Evaluation of Bio-red Pigment Extraction from *Monascus purpureus* FTC5357

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Abstract. A suitable extraction technique helps to increase the extraction yield and stimulate higher quality of pigments. Therefore, investigating the effect of different extraction solvents on red pigment produced via solid-state fermentation (SSF) by *Monascus purpureus* FTC 5357 are essential. In this study, oil palm frond (OPF) was used as a substrate for the fermentation process. The fermentation was conducted at 30 °C for eight days. Variation of solvents (95% ethanol, 60% ethanol and distilled water), pH and time of extractions were applied on the fermented product. The extracted pigment was then analysed using spectrophotometer at 500 nm, for red pigment. Combination of pH 6 and 60% ethanol at 16 h pronounced to be the best conditions to extract the pigment, with an absorbance value of 207 AU/g.d. The advantage of the ethanol as a solvent extraction is cheap and non-toxic. Later, the extracted pigment is safe to be used in food applications.

1 Introduction

Customarily, the manufactured food will be imposed with colorants to amplify its commercial values [1]. According to Martins et al. (2016), pleasing colours might affect the product acceptance. There are two categories of food colorant such as natural food colorant and synthetic food colorant. Recently, the awareness on the application of the former to the food product has increased due to the harmful effect caused by the latter [2-4].

Natural pigments are coloured compounds extracted from living organisms; such as from plant [5], animal [6, 7] and fungus [8-10] and most of the available natural pigment was extracted from plant [11-13]. Despite the popularity of pigment extracted from plant, pigment produced by microorganisms hold a promising potential to meet present day challenges. *Monascus* species is known be able to produce an edible pigment and it is highly safe [14, 15]. Furthermore, *Monascus* pigments not only improve the marketability of the product but also have varied biological activities such as anti-inflammatory [16, 17], anti-tumor [18, 19], anti-oxidant [20-22] and regulation of cholesterol levels characteristics [23, 24].

Generally, pigment production in industrial scale has been carried out using submerged fermentation (SmF) [25]. However, solid state fermentation (SSF) has emerged as an effective way due to the high production yield [26]. In addition, by SSF process, a relative low-cost process can be achieved, especially when agro-industrial wastes are used as substrate [27].

In Malaysia, there are more than 4.98 million hectares of oil palm plantations [28]. The main problem in the oil palm tree cultivation and its related industries is its substantial amount of biomass
wastes. Oil palm frond (OPF) is one of the biomass waste generated. However, the utilization of OPF is limited. Previous study were done on the usage of petiole and leaflet of OPF as a substrate by SSF to produce red pigment [29-31]. Yet, the challenging occurred on how to extract the red pigment from the fermented OPF via SSF, since the extraction is one of the most expensive steps in the production of natural colorants [32].

Solid liquid extraction (SLE) is the most common technique for the removal of pigment from fermented substrate by SSF [33-36]. A suitable extraction technique helps in increasing the extraction yield, besides prevent the degradation of the extracted pigments [37].

A number of researchers have proposed various extraction methods, however, most of the methods are on plant material [5, 38-41]. Due to the limitation of previous study in extracting the natural pigment from fungi via SSF, thus, this study aims to investigate the performance of red pigment extracted from Monascus purpureus FTC 5357. Extraction process is generally affected by several factors such as temperature, time and solvent type [32, 42, 43]. Hence, the above mentioned factors are identified on the efficiency to extract the red pigment produced by different extraction solvents under various conditions.

2 Methodology

2.1 Culture and Solid State Fermentation

Monascus purpureus FTC 5357 was purchased from culture collection Malaysian Agricultural Research and Development Institute (MARDI), Malaysia. Petiole oil palm fronds (OPFs) were obtained from agricultural fields, Felda Lepar Hilir, Gambang, Pahang, Malaysia. The fresh OPFs were cut into smaller pieces and dried in an oven for 1 day, ground and sieve to get 1 mm particle size using a sieve shaker (Retsch AS 200 Basic, Germany) [30]. Later, the OPFs powder were autoclaved with distilled water in a 1:18 ratio (w/v) at 121 °C, for 15 min and cooled at room temperature [44, 45]. The pre-treated OPFs were filtered and washed with distilled water, before being oven dried at 60 °C for 24 h [30]. The pre-treated OPFs were mixed with distilled water to get approximately 75% initial moisture content, adjusted to pH 8 and 4% (w/w) of peptone. The medium was autoclaved at 121 °C for 20 min. Then, the sterilized OPFs were inoculated with 1.0x10⁷ spores/mL and incubated at 30 °C, for 8 days.

2.2 Extraction Methods

Fermented OPFs were dried in an oven at 60 °C for 24 h. In order to determine the performance of different extraction conditions, three different solvent mixtures were evaluated which are distilled water, 60% ethanol (v/v) and 95% ethanol (v/v). A 0.5 g of dried fermented OPFs were placed in 250 mL Erlenmeyer flasks and mixed with different solvents in a ratio of 1:160 (g/ml). The solvent extraction was performed in an incubator shaker at 180 rpm, 30 °C for 1 h. After that, another experiment was repeated with different pH (i.e. pH 2, 4, 6 and 8) using the best solvent obtained in the previous experiment in a ratio of 1:160 (g/ml) at 180 rpm, 30 °C for 2 h. The pH of the solvent was adjusted using hydrochloric acid (HCl) and sodium hydroxide (NaOH). Later, with the optimum condition found earlier, six individuals Erlenmeyer flasks were exposed to six different extraction times (i.e. 4, 8, 12, 16, 20 and 24 h).

