Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
7(4): 331–345 (2017) ISSN 2229-2225

www.creamjournal.org

Article
Doi 10.5943/cream/7/4/10

Red yeast as a powerful stable biopigment producer under various growth conditions

Ghada AEM[1*] and Walid MAA[2]

[1] Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut 71516, Egypt
[2] Central Laboratory of Organic Agriculture, Agricultural Research Center, Minia 61642, Egypt.

Ghada AEM, Walid MAA 2017 – Red yeast as a powerful stable biopigment producer under various growth conditions. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 7(4), 331–345, Doi 10.5943/cream/7/4/10

Abstract

Most of the synthetic dyes found to be hazardous to human health therefore; we need to develop alternative source of natural food colorants. The current study aimed to study different factors affecting on pigment production by *Monascus purpureus*. The optimal growth and pigment production factors were carbon and nitrogen source (Corn starch & yeast extract), medium pH 6 after fermentation time 12 days under shaking (150 rpm) at 30 ± 1 °C. The impact of testing different amino acids, metal ions and vitamins addition to the medium was explored by applying (100 µg/l) for each of them on the medium. The addition of Tryptophan was effective and enhanced both growth and production (3.9 g/l dry mass and 2.376 A500), Manganese improve growth and production with 4.6 g/l dry mass and 1.997 A500 and the addition of ascorbic acid also increase the production (4 g/l dry mass and 2.652 A500). The isolate seems to be tolerated the salinity stress until 2% for growth and production then production decrease until it stops completely at 6% NaCl. Maximum optical density values of red yeast pigment recorded at wavelength 500nm followed by 460nm. Red pigment shows various optical density values in different solutions (salicylic acid; citric acid and ascorbic acid. Testing different factors affecting on production is an efficient approach for the production of pigment through microbial fermentation by *Monascus purpureus* and could be utilized in industrial application.

Key words – Angkak – *Monascus purpureus* – Pigment – Production

Introduction

Microorganisms have been produced several substances with industrial importance. Filamentous fungi demonstrated to be useful for their ability to produce primary and secondary metabolites such as enzymes, organic acids, proteins, antibiotics, and pigments (Hajjaj et al. 1999). *Monascus purpureus*, edible fungus, has been used by people for centuries in Asian countries (China, Japan and South East) in red rice production. Red yeast rice is commercially available in capsules and should be taken in 1.2-2.4 g (5-10 mg monacolins) per day in divided doses for a trial period of up to 12 weeks (Wang et al. 1997, Heber et al. 1999). Characterize by production of colorless to pale brown cleistothecia and aleurioconidia. Each cleistothecium is borne on a well defined stalk. Asci break down rapidly when ascospores are mature so the impression under the microscope is of a sac filled with a mass of ellipsoidal; smooth walled, and retractile spores (Pitt & Hocking 2009, Feng et al. 2012).

Submitted 7 September, Accepted 29 November 2017, Published 4 December 2017
Corresponding Author: Dr.Ghada Abd-Elmonsef Mahmoud – e-mail – ghada_botany@yahoo.com

Addition of color to food maintains the original food appearance even after processing and storage, to maintain its quality and to increase its acceptability. Food colors can be classified into four parts: natural, nature-identical, synthetic and inorganic colors. Natural pigments are obtained from many sources such as plants, insects, and microorganisms. Microorganisms have advantages of productivity and versatility. It has important properties by playing a significant role in the human lifestyle and environment in food production, health products and recycling of complex compounds in our biosphere (Hajjaj et al. 2000).

Monascus has the ability of producing some polyketones secondary metabolites used in many industries. *Monascus* species produce orange, yellow and red pigments which have anticancer, antimicrobial, anti-obesity, anti-inflammation and anti-diabetes prosperities (Mapari et al. 2005, Feng et al. 2012). Microorganisms provide a significant alternative source of synthetic due to demand for natural colorants, environmental and toxicity issues associated with synthetic pigments (Méndez et al. 2011). Moreover we use these pigments traditionally in food products e.g. cheese, meat, wine, tofu and fish in food coloring, medical and pharmaceutical industries. These pigments involved many good properties like high safety, easy production, good solubility and can replace synthetic pigments in the food industry (Chen et al. 2015, Vendruscolo et al. 2016, Ning et al. 2017).

Numerous reports have been indicating that adjusting the composition of growth media and culture conditions increase health-related compounds such pigments (Wang & Pan 2003). Solid state fermentation was mostly used in pigment production by *Monascus*, however low yield obtained make it not economical for industrial process, so researchers focused now on submerged fermentation. For increasing the production many techniques used e. g. medium composition (carbon and nitrogen sources), pH, agitation speed and addition of nutrient (Johns & Stuart 1991, Kim et al. 2002, Miyake et al. 2008, Mukherjee & Singh 2011).

Monascus is recorded as a rich source of natural color and produces chemical species that give a red color. Colorants are often added to fruit flavored yoghurt to enhance or replace the natural color of the fruit. Pigments produced by the mold, *Monascus purpureus*, offer a possible alternative way to certified food dyes or natural pigments now used (Koehler 2001, Dweck 2002). Based on the aspects cited above, the main objective here is to study factors affecting on the red pigment production and stability by *Monascus purpureus* to assess the effects of the process variables and optimize the production of red pigments.

Materials & Methods

Microorganism

Monascus purpureus (Went) a saprophytic fungus, was obtained from the Agriculture Research Center, Egypt (ARC). The stock culture was maintained aerobically on potato dextrose agar medium (PDA) and stored at 4± 1 °C. Prior to pigment production experiments, the fungus was grown aerobically on PDA medium at 28± 1 °C for one week.

Production medium and conditions

Corn starch medium (CSM) was used as a production medium containing (g/l): Corn starch, 20.0; NH_4NO_3, 1.0; KH_2PO_4, 1.5 and $MgSO_4.7H_2O$, 0.5. These contents were dissolved in 1000 ml distilled water with initial pH adjusted to 5.5 before autoclaving. After sterilization chloramphenicol, 250 mg/ml was sterilized separately by membrane filtration, using a membrane of pore size 0.22 mm and added as bacteriostatic agent. Homogeneous spore suspensions of *M. purpureus* was prepared by scrapping fungal hyphae from 7 days PDA plates and suspended in sterilized distilled water containing 0.01% (v/v) tween 80 and stirred for 30 min until final concentration 2.5×10^6. One ml of the inoculums was transferred to an Erlenmeyer flask containing 50 ml of production medium. Incubation was carried out at 28 ± 1 °C on a rotary shaking (150 rpm). After 7 days, fungal mycelium was recovered by filtration through dried Whatman filter

paper (No. 113) for intracellular red pigment and dry weight determination. The filtrate was used as the crude extracellular red pigment.

Optimization of red pigment production

Effect of different carbon sources

Different carbon sources were examined to determine their effect on pigment production namely, L- sorbose, Dextrose, Fructose, Sucrose, Maltose, Lactose, Cellulose and Soluble starch. *Monascus purpureus* was grown on mineral medium supplemented with the carbon source, fungal mass harvested and pigment production was determined.

Effect of different nitrogen sources

Different nitrogen sources were examined to determine their effect on pigment production including non organic nitrogen sources ($NaNO_3$, KNO_3, $(NH_4)_2SO_4$, $(NH_4)_2PO_4$ and $(NH_4)_2FeSO_4$) and organic nitrogen sources (Malt extract, Beef extract, Yeast extract, Peptone and Casein). *Monascus purpureus* was grown on corn starch medium supplemented with the different nitrogen source, fungal mass harvested and pigment production was determined.

Effect of initial pH on pigment production

Monascus purpureus was grown on corn starch medium at different initial pH ranging from 2 to 12. The initial pH was adjusted by 0.1 M HCl or 0.1 M NaOH. After 7 days the fungal mass was harvested and pigment production was determined.

