



Optimization of Production of *Monascus Ruber* Pigments on Broth Medium and Their Application in Flavored Yogurts

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THE AIM of the present study was to optimize production conditions of yellow, orange and red pigments from the fungus *Monascus ruber* Went AUMC 5705 culture through submerged fermentation (SMF), then separation and application of the produced pigments individually in flavored yogurts. Maximum pigments productivity was achieved at incubation temperature of 30°, inoculum size by 4% (V/V) with (36×10^4 spores /ml of inoculum), pH of 6.5 and incubation period of 11 days. The favorable carbon and nitrogen sources were Dextrin and monosodium glutamate at 4% (W/V) and 1.5% (W/V) concentrations respectively. The present study has also revealed that the yogurt preparations supplemented with these pigments received highly acceptability. To the best of our knowledge, this is the first report on application of yellow, orange and red *Monascus* pigments individually in flavored yogurts. It is fervently hoped that in the future, these fungal pigments could receive greater attention in the field of food industries.

Keywords: *Monascus ruber* Went AUMC 5705, Pigments, Flavored yogurts, SMF, Food.

Introduction

Researches on *Monascus* pigments (MPs) include methods used for the isolation and identification of new pigments and application in varying objects especially in food industries has been very rapidly progressed. Application of the natural MPs to some foodstuffs with the aim to improve their quality, durability, storage, nutritious value and visual attraction also promotes positive health aspects of these foods (Mafa et al., 2010).

Monascus represents a genus of small, filamentous, saprophytic fungi, belonging to Eumycophyta, Ascomycotina, Plectomycetes, Eurotiales, Monascaceae (Yang et al., 2016). *Monascus* strains are a source of various secondary bioactive metabolites including pigment (Patakova et al., 2015). *Monascus* pigments (MPs) contain six main compositions, namely, two yellow

pigments (ankaflavin and monascin), two orange pigments (monascorubin and rubropunctatin) and two red pigments (monascorubramine and rubropunctamine) (Patakova, 2013).

MPs have long been used as natural food colorants, in South China. They are also used for production of red yeast rice, which is rice fermented by a red *Monascus* species which can be used to dye yoghurt, bacon, sausage, and for the preservative of fruits, vegetables, and fish products (Singgih and Julianti, 2015).

MPs have been used as a functional food additive for several thousands of years. Modern research found that MPs have many applications such as coloring agents in foodstuffs and texture industries, pharmacology, medicine and cosmetics (Mostafa and Abbady, 2014). Moreover, MPs possessed a range of biological activities, such

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as antimutagenic and anticancer properties (Hsu et al., 2011), antidiabetic effects (Shi and Pan, 2011 and Lee et al., 2011) antimicrobial activities (Martlnková et al., 1995; Kim et al., 2006 and Vedruscolo et al., 2015), potential anti-obesity characteristics (Feng et al., 2012), and antioxidant (Tseng et al., 2006; Yang et al., 2006 and Pyo & Lee, 2007).

According to literature, substantial effort has been made to evaluate factors that effect of MPs production, such as pH, temperature, incubation period, dissolved oxygen, and nutritional requirements such as carbon and nitrogen sources to cultivate and to use this information to optimize the culture conditions on both submerged and solid state fermentation (Zeng et al., 2018).

The present work, deals with optimization of physical and nutritional conditions in the pigments production from *Monascus ruber* Went AUMC 5705 by using submerged culture, separation of red, orange and yellow *Monascus* pigments from optimized culture and application of the separated pigments individually in flavored yogurt were also main aims.

Materials and Methods

Culture

Non mycotoxin producer culture of *Monascus ruber* Went AUMC 5705 obtained from Assuit University Mycological center (AUMC), Assuit, Egypt, was used in the present study. The fungal culture was maintained on Yeast Extract-Peptone-Dextrose agar (YEPD) medium, at 4°C and subculture spored every three weeks, as described by Verma et al. (2000).

Inoculum preparation

M. ruber Went AUMC 5705 was grown on YEPD slant agar in the dark at 30°C under static conditions. To fully sporulated (6-8 days old) agar slope culture, 10ml of sterile distilled water was added and the spores were scraped under strict aseptic conditions. The spore suspension obtained was used as inoculum (approximately 36×10^4 spores per ml) as described by Babitha et al. (2007b)

Fermentation medium

The fermentation medium consisted of glucose, 20 g; monosodium glutamate, 5g; K_2HPO_4 , 5g, KH_2PO_4 , 5g, $CaCl_2$, 0.1g, $MgSO_4 \cdot 7H_2O$ 0.5g, $FeSO_4 \cdot 7H_2O$ 0.01g, $ZnSO_4 \cdot 7H_2O$ 0.01g, $MnSO_4 \cdot H_2O$ 0.03 g and 1000 ml of distilled water. One hundred milliliters of broth of this fermentation medium were dispensed into a 500 ml Erlenmeyer flask, adjusted to pH 6.5 and autoclaved at 121°C for 15 min. After cooling, the medium was inoculated with 1 ml spore suspension (36×10^4 spores / ml)

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and incubated at 30 °C for 7 days under static condition (Ahmad et al., 2009).

Determination of optimized conditions for pigments production

For optimization of fermentation temperature the pigments broth production media which inoculated with the selected organism were incubated at temperatures viz. 20, 25, 30, 35 and 40°C ($\pm 1^\circ C$) for one week. To check the effect of inoculum size on the yield of red, orange and yellow pigments fungus strain was inoculated in the pigment broth production medium with different spore suspension concentrations (1.0%, 2.0%, 3.0%, 4% and 5% V/V) and incubated for one week at the optimum temperature known from the previous experiment. For optimization of the pH, fungus strain was inoculated at the optimum inoculum size in broth media adjusted to different pH values (viz. 4.5, 5.5, 6.5, 7.5 and 8.5) and incubated at the optimum temperature for one week.

For optimization of incubation time the organism was incubated for different time interval viz. 5, 7, 9, 11, 13, 15, 17 and 19 days at the optimum conditions obtained from the previous experiments. Experiments were also performed to evaluate the effect of addition of different carbon sources such as glucose, sucrose, maltose, lactose, dextrin and soluble starch at 1.0% concentration of each. For optimization of incubation time the organism was incubated for different time interval viz. 5, 7, 9, 11, 13, 15, 17 and 19 days at the optimum conditions obtained from the previous experiments. Experiments were also performed to evaluate the effect of addition of different carbon sources such as glucose, sucrose, maltose, lactose, dextrin and soluble starch at 1.0% concentration of each.

Experiments were also performed to evaluate the influence of the addition of different organic and inorganic nitrogen sources such as monosodium glutamate, peptone, yeast extract, beef extract, ammonium nitrate, ammonium sulfate, ammonium chloride, potassium nitrate and urea at 1.0% concentration of each. The optimum concentration of the selected nitrogen source (monosodium glutamate) which stimulates pigment production was estimated by adding it at different concentrations (1.0, 1.5, 2.0, 2.5 and 3.0%) to the production medium. All the experiments were carried out in triplicates and mean yield was calculated for each observation.

Pigment extraction and quantification

At the end of the incubation period of each experiment, the contents of each flask were filtered using Whatman No. 1 filter paper and washed with distilled water. The washed mycelia

were kept aside for measuring the intracellular pigment. The filