THE EFFECT OF INDEPENDENT VARIABLES ON MONASCUS MUSHROOM PIGMENT PRODUCTION

Ehsan Hakimi[1], Hanie Nuriun[2]

Abstract

Considering the advantages of natural colors over artificial colors, extracting color from microorganism sources is beneficial to mankind. Monascus purpureus is a microscopic color-producing fungus belonging to the Monascus family and the most famous mushroom for producing red, orange and yellow pigments. Monascus mushroom has many properties and uses, including food preservative, coloring, flavoring and cholesterol lowering. In general, the purpose of conducting this research is to cultivate and produce pigment from Monascus purpuras mushroom by submerged culture fermentation method and then investigate and compare the effect of five parameters (PH, type of carbon source and its concentration, nitrogen source concentration and surfactant concentration) on it is pigment production. This research was conducted in the form of response level and data analysis in the form of 38 tests with different values by Design expert software. After conducting the experiments for ten days at a temperature of 30 degrees Celsius and a revolution of 150 rpm, they were affected by 5 different parameters inside the shaker incubator. After 10 days, the pigments were measured. The results showed that the highest concentration of pigment production was created when pH = 5, the carbon source was wheat starch with a concentration of 18 g/l, nitrogen concentration of 3 g/l and surfactant concentration of 5 g/l, in which case the highest amount of production The pigment was 15.089 g/l and the lowest at pH=7, the corn starch source was with a concentration of 16 g/l, nitrogen concentration of 3 g/l and surfactant concentration of 15 g/l with a pigment production rate of 8 g/l.

Keywords: pigment, corn starch, wheat starch, yeast extract, surfactant, PH, bacteria, agricultural waste

[1] Chemical Engineering, Islamic Azad University, South Tehran Branch
Ehakimi13011@gmail.com
[2] Master's degree in materials engineering, identification and selection of engineering materials, Hamedan University of Technology, Hamedan, Iran.
Hn.1370@ymail.com

Introduction

One of the most important qualitative properties of the appearance of the product that can be seen by the consumer first is the color. Color is one of the factors that is received through the sense of sight and is effective in attracting attention and choosing food. According to the FDA (US Food and Drug Administration) definition, the so-called food color is: "A color additive includes any color, pigment, or other matter that is made by the process of synthesis or similar methods, or from a plant, animal, The mineral and other sources or intermediates have been extracted, isolated and derivatized and when used for food, medicine, cosmetics or any other part, it can cause and add color to it" which is to create color in Food, drink and cosmetics for human consumption will be added to them. According to this definition, dyes are divided into two categories with a license for consumption (colors of natural origin, meaning that a license is required for edible consumption) and without a license for consumption (used for dyeing fabric and carpets, meaning that a license is not required for consumption). Are divided Due to the sensitivity of some artificial colors in food, such as azorobin or tartrazine, their use in various food industries has been limited [Pattanagul et al., 2008]. Some artificial colors have carcinogenic and mutagenic effects, for example, the color used in the meat industry causes the formation of nitrosamine, which is a combination of nitrate and nitrite and is formed with an amine agent [Dikshit and Tallapragad, 2013].

Natural colors are the result of two important sources of plants and microorganisms. Dyes obtained from fungal sources have many and very important applications in the nutrition and economy of human society [Surawut, et al., 2021]. Allowed edible and natural pigments of plant origin have many problems such as instability to light, heat, low or high acidity, low solubility and often lack of easy access throughout the year [Mehrabi and Hekaran, 2010]. The reason for the natural production of coloring agents from microorganisms and some pigments produced from Monascus, Streptomyces, Seraita is of interest: because pigment production from microorganisms due to fast and easy growth, easier extraction, cheap culture medium, lack of dependence on atmospheric conditions and expansion Color diversity has more advantages than other biological sources. Monascus mushroom can be used as a food color by producing non-toxic pigment. The pigments extracted from this mushroom have a wide range of biological activities such as anti-cancer, anti-microbial and anti-fungal properties in addition to their coloring properties, and also have the ability to produce a variety of colors from yellow to red [Vidyalakshmi, 2009]. Pigments synthesized by Monascus are traditionally used in Asia to color and preserve canned foods, such as red soybean curd, meat, and vegetables. The growth, amount and types of secondary metabolites of mushrooms can be directly or indirectly affected by the culture medium and culture method. In order to maintain the productivity and maximize the Monascus pigment, the cultivation conditions should be optimized. Many measures such as genetic manipulation, change in pH [Orozco and Kilikian, 2008], oxygen [Pereira et al., 2008] and various nutritional elements [Sabri and Hekaran, 2010] are performed to increase the pigment and biomass weight.

Pigments have been used in food since ancient times for coloring, increasing appetite and attracting customers. However, now most of the food and cosmetic manufacturers of third world countries use artificial colorants, most of which are toxic and carcinogenic, to attract consumers. For this reason, natural pigments from plants, animals and microorganisms attract attention among people. Currently, microbial pigment production is one of the emerging fields of research in food, cosmetic and textile industrial applications [Sharmin Suraiya et al., 2018]. The new found awareness of human safety and environmental preservation has a renewed interest in natural sources of dyes. It is believed that natural dyes or dyes obtained from plants and animals in nature are safe and non-toxic, non-carcinogenic and degradable [Wagh and Mane, 2017]. Despite the popularity of plant-derived pigments, pigments produced by microorganisms have a promising potential to meet today's challenges. Monascus species are known to produce an edible pigment and are very safe [Abdel Khafar, et al., 2022]. Monascus pigments not only increase the marketing of the product, but also have various biological activities such as anti-inflammatory, anti-tumor, antioxidant and regulating cholesterol properties [Daud et al., 2020]. Natural pigments have good potential to be used as colorants in food and cosmetics [Farhan et al., 2014]. A lot of evidence shows that the yellow pigments in Monascus are beneficial to human health in appropriate doses and may even be used by the food and pharmaceutical industries

[Meihua Wang et al., 2016]. Some artificial colors contain possible colon carcinogens, and people are concerned about the health effects of artificial colors, especially in food, clothing, cosmetics, and pharmaceuticals. This encourages interest in creating non-toxic dyes and expanding sources of natural pigments. The market of natural colors began to grow. The market price of natural dyes is high due to their low production level. There are several other problems such as lack of stability or dependence on pH. Researchers proposed to overcome these obstaclesthat microorganisms, including bacteria or fungi, should be developed and used as alternative sources of natural pigments [Brahma, et al., 2022]. Filamentous fungi are known to produce various secondary metabolites that are beneficial to humans [Young Mok Heo et al., 2018]. Factors affecting the growth and production of pigment are as follows: culture medium, temperature, presence of oxygen and aeration, pH, nitrogen source, rheology of culture medium and morphology [Rose Marie Meinicke Bühler et al., 2015]. Pigments come in a variety of colors and some of them are water soluble. For this reason, many of these compounds have been produced, isolated and characterized [Alejandro MÉNDEZ et al., 2011]. The use of food colors as additives in the food industry to determine the acceptance of processed food is one of the important production and consumer factors. It is likely that many consumers are not aware of the exotic sources of some of the currently permitted so-called natural dyes [Iffat Sayyed and Devipriya, 2015]. East Asia has been used to prepare rice wine, soy cheese and red rice (Anka) [Pisareva and Kujumdzieva, 2010]. Based on cultivation characteristics, 9 species of Monascus have been recognized internationally. These are M. pilosus, M. ruber, M. purpureus, M. floridanus, M. eremophilus, M. pallens, M. sanguineus, M. lunisporas and M. argentinensis. However, more than 20 species of Monascus have been recorded since the introduction of the genus Monascus in 1884 [Dikshit Rashmi and Tallapragada Padmavathi, 2013]. Monascus species are an important source of natural food pigments. Currently, more than 50 patents on the use of Monascus pigments in food have been issued in Japan, the United States, France, and Germany [Hassan Hajjaj et al., 2012]. The main question about the safety and usefulness of food colors using Monascus pigments is their possible contamination with mycotoxins and citrinin, which can be produced together with pigments [Matej Patrovsky et al., 2017]. Monascus is known to produce at least six pigment molecular structures, which can be classified into three groups depending on their color [Chuannan Long et al., 2019]. Among the pigments produced by Monascus, red pigment (monascorobamine and rubopunctamine) is in high demand, especially when produced in red rice, red pigment has potential for therapeutic use [Gunjan Mukherjee et al., 2011].

