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

IOP Conf. Series: Materials Science and Engineering **736** (2020) 022084 doi:10.1088/1757-899X/736/2/022084

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

**N F S Daud[1], F M Said[1] M Ramu[1] and N M H Yasin[1]**

[1]Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak 26300 Gambang, Pahang, Malaysia

farhan@ump.edu.my

**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 $1.0x10^7$ 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 ($C_2H_5$) 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.

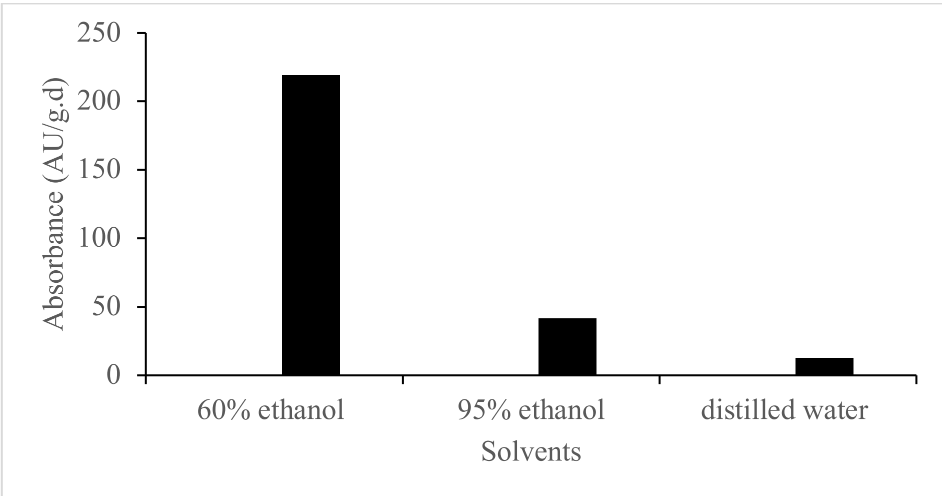


**Figure 1.** Absorbance of red pigments extracted using different solvents.

### *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.

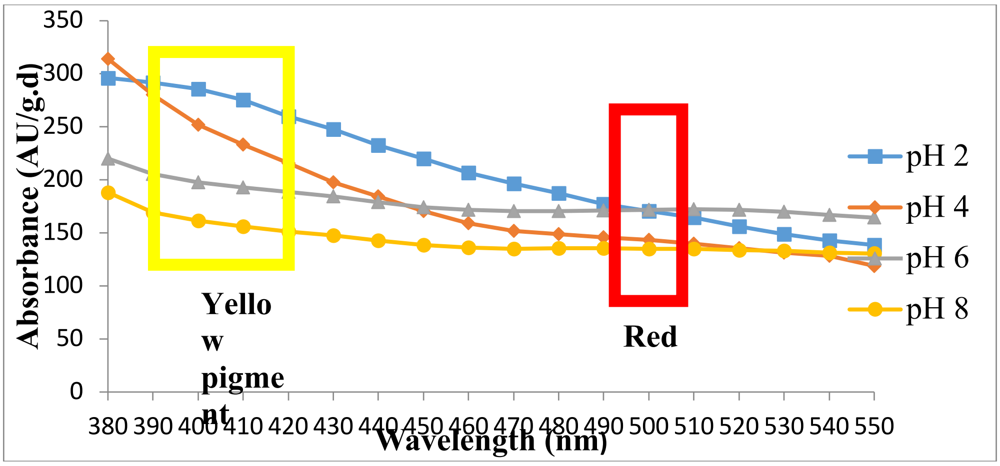


**Figure 2.** Effect of pH on extraction.

*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.

