian ini bertujuan untuk mengkaji pengaruh pH terhadap stabilitas ekstrak pigmen MpRYR. MpRYR diperoleh dari pemasok herbal Raja Godhong. Ekstraksi dilakukan menggunakan etanol 70% selama 3 × 24 jam. Ekstrak MpRYR yang diperoleh kemudian diberikan perlakuan pada berbagai kondisi pH, dilakukan pengukuran pH dan absorbansi, serta dihitung laju degradasinya. pH awal ekstrak MpRYR adalah 5,75 dan meningkat menjadi 5,97 (+3,82%) setelah 3 jam. Ekstrak MpRYR menunjukkan stabilitas yang lebih baik pada medium asam dengan laju degradasi sebesar 3,80% dalam 3 jam. Hasil penelitian menunjukkan bahwa kondisi asam lebih disarankan untuk penyimpanan dan formulasi produk berbasis MpRYR guna mempertahankan khasiat fungsionalnya.

**Kata Kunci:** angkak, beras fermentasi, komposisi nutrisi, *Monascus*, proksimat

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## 1. Introduction

The natural yellow, orange, and red pigments produced by *Monascus sp.* have garnered interest due to their multifunctional properties, including serving as food colorants and exhibiting numerous pharmacological activities. *Monascus* pigments have demonstrated antioxidant, anti-inflammatory, anticancer, antidiabetic, and anti-obesity properties.<sup>1-5</sup> Of the yellow, orange, and red pigments, the red *Monascus* pigments are the most promising food colorants to be further explored because of their good solubility in water.<sup>6</sup> Unfortunately, the red *Monascus* pigments are sensitive to numerous factors, e.g., acidity, temperature, light, and process oxidation. These pigments were found to be unstable during storage and processing.<sup>7</sup> Therefore, preparation for *Monascus* red pigments embedded in liposomes was performed. Long et al.<sup>8</sup> reported that *Monascus* red pigments embedded in liposomes were stable in a pH range between 4 and 8, with a degradation rate of approximately 40–50% when stored at 4°C in the dark for up to 30 days.<sup>6</sup>

*Monascus purpureus* red yeast rice (MpRYP), popularly known as angkak, is a Chinese fermented rice product that decreases cholesterol and triglyceride levels in human blood because of its monacolin K, which is structurally identical to lovastatin, an inhibitor of HMG-CoA reductase.<sup>8,9</sup> Red yeast rice supplementation could reduce LDL-cholesterol levels by approximately 15–34%.<sup>10</sup> Similar to the *Monascus* red pigment, monacolin K is also unstable during storage. This statin analog is sensitive to temperature, moisture, light, and oxygen, and degrades to form dehydromonacolin K. Storage of red yeast rice powder at 4°C under a vacuum container could increase the retention of monacolin K.<sup>11</sup> Ou et al. (2009) reported that monacolin K in red yeast rice solution was easily degraded under thermal treatment at 85–121°C. Only 53.29% of monacolin K residues were recovered after being heated at 121°C for 90 min.<sup>12</sup> Furthermore, a previous study by Priatni<sup>13</sup> reported the stability of *Monascus* red yeast extract. The absorbance of the red yeast extract was reported to increase at an alkaline pH, and the absorbance increased gradually during heating up to 70°C.<sup>13</sup> Taking everything into consideration, this work aimed to study the effects of pH on the stability of MpRYP extract.

## 2. Materials and methods

### 2.1. Tools

Instruments used were Buchi R-215 rotavapor system, Buchi B-491 heating bath 120V, Buchi V-700 vacuum pump, Buchi distillation water chiller B-741, Buchi evaporator glassware, digital analytical balance

(Mettler Toledo AB204-S), oven (Mettler UN110), furnace (Thermo Scientific Thermolyne F47925), Soxhlet apparatus (Buchi E-812), Kjeldahl apparatus (VELP Scientifica DK 6), desiccator (Kartell 2446), pH meter (Toledo), spectrophotometer ultraviolet-visible (Thermo Scientific type Genesys-50).

### 2.2. Materials

*Monascus purpureus* red yeast IR-64 rice (MpRYP) was purchased from Raja Godhong herbal supplier, technical grade ethanol (Brataco), hydrochloric acid (HCl) (Merck 1.00731), sodium hydroxide (NaOH) (Merck 1.06498), distilled water (Brataco), filter paper (Whatman No. 1).

### 2.3. Methods

#### 2.3.1. Organoleptic observation and preparation of the extract

MpRYP was observed for its morphology, smell, and taste, then weighed (3.0 kg), ground, and the resulting powder was passed through a 40-mesh sieve to obtain approximately 2.9 kg of powder.

The powder was macerated in 70% ethanol for 24 hours and then filtered. The residue was further macerated using the same solvent for 2 × 24 h. The macerates were collected and filtered, and the solvent was evaporated at 70°C to a thick consistency.

#### 2.3.2. Effects of pH on the stability of MpRYP extract

Approximately 1 g of MpRYP extract was put in a 25 mL volumetric flask. The extract was added and dissolved in distilled water to obtain a concentration of 40,000 µg/mL.

The extract of MpRYP (40,000 µg/mL) was put in 9 volumetric flasks and diluted with distilled water and HCl 0.1 N (pH of 1.39) or NaOH 0.1 N (pH of 12.04) as follows: A total of nine flasks were prepared to assess the effect of pH on MpRYP pigment extract. Flask 1 contained 10 mL of MpRYP pigment extract without any addition. Flasks 2 to 5 contained mixtures of 8, 6, 4, and 2 mL of MpRYP pigment extract, respectively, each diluted with 2, 4, 6, and 8 mL of 0.1 N HCl to adjust the acidity. Similarly, flasks 6 to 9 were prepared by mixing 8, 6, 4, and 2 mL of MpRYP pigment extract with 2, 4, 6, and 8 mL of 0.1 N NaOH, respectively, to create alkaline conditions.

All solutions were scanned in a UV-visible spectrophotometer, the wavelength range was set at 400 to 800 nm, and the absorbance of the solutions was recorded at 0 h, 1 h, 2 h, and 3 h. The absorbance

of extract pigment solutions was plotted against the concentration of MpRYR extracts to obtain the calibration curves. The degradation rate (DR) was calculated using the following formula:

$$DR (\%) = C_0 - C_t / C_0 \times 100 \dots (1)$$

Where  $C_t$  is the concentration of MpRYR extract during different treatments (calculated using the linear regression equation of the calibration curve), and  $C_0$  is the initial concentration of MpRYR extract.

Moreover, the pH of the solutions was also measured using a pH meter.

### 3. Result

#### 3.1. Organoleptic observation and preparation of the extract

MpRYR had a reddish-brown color with a fresh rice aroma and a bitter taste. The extraction yield of MpRYR is 9.02 %.

#### 3.2. Effects of pH on the stability of MpRYR extracts

The pH of MpRYR extracts at 0 h, 1 h, 2 h, and 3 h was depicted in Figure 1. The initial pH of the MpRYR extract (flask 1) was 5.75, which increased to 5.97 at 3 hours. The pH of MpRYR extracts at 3 h measurement showed a slight increase in an acid medium (flasks 2, 3, 4, 5) and a slight decrease in an alkaline medium (flasks 6, 7, 8, 9).

Figure 2 illustrates the spectral response of the MpRYR extract in acidic conditions. The absorbance at 486 nm decreased as the amount of HCl increased (from flask 1 to flask 5), indicating that higher acidity results in slightly reduced pigment intensity. This trend suggests that while MpRYR extract remains relatively stable in acidic media, extreme acidity may slightly affect pigment solubility or interaction.

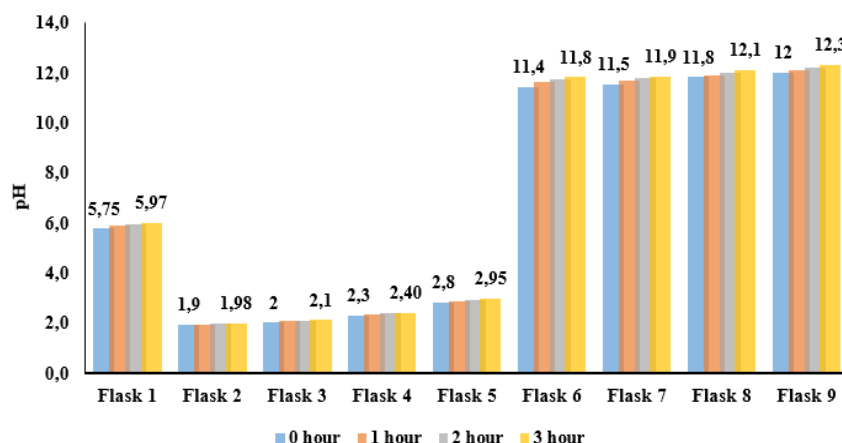


Figure 1. The pH (y-axis) of MpRYR pigment extract solutions at 0 h, 1 h, 2 h, and 3 h.

Figure 3 shows the spectra under alkaline conditions. The absorbance at 486 nm significantly declined from flask 6 to flask 9 as the concentration of NaOH increased, indicating that MpRYR pigments are more prone to degradation in basic environments. The pronounced drop in absorbance under alkaline conditions implies that pH elevation causes structural breakdown of the pigment compounds, particularly azaphilones, which are known to be unstable in high pH media.