Monascus pigments are mainly produced in cell attachment, have low water solubility, are sensitive to heat, are unstable in the pH range from 2 to 10, and fade when exposed to light [Hassan Hajjaj et al., 2012]. Monascuses produce various biologically active compounds such as enzymes, lipids, organic acids, pigments, monaculins and methylketones [Pisareva and Kujumdzieva, 2010. They have many advantages such as easy production on pressure-free substrates, good solubility in water and ethanol, bioactive metabolites. They have countless during production and are completely safe under certain conditions [Francielo Vendruscolo et al., 2016]. Monascus is not approved for use as a food additive in the European Union or the United States, although it is currently permitted in Japan. Monascus pigments were used as coloring materials in the preparation of sausage, ham, fish and tomatoes [Mohan Kumari et al., 2011]. At different stages of the life cycle, light can be stimulatory, inhibitory or regulatory, and some of the effects reported as a result of light exposure include suppression of spore release [Hilares et al., 2017]. Monascin (MS) and Ankaflavin (AK) are two the classic yellow pigment is produced by Monascus. MS and AK have anti-cancer, anti-inflammatory [Zhu et al., 2019], anti-obesity and anti-diabetic properties, and at the same time, they regulate blood cholesterol levels [Rose Marie Meinicke et al., 2012]. Fungi have been reported as strong pigment producing microorganisms [Velmurugan et al., 2009]. Filamentous fungi are readily available raw materials that can provide microbial cell factories for the production of food pigments due to their chemical and color versatility in pigment profile, easier production on a larger scale, and a long history of well-known production strains. The production of other types of biochemicals, including dyes, microorganisms belonging to the genus Aspergillus and Penicillium have also been studied as potential producers of natural pigments. Recently, the production of Monascus-like pigments from Penicillium strains with potential use in the food industry has been reported [Majid Afshari et al.,

2015], these pigments have potential use in the food industry because they are not related to citrinin production. They are homologues of Monascus pigments, which have polykidophores similar to the chromophore, and of the fungal species Epicoccum nigrum, which produce yellow pigments [Alejandro MÉNDEZ et al., 2011]. Monascus purpurues is a hemothallic fungus. The most important feature of this fungus is the ability to produce secondary metabolites of polypeptide structure. Therefore, the development of low-cost processes is required. So far, several materials such as jackfruit seed powder, sesame oil cake, coconut oil, wheat bran, date kernel cake and grape waste have been studied as substrates in solid state fermentation (SSF) [Pongrawee Nimnoi and Saisamorn Lumyong, 2011]. Artificial yellow pigments allowed for use in food processing are limited to tartazine. The health toxicity of both pigments is currently still in question, while natural yellow pigments from animals, plants or microorganisms have received more favorable attention in recent years. It produces yellow pigment in immersion culture using wheat starch and soybean flour as the main substrates [Xijun Lian et al., 2014]. Among microbes, bacteria have a high potential to produce various pigments. A variety of growth media can be used to produce pigments, but they are expensive, so they cannot be used. Agricultural waste can reduce the cost of fermentation for pigment production and increase the production of red pigment [Wagh and Mane, 2017]. There are different types of pigments in the Monascus product, some of which are still unknown [Gunjan Mukherjee and Sanjay Kumar Singh, 2010]. Some natural substrates that have already been tested, in addition to rice and other grains, are wheat starch, wheat bran, wheat meal, bread meal, cornmeal, and dairy milk. Currently, several companies are selling powdered and fermented dried rice products as a food ingredient and as a food supplement with the ability to lower cholesterol levels, and others are selling the dried product or pure extracts as food coloring. They sell [Sumathy Babitha et al., 2006]. Microorganisms provide a readily available alternative source of naturally derived pigments. In addition, pigments produced by microorganisms are of industrial interest due to the fact that the microorganism can grow rapidly under controlled conditions, resulting in high productivity and product availability throughout the year, and batch changes to it minimizes a batch [Thiyam Genera et al., 2013]. The function of fungal pigments increases their value. In particular, pigments with high antioxidant activity are very useful and easily used in food and cosmetic industries [Young Mok Heo et al., 2018]. Synthetic dyes cause dangerous effects on the environment, human health, animals and plants because they are formed from a petrochemical source through a dangerous chemical process. Also, they are non-biodegradable, renewable, and have a high single-use problem. Natural colors are always potential alternatives to artificial colors [Jyoti Chauhan et al., 2020]. My best Different carbon sources were glucose, sucrose and starch used for Monascus. The best growth is generally observed in glucose [Rose Marie Meinicke Bühler et al., 2015]. Industrial use of agricultural products attracts a lot of attention due to its high nutritional value and low cost. Crops and by-products of agricultural processes have been used as substrates for the production of various biological products, including pigments, biopolymers, organic acids, solvents and enzymes. Sweet potato is an ideal source of glucose for several industrial applications because it contains 20-30% starch [Prateek Srivastav et al., 2015]. The composition of pigments varies significantly depending on the type of nutrients available such as nitrogen sources and the strain used. Therefore, prior to pigment isolation, it is important to optimize various physical and nutritional parameters for maximum culture growth and pigment production by M. perpureus in immersion fermentation [Gunjan Mukherjee and Sanjay Kumar Singh, 2010]. BSG is the most important waste produced by factories and water catchments, it includes 85% of the total waste produced in beer production. Because BSG is cheap and abundant, these properties contribute to economics in any process that uses this waste biomass. Therefore, it will be beneficial to use BSG as a substrate for pigment production [Selim Silbir and Yekta Goksungur, 2019]. The highest amount of pigments used to enhance the light pink color is due to insufficient meat consumption in sausage [Xijun Lian et al., 2014]. Monascus pigments do not cause any allergic reactions and are not dangerous to health. In addition, in the food and beverage industry, these pigments have been used as preservatives due to their antimicrobial properties and also to increase the level of platelets in hemorrhage (DHF).) are used [Sharmin Suraiya et al., 2018.[

Monascus mushroom has been well introduced for a long time in Asian countries, especially in Korea, China, Indonesia, Japan, Thailand, and the

Philippines, which are mainly associated with the production of Monascus fermented rice. Monascus Fermented Rice or RYR has been introduced under different names, for example, Honggu in China, Benny-Koji in Japan and Ancak in the Philippines, which are mostly food colorings that have beneficial effects on human health, especially its beneficial effects that promote digestion. And it also helps to deal with cardiovascular diseases.

Red koji (red rice is usually used for Monascus fermentation substrate) was used in food fermentation as a source of hydrolytic enzymes and active fungi, which products such as red rice vinegar, red rice wine, fermented tofu (sofu tofuyo) or sauce Fish (e.g. begong) have been produced by this method. The effect of red rice is mostly due to the presence of monacolinca, a statin with a similar structure to lovastatin (movinolin), which works together with other compounds in red rice (pigments, various monacolins).

In order to control its harmful effects, the permissible limit of using citrinin in Monascus pigments in China and Japan is 0.1 and 0.2 mg/kg, respectively [Feng et al., 2012].

Monascus mushroom has been enthusiastically studied in Asian countries, especially in Japan and China, unfortunately, not all the results of Asian researchers have been published in English, and because of this, non-native people have been deprived of this information. The genomes of three of the most famous species of Monascus piloses, Raber and Purpureus have been sequenced recently [Yang et al., 2015; Chen et al., 2015], which has provided the possibility to know more about the physiology of Monascus and reveal new approaches to improve the principle of this fungus.

In the meantime, colors of fungal origin (including Monascus purpureus colors) not only have wide and very important applications in the economy of human society, health and nutrition, but they are able to generate about 30 billion dollars per year in the process of commercial industry. The variety of colors in mushrooms reaches more than 1,000, which are not found in other organisms or are present in a very small amount, which can be referred to the specific colors produced by specific strains of Monascus mushroom. The colors produced by this mushroom have been used since 2800 years ago in Southeast Asian countries, especially in China, due to their edible, hygienic, cosmetic and medicinal values [Sarmi et al., 2008].

So far, more than 200 strains of the Monascus genus, whose colors have been isolated for various health, food, cosmetic, medicinal, etc. uses, have been registered in Asian, European, and American countries (United States Trademark and Exclusive Copyright Office). Due to the high amount of alpha-amylase, this fungus has the ability to change starch to glucose and has been used as a process starter (promoter) in fermentation factories [Malik et al., 2012].

The availability and cheapness of its raw material (rice) and the appropriate efficiency of color production are the advantages that have caused global acceptance for the use of these colors. The production of this paint in Jajie Company in Shanghai, China is 3000 tons per year. Also, in 1992, 600 tons of this paint worth about 1.5 million dollars were produced and sold in Japan.