Effect of incubation temperature

The effect of incubation temperature on pigment production was studied in range of 10 to 55 ± 1˚C. *Monascus purpureus* was grown on corn starch medium for 7 days. Subsequently fungal mass harvested and pigment production was determined.

Effect of incubation period

The effect of incubation period on pigment production was studied over range of 2 to 14 days. *Monascus purpureus* was grown on corn starch medium. Subsequently fungal mass harvested and pigment production was determined.

Effect of incubation type

For investigating the optimal conditions for red pigment production both shaking (150 rpm) as well as static conditions was monitored.

Factors affecting on red pigment production

Effect of ionic strength

The effect of ionic strength on red pigment production was studied at different salt concentrations in range of 0.0 to 10% NaCl. *Monascus purpureus* was grown on corn starch medium for 7 days then fungal mass harvested and pigment concentration was determined.

Effect of different amino acids addition

Different amino acids were tested for their effect on pigment production by *Monascus*. Arginine, L-Asparagines, glycine, L-tryptophan, L-cystiene and phenylalanine sterilized separately using by membrane filtration using a membrane of pore size 0.22 µm and (100 µg /l) were add to medium after sterilization. *Monascus* was grown on corn starch medium supplemented with amino acid for 7 days then fungal mass harvested and pigment concentration was determined.

Effect of different metal ions addition

Different trace elements (Fe^{2+}, Mn^{2+} , Co^{2+} and Zn^{2+}) were tested for their effect on pigment production by *M. purpureus*, 100 µg/l were add to medium. After incubation for 7 days then fungal mass harvested and pigment concentration was determined.

Effect of different vitamins addition

Different water soluble vitamins namely; Thiamine (Vitamin B1), Riboflavin (Vitamin B2), Folic acid (Vitamin B) and Ascorbic acid (Vitamin C) were tested for their effect on pigment production by *M. purpureus*. They sterilized separately using by membrane filtration using a membrane of pore size 0.22 µm and (100 µg/l) were added to medium after sterilization. After incubation for 7 days then fungal mass harvested and pigment concentration was determined.

Pigment optical density in different solutions

After red pigment extracted from red yeast using ethanol, pigment optical denisty measured at 380-700 nm using a spectrophotometer. Red pigment treated with different solutions adjusted to (1:1) red yeast pigment in ethanol: water; salicylic acid (1, 1.5 and 2mM); citric acid (0.3, 0.5 and 1%) and ascorbic acid (100, 300 and 500ppm) solutions.

Pigment analysis

At the end of cultivation, the culture filtrate centrifuged (4,000×g for 15 min) and the supernatant was used for extracellular red pigment estimation. For the intracellular red pigment estimation, one gram of fungal biomass was suspended in 10 ml of ethanol aqueous solution 95% and incubated overnight in room temperature then centrifuge for 10 min and the supernatant used as intracellular red pigment (Babitha et al. 2007). The analysis of pigment was measured at 500 nm using a spectrophotometer covers a wavelength range of 190–1100 nm. The red pigment produced by *Monascus* was expressed in optical density units (UA500/ml). Also the analysis of the absorbance spectra (350–700 nm) of the pigment solution was performed (Kang et al. 2014).

Results

Morphological description of *Monascus purpureus*

Monascus purpureus (Went) is a homothallic fungus belongs to the group of Ascomycetes (family Monascaceae). Growth on potato dextrose agar medium (PDA), mycelium is white in the early stage then rapidly changes to a rich pink with soluble pigment diffuse in medium (A). Pedicellate ascomata with ascospores (B, C). *Monascus purpureus* produced spherical ascospores of 5 microns in diameter (D) as shown in fig.1.

Spectral analysis of *Monascus purpureus* red pigment

Spectral analysis of pigments produced by *M. purpureus* on corn starch medium shows absorbance peaks at 420–430 and the highest peak was at 500nm (Fig. 2).

Optimization of red pigment production

Various factors, including medium composition (carbon and nitrogen sources), pH, temperature, incubation period and type of fermentation were employed to screen red pigment production by *M. purpureus*. *Monascus* first utilize carbon and nitrogen sources for primary metabolites synthesis, and then pigments produced at the end of fungal growth as secondary metabolites. Among the carbon sources tested, corn starch gave a maximum red pigment absorbance (A_{500}) 3.314 ± 0.02 and maximum dry weight (3 ± 0.12 g/l) followed by soluble starch (3.07 ± 0.01). *Monascus purpureus* assimilated small part of cellulose giving 0.7 A_{500} and 0.42 g/l dry mass (Fig. 3).

Data depicted in Fig. 4, illustrated effect of different nitrogen sources on red pigment production. In this context, yeast extract was excellent nitrogen source for both biomass (3.73 ±0.19 g/l) and red pigment (6.116 ± 0.15 A_{500}). Red pigment production was evaluated under

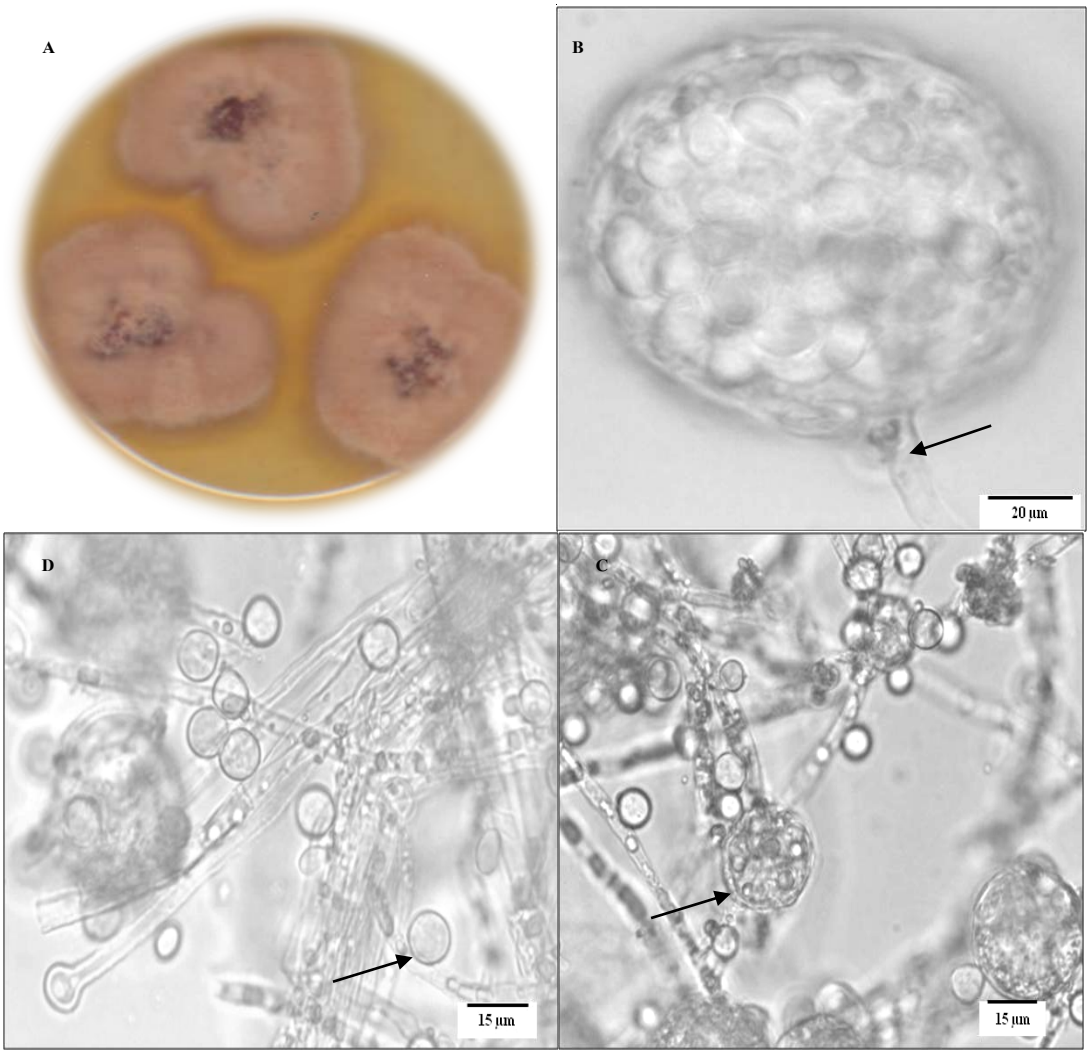

Fig. 1 – *Monascus purpureus* growth on PDA medium A Pedicellate ascomata with ascospores. B, C Spherical ascospores of 5 microns in diameter D.