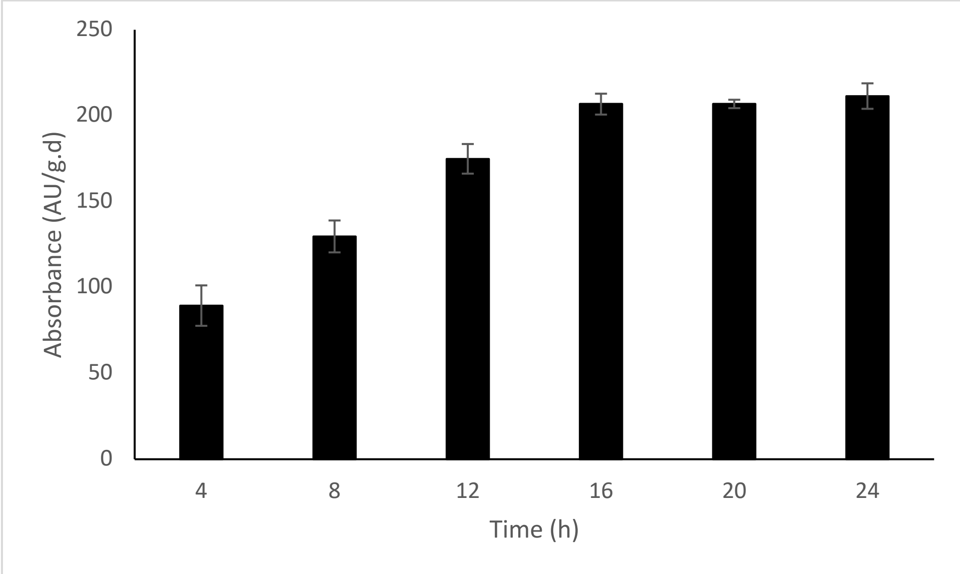


**Figure 3.** Effect of time on extraction.

## 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.

## ACKNOLEGDEMENTS

The authors would like to thank to Fundamental Research Grant Scheme (FRGS) FRGS/1/2015/SG05/UMP/03/2 (RDU150105) granted by the Ministry of Higher Education (Malaysia) for fully funding the work.

## References

[1] Kumar, S.N.A., S.K. Ritesh, G. Sharmila and C. Muthukumaran, Extraction optimization and characterization of water soluble red purple pigment from floral bracts of *Bougainvillea* glabra. *Arabian Journal of Chemistry*, 2017. **10**: p. S2145-S50.

[2] Basu, A. and G. Suresh K, Binding of carmoisine, a food colorant, with hemoglobin: Spectroscopic and calorimetric studies. *Food Research International*, 2015. **72**: p. 54-61.

[3] Basu, A. and G.S. Kumar, Interaction of toxic azo dyes with heme protein: biophysical insights into the binding aspect of the food additive amaranth with human hemoglobin. *Journal of hazardous materials*, 2015. **289**: p. 204-09.

[4] Basu, A. and G. Suresh Kumar, Multispectroscopic and calorimetric studies on the binding of the food colorant tartrazine with human hemoglobin. *Journal of Hazardous Materials*, 2016. **318**: p. 468-76.

[5] Tan, J.B.L., Y.Y. Lim and S.M. Lee, *Rhoeo spathacea* (Swartz) Stearn leaves, a potential natural food colorant. *Journal of Functional Foods*, 2014. **7**: p. 443-51.

[6] Mapari, S.A.S., U. Thrane and A.S. Meyer, Fungal polyketide azaphilone pigments as future natural food colorants? *Trends in Biotechnology*, 2010. **28**(6): p. 300-07.

[7] Van, A. Huis, J. Van Itterbeeck, H. Klunder, E. Mertens, A. Halloran, G. Muir and P. Vantomme, *Edible insects: future prospects for food and feed security*. 2013: Food and Agriculture Organization of the United Nations.

[8] Mapari, S.A.S., K.F. Nielsen, T.O. Larsen, J.C. Frisvad, A.S. Meyer and U. Thrane, Exploring fungal biodiversity for the production of water-soluble pigments as potential natural food colorants. *Current Opinion in Biotechnology*, 2005. **16**(2): p. 231-38.

[9] Velmurugan, P., H. Hur, V. Balachandar, S. Kamala-Kannan, K.-J. Lee, S.-M. Lee, J.-C. Chae, P.J. Shea and B.-T. Oh, *Monascus* pigment production by solid-state fermentation with corn cob substrate. *Journal of Bioscience and Bioengineering*, 2011. **112**(6): p. 590-94.

[10] Orozco, S.F.B. and B.V. Kilikian, Effect of pH on citrinin and red pigments production by *Monascus purpureus* CCT3802. *World Journal of Microbiology and Biotechnology*, 2008. **24**(2): p. 263-68.

[11] Aberoumand, A., A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry. *World J Dairy Food Sci*, 2011. **6**(1): p. 71-78.