In 1994, about 700 tons of this color were used in China. In general, according to average statistics, the consumption of this color per person per day in Asia is about 14 to 55 grams. According to the explanations given, many companies in Asia are producing it due to the high consumption of these colors (on a scale of 5000-2000 tons).

Materials and methods

Preparation of Monascus purpureus mushroom strain

The desired strain of Monascus purpureus was obtained from Iran Scientific and Industrial Research Organization.

Tools and devices

The tools and devices used in this research are presented respectively in Table 1:

Table 1: Equipment used for pigment cultivation and extraction

Model devices and tools
AV413 digital scale
Autoclave 87419
BIOSAFE biological hood
Incubator IP100
Incubator shaker DKSI060
PH meter 827
Spectrophotometer i3
EBA 20 centrifuge
Avon Memmert

Mushroom cultivation environments

Monascus purpureus can grow in many fungal culture media such as PDA and MDA. Also, MEPAG and YPSS culture media are used

specifically for this fungus. PDA and YPSS were used in this experiment.

-Preparation and preservation of the main mushroom

To preserve Monascus purpureus, its mycelium was cultured on PDA and YPSS media and incubated in an incubator for 7 days at 30°C. After 7 days, it was taken out of the incubator Results

Monascus purpureus mushroom cultivation environments

The method and method of cultivation of solid mediums has been fully explained in the previous chapter, in Figure 2 (a) the growth of this fungus is shown in YPSS medium and part (b) in PDA medium, after 7 days of their cultivation inside the incubator, as It is clear in the figure that it has grown more in the PDA culture medium than in the YPSS culture medium. However, due to the high price of PDA culture medium, YPSS medium was used to cultivate this mushroom.

Mushroom cultivation in YPSS medium b) Mushroom cultivation in PDA medium

Pigment extraction

After performing the necessary steps to extract the pigment, the pigment obtained after 72 hours of drying was as follows. Part (a) of Figure 3 shows the solid material obtained after dissolving it with ethanol and centrifuging it three times. 13.0 grams/liter of dried colored solid material was obtained, and 10 grams/liter of this dried material was used to make the standard solution.

-Experimental design and statistical analysis

Obtaining the optimal percentages of the main components of the environment can be one of the ways to produce a high-quality product and reduce rework. To find the optimal percentage of these compounds and to optimize the response variable, a method called response surface methodology (RSM) can be used. RSM is a set of effective statistical and mathematical methods that determine several variables at the same time with minimal resources and quantitative data and with a suitable experimental design. Also, this method is used to improve and optimize processes where the desired response is determined by a number of variables under be affected, and its purpose is to describe the relationship between the response and independent variables by mathematical models and to optimize this response. When many factors and relationships have an effect on the response variable, the RSM method is one of the effective tools for process optimization. The optimization process by RSM can be divided into three stages. The first step is to specify the independent parameters and their levels, the second step is to select the experimental design and predict and evaluate the relationship of the model, and the final step is to obtain and draw the shape of the response surface as a function of the independent parameters and determine the optimal points.

-color materials obtained from the design of experiments

The environments that were cultured using the experimental design were taken out of the incubator shaker after 10 days, then each one was smoothed using Whatman filter paper, after smoothing the samples, they were centrifuged to use the spectrophotometer device. It was set at 5000 rpm for 10 minutes.and transferred to the refrigerator at a temperature of 4 °C for storage, and this process was repeated on the culture medium every 30 days.

Preparation of primary culture medium

After preparing PDA and YPSS culture media in 250 ml Erlenmeyer flasks, it was sterilized for 20 minutes in an autoclave at a temperature of 121 degrees Celsius and a pressure of 15 pounds per square meter. After reaching the ambient temperature, in completely sterilized conditions, under the hood as well as next to the flame, pour them into the test plate and tube so that 2/3 of the surface of the plate and 1/2 of the test tube are filled. When the plate and the test tube were completely closed, the mycelium of the mentioned fungus was transferred from the primary culture medium using a loop (it was placed on the flame to disinfect the loop before doing the work) to the center of the plate and the test tube containing the culture medium. New PDA and YPSS were transferred.

Solid culture medium

In order to use the fungus, it must be activated first, for this reason, YPSS culture medium, which was suggested to be DSMZ, was poured into several test tubes and was previously autoclaved and sterilized. The cultures were placed in an incubator at a temperature of 30°C for 7 days. became.

Table 2: Compositions of 1 liter of YPSS culture medium

Agar $MgSo_4.7H_2O$ K_2HPO_4 starch soluble yeast

extract distilled water

Liquid culture medium

The composition of the liquid culture medium for inoculation of the main medium per 1 liter of distilled water was as follows:

100 cc of liquid culture medium was prepared and poured into a 250 ml Erlenmeyer flask and autoclaved. The size of a mushroom was removed and inoculated. This environment was placed in a shaker incubator at a temperature of 30-32 degrees Celsius and 150 rpm for 8 days. It should be noted that after 72 hours the medium has grown and the color change occurs after the growth of the medium. It is shown in Figure 1.

How to extract pigments

Color separation from biomass

First, the cultures that turned red were taken out of the shaker, then for every 2.5 ml of the colored substance obtained, it was diluted with 5 ml of 96% ethanol, the obtained substances were kept in the incubator shaker for 30 minutes. They were placed at 150 rpm. After removing the materials from the incubator shaker, centrifugation was performed in three steps:

First step: the materials were centrifuged at 5000 rpm for 15 minutes.

The second step: the supernatant was discarded and 3.5 ml of 96% ethanol was added to it again for 1.5 ml of the material obtained, and then it was centrifuged at 5000 rpm for 5 minutes.

The third step: As in the previous step, the supernatant was discarded, then 5 ml of 96% ethanol was added to it for 2.5 ml of the obtained material, and then centrifuged again at 5000 rpm for 15 minutes.

After completing the centrifugation process, the obtained solid material was poured into the container, then it was kept in the oven at a temperature of 45°C for 70 hours until the obtained material was completely dry.

Measurement of biomass dry weight

After finishing the work and complete drying of the biomass, it was measured, for 400 ml of liquid pigment, after performing the above steps, 13.58 grams/liter of dry solid matter was obtained.

Model analysis using ANOVA table and objective function

The usual term is the sum of squares in the ANOVA (SS) table, which is useful for determining the quality of variability. It can be divided into smaller parts.

SS_tot= SS_reg+ SS_resid

SS_tot in its first term indicates the overall change in response. Using the term SS_reg we can model a number of changes. The SS_resid term is also for values that cannot be modeled. A model with a high SS_reg as well as a low SS_resid can be well expressed. This possibility can be investigated by performing a test. It is also possible to use MS_reg and 〖MS〗_resid by dividing the estimated SS by the degree of freedom DF. Using F-test, you can measure the probability percentage:

F = MS_reg/MS_resid

Using statistical tables, the corresponding p value can be obtained. The maximum value of p which is the critical limit can be 0.05. To have a suitable model, p must be less than 0.05. If we repeat the tests, we will have:

SS_resid= SS_LOF+ SS_PE

DF_resid= (n-p)

SS_PE= ∑▒k_i 〖(C_ki-C_k)〗^2

DF_PE= ∑_k▒ 〖(n_k-1)〗

DF_LOF= n-p-∑_i▒ 〖(n_k 〗-1)

= number of iterations an_k

n = number of trials

C_k = average of n_k residues in a set that has repetitions.

To know the good match of the model with the experimental data, the following relationship can be used:

F_LOF= (SS_LOF⁄DF_LOF)/(SS_PE⁄DF_PE)

SS_resid can be decomposed into two separate terms in the ANOVA table:

SS_resid=SS_(model error)+SS_(replicate error)

When the model fails, the first term is created and the error is caused by the repeatability of the second term. We use the following formula to determine the probability percentage:

= MS_model/MS_replicate F

DF= n-p-∑_k▒ 〖(n_k-1)〗

n_k= number of repetitions

P = the number of terms of the model, which includes the constant

n = number of trials

A good model is LOF whose p is greater than 0.05 [Myers et al., 2016, Mary Ladidi Abu et al.,

2020].