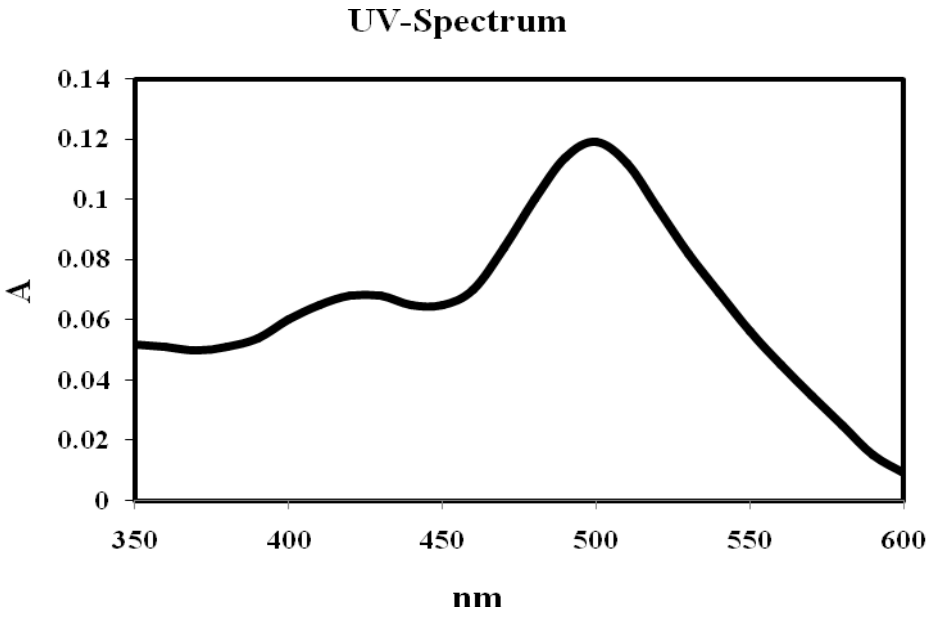

Fig. 2 – UV-spectrum of *Monascus purpureus* pigments.

both static and shaken culture conditions. It was observed that cultures incubated under static were low in both growth and production (1.85± 0.09 A_{500} and 0.6 ± 0.05 g/l, respectively). The effect of different initial pH values on red pigment production by *M. purpureus* cleared in Fig. 5. The results showed that the fungal strain was able to grow in the pH range from 2 to 10 achieve maximum production (3.74± 0.09 A_{500}) and maximum cell mass (3.3 ± 0.18 g/l) at pH 6.

The effect of incubation temperature on pigment production was studied in range of 10 to 50 °C (Fig. 6). Red pigment production was proportional to growth rate in the stationary phase and reached its maximum value at 30°C.Temperature plays a pivotal role in cell metabolism and consequently influencing the pigment production**.** Red pigment production was detected over range of 2 to 20 days. It was observed that maximum pigment production was (4.8 ± 0.05 A_{500}) and maximum dry cell mass was (7.2 ± 0.19 g/l) recorded after 12 days (Fig. 7).

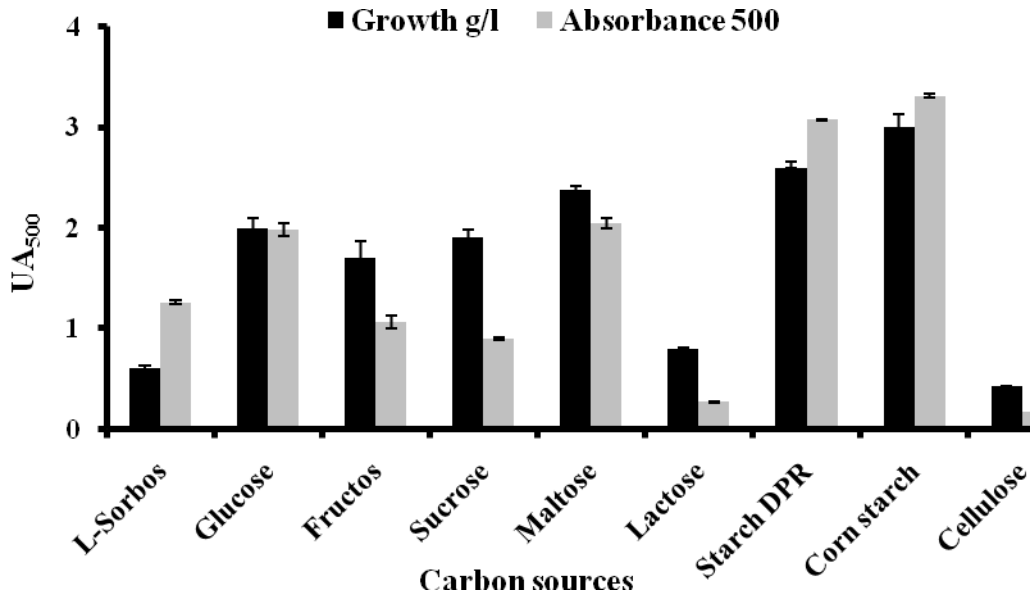

Fig. 3 – Effect of different carbon sources on red pigment production by *M. purpureus.*

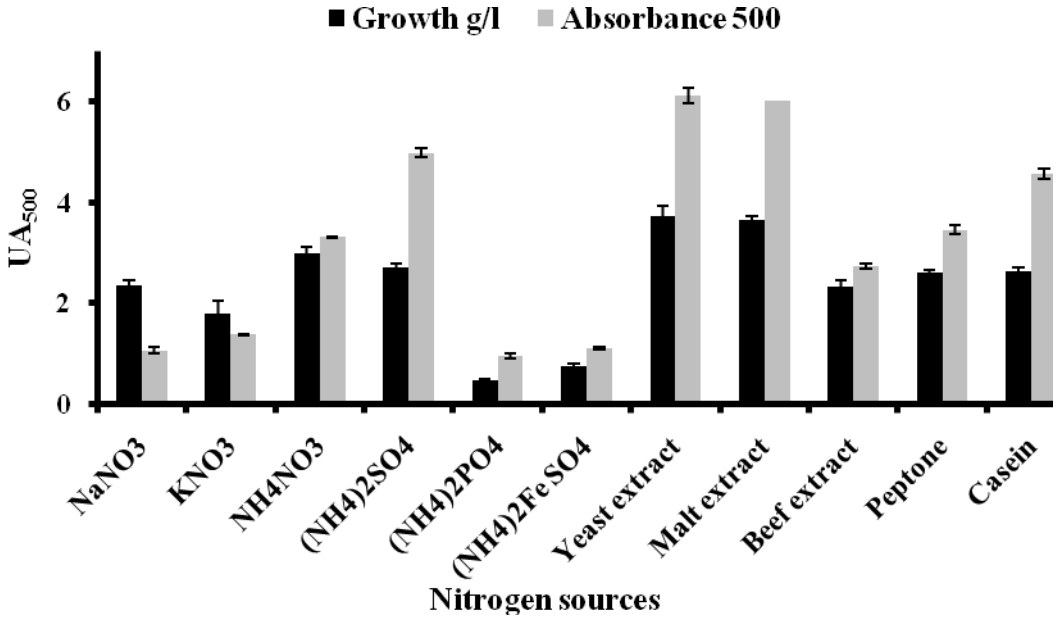

Fig. 4 – Effect of different nitrogen sources on red pigment production by *M. purpureus*.