The degradation rate of MpRYR extracts in both acidic and alkaline pH is summarized in the combined Figure 4. In acidic medium, the degradation rate increased moderately over time, from 0% at 0 h to 3.7975% at 3 h. Meanwhile, in alkaline medium, degradation occurred more rapidly, reaching 4.9501% at 3 h. This trend highlights that MpRYR extract is more stable under acidic conditions.

This behavior is likely due to the stability of *Monascus* pigments, particularly azaphilone compounds, which are known to degrade more easily in basic environments. Our findings are consistent with previous studies, such as Long et al. (2023), which reported enhanced pigment stability at pH 5–6, with higher degradation observed in more alkaline conditions. The data support the conclusion that maintaining a slightly acidic environment is beneficial for preserving MpRYR pigment stability during formulation and storage.

### 4. Discussion

As shown in Figure 1, the initial pH of MpRYR extract was 5.75, which increased to 5.97 (+3.82%) at 3 h. The pH of the MpRYR water extract was almost similar to the ethanol extract of red yeast rice reported by Yuan et al. (2023), which was 5.5.<sup>14</sup> However, it is higher than that reported by Husakova et al. (2021), which was 4.9.<sup>15</sup> The stability of key secondary metabolites in *Monascus purpureus* red yeast rice (MpRYR), such as

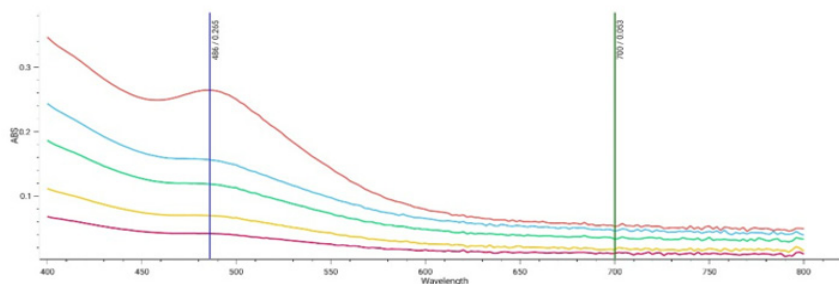


Figure 2. The spectra of MpRYR pigment extract solutions at 0 h show one maximum at 486 nm. The red spectrum belongs to the MpRYR extract without the addition of HCl 0.1 N (flask 1). A decrease in pH correlates positively with the absorbance. The blue spectrum is the solution in (flask 2); the green spectrum is the solution in (flask 3); the yellow spectrum is the solution in (flask 4); and the violet spectrum is the solution in (flask 5).

monacolin K and red *Monascus* pigments (monascin, rubropunctatin, ankaflavin, etc.), is highly influenced by the pH of the medium. These compounds are known to undergo structural or functional degradation outside optimal pH conditions.<sup>16</sup>

Monacolin K, a natural statin, is particularly unstable under alkaline conditions. At higher pH levels, it can undergo lactone ring hydrolysis to form its hydroxy acid, thereby reducing its pharmacological activity and altering its therapeutic index.<sup>9,17,18</sup> Under acidic conditions, the lactone form is more stable, which aligns with your results showing a lower degradation rate of 3.80% at pH 5.75 after 3 hours.

Similarly, *Monascus* pigments are highly sensitive to pH. Studies show that the red pigments (e.g., rubropunctamine, monascorubramine) are more stable under mildly acidic environments, while degradation or transformation to yellow/orange pigments occurs at higher pH levels.<sup>19,20</sup> These pH-induced transformations may influence not only stability but also color intensity and antioxidant capacity.

Thus, maintaining an acidic medium is essential not only for physical stability but also for preserving the bioactivity and integrity of the functional metabolites in MpRYR products.

As shown in Figure 2, the spectra of MpRYR extract solutions indicate a decrease in the absorbance with

pH, and an increase in pH correlates inversely with the absorbance. Similar to our results, a study by Priatni (2014) reported that the absorbance of *Monascus* fermented rice pigments lessened under acidic conditions.<sup>13</sup>

The spectrum of MpRYR (Figure 3) shows a maximum of 486 nm, which is lower than an earlier study by Husakova et al. (2021), who confirmed a value of 500 nm.<sup>15</sup>

Our study indicates that MpRYR extracts are more stable in an acidic medium, as proven by the degradation rate of 3.7975% at 3 h (Figure 4), while the degradation rate of MpRYR extracts in alkaline pH is 4.9501% at 3h (Figure 5). This higher stability in acidic conditions is likely due to the chemical structure of the pigments in *Monascus*, such as azaphilone compounds, which are known to exhibit greater stability in acidic environments compared to alkaline ones. Similar to our results, Long et al. (2023) described that *Monascus* red pigments exhibited better stability in slightly acidic or neutral mediums. It was reported that the degradation rate of *Monascus* red pigments at pH 5 was 30.2%, and at pH 6 was 19.75%.<sup>6</sup>