-Obtaining optimal points in variables

The optimal points of the variables can be obtained from the first derivative of the regression equation. The following example is for a variable with two regressions:

$y= \beta_0+ \beta_1 x_1+ \beta_2 x_2+ \beta_{11} x_1^2+ \beta_{22} x_2^2+ \beta_{12} x_1 x_2+$ Residual

$\partial y/〖\partial x〗_1 = \beta_1+2\beta_{11} x_1+\beta_{12} x_2 =0$ (1-13)

$\partial y/〖\partial x〗_2 = \beta_2+2\beta_{22} x_2+\beta_{12} x_1 =0$ (1-14)

The value of x_1 and x_2 can be obtained by solving this device [Myers et al., 2016, Mary Ladidi Abu et al., 2020].

-Table of experiments design

Experiments were designed with Design Expert 11 software based on Table 4, for which 5 different parameters were considered:

Table 4: Type and amount of variables in the design of experiments

Type of carbon source, concentration of nitrogen source

(g/l) Carbon source concentration

(g/l) surfactant

(g/l) pH row

Wheat starch 2.4 8.14 11 7 1

Wheat starch 3 2.13 15 5 2

Corn starch 3 18 15 5 3

Wheat starch 3.5 12 7.7 5 4

Corn starch 2.3 12 15 6.5 5

Corn starch 4/6 18 1/11 6/2 6

Wheat starch 2.4 8.14 11 7 7

Corn starch 6 12 8.7 5.5 8

Wheat starch 3 12 6/7 6/5 9

Wheat starch 6 14/9 10/9 5/8 10

Corn starch 16/6 4/7 7 11

Wheat starch 3.5 12 15 6.5 12

Corn starch 5/2 1/12 8/4 7 13

Corn starch 4/6 14/5 10/9 5 14

Wheat starch 4/9 18 6/8 6/3 15

Corn starch 17.4 9.5 1.5 16

Wheat starch 3/9 15/6 11/8 5/7 17

Wheat starch 6 12 5 7 18

Corn starch 6 12 15 1/5 19

Corn starch 4.5 12 15 7 20

Wheat starch 2.5 7.17 15 5 21

Corn starch 4/5 14/6 15 6/2 22

Corn starch 3 9/15 6/8 5/7 23

Corn starch 16/6 4/15 5/5 24

Corn starch 2/3 2/16 15 7 25

Corn starch 2/3 12 8 7 26

Corn starch 4/6 14/3 6/4 6/2 27

Corn starch 3 18 5 7 28

Wheat starch 17/3 7/15 6/4 29

Wheat starch 6 18 5 5 30

Corn starch 6 18 5 5 31

Wheat starch 6 9/14 9/10 5/8 32

Wheat starch 6 18 15 7 33

Wheat starch 17/3 7 8 5 34

Wheat starch 3 18 5 7 35

Corn starch 3 12 5 5 36

Corn starch 3 9/15 6/8 7/5 37

Wheat starch 2.4 8.14 5 8.5 38

Results

-Monascus purpureus mushroom cultivation environments

The method and method of cultivation of solid mediums has been fully explained in the previous chapter, in Figure 2 (a) the growth of this fungus is shown in YPSS medium and part (b) in PDA medium, after 7 days of their cultivation inside the incubator, as It is clear in the figure that it has grown more in the PDA culture medium than in the YPSS culture medium. However, due to the high price of PDA culture medium, YPSS medium was used to cultivate this mushroom.

-pigment extraction

After performing the necessary steps to extract the pigment, the pigment obtained after 72 hours of drying was as follows. Part (a) of Figure 3 shows the solid material obtained after dissolving it with ethanol and centrifuging it three times. 13.0 grams/liter of dried colored solid material was obtained, and 10 grams/liter of this dried material was used to make the standard solution.

-Experimental design and statistical analysis

Obtaining the optimal percentages of the main components of the environment can be one of the ways to produce a high-quality product and

reduce rework. To find the optimal percentage of these compounds and to optimize the response variable, a method called response surface methodology (RSM) can be used. RSM is a set of effective statistical and mathematical methods that determine several variables at the same time with minimal resources and quantitative data and with a suitable experimental design. Also, this method is used to improve and optimize processes where the desired response is determined by a number of variables under be affected, and its purpose is to describe the relationship between the response and independent variables by mathematical models and to optimize this response. When many factors and relationships have an effect on the response variable, the RSM method is one of the effective tools for process optimization. The optimization process by RSM can be divided into three stages. The first step is to specify the independent parameters and their levels, the second step is to select the experimental design and predict and evaluate the relationship of the model, and the final step is to obtain and draw the shape of the response surface as a function of the independent parameters and determine the optimal points.

-color materials obtained from the design of experiments

The environments that were cultured using the experimental design were taken out of the incubator shaker after 10 days, then each one was smoothed using Whatman filter paper, after smoothing the samples, they were centrifuged to use the spectrophotometer device. It was set at 5000 rpm for 10 minutes.

-The effect of independent variables on Monascus mushroom pigment production in the design of experiments

-The effect of pH on pigment production and growth

One of the important factors in the synthesis of red pigments by Monascus species is the pH of the fermentation environment, because high pH values and the presence of a suitable nitrogen source lead to the production of extracellular and water-soluble red pigments (125). pH plays an important role in activating key enzymes in pigment production by Monascus purpureus, because the chemical modification of extracellular and water-soluble red pigments are produced at high pH values in the presence of a suitable nitrogen source. Because the pH of the culture medium may affect the transport of certain compounds such as carbon and nitrogen sources, therefore, pigment production increases with a decrease in pH. Changes in pH during microorganism cultivation depend more on nitrogen and less on carbon. The maximum red pigment production (2.46 g/L) was obtained at pH=5 and 24°C [Alejandro MÉNDEZ et al., 2011].

In this research, the red pigment had the highest efficiency at pH=5 and the maximum absorbance of 500 nm, acidic pH produces yellow pigment, in addition, alkaline pH increases the stability of this pigment more than acidic values. As mentioned, the amount of pigment production was at pH=5, which may be due to the characteristics of the used strain, which produces pigment at this particular pH.

In Figure 5, with the constant carbon source, wheat starch at a concentration of 18 g/L, nitrogen source at a concentration of 3 g/L, and surfactant at a concentration of 5 g/L had the highest pigment production when their pH was set to 5 As it is clear from the graph, the lowest amount of pigment production was when the pH was set at 5.6.

Effect of pH on red pigment concentration

-Carbon sources and their preparation method as substrate

-Using corn and wheat starch as mushroom cultivation medium

Growth of Monascus species is directly affected by starch composition and type of carbon sources. Carbon plays an important role in cell metabolism and organism growth, simultaneously affecting pigment production. In the present study, the results of the effect of different carbon sources showed that corn starch with an amount of 18 g/liter was a suitable source for maximum pigment production among the tested cases. The overall results of this research on the carbon source show that the highest growth rate and pigment production occur in the presence of wheat starch carbon source. Starch can be a promising alternative source for the production of natural pigments of Monascus purpureus. Wheat starch was more effective for red color.

As it is clear in Figure 6, by comparing two types of carbon sources, wheat starch and corn starch, it can be seen that the carbon source that used wheat starch produced much more red pigment than corn starch.

Based on Figure 7, by comparing the effect of surfactant concentration for two carbon sources with the same concentrations, it had the greatest effect at a concentration of 5 g/liter, but it did not

have much effect for corn starch. However, with the addition of surfactant, the production of color by both carbon sources decreased.

Comparison of the effect of surfactant concentration with two types of carbon sources on red pigment concentration

Figure 8 shows the effect of nitrogen source on two types of starch carbon source for pigment production, nitrogen has the most effect for wheat starch at 3 g/l, but for corn starch at 5.5 g/l. It can be said that the nitrogen source has the opposite effect for wheat starch and corn starch.

Figure 9: The effect of wheat starch on pigment production has been investigated. Sufficient amount of starch must be available in the cell for the growth of the microorganism. At pH = 5 and nitrogen concentration of 3 g/L and surfactant amount of 5 g/L, the highest concentration of red pigment was obtained when the amount of starch was 18 g/L. The lower the amount of starch, the less the production of red pigment.

Figure 9: The effect of wheat starch on red pigment concentration

Figure 10 shows the two-dimensional effect of the concentration of carbon source and surfactant for the production of red pigment, when the value of pH = 5 and the concentration of nitrogen was equal to 3 g/liter, the amount of carbon from 15 g/liter to 18 g/liter of pigment production It has red, the most red pigment was produced when the type of carbon source was wheat starch and the amount of 18 g/l of it with 8 g/l of surfactant produced the most red pigment.

Figure 11 shows this diagram in three dimensions.