[12] Rajeswari, T.R., V. Ponnusami and K. Sugumaran, Production of *Monascus* Pigment in low cost fermentation. *International Journal of ChemTech Research*, 2014. **6**: p. 2929-32.

[13] Sinha, K., P.D. Saha and S. Datta, Extraction of natural dye from petals of Flame of forest (*Butea monosperma*) flower: Process optimization using response surface methodology (RSM). *Dyes and Pigments*, 2012. **94**(2): p. 212-16.

[14] Tuli, H.S., P. Chaudhary, V. Beniwal and A.K. Sharma, Microbial pigments as natural color sources: current trends and future perspectives. *Journal of food science and technology*, 2015. **52**(8): p. 4669-78.

[15] Babitha, S., *Microbial Pigments*, in *Biotechnology for Agro-Industrial Residues Utilisation: Utilisation of Agro-Residues*, P. Singh nee' Nigam and A. Pandey, Editors. 2009, Springer Netherlands: Dordrecht. p. 147-62.

[16] Hsu, L.-C., Y.-H. Liang, Y.-W. Hsu, Y.-H. Kuo and T.-M. Pan, Anti-inflammatory properties of yellow and orange pigments from *Monascus purpureus* NTU 568. *Journal of agricultural and food chemistry*, 2013. **61**(11): p. 2796-802.

[17] Wu, H.-C., M.-J. Cheng, M.-D. Wu, J.-J. Chen, Y.-L. Chen and H.-S. Chang, Three new constituents from the fungus of *Monascus purpureus* and their anti-inflammatory activity. *Phytochemistry Letters*, 2019. **31**: p. 242-48.

[18] Cheng, C.-F. and T.-M. Pan, Ankaflavin and Monascin Induce Apoptosis in Activated Hepatic Stellate Cells through Suppression of the Akt/NF-κB/p38 Signaling Pathway. *Journal of agricultural and food chemistry*, 2016. **64**(49): p. 9326-34.

[19] Tan, H., Z. Xing, G. Chen, X. Tian and Z. Wu, Evaluating Antitumor and Antioxidant Activities of Yellow *Monascus* Pigments from *Monascus ruber* Fermentation. *Molecules*, 2018. **23**(12): p. 3242.

[20] Lee, Y.L., J.H. Yang and J.L. Mau, Antioxidant Properties of Ethanolic and Methanolic Extracts from *Monascus*-Fermented Soybeans *Journal of food biochemistry*, 2009. **33**(5): p. 707-27.

[21] Zeng, H., Q. Jie, Z. Xin, X. Dayong, X. Minghua, L. Feng, S. Jianfan, J. Xuan and D. Chuanyun, Optimization of submerged and solid state culture conditions for *Monascus* pigment production and characterization of its composition and antioxidant activity. *Pigment & Resin Technology*, 2019. **48**(2): p. 108-18.

[22] Martins, Natália, C.L. Roriz, P. Morales, L. Barros and I.C. Ferreira, Food colorants: Challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. *Trends in Food Science & Technology*, 2016. **52**: p. 1-15.

[23] Kim, D. and S. Ku, Beneficial Effects of *Monascus* sp. KCCM 10093 Pigments and