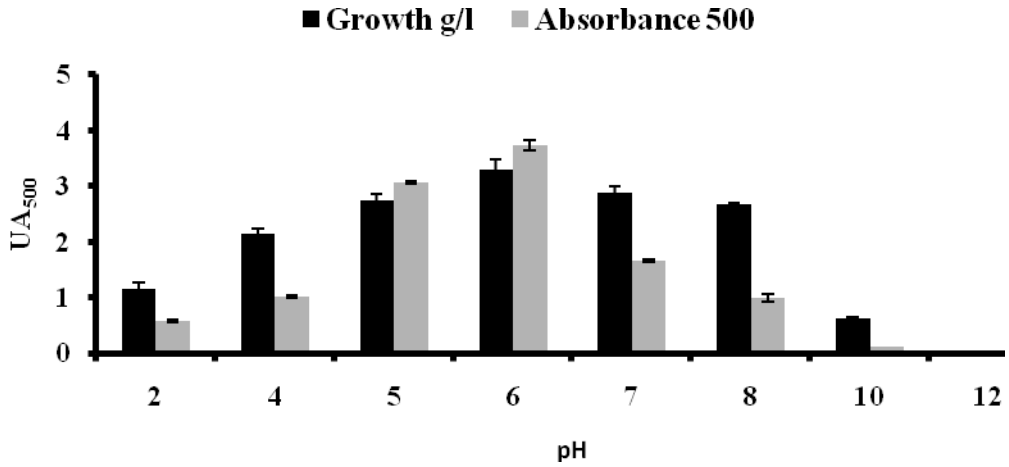

Fig. 5 – Effect of initial pH on red pigment production by *M. purpureus*.

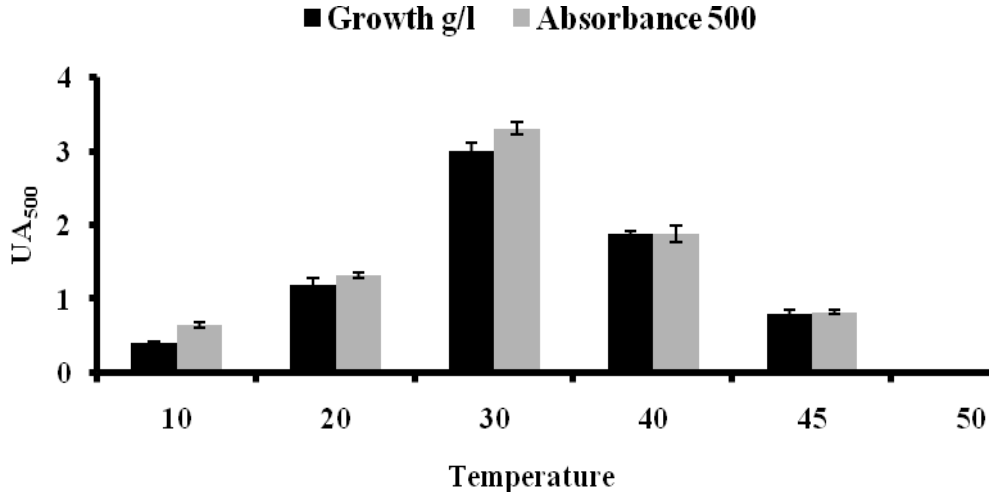

Fig. 6 – Effect of incubation temperature on red pigment production by *M. purpureus*.

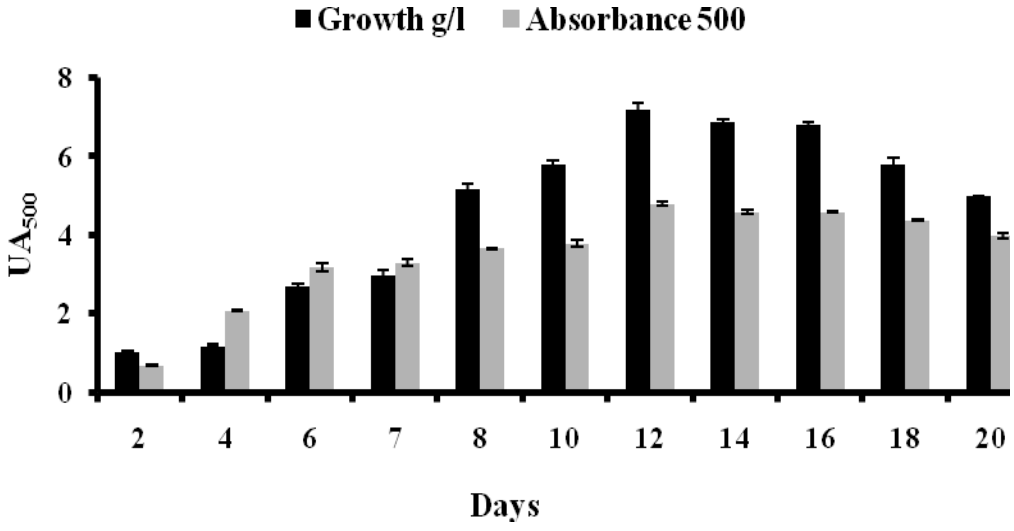

Fig. 7 – Effect of incubation period on red pigment production by *M. purpureus*.

Factors affecting on red pigment production

The additions of different salt concentrations increase both growth and red pigment production in low concentration only, the fungus could tolerate high levels of ionic strength until 6% NaCl without red pigment production (Fig. 8). The highest growth and red pigment production obtained from medium containing amino acid was L- tryptophan (4.75 ± 0.09 A_{500}) and maximum dry cell mass was (3.9 ± 0.02 g/l) as shown in fig. 9. Metal ions additions showed a great effect on *Monascus* both growth and red pigment production (Fig. 10). The highest red pigment production obtained after Zn^{2+} addition giving 5.99 ± 0.04 A_{500} and 4.6 ± 0.09 g/l dry cell mass. Fortification the medium with vitamins also produced great stimulatory effect by all tested vitamins (Fig. 11). The highest growth and red pigment production obtained by adding ascorbic acid (vitamin C) to medium 5.3 ± 0.1 A_{500} and 4 ± 0.14 g/l dry cell mass.

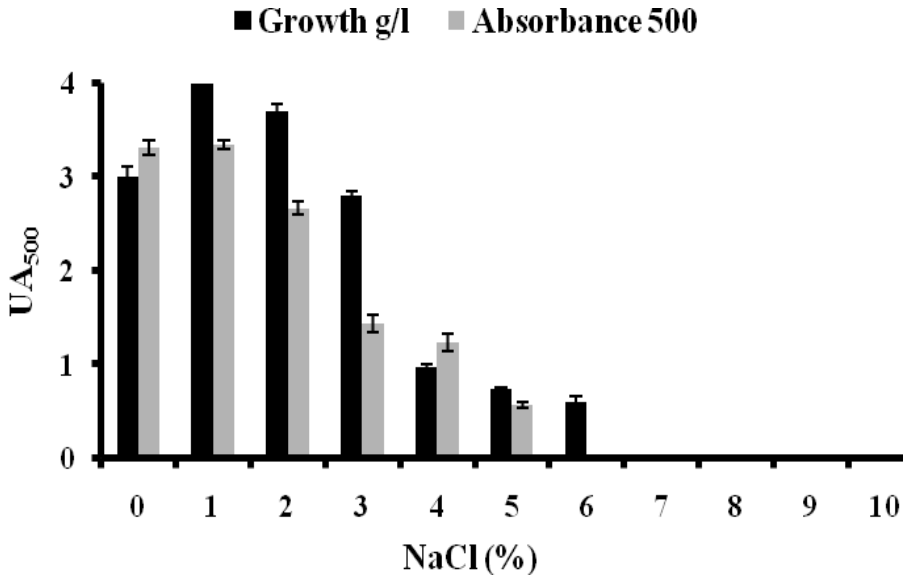

Fig. 8 – Effect of ionic strength on red pigment production by *M. purpureus*.