Figure 10: Two-dimensional representation of the effect of carbon source concentration and surfactant concentration on pigment production

Figure 11: Three-dimensional diagram of the effect of surfactant and carbon source concentration on red pigment production

-Investigating the effect of nitrogen source concentration

Figure 12. At pH = 5 and the carbon source of wheat starch, the concentration of which was 18 g/liter, together with 5 g/liter of surfactant, the production of red pigment was the highest at the carbon source concentration of 18 g/liter, increasing the concentration of the nitrogen source with the production of pigment. Red has a photo relationship.

Figure 12: The effect of nitrogen source concentration on red pigment production

Figure 13. At pH = 5 and wheat starch carbon source 18 g/l, nitrogen source concentration from 3 to 4 g/l and surfactant concentration from 5 to almost 8.5 g/l produced red pigment. Nitrogen amount of 3 g/L and surfactant 5 g/L have produced the highest amount of red pigment.

Figure 14 shows the following diagram in three dimensions.

Figure 13: Two-dimensional representation of the effect of nitrogen source concentration and surfactant on red pigment production

Figure 14: Three-dimensional diagram of the effect of surfactant and nitrogen concentration on the production of red pigment

Investigating the effect of variables on pigment production

In order to investigate the effect of independent variables on the tested traits, response level shapes were drawn for each trait. As shown in the above figures, the obtained and predicted laboratory results are in good agreement. In each figure, the effect of two variables has been examined in the state where the rest of the variables are in the average state. The optimization of the process variables has been done to achieve the maximum response values, after conducting the experiments and obtaining the model, the desired software determines the optimal points. A response surface diagram is a geometric diagram and is actually a plot of the response variable as a function of one or more independent variables.

-Estimation of coefficient and standard error

Table 5 shows the standard error and standard coefficient of each parameter. The standard coefficients are obtained according to the table below, but these coefficients can be between the two numbers obtained in the highest and lowest cases.

Table 5: Standard coefficients and standard error

Factor Coefficient Estimate df Standard Error 95% CI Low 95% CI High

Intercept 1/85 1 0/2184 1/38 2/32

A-PH -0/8532 1 0/1459 -1/16 -0/5423

B-Carbon Source Concentration 1/56 1 0/1472 1/24 1/87

C-Nitrogen Source Concentration -0/4166 1 0/1421 -0/7195 -0/1136

D-Surfactant Concentration -0/4872 1 0/1492 -0/8052 -0/1692

E-Carbon Source Type -1/11 1 0/2587 -1/67 -0/5628

AD 0/5026 1 0/1785 0/1221 0/883

AE -0/3019 1 0/1438 -0/6083 0/0045

BD -0/3894 1 0/1754 -0/7632 -0/0155

CD 0/9256 1 0/1752 0/5521 1/3

CE -0/7667 1 0/1418 -1/07 -0/4645

DE -0/4954 1 0/1536 -0/8228 -0/168

A^2 1/03 1 0/268 0/4571 1/6

B^2 1/27 1 0/2726 0/6888 1/85

ABE -0/6322 1 0/1756 -1/01 -0/258

ACE 0/3584 1 0/1754 -0/0155 0/7324

BCE -1/23 1 0/1714 -1/6 -0/8671

BDE -0/5777 1 0/1792 -0/9597 -0/1958

CDE 0/5303 1 0/1806 0/1454 0/9153

A^2E 1/47 1 0/2702 0/8972 2/05

B^2E -1/45 1 0/289 -2/06 -0/8299

C^2E 1/76 1 0/2676 1/19 2/33

-Optimum conditions for pigment production

The optimal value obtained from the experiments according to Table 6 is shown that at pH = 5.008, the carbon source of wheat starch with a concentration of 17.921 g/liter, the concentration of the nitrogen source (yeast) is 30.63 g/liter and the concentration Surfactant is 50.92 g/liter, and in these optimal conditions, the maximum production of pigment is 15.089 g/liter, and these optimal conditions for pigment selection are shown in this table. According to the process conditions, we can choose other optimums from Table 7.

Table 6: Comparison of past work with current work

Microorganism optimal amount of culture medium (g/l) pH source

Monascus U 11/3 3 malt extract 5/5 [Sandra et al., 2008] purpureus 5 peptone

CCT3802 10 Glucose

30 ,°C 300 rpm

Monascus ODU/ml488/4 3/34 potato starch ===] et al., 2015 [Prateek Srivastav

purpureus 0.082 K_2HPO_4

MTCC 369 0.033 $MgSO_4.7H_2O$

30 ,°C 150 rpm

Monascus 〖UA〗_500 25/22 8 monosodium glutamate 5/6 [Selim Silbir and Yekta Goksungur, 2019]

purpureus 0.01 $ZnSo_4.7H_2O$

30 ,°C 350 rpm

Monascus ODU/ml 12/47 28 glucose 8 [Vimal et al., 2013] purpureus 1 tryptone

MTCC 410 30°C, rpm 120

Monascus ODU/ml 2.05 2.75 yeast extract 6 [Seyedin et al., 2015] purpureus 1.5K_2HPO_4

PTCC 5303 20 Glucose

0.3 % monosodium glutamate

30 ,°C 130 rpm

Microorganism optimal amount of culture medium (g/l) pH source

Monascus AU/g 47% 3 Peptone 3 [Said and Hamid, 2018]

purpureus 55% initial humidity

FTC 5356 30°C

Monascus AU/ml 4/54 20 Glucose 6 [Lee et al., 2002] Purpureus 3 Malt extract

ATCC 16362 3 yeast extract

5 Peptone

30°C

Monascus g/l 15/089 18 wheat starch 5 present work

purpureus OD 9625/3 3 Yeast

DSM 1603 3 surfactant

General conclusion

Pigments are very important in the biotechnology

industry. Although various sources such as plants and microorganisms are able to produce these pigments. Monascus mushroom is generally of high value from the practical and industrial aspect. The application of this pigment in the industry is very diverse and it is used in a large number of industrial processes, including food, textile, dye, detergent, pharmaceutical, papermaking, etc. Compared to plants, this pigment has various properties in terms of stability and resistance in different pH, thermal stability, temperature and optimal pH of activity.

In this research, the growth rate of Monascus purpureus and its pigments in liquid medium under the influence of different parameters (PH, nitrogen source concentration, carbon source concentration, type of carbon source and surfactant concentration) was investigated at 30 degrees Celsius. Then the effect of each parameter was investigated separately. To check the best type of carbon source, chickpea flour, beet molasses, wheat starch and corn starch were first compared, but considering that two types of wheat and corn starch had the highest amount of pigment production, these two sources were used to design the experiments. Also, the effect of other parameters such as pH, nitrogen source and surfactant on pigment production from Monascus purpureus was investigated.

The results obtained from this research showed that the highest production rate of red pigment with the carbon source of wheat starch was 15.089 g/liter, which was grown in a shaker at 150 rpm and in water at 30°C. The suitable conditions for the production of maximum red pigment were when the five influencing parameters were selected, respectively: pH = 5, its carbon source concentration was 18 g/l, nitrogen source concentration was 3 g/l, and the surfactant concentration was set to 5 g/l. They were. From the two carbon sources that were selected, the production of red pigment using wheat starch was much higher than that of corn starch.

Suggestions

Other agricultural waste can be used as a carbon source to increase growth and pigment production; Identifying and investigating other species that have the ability to produce pigments and can be used to produce natural pigments; Examining the use cases of pigment production in textile, food and pharmaceutical industries, also other uses for pigment have recently been proposed, which can be used as a stimulant for digestive digestion, treatment or prevention of osteoporosis, Alzheimer's, stroke, cancer, etc. . Considering the wide applications of this mushroom, it is possible to use this mushroom's applications in the treatment of diseases with research in this field.

References

- Abdel Khafar, E. A., Darwish, D. B., Al-Jahani, G. M., & Anean, H. E. A. (2022). Bacterial Nano-polymer Production to Produce Edible Coating and Films. *International Journal of Pharmaceutical Research and Allied Sciences, 11*(2), 13-23.

- Alejandro MÉNDEZ, Catalina PÉREZ, Julio Cesar MONTAÑÉZ, Gabriela MARTÍNEZ, Cristóbal Noé AGUILAR. Red pigment production by Penicillium purpurogenum GH2 is influenced by PH and temperature. Méndez et al. / J Zhejiang Univ-Sci B (Biomed & Biotechnol) 2011 12(12):961-968. doi:10.1631/jzus.B1100039.