Derivatives: A Mini Review. *Molecules*, 2018. **23**(1): p. 98.
[24] Puttananjaiah, M.-K.H., M.A. Dhale, V. Gaonkar and S. Keni, Statins: 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors demonstrate anti-atherosclerotic character due to their antioxidant capacity. *Applied biochemistry and biotechnology*, 2011. **163**(2): p. 215-22.
[25] Babitha, S., C.R. Soccol and A. Pandey, Jackfruit seed-a novel substrate for the production of *Monascus* pigments through solid-state fermentation. *Food Technology and Biotechnology*, 2006. **44**(4): p. 465-71.
[26] Feng, Y., Y. Shao and F. Chen, *Monascus* pigments. *Applied Microbiology and Biotechnology*, 2012. **96**(6): p. 1421-40.
[27] Subhasree, R., P.D. Babu, R. Vidyalakshmi and V.C. Mohan, Effect of carbon and nitrogen sources on stimulation of pigment production by *Monascus purpureus* on jackfruit seeds. *Intl J of Microbiological Res*, 2011. **2**(2): p. 184-87.
[28] Ng, W.P.Q., H.L. Lam, F.Y. Ng, M. Kamal and J.H.E. Lim, Waste-to-wealth: green potential from palm biomass in Malaysia. *Journal of Cleaner Production*, 2012. **34**: p. 57-65.
[29] Hamid, N. and F. Said, Factorial design screening for the red pigment production by *Monascus purpureus* FTC 5356. *Jurnal Teknologi*, 2016. **78**: p. 13-17.
[30] Razali, M.A.A. and F.M. Said, Red pigment production by *monascus purpureus* in stirred-drum bioreactor. *Galeri Warisan Sains*, 2017. **1**(1): p. 13-15.
[31] Guangul, F.M., S.A. Sulaiman and A. Ramli, Gasifier selection, design and gasification of oil palm fronds with preheated and unheated gasifying air. *Bioresource Technology*, 2012. **126**: p. 224-32.
[32] Karasu, S., Y. Bayram, K. Ozkan and O. Sagdic, Extraction optimization crocin pigments of saffron (*Crocus sativus*) using response surface methodology and determination stability of crocin microcapsules. *Journal of Food Measurement and Characterization*, 2019. **13**(2): p. 1515-23.
[33] Capozzi, V., M. Fragasso, R. Romaniello, C. Berbegal, P. Russo and G. Spano, Spontaneous Food Fermentations and Potential Risks for Human Health. *Fermentation*, 2017. **3**(4): p. 49.
[34] Carvalho, J.C. de, B.O. Oishi, A. Pandey and C.R. Soccol, Biopigments from *Monascus*: strains selection, citrinin production and color stability. *Brazilian Archives of Biology and Technology*, 2005. **48**: p. 885-94.
[35] Johns, M.R. and D.M. Stuart, Production of pigments by *Monascus purpureus* in solid culture. *Journal of Industrial Microbiology*, 1991. **8**(1): p. 23-28.
[36] Nimnoi, P. and S. Lumyong, Improving Solid-State Fermentation of *Monascus purpureus* on Agricultural Products for Pigment Production. *Food and Bioprocess Technology*, 2011. **4**(8): p. 1384-90.
[37] Wang, Y., V. Herdegen, X. Li and J.-U. Repke, Numerical study and evaluation of solid-liquid extraction of Montan wax in stirred tanks on different scales. *Separation and Purification Technology*, 2018. **204**: p. 90-97.
[38] Alexandra, E. Pazmiño-Durán, M.M. Giusti, R.E. Wrolstad and M.B.A. Glória, Anthocyanins from banana bracts (*Musa X paradisiaca*) as potential food colorants. *Food Chemistry*, 2001. **73**(3): p. 327-32.
[39] Assous, M.T.M., M.M. Abdel-Hady and G.M. Medany, Evaluation of red pigment extracted from purple carrots and its utilization as antioxidant and natural food colorants. *Annals of Agricultural Sciences*, 2014. **59**(1): p. 1-7.
[40] Gengatharan, A., G.A. Dykes and W.S. Choo, Betalains: Natural plant pigments with potential application in functional foods. *LWT - Food Science and Technology*, 2015. **64**(2): p. 645-49.
[41] Silva, S., E. Costa, C. Calhau, R. Morais and M. Pintado, Anthocyanin Extraction from Plant Tissues: A Review. *Critical reviews in food science and nutrition*, 2015. **57**(14): p. 3072-83.
[42] Karacabey, E. and G. Mazza, Optimization of Solid− Liquid Extraction of Resveratrol and