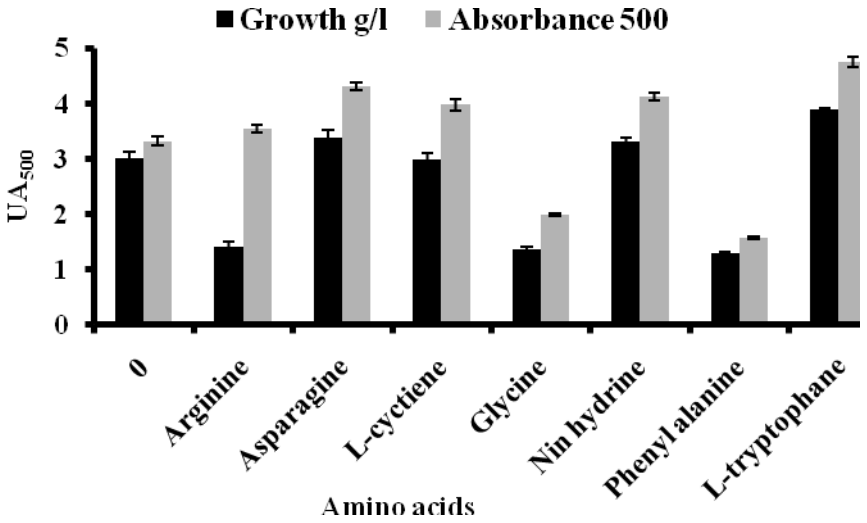

Fig. 9 – Effect of different amino acids on red pigment production by *M. purpureus*.

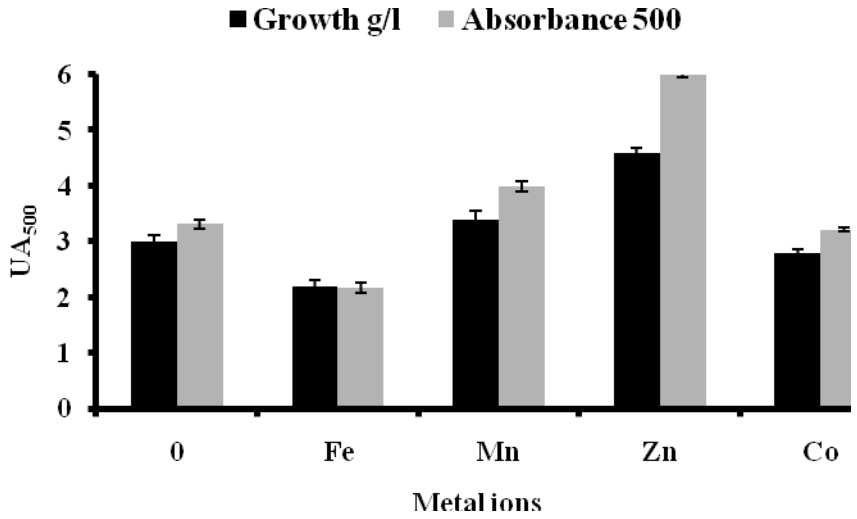

Fig 10 – Effect of different metal ions on red pigment production by *M. purpureus*.

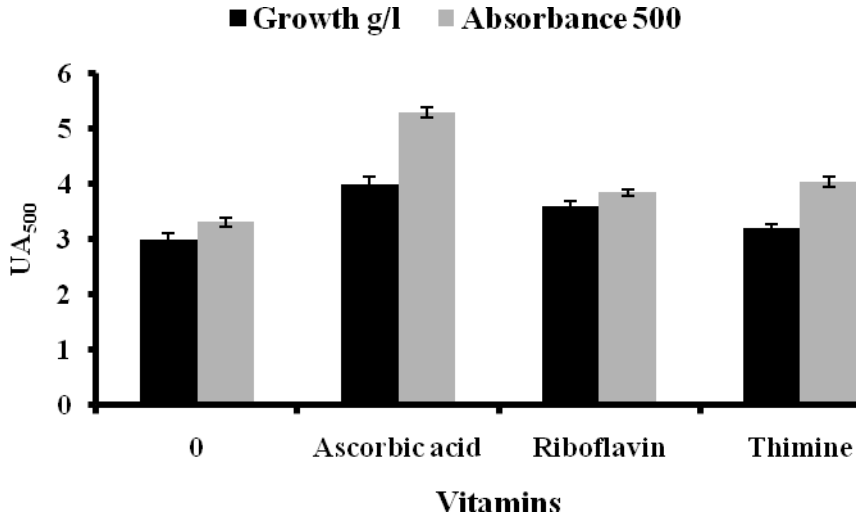

Fig 11 – Effect of different vitamins on red pigment production by *M. purpureus*.

Pigment optical density in different solutions

Pigment optical density is one of the most important factors that influence its application highly. Date given in figs 12–15 showed that the optical density values of red yeast pigment in different solutions at wavelength from 380 to 700 nm was ranged from 0.05±0.001 in ethanol solution at 700 nm to 4.78±0.03 in Citric acid 1% solution. Maximum optical density values of red yeast pigment recorded at wavelength 500 nm followed by 460 nm. However the minimum optical density values showed at wavelength 700 nm followed by 660 nm.

Citric acid solutions (0.3, 0.5 and 1%) have the highest optical density values of red yeast pigment at 500 nm 4.31, 4.72 and 4.78, respectively followed by vitamin C 300 ppm solution (3.16) and salicylic1.5 mM (3.00). However water solution at 500 nm recorded the lowest optical density values of red yeast pigment (2.16).In the same line citric acid solutions (0.3, 0.5 and 1%) have the highest optical density values of red yeast pigment at 460 nm (4.44,4.43 and 4.44, respectively) followed by vitamin C solutions (100,300 and 500 ppm) giving 3.15,3.29 and 3.30, respectively. Water solution at 460 nm recorded the lowest optical density values of red yeast pigment (2.83±0.04).

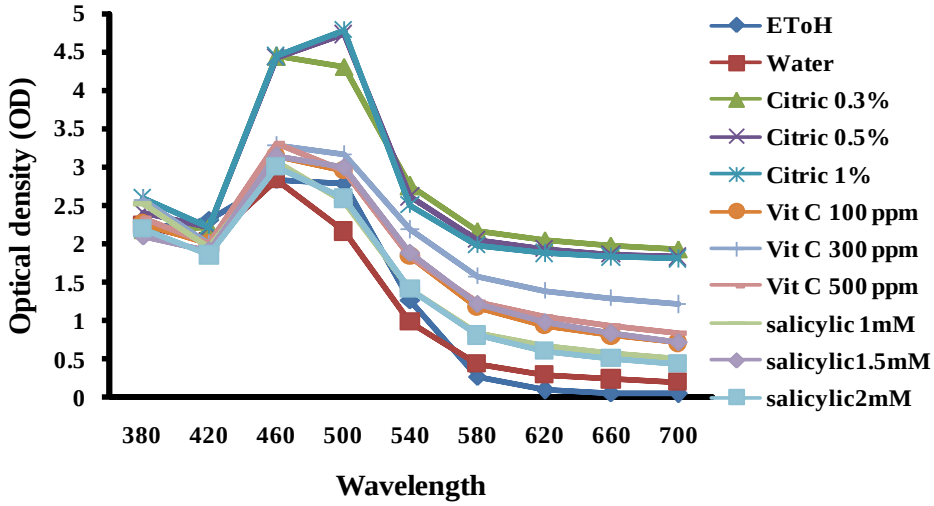

Fig. 12 – Optical density of red yeast pigment in different solutions at wavelength from 380 to 700 nm.