- Antonio, B. 2006. Extracts of Monascus purpureus. Avalible: htt://www.renacoitalia.com/prodotti/eng monascus.html.

- Babitha, S. 2009. Biotechnology for Agro-Industrial Residues Utilisation: Microbial Pigments. Springer Sciencet Busness Media. P: 148-160.

- Brahma, S., Chatterjee, S., Pal, G. S., & Hazra, N. (2022). Larval Food Analysis and Qualitative Determination of Exoenzyme-Producing Gut Bacteria in Adult Ceratopogonid Midges (Diptera). *Entomology and Applied Science Letters*, *9*(2), 38-47.

- Boonyapanai, K., Tung Prodit, R., Lhieochaiphant, S. and Phutrakul, S. 2008. Optimization of submerged culture for the production of naphthoquinones pigment by Fusarium verticillioides. Chiang Mai J.Sci. 35: 457-466.

- Carvalho, D.J., Oishi, B.O., Pandey, A. and Soccol, C.R. 2005. Biopigments from Monascuse: Strains Selection, Citrinin Production and color stability. Brazilian Archives of Biology and Technology. 48:885-894.

- Chai, C.N., Sheu, F., Wang, C.L and Shyu, Y.T. 2004. Fermentation of Monascus purpureus on agri-by-products to make colorful and functional bacterial cellulose (NATA). Technical Bulletin

Food and Fertilizer Technology Center, 168.

- Chen, K and chen, T.T.2004. Hong Qu (Monascus): Chinese medical herbology and pharmacology. Art of Medicine Press.

- Chen, W., He, Y., Zhou, Y., Shao, Y., Feng, Y., Li, M., and Chen, F. (2015). Edible Filamentous Fungi from the Species Monascus: Early Traditional Fermentations, Modern Molecular Biology, and Future Genomics. Comprehensive Reviews in Food Science and Food Safety, 14(5), 555-567.

- Chiu, C.,Ni,K., Guu, Y. and Pan, T. (2006). Production of red mold rice using a modified Nagatatype Koji maker. Journal of Applied Microbiology and Biotechnology. 73,297-304.

- Cho, Y. J., Park, J. P., Hwang, H. J., Kim, S. W., Choi, J. W., & Yun, J. W. (2002). Production of red pigment by submerged culture of Paecilomyces sinclairii. Letters in applied microbiology, 35(3), 195-202.

- Chuannan Long, Xu Zeng, Jian Xie, Yumei Liang, Jingjing Tao, Qingqing Tao, Mengmeng Liu, Jingjing Cui, Zhiwei Huang, Bin Zeng. High-level production of Monascus pigments in Monascus ruber CICC41233 through ATP-citrate lyase overexpression. March 2019.DOI: https://doi.org/10.1016/j.bej.2019.03.007.

- Dikshit, R., and Tallapragad, P. (2013). Comparative study of Monascus sanguineus and Monascus purpureus for red pigment production under stress condition. International Food Research Journal, 20(3): 1235-1238.

- Dikshit, R. and Tallapragada, P. (2013). Exploring Monascus sanguineus as a potential natural source for pigment production. International Research Journal Biological Science, 2(5): 59-67.

- Dikshit Rashmi and Tallapragada Padmavathi. Exploring Monascus sanguineus as a Potential Natural Source for Pigment Production. ISSN 2278-3202. Vol. 2(5), 59-67, May (2013).

- Dilara Mehri, N. Altinay Perendeci and Yekta Goksungur. (2021). Utilization of Whey for Red Pigment Production by Monascus purpureus in Submerged Fermentation. 2021, 7, 75.
https://doi.org/10.3390/fermentation7020075

- Doughari, J.H. (2015). The Occurrence, Properties and Significance of Citrinin Mycotoxin. J Plant Pathol Microbiol, 6(321), 2.

- Dufosse, L. (2006). Microbial production of food grade pigments. Food Technology and Biotechnology. 44, 313-321.

- Dufosse L. (2009). Pigments, Microbial. Encyclopedia Microbiol, 4: 457-471.

- E. I. Pisareva & A. V. Kujumdzieva (2010) Influence of Carbon and Nitrogen Sources on Growth and Pigment Production by MonascusPilosus C1 Strain, Biotechnology & Biotechnological Equipment, 24:sup1, 501-506, DOI: 10.1080/13102818.2010.10817890.

- Eisenbrand, G. 2006. Toxicological evaluation of red mould rice. Molecular Nutrition and Food Research. 50(3), 322-327.

- Erdogrul, O. and Azirak, S. 2004. Reviw of the studies on the red yeast rice (Monascus purpureus). Turkish Electronic Journal of Biotechnology. 2:37-49.

- Fanelli, F., Geisen, R., Schmidt-Heydt, M., Logrieco, A.F., and Mule, G (2016). Light Regulation of Mycotoxin Biosynthesis: New Perspectives for Food Safety. World Mycotoxin Journal,9(1): 129-146.

- Farhan M. Said • John Brooks • Yusuf Chisti. Optimal C:N ratio for the production of red pigments by Monascus ruber. May 2014. DOI 10.1007/s11274-014-1672-6.

- Feng, Y., Shao, Y., and Chen, F. (2012). Monascus Pigments. Applied Microbiology and Biotechnology, 96(6), 1421-1440.

-Francielo Vendruscolo & Rose Marie Meinicke Bühler & Júlio Cesar de Carvalho & Débora de Oliveira & Denise Estevez Moritz& Willibaldo Schmidell & Jorge Luiz Ninow. Monascus: a Reality on the Production and Application of Microbial Pigments. Appl Biochem Biotechnol (2016) 178:211–223. DOI 10.1007/s12010-015-1880-z.

- Gunjan Mukherjee, Sanjay Kumar Singh. Purification and characterization of a new red pigment from Monascus purpureus in submerged fermentation. Process Biochemistry 46 (2011) 188–192. doi:10.1016/j.procbio.2010.08.006.

- Hadiseh Keivani, Mahshid Jahadi, Nafiseh Ghasemisepero. (2020). Optimizing Submerged Cultivation for the Production of Red Pigments by Monascus purpureus on Soybean Meals using Response Surface Methodology. APPLIED FOOD BIOTECHNOLOGY, 7 (3):143-152.

- Hassan Hajjaj, Jean-Marie Franc¸ois, G´erard Goma, and Philippe J. Blanc. Effect of Amino Acids on Red Pigments and Citrinin Production in Monascus ruber. JournalofFoodScience Vol.77,Nr.3,2012. doi:10.1111/j.1750-3841.2011.02579.x.

- Hilares, R.T., de Souza, R.A., Marcelino, P.F.,

da Silva, S.S., Dragone, G., Mussatto, S.I., César Santos, J., Sugarcane bagasse hydrolysate as a potential feedstock for red pigment production by Monascus ruber, Food Chemistry (2017), doi: https://doi.org/10.1016/j.foodchem.2017.11.111.

- Iffat Sayyed and Devipriya R. Majumder. Pigment Production from Fungi. 2319-7706 Special Issue-2 (2015) pp. 103-109.doi: http://www.ijcmas.com.

- Jyoti Chauhan, Suhani Parekh, Hitesh A. Solanki. Isolation and optimization of pigment production from Penicillium sp. use as natural dyestuff. Vol 11, Issue 6,June/ 2020 ISSN NO: 0377-9254.

- Keller, N.P., Turner, G., and Bennett, J.W.(2005). Fungal se Condary metabolism-from biochemistry to genomics. Nature Reviews Microbiology, 3(120): 937-947.

- Koppen, R., Koch, M., Siegel, D., Merkel, S., Maul, R., and Nehls, I. (2010). Determination of Mycotoxins in Foods: Current State of Analytical Methods and limitations. Applied Microbiology and Biotechnology, 86(6): 1595-1612.

- Kumari, H.M., Naidu, K.A., Vishwanatha, S., Narasimhamurthy K., and Vijayalakshmi, G. (2009). Safety evaluation of Monascus purpureus red mould rice in albino rast. Food and Chemical Toxicology, 47(8): 1739-1746.

- Lee, B. K., Piao, H. Y., & Chung, W. J. (2002). Production of red pigments byMonascus purpureus in solid-state culture. Biotechnology and Bioprocess Engineering, 7(1), 21-25.

- Lee, C.H., Lee, C.L., and Pan, T.M. (2010). A 90-D Toxicity Study of Monascus Fermented Products Including High Citrinin Level. Journal of Food Science, 75(5): 91-97.