The linear regression equation and coefficient of correlation ( $R^2$ ) of MpRYR extracts in acidic and alkaline pH (Table 1) present the linear regression equations and the coefficients of determination ( $R^2$ ) of *Monascus purpureus* red yeast rice (MpRYR)

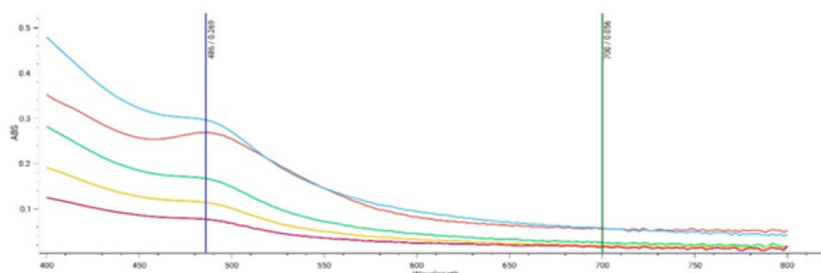
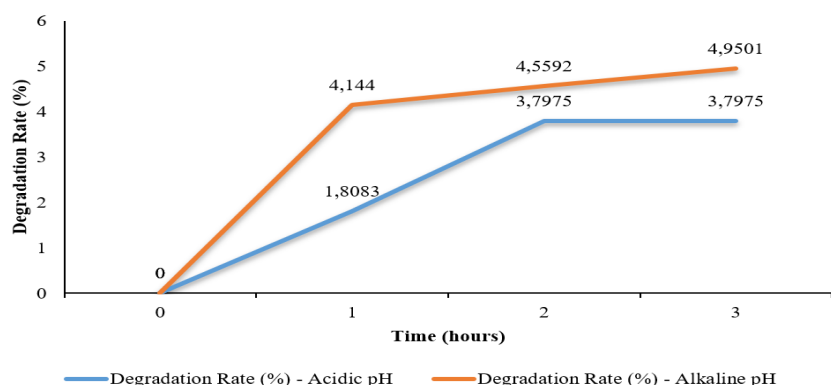


Figure 3. The spectra of MpRYR pigment extract solutions at 0 h with one maximum at 486 nm. The red spectrum belongs to the MpRYR extract without the addition of NaOH 0.1 N (flask 1). An increase in pH correlates inversely to the absorbance. The blue spectrum is the solution in (flask 6); the green spectrum is the solution in (flask 7); the yellow spectrum is the solution in (flask 8); and the violet spectrum is the solution in (flask 9).





**Figure 4.** The degradation rate of MpRYR extracts in acidic and alkaline pH at 0 to 3 h.

extract under acidic and alkaline pH conditions over a 0–3h period. These regression values reflect changes in a measured parameter (presumably absorbance or concentration of active compounds) concerning storage time at each pH level.<sup>21</sup>

Under acidic pH conditions, all  $R^2$  values indicate a very strong correlation ( $\geq 0.87$ ), with the highest value observed at 0 hours ( $R^2 = 0.9331$ ). This suggests that the stability of the bioactive compounds under acidic conditions tends to remain more consistent over time. The slope of each regression equation, which is negative, reflects a declining trend in the measured parameter, indicating a gradual degradation of bioactive compounds. This degradation may be attributed to the breakdown of pigments such as monascorubrin and monascin, which are known to be sensitive to both time and acidic environments.<sup>22</sup>

In contrast, under alkaline pH conditions, the slopes are positive (indicating an increasing trend), but the  $R^2$  values are generally lower (ranging from 0.8202 to 0.8787), suggesting greater variability in the stability of the active compounds. These findings align with previous studies reporting that alkaline environments can induce pigment degradation and structural changes in active compounds of MpRYR, particularly phenolic constituents and *Monascus* pigments.<sup>23</sup> Overall, these results indicate that MpRYR extract exhibits greater kinetic stability in acidic pH compared to alkaline pH. This higher stability is particularly relevant if MpRYR products are intended for oral delivery systems that pass through the acidic gastric environment. Moreover, the regression profiles may serve as predictive models to estimate the stability duration of bioactive compounds during early storage.

## 5. Conclusion

The pH of *Monascus purpureus* red yeast IR-64 Rice (MpRYR) extract is 5.75, which increases to 5.97 (+3.82%) at 3 h. MpRYR extract is more stable in an acidic medium with a degradation rate of 3.8% at 3 h.

## Conflict of Interest

The authors declare no conflicts of interest.

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