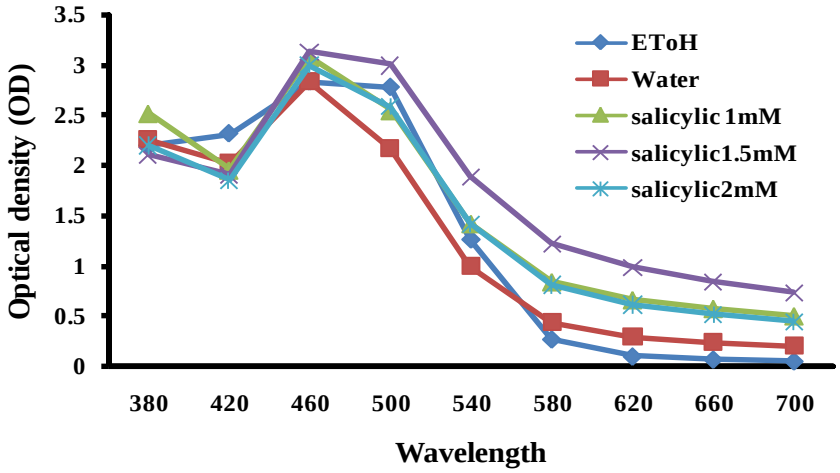

Fig. 13 – Optical density of red yeast pigment in ethanol, water and salicylic acid (1, 1.5 and 2mM) solutions at wavelength from 380 to 700 nm.

Discussion

Spectral analysis of pigments produced by *M. purpureus* shows absorbance peaks at 420–430 and the highest peak was at 500nm. In agreement with our results; Yongsmith et al. (1993) proved that the absorption spectra of pigments produced by *Monascus* sp. KB9 on solid rice cultures showed double peaks of absorption at 420 and 500 nm. According to Domı´nguez-Espinosa & Webb (2003) *Monascus purpureus* Went (IMI-210765) gives two absorption pigments peaks 400–410 and 490–500 nm on wheat flour medium.

Optimization of red pigment production

Various factors, including medium composition (carbon and nitrogen sources), pH, temperature, incubation period and type of fermentation were employed to screen red pigment production by *M. purpureus*. The literature on these factors shows a variety of approaches; Juzlova´ et al (1996) found that biomass measurement is significant for production of red pigments as it is

linked to growth. *Monascus* first utilize carbon and nitrogen sources for primary metabolites synthesis, and then pigments produced at the end of fungal growth as secondary metabolites. Carbon and nitrogen sources considered as the most effected parameters on both growth and metabolic production (Easterling et al. 2009).

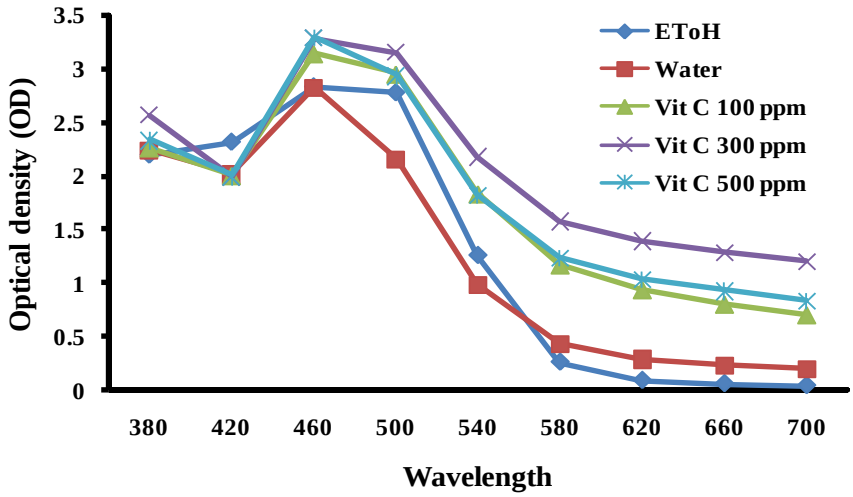

Fig. 14 – Optical density of red yeast pigment in ethanol, water and ascorbic acid (100, 300 and 500 ppm) solutions at wavelength from 380 to 700 nm.

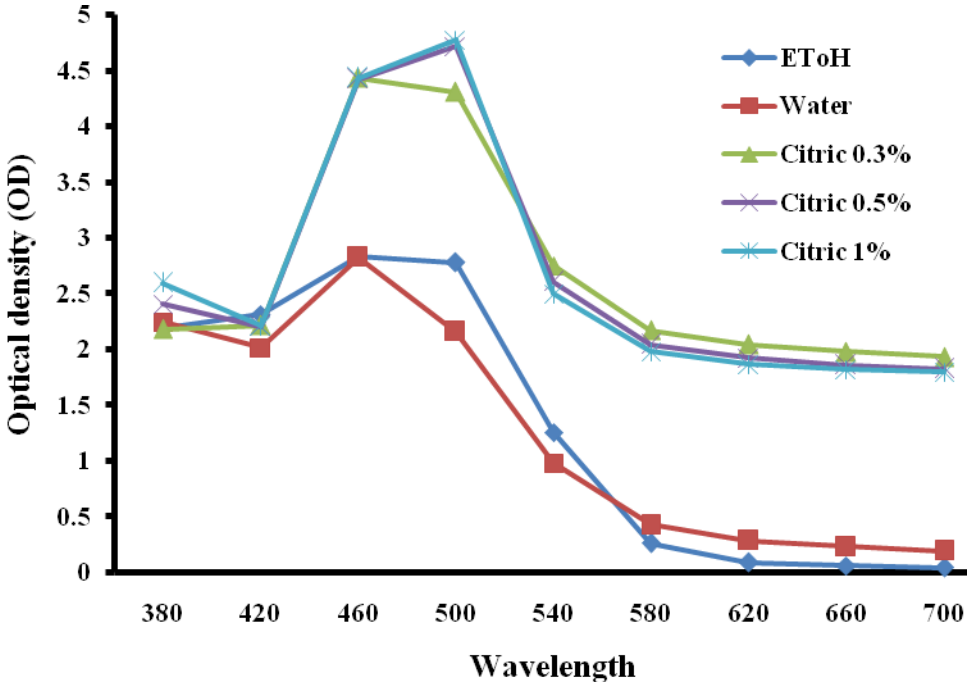

Fig. 15 – Optical density of red yeast pigment in ethanol, water and citric acid (0.3, 0.5 and 1%) solutions at wavelength from 380 to 700 nm.

Among the carbon sources tested, corn starch gave a maximum red pigment absorbance followed by soluble starch. According to Juzlova´ et al (1996) *Monascus* pigments produced on different carbon substrates e.g. glucose, cellobiose, maltose and fructose. However, glucose was the most used and studied substrate in the production of pigments (Dufosse´ et al. 2005, Mukherjee & Singh 2011). Nimnoi & Lumyong (2011) tested *M. purpureus* pigment production on agricultural

products, found the best production taken on corn as substrate. Yeast extract was excellent nitrogen source for both biomass and red pigment. Weinberg (1989) found that pH changed during cultivation depends on the nitrogen source rather than carbon source. Lin & Demain (1994) suggested that organic nitrogen sources are better in metabolic production than inorganic nitrogen compounds. Addition of different nitrogen source like yeast extract, soybean and chitin on the production medium repressed the sexual cycle, increased biomass production and enhanced the production of pigment (Juzlova et al. 1996). Moreover, Miyake et al (2008) stated that the type of medium constituents (particularly nitrogen sources) significantly influences on the pattern and quality of the produced pigments.