2.3 Pigment Assay

In all cases, at the end of extraction period, the mixtures were allowed to stand for 15 min at room temperature and filtered through Whatman no.1 filter paper [46-48]. The supernatants were analyzed by a spectrophotometer at a wavelength of 500 nm, for red pigment, taking into consideration the dilution factor of the sample [49]. The results were expressed as absorbance units per g of dried solid (AU/g.d).
3 Results and Discussion

3.1 The effect of the extraction solvent

The solvent selection is very important to determine the affiliation of the solvent composition to the particles to be extracted [50]. Extraction by different types of solvent mixture were investigated on the dried fermented OPF, separately. Figure 1 shows that all the solvents tested were able to extract the red pigment. Among the solvents tested, 60% ethanol shows the best extraction with an absorbance value of 219.2 AU/g.d, followed by 95% ethanol (41.6 AU/g.d) and distilled water with an absorbance value of 12.8 AU/g.d. The absorbance value of 95% ethanol and distilled water are lower than 60% ethanol by 81% and 94%, respectively, due to the polarity of solvent. Where, the ethanol is able to react with both polar and non-polar compounds due to its unique structure molecule. The hydroxyl (OH) group with the high electronegativity of oxygen allow the hydrogen bonding to take place known as polar compound, while the ethyl (C2H5) group acted as non-polar compound. At lower ethanol concentration (60%), the polarity of the solvent was quite higher when compared to the 95%. Where, at 60% ethanol the hydrogen bond (OH) in water was mixed together in ethanol. Too high polar solvent (i.e. distilled water) did not promote to better pigment extraction because it consists of only OH group.

Karacabey & Mazza (2008) and Carvalho et al. (2007) reported that moderate polar compounds were suited to be extracted with 50-70% ethanol concentration. The efficiency of the extraction is based on the selectivity of the solvent to the compound that need to be extracted. The result obtained is compatible with previous research which reported that water has the lowest yield of Monascus pigment due to high polarity of distilled water [51]. Ethanol 60% appeared to be the best solvent for red pigment extraction due to the close on the polarity of the red pigment produced by Monascus and the solvent [52, 53]. Thus 60% ethanol was used for the next experiment.

![Figure 1. Absorbance of red pigments extracted using different solvents.](image)

3.2 Effect of pH on extraction

The pH value plays a crucial role in the extraction process. Next a series of experiments at different pH value were studied. Monascus pigment was extracted at different pH of ethanol (60%) ranging from pH 2 to 8. The wavelength of 500 nm denoted as red pigment as agreed by many researchers [36, 45, 47, 54-56]. On the other hand, the wavelength of 400-420 nm, indicated yellow pigment [57, 58]. Figure 2 shows that pH 2 (170 AU/g.d) and pH 6 (172 AU/g.d) produced high red pigment compared to pH 4 (143 AU/g.d) and pH 8 (135 AU/g.d). But, pH 2 produced higher yellow pigment compared to the red pigment. While, at pH 6, the absorbance value for yellow and red pigments were almost
comparable. It was observed that, the pH solvent for extraction was comparable with the pH medium for *Monascus* to growth. It has been reported that when the pigment produced at lower pH (pH<6), there was predominance of yellow pigment and at higher pH (pH≥6), there was predominance of red pigment [46, 47, 59, 60]. Feng et al. (2012) and Orozco et al. (2008) reported that the pH ranged from 5.5 to 8.0 are shown to stimulate *Monascus* growth and the red pigment production. As shown in Figure 2, the best conditions to extract the red pigment occurred at pH 6 and yellow pigment at pH 2, using 60% of ethanol. Thus, pH 6 was used for the next experiment.

![Figure 2. Effect of pH on extraction.](image_url)

3.3 Effect of time on extraction

*Monascus* pigment was soaked in the best pH (pH 6) using 60% of ethanol solution as discussed in previous section, at different soaking time from 4 h to 24 h. As shown in Figure 3, the trend was increased as the soaking time extended to 16 h, with the absorbance values of 207 AU/g.d. At 16 h of extraction, an absorbance value was increased up to 2.3–fold when compared to the 4 h extraction. No further increased of the pigment value when the extraction time increased to more than 16 h. The result indicated that the longer the exposure of solute to the solvent, the greater the pigment can be extracted from the solid substance (fermented OPF). At longer soaking time, the contact time between the fermented OPF to the solvent is greater, allowing the phase equilibrium to be established [50]. Hence, the reaction complete, as a result more pigment is extracted from the fermented OPF. Similar findings were reported by Henriques et. al. (2007), Kumar et. al (2017) and Sinha et al., (2012), where the pigments extracted from marine microalga, *Bougainvillea glabra* and *Butea monosperma*, respectively, improved at longer time.
4 Conclusion
The key point in pigment extraction is the selectivity of the solvent. Ethanol was used as extraction solvent due to its characteristic such as non-toxic and volatile, which could be significant point to be used in food industry. It was confirmed that red Monascus pigment yield can be improved: (i) by using 60% ethanol, (ii) applying pH 6 of ethanol and (iii) extraction time of 16 h. In order to analyze more about extraction method in extracting the red pigment by Monascus purpureus on OPF, an optimization of parameters in speed of extraction and extraction temperature need to be investigated in the future. This will in turn provide more important information in order to apply in industrial applications.

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