- Lee, C.L., Kuo, T.F., Wang, J.J. and Pan, T.M. (2007). Red mold rice ameliorates impairment of memory and learning ability in intracerebroventricular amyloid beta-infused rat by repressing amyloid beta accumulation. Journal of Neuroscience Resserch. Jul 30:17663476 (P,S,G,E,B,D).

- Lee, Y.K. and Chen, D. (1998). Application of Monascus culture and application, 8-10 July, Toulouse, France.

- Lin,Y.L., Wang, T.H. and Lee, M.H. 2008. Biologically active components and nutraceuticals in the Monascus-fermented rice: a review. Apple Microbial Bitechnol. 77: 965-973.

- Liu, L., Zhao, S.P., Cheng, Y.C. and Li, Y.L. (2003). Xuezhikang decreases serum lipoprotein (a) and C-reactive protein concentrations in patients with coronary heart disease. Clinical Chemistry. 49, 1347-1352.

- Li, Y.P., Pan, Y.F., Zou, L.H., Xu, Y., Huang, Z.B., and He, Q.H. (2013). Lower Citrinin Production by Gene Disruption of CtnB Involved in Citrinin Biosynthesis in Monascus aurantiacus Li AS3. 4384. Journal of Agricultural and Food Chemistry, 61(30), 7397-7402.

- Majid Afshari, Fakhri Shahidi, Seyed Ali Mortazavi, Farideh Tabatabai and Zarin Es'haghi. Investigating the influence of PH, temperature and agitation speed on yellow pigment production by Penicillium aculeatum ATCC 10409. Natural Product Research, 2015.doi: http://dx.doi.org/10.1080/14786419.2014.999059.

- Malik, K., Tokkas, J., and Goyal, S. (2012). Microbial pigments: a review. International Journal of Microbial Resource Technology, 1(4): 361-365.

- Mary Ladidi Abu, Rosfarizan Mohammad, Siti Nurbaya Oslan, Abu Bakar Salleh. (2020). The use of response surface methodology for enhanced production of a thermostable bacterial lipase in a novel yeast system. Published online: 17 Sep 2020. 2021;51(4):350-360. https://doi.org/10.1080/10826068.2020.1818256

- Matej Patrovsky, Kristyna Sinovska, Barbora Branska, Petra Patakova. Effect of initial PH, different nitrogen sources, and cultivation time on the production of yellow or orange Monascus purpureus pigments and the mycotoxin citrinin. 20SVV/2017 and 8H17043. DOI: 10.1002/fsn3.1197.

-Meihua Wang & Tao Huang & Gong Chen & Zhenqiang Wu. Production of water-soluble yellow pigments via high glucose stress fermentation of Monascus ruber CGMCC 10910. December 2016. DOI:10.1007/s00253-017-8106-y.

- Meinicke, R.M., Vendruscolo, F., Moritz, D.E., de Oliveira, D., Schmidell, W., Samohyl, R.W., and Ninow, J.L. (2012). Potential Use of Glycerol as Substrate for the Production of Red Pigments by Monascus ruber in Submerged Fermentation. Biocatalysis and Agricultural Biotechnology, 1(3), 238-242.

- Ming-Ho Chen, Michael R. Johns. (1993).

Effect of PH and nitrogen source on pigment production by Monascus purpureu. Appl Microbiol Biotechnol (1993) 40:132-138.

- Mohan-Kumari Honganoor Puttananjaiah, Mohan Appasaheb Dhale, Vijayalakshmi Govindaswamy. Non-Toxic Effect of Monascus purpureus Extract on Lactic Acid Bacteria Suggested Their Application in Fermented Foods. Food and Nutrition Sciences, 2011, 2, 837-843. doi:10.4236/fns.2011.28115.

- Mohankumari H.P., Akhilender Naidu K,. Vishwanatha S., Narasimhamurthy K., Vijayalak shim G (2009). Safety evaluation of Mnascus purpureus red mould rice in albino rats. Food chemtoxicol 47:1739-1746.

- Muhammad Safiullah Virk, Rabia Ramzan, Muhammad Abdulrehman Virk, Xi Yuan and Fusheng Chen. Transfigured Morphology and Ameliorated Production of Six Monascus Pigments by Acetate Species Supplementation in Monascus ruber M7. Microorganisms 2020, 8, 81; doi:10.3390/microorganisms8010081.

- Mukherjee, G., & Singh, S. K. (2011). Purification and characterization of a new red pigment from Monascus purpureus in submerged fermentation. Process Biochemistry, 46(1), 188-192.

- Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2016). Response surface methodology: process and product optimization using designed experiments. John Wiley & Sons.

- Nanis H. Gomah; H. E. F. Abdel-Raheam and T. H. Mohamed. (2017). Production of Natural Pigments from Monascus ruber by Solid State Fermentation of Broken Rice and its Application as Colorants of Some Dairy Products. J. Food and Dairy Sci., Mansoura Univ., Vol. 8 (1): 37- 43.

- Nazli Pinar Arslan, Aysenur Yazici, Senba Komesli, Nevzat Esim, Serkan Ortucu. (2020). Direct conversion of waste loquat kernels to pigments using Monascus purpureus ATCC16365 with proteolytic and amylolytic activity. https://doi.org/10.1007/s13399-020-01139-5.

- N F S Daud, F M Said M Ramu and N M H Yasin. Evaluation of Bio-red Pigment Extraction from Monascus purpureus FTC5357. IOP Conf. Series: Materials Science and Engineering736 (2020) 022084. doi:10.1088/1757-899X/736/2/022084.

- Nimnoi, P., & Lumyong, S. (2011). Improving solid-state fermentation of Monascus purpureus on agricultural products for pigment production. Food and Bioprocess Technology, 4(8), 1384-1390.

- Orak T, Caglar O, Ortucu S, Ozkan H, Taskin M, Chicken feather peptone: A new alternative nitrogen source for pigment production by Monascus purpureus, Journal of Biotechnology (2010), https://doi.org/10.1016/j.jbiotec.2018.02.010.

- Orozco, S. F. B., and Kilikian, B.V. (2008). Effect of PH on citrinin and red pigments production by Monascus purpureus CCT3802. World Journal of Microbiology and Biotechnology, 24(2): 263-268.

- Patakova, P., Branska, B., and Patrovsky, M. (2017). Monascus Secondary Metabolites. Fungal Metabolites, 821-851.

- Pagano, M. C., & Dhar, P. P. (2015). Fungal pigments: an overview. Fungal Biomolecules: Sources, Applications and Recent Developments; Gupta, VK, Mach, RL, Sreenivasaprasad, S., Eds, 173-181.

- Pattanagul, P., Pinthong, R., Phianmongkhol, A., and Leksawasdi, N. (2008). Review of angkak production (Monascus purpureus). International Journal of Food Microbiology, 34(3): 319-328.

- Pekkanen, A.N., Punsar, S. and Karvonen, M.J. (1992). Short and long term association of serum cholesterol with mortality. American Journal of Epidemiology 135, 1252-1258.

- Pereira, D. G., Tonso, A., and Kilikian, B. V. (2008). Effect of dissolved oxygen concentration on red pigment and citrinin production by Monascus purpureus ATCC36928. Brazilian Journal of chemical Engineering, 25(2): 247-253.

- Pinar Atalay, Sayit Sargin, Yekta Goksungur. (2020). UTILIZATION OF RESIDUAL BEER FOR RED PIGMENT PRODUCTION BY MONASCUS PURPUREUS IN SUBMERGED FERMENTATION. Volume 29 – No. 02/2020 pages 1025-1034

- Please cite this article in press as: Velmurugan, P., et al. Natural pigment extraction from five filamentous fungi for industrial applications and dyeing of leather. Carbohydrate Polymers (2009), doi:10.1016/j.carbpol.2009.07.058.

- Pongrawee Nimnoi & Saisamorn Lumyong. Improving Solid-State Fermentation of Monascus purpureus on Agricultural Products for Pigment Production. Food Bioprocess Technol (2011) 4:1384–1390. DOI 10.1007/s11947-009-0233-8.

- Prateek Srivastav, Vivek Kumar Yadav,

Sharmila Govindasamy, Muthukumaran Chandrasekaran. Red pigment production by Monascus purpureus using sweet potato-based medium in submerged fermentation. Nutrafoods (2015) 14:159-167. DOI 10.1007/s13749-015-0032-y.

- P.R. Wagh & R.S. Mane 2017. Identification And Characterization Of Extra Cellular Red Pigment Producing Neisseriasppis Olated From Soil Sample. ISSN: 2348-1390.