Monascus cultures incubated under static were low in both growth and production. Turner (1971) indicated that *Monascus* pigments are polyketides and there for oxygen is very important substrate for their biosynthesis. Cultivation under shaking at optimal agitation speed improves both mycelia and pigment yield (Su & Huang 1980). Vendruscolo et al. (2010) stated that the benefits of using submerged technique in *Monascus* pigment production are to minimize the problems of scale, space and process control. *Monascus* strain was able to grow in the pH range from 2 to 10 achieve maximum production and maximum cell mass at pH 6. Wong et al. (1981) and Lee et al. (2001) have demonstrated that high quality pigments produced by *Monascus* sp. obtain in acidic and towards alkaline pH and higher than pH 7 stimulate the conversion of orange pigment to red pigment. However, *Monascus* growth and production found to be better at pH low than 7. *Monascus* red pigment obtained higher in medium with pH 6.5; whereas orange pigments with pH 2.5. Orozco & Kilikian (2008) declared that initial pH plays a significant role in activating the enzymes involved in pigment production and by *M. purpureus* CCT3802.

Red pigment production was proportional to growth rate in the stationary phase and reached its maximum value at 30°C.Temperature plays a pivotal role in cell metabolism and consequently influencing the pigment production. In agreement with our results, optimum growing and pigment production temperature of *Monascus* sp. stated by other researchers were 31 °C (Zhou et al. 2009), 32 °C (Mohamed et al. 2009) and 25–30 °C (Hu et al. 2012). Maximum pigment production and maximum dry cell mass recorded after 12 days. In the stationary phase apart of the produced metabolites degraded commonly occurs in fungal cultures (Johns et al. 1982). Biomass measurement considered significant as the production of red pigments linked to the growth (Juzlova´ et al. 1996).

Factors affecting on red pigment production

The additions of different salt concentrations increase both growth and red pigment production in low concentration only; the fungus could tolerate high levels of ionic strength until 6% NaCl without red pigment production. The optimum ionic strength for red pigment production by *M. purpureus* was 1% NaCl. These results may be due to the increasing of electrolyte concentrations in saline environments tending to inhibit metabolic functions of the cells (Adler et al. 1982).

The highest growth and red pigment production obtained from medium containing amino acid was L- tryptophan and maximum dry cell mass. Juzlova et al. (1996) described that amino acid addition in growth medium of *Monascus* leads to increase red pigment production than other pigment. Metal ions additions showed a great effect on *Monascus* both growth and red pigment production. Weinberg (1989) stated that trace metals have stimulatory effects on secondary metabolism. Metal ions, Fe^{2+}, Zn^{2+} and Mg^{2+} showed strong effect on both growth and pigments production of *Monascus* sp. (Lin & Demain 1993, Lee et al. 2001).The highest red pigment production obtained after Zn^{2+} addition. Zn^{2+} considered being involved in inter-relationships between carbohydrate and nitrogen of *M. purpureus* (Mchan & Johnson 1970).

Moreover, Combination of amino acids and metal ion such as Zn^{2+} improve the transferring coefficient of carbon source like starch during *Monascus* pigment production (Johnson & Mchan 1975). Adding Zn^{2+} with nitrogen source to pigment production medium increased the production by *Monascus* sp. which indicates that Zn^{2+} was probably work as a co-factor for a metal dependent

enzymes involved in the metabolism (Mchan & Johnson 1979). Bau & Wong (1979) reported that pigment production by *M. purpureus* N11S was promoted by 5×10^{-5} M of zinc. Fortification the medium with vitamins also produced great stimulatory effect by all tested vitamins. The highest growth and red pigment production obtained by adding ascorbic acid (vitamin C) to medium. Han & Mudgett (1992) reported that increasing in growth associated with increasing the production of pigments.

Pigment optical density in different solutions

Pigment optical density is one of the most important factors that influence its application highly. Microbial pigment considered as an important technological tool by food processors needed to apply pigments as food colorants (Jespersen et al. 2005). Maximum optical density values of red yeast pigment recorded at wavelength 500 nm followed by 460 nm. However the minimum optical density values showed at wavelength 700 nm followed by 660 nm. Citric acid solutions have the highest optical density values of red yeast pigment at 500nm followed by vitamin C 300 ppm solution and salicylic1.5 mM. These results are in accordance with many researchers e.g. Lin & Demain 1991, Santerre et al. 1995, Ding et al. 2008; they indicated that the total *Monascus* pigments (MPs) contents, which defined as total optical density values at a given wavelength per milliliter or gram might be measured at 500 nm, 505 nm and 480 nm respectively. Velmurugan et al. (2010) reported that maximum red pigment (36.75 OD500/g) was observed when *M. purpureus* was cultured in darkness, the minimum (5.9 OD500/g) in white unscreened light and the maximum intracellular red pigment (18.27OD500/g) was obtained when *M. purpureus* was cultured in darkness, the minimum (8.03 OD500/g) in yellow light. Hajjaj et al. 2012 & Hu et al. 2012 demonstrated that the MPs of different color could be detected at different wavelengths the extracting or extract-liquor of unfermented substrate was always used as the blank.

References

Adler L, Pedersen A, Tunblad JI. 1982 – Polyol accumulation by two filamentous fungi grown at different concentrations of NaCl. Physiologia Plantarum 56, 139–142.

Babitha S, Soccol CR, Pandey A. 2007 – Solid-state fermentation for the production of *Monascus* pigments from jackfruit seed, Bioresource Technology, 98, 1554–1560.

Bau YS, Wong HC. 1979 – Zinc effects on growth pigmentation and antibacterial activity of *Monascus purpureus*. Physiology Plantarum 46, 63-67.

Chen W, He Y, Zhou Y, Shao Y et al. 2015 – Edible filamentous fungi from the species *Monascus*: early traditional fermentations, modern molecular biology, and future genomics. Comprehensive Review in Food Science and Food Safety 14 (5), 555–567.

Ding G, Zhao JX, Zhang W, Yao JC et al. 2008 – National standard GB4926-2008: food additive-red kojic rice (powder) AQSIQ 1–5.

Domı´nguez-Espinosa RM, Webb CRM. 2003 – Submerged fermentation in wheat substrates for production of *Monascus* pigments. World Journal of Microbiology and Biotechnology 19(3), 329–336.

Dufosse´ L, Galaup P, Yaron A, Arad SM et al. 2005 – Microorganisms and microalgae as sources of pigments for food use: a scientific oddity ran industrial reality. Trends on Food Science &Technology 16 (9), 389–406.

Dweck AC. 2002 – Natural ingredients for coloring and styling. International Journal of Cosmetic Science 24, 1-16.

Easterling ER, French WT, Hernandez R, Licha M. 2009 – The effect of glycerol as a sole and secondary substrate on the growth and fatty acid composition of *Rhodotorula glutinis*. Bioresource Technology 100,356–361.

Feng Y, Shao Y, Chen F. 2012 – *Monascus* pigments. Applied Microbiology and Biotechnology 96 (6), 1421–1440.

Hajjaj H, Blanc PJ, Groussac E, Goma G et al. 1999 – Improvement of red pigment/citrinin production ratio as a function of environmental conditions by *Monascus ruber*. Biotechnology and Bioengineering 64, 497–501.

Hajjaj H, Blanc P, Groussac E, Uribelarrea JL et al. 2000 – Kinetic analysis of red pigment and citrinin production by *Monascus ruber* as a function of organic acid accumulation. Enzyme and Microbial Technology 27, 619–625.