Other Phenolic Compounds from Milled Grape Canes (*Vitis vinifera*). *Journal of agricultural and food chemistry*, 2008. **56**(15): p. 6318-25.
[43] Patsea, M., I. Stefou, S. Grigorakis and D.P. Makris, Screening of Natural Sodium Acetate-Based Low-Transition Temperature Mixtures (LTTMs) for Enhanced Extraction of Antioxidants and Pigments from Red Vinification Solid Wastes. *Environmental Processes*, 2017. **4**(1): p. 123-35.
[44] Chen, M.-H. and M.R. Johns, Effect of pH and nitrogen source on pigment production by *Monascus purpureus*. *Applied Microbiology and Biotechnology*, 1993. **40**(1): p. 132-38.
[45] da Costa, J.P.V. and F. Vendruscolo, Production of red pigments by *Monascus ruber* CCT 3802 using lactose as a substrate. *Biocatalysis and Agricultural Biotechnology*, 2017. **11**: p. 50-55.
[46] Babitha, S., C.R. Soccol and A. Pandey, Solid-state fermentation for the production of *Monascus* pigments from jackfruit seed. *Bioresource Technology*, 2007. **98**(8): p. 1554-60.
[47] Dikshit, R. and P. Tallapragada, *Monascus purpureus*: A potential source for natural pigment production. *Journal of Microbiology and Biotechnology Research*, 2017. **1**(4): p. 164-74.
[48] Suraiya, S., M.P. Siddique, J.-M. Lee, E.-Y. Kim, J.-M. Kim and I.-S. Kong, Enhancement and characterization of natural pigments produced by *Monascus* spp. using *Saccharina japonica* as fermentation substrate. *Journal of Applied Phycology*, 2018. **30**(1): p. 729-42.
[49] Carvalho, J. C, A. Pandey, S. Babitha and C.R. Soccol, Production of *Monascus* biopigments: an overview. *Agro Food Industry Hi Tech*, 2003. **14**(6): p. 37-43.
[50] Henriques, M., A. Silva and J. Rocha, Extraction and quantification of pigments from a marine microalga: a simple and reproducible method. *Communicating Current Research and Educational Topics and Trends in Applied Microbiology Formatex*, 2007. **2**: p. 586-93.
[51] Carvalho, J. C, B.O. Oishi, A.L. Woiciechowski, A. Pandey, S. Babitha and C.R. Socco, Effect of substrates on the production of *Monascus* biopigments by solid-state fermentation and pigment extraction using different solvents. *Indian Journal of Biotechnology*, 2007. **6**: p. 194-99.
[52] Dufossé, L., P. Galaup, A. Yaron, S.M. Arad, P. Blanc, K.N. Chidambara Murthy and G.A. Ravishankar, Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? *Trends in Food Science & Technology*, 2005. **16**(9): p. 389-406.
[53] Shi, K., R. Tang, T. Huang, L. Wang and Z. Wu, Pigment fingerprint profile during extractive fermentation with Monascus anka GIM 3.592. *BMC biotechnology*, 2017. **17**(1): p. 46.
[54] Bühler, R.M.M., A.C. Dutra, F. Vendruscolo, D.E. Moritz and J.L. Ninow, *Monascus* pigment production in bioreactor using a co-product of biodiesel as substrate. *Food Science and Technology (Campinas)*, 2013. **33**: p. 9-13.
[55] Gunasekaran, S. and R. Poorniammal, Optimization of fermentation conditions for red pigment production from *Penicillium* sp. under submerged cultivation. *African journal of Biotechnology*, 2008. **7**(12).
[56] Long, C., M. Liu, D. Zhang, S. Xie, W. Yuan, N. Gui, J. Cui and B. Zeng, Highly efficient improvement of *Monascus* pigment production by accelerating starch hydrolysis in *Monascus ruber* CICC41233. *3 Biotech*, 2018. **8**(8): p. 329.
[57] Mukherjee, G. and S.K. Singh, Purification and characterization of a new red pigment from *Monascus purpureus* in submerged fermentation. *Process Biochemistry*, 2011. **46**(1): p. 188-92.
[58] Srianta, I., E. Zubaidah, T. Estiasih, M. Yamada and Harijono, Comparison of *Monascus purpureus* growth, pigment production and composition on different cereal substrates with solid state fermentation. *Biocatalysis and Agricultural Biotechnology*, 2016. **7**: p. 181-86.
[59] Kang, B., X. Zhang, Z. Wu, H. Qi and Z. Wang, Effect of pH and nonionic surfactant on profile of intracellular and extracellular *Monascus* pigments. *Process Biochemistry*, 2013. **48**(5): p. 759-67.
[60] Kang, B., X. Zhang, Z. Wu, Z. Wang and S. Park, Production of citrinin-free *Monascus*

IOP Conf. Series: Materials Science and Engineering **736** (2020) 022084 doi:10.1088/1757-899X/736/2/022084

pigments by submerged culture at low pH. *Enzyme and Microbial Technology*, 2014. **55**: p. 50-57.