- Jensen, W. A. (2017). Response surface methodology: process and product optimization using designed experiments. Journal of Quality Technology, 49(2), 186.

- Retterstol, L. and Bohn, M. (2002). C-reactive protein predicts death in patients with previous premature myocardial in farction: a 10 Year follow-up study. Atherosclerosis. 160, 433-440.

- Rose Marie Meinicke Bühler & Bruna Luíse Müller & Denise Esteves Moritz & Francielo Vendruscolo & Debora de Oliveira & Jorge Luiz Ninow. Influence of Light Intensity on Growth and Pigment Production by Monascus ruber in Submerged Fermentation. Appl Biochem Biotechnol (2015) 176:1277–1289 DOI:10.1007/s12010-015-1645-8.

- Rose Marie Meinicke, Francielo Vendruscolo, Denise Esteves Moritz, De ´bora de Oliveira, Willibaldo Schmidell, Robert Wayne Samohyl, Jorge Luiz Ninow. Potential use of glycerol as substrate for the production of red pigments by Monascus ruber in submerged fermentation. Biocatalysis and Agricultural Biotechnology 1 (2012) 238–242. doi:10.1016/j.bcab.2012.03.001.

- Said, F. M., & Hamid, N. F. B. (2018). Optimization of red pigment production by Monascus purpureus FTC 5356 using response surface methodology. IIUM Engineering Journal, 19(1), 34-47.

- Sanae, P., Fernanda, S. and Vahan, B. (2005). Concentration determination of extracellular and intracellular red pigments produced by Monascus sp. Brazilian Archives of Biology and Technology. 48, 43-49.

- Sandra Fernanda Bilbao Orozco Æ Beatriz Vahan Kilikian. Effect of PH on citrinin and red pigments production by Monascus purpureus CCT3802. World J Microbiol Biotechnol (2008) 24:263–268. DOI 10.1007/s11274-007-9465-9.

- Segal, B.H., Herbrecht, R. and Stevens, D.A. 2007. Doctor fungus web site. Avalible: http://www.doctorfungus.org.

- Selim Silbir and Yekta Goksungur. Natural Red Pigment Production by Monascus Purpureus in Submerged Fermentation Systems Using a Food Industry Waste: Brewer's Spent Grain. Foods 2019, 8, 161; doi:10.3390/foods8050161.

- Seyedin, A., Hatamian-Zarmi, A., Rasekh, B., & Mirderikvand, M. (2015). Natural pigment production by Monascus purpureus: Bioreactor yield improvement through statistical analysis. Applied Food Biotechnology, 2(2), 23-30.

- Sharmin Suraiya & Mahbubul Pratik Siddique & Jong-Min Lee & Eun-Young Kim & Jin-Man Kim & In-Soo Kong. Enhancement and characterization of natural pigments produced by Monascus spp. using Saccharina japonica as fermentation substrate. J Appl Phycol (2018) 30:729–742. DOI 10.1007/s10811-017-1258-4.

- Shazwani, N. 2012. The optimization of red pigment by Monascus purpureus FTCB5356 in solid state fermentation. MSc Thesis, Universiti Malaysia Pahang.

- Shi, Y.C., and Pan, T.M. (2011). Beneficial effects of Monascus purpureus NTU 568-fermented products: a review. Applied Microbiology and Biotechnology, 90(4): 1207-1217.

- Silveira, S. T., Daroit, D. J., Sant'Anna, V., & Brandelli, A. (2013). Stability modeling of red pigments produced by Monascus purpureus in submerged cultivations with sugarcane bagasse. Food and Bioprocess Technology, 6(4), 1007-1014.

- Stchigel, A.M., Cano, J., Samir, K.A. and Guarro, J. 2004. New and interesting species of Monascus from soil with a Key to known species. Studies in mycology. 50,299-307.

- Sumathy Babitha, Carlos R. Soccol and Ashok Pandey. Jackfruit Seed – A Novel Substrate for the Production of Monascus Pigments through Solid-State Fermentation. Biotechnol. 44 (4) 465–471 (2006).doi: https://www.researchgate.net/publication/228508498.

- Sumathy Babitha, Julio C. Carvahlo, Carlos R. Soccol, Ashok Pandey. (2008). Effect of light on growth, pigment production and culture morphology of Monascus purpureus in solid-state fermentation. https://www.researchgate.net/publication/225587610.

- Surawut, S., Nak-eiam, S., Kunsook, C., Kamhaengkul, L., Kanjanavas, P., & Yasawong,

M. (2021). Diversity and the Molecular Identification of Some Ascomycetes Macrofungi Found in the Para Rubber Plantation, Thailand. *Journal of Biochemical Technology, 12*(4), 50-56.

- Temudo, M.F. Kleerebezem, R., and Loosdrecht, M. (2007). Influence of the PH on (open) mixed Culture fermentation of glucose: A chemostat study. Journal of Biotechnology and Bioengineering, 98,69-79.

- Thiyam General & Hye-Jin Kim & Binod Prasad & Hoang Tuan Anh Ngo & Nithya Vadakedath & Man-Gi Cho 23 September 2013. Fungal utilization of a known and safe macroalga for pigment production using solid-state fermentation. DOI 10.1007/s10811-013-0168-3.

- Vidyalakshmi, R., Paranthaman, R., Murugesh, S., and Singaravadivel, K. (2009). Stimulation of Monascus pigments by intervention of different nitrogen sources. Global Journal Biotechnol Biochem, 4(1): 25-28.

- Vimal S. Prajapati, Nidhi Soni, Ujjval B. Trivedi, Kamlesh C. Patel, An enhancement of red pigment production by submerged culture of Monascus purpureus MTCC 410 employing statistical methodology, Biocatalysis and Agricultural Biotechnology, http://dx.doi.org/10.1016/j.bcab.2013.08.008.

- Wang, J.J., Shieh, M.J., Kuo, S.L. and Lee , C.L. (2006). Effect in red mold rice on antifatigue and exercise-related changes in lipid peroxidation in endurance exercise. Appled Microbiology and Biotechnology. 70, 247-253.

- Wong, H.C and Bau, Y.S. 1977. Pigmentation and antibacterial activity of fast neutron and X-ray-induced strains of Monascus went. Plant physiology. 60, 578-581.

- Wong, H. C., Lin, Y. C., & Koehler, P. E. (1981). Regulation of growth and pigmentation of Monascus purpureus by carbon and nitrogen concentrations. Mycologia, 73(4), 649-654.

- Wu, G.F and Wu, X.C. (2000). Screening Dpph radical Scavengers from Monascus sp. Acta Microbiologica Sinica. 40,394-399.

- Xijun Lian · Lizeng Liu · Shirui Dong · Hong Wu · Jixing Zhao · Yanjun Han. Two new Monascus red pigments produced by Shandong Zhonghui Food Company in China. November 2014. DOI:10.1007/s00217-014-2376-8.

- Young Mok Heo, Kyeongwon Kim, Sun Lul Kwon, Joorim Na, Hanbyul Lee, Seokyoon Jang, Chul Hwan Kim, Jinho Jung & Jae-Jin Kim (2018) Investigation of Filamentous Fungi Producing Safe, Functional Water-Soluble Pigments, Mycobiology, 46:3, 269-277, DOI: 10.1080/12298093.2018.1513114.

- Yang, Y., Liu, B., Du, X., Li, P., Liang, B., Cheng, X., Du, L., Huang, D., Wang, L. and Wang, S. (2015). Complete Genome Sequence and Transcriptomics Analyses Reveal Pigment Biosynthesis and Regulatory Mechanisms in an Industrial Strain, Monascus purpureus YY-1. Scientific Reports, 5, 8331.

- Yasukawa, K., Takahashi, M., Yamanochi, S. and Takido, M. (1996). Inhibitiry effect of oral administration of Monascus Pigment on tumor promotion in two-stage carcinogenesis in mouse skin Oncology. 53, 247-249.

- Yonghui Wang, Bobo Zhang, Liping Lu, Yan Huang and Ganrong Xu. Enhanced production of pigments by addition of surfactants in submerged fermentation of Monascus purpureus H1102. JSciFoodAgric 2013;93:3339–3344. DOI10.1002/jsfa.6182

- Zhu, Y., Shang, X., Yang, L., Zheng, S., Liu, K., Li, X., Purification, Identification and properties of a new blue pigment produced from Streptomyces sp. A1013Y, Food Chemistry (2019), doi: https:// doi.org/10.1016/j.foodchem.2019.125600.