Hajjaj H, François JM, Goma G, Blanc PJ. 2012 – Effect of amino acids on red pigments and citrinin production in *Monascus ruber*. Journal of Food Science 77,156–159.

Han O, Mudgett RE 1992 – Effects of oxygen and carbon dioxide partial pressures on *Monascus* growth and pigment production in solid-state fermentations. Biotechnology Progress 8:5–10.

Heber D, Yip I, Ashley JM, Elashoff DA et al. 1999 – Cholesterol-lowering effects of a proprietary Chinese red yeast rice dietary supplement. American Journal of Clinical Nutrition 69:231-236.

Hu ZQ, Zhang XH, Wu ZQ, Qi HS et al. 2012 – Perstraction of intracellular pigments by submerged cultivation of *Monascus* in nonionic surfactant micelle aqueous solution. Applied Microbiology & Biotechnology 94,81–89.

Jespersen L, Stromdahl LD, Olsen K, Skibsted LH. 2005 – Heat and light stability of three natural blue colorants for use in confectionery and beverages. European Food Research and Technology 220, 261-266.

Johns MR, Stuart DM. 1991 – Production of pigments by *Monascus purpureus* in solid culture. Journal of Industrial Microbiology 8, 23–28.

Johns MR, Chong R, Maddox IS. 1982 – Hydrolysis of some natural and synthetic bile acid conjugates by *Cerospora melonis*. Canadian Journal of Microbiology 28, 457–461.

Johnson GT, Mchan F. 1975 – Some effects of zinc on the utilization of carbon sources by *Monascus purpureus*. Mycologia 67, 806–816.

Juzlova´ P, Martinkova´ L, Kren V. 1996 – Secondary metabolites of the fungus *Monascus*: a review. Journal of Industrial Microbiology 16, 163–170.

Kang B, Zhang X, Wu Z, Qi H et al. 2014 – Production of citrinin-free *Monascus* pigments by submerged culture at low pH, Enzyme Microbial Technology 55, 50–57.

Kim HJ, Kim JH, Oh HJ, Shin CS. 2002 – Morphology control of *Monascus* cells and scaleup of pigment fermentation. Process Biochemistry 38, 649–655.

Koehler PE. 2001 – *Monascus purpureus* pigments as yoghurt colorants. Department of Food Science and Technology, University of Georgia, Athens, GA 30602 and W. B. West.

Lee BK, Park NH, Piao HY, Chung WZ. 2001 – Production of red pigments by *Monascus purpureus* in submerged culture. Biotechnology and Bioprocess Engineering 6, 341–346.

Lin TF, Demain AL. 1991 – Effect of nutrition of *Monascus* sp. on formation of red pigments. Applied Microbiology & Biotechnology 36(1), 70–75.

Lin TF, Demain AL. 1993 – Resting cell studies on formation of water-soluble red pigments by *Monascus* sp. Journal of Industrial Microbiology 12,361–367.

Lin TF, Demain AL. 1994 – Leucine interference in the production of water-soluble red *Monascus* pigements. Archive of Microbiology 162,114–119.

Mapari SAS, Nielsen KF, Larssen TO. 2005 – Exploring fungal biodiversity for the production of water-soluble pigments as potential natural food colorants. Current Opinion in Biotechnology 16, 231–238.

Mchan F, Johnson GT. 1970 – Zinc and amino acids: important components of a medium promoting growth of *Monascus purpureus*. Mycologia 62,1018–1031.

Mchan F, Johnson GT. 1979 – Some effects of zinc on the utilization of nitrogen sources by *Monascus purpureus*. Mycologia 71,160–169.

Méndez A, Pérez C, Montañéz JC, Martínez G et al. 2011 – Red pigment production by *Penicillium purpurogenum* GH2 is influenced by pH and temperature. Journal of Zhejiang University Science B 12, 961-968.

Miyake T, Isato K, Nobuyuki N, Sammoto H. 2008 – Analysis of pigment compositions in various *Monascus* cultures. Food Science & Technology Research 14, 194–197.

Mohamed MS, Mohamad R, Manan MA, Ariff AB. 2009 – Enhancement of red pigment production by *Monascus purpureus* FTC 5391 through retrofitting of helical ribbon impeller in stirred-tank fermenter. Food Bioprocess Technology 5,80–91.

Mukherjee G, Singh SK. 2011 – Purification and characterization of a new red pigment from *Monascus purpureus* in submerged fermentation. Process Biochemistry 46,188–192.

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.

Ning ZQ, Cui H, Xu Y, Huang ZB et al. 2017 – Deleting the citrinin biosynthesis-related gene, ctnE, to greatly reduce citrinin production in *Monascus aurantiacus* Li AS3.4384. International Journal of Food Microbiology 241, 325–330.

Orozco SFB, Kilikian BV. 2008 – Effect of pH on citrinin and red pigments production by *Monascus purpureus* CCT3802. World Journal of Microbiology and Biotechnology 24, 263–268.

Pitt JI, Hocking AD. 2009 – Fungi and food spoilage. 3ed (p. 535). London: Springer.

Santerre AL, Queinnec I, Blanc PJ. 1995 – A fed batch strategy for optimal red pigment. Bioprocess Engineering 13,245–250

Su YC, Huang H. 1980 – Fermentative production of anka-pigments (*Monascus*-pigments). Proceedings of the National Science Council, Republic of china 4, 201–215.

Turner WB. 1971 – Fungal Metabolites. 2nd ed., pp. 136–139. Academic Press, London, UK.

Velmurugan P, Lee YH, Venil CK, Lakshmanaperumalsamy P et al. 2010 – Effect of light on growth, intracellular and extracellular pigment production by five pigment-producing filamentous fungi in synthetic medium. Journal of Bioscience and Bioengineering 109,346–350.

Vendruscolo F, Meinicke Buhler RM, Cesar de Carvalho J et al. 2016 – *Monascus*: a reality on the production and application of microbial pigments. Applied Biochemistry and Biotechnology 178 (2), 211–223.

Vendruscolo F, Pitol LO, Carciofi BAM, Moritz DE et al. 2010 – Construction and application avane systemina rotational rheometer for determination of the rheological properties of Monascus ruber CCT 3802. Journal of Biorheology 2, 29–35.

Wang JJ, Lee CL, Pan TM. 2003 – Improvement of monacolin K, γ-aminobutyric acid and citrinin production ratio as a function of environmental conditions of *Monascus purpureus* NTU 601. Journal of Industrial Microbiology & Biotechnology 30, 669–676.

Wang J, Lu Z, Chi J. 1997 – Multicenter clinical trial of the serum lipid-lowering effects of a *Monascus purpureus* (Red Yeast) rice preparation from traditional Chinese medicine. Current Therapeutic Research 58(12), 964-978.

Weinberg ED. 1989 – Roles of micronutrients in secondary metabolism of Actinomycetes. pp. 239–261. In: Shapiro, S. (ed.). Regulation of Secondary Metabolism in Actinomycetes. CRC Press, Boca Raton, USA.

Wong HC, Lin YC, Koehler PE. 1981 – Regulation of growth and pigmentation of *Monascus purpureus* by carbon and nitrogen concentrations. Mycologia 73, 649–654.

Yongsmith B, Tabloka W, Yongmanitchai W, Bavavoda R. 1993 – Culture conditions for yellow pigments formation by *Monascus* sp. KB10 grown on *cassava* medium. World Journal of Microbiology and Biotechnology 9, 85–90.

Zhou B, Wang J, Pu Y, Zhu M et al. 2009 – Optimization of culture medium for yellow pigments production with *Monascus* anka mutant using response surface methodology. European Food Research and Technology 228, 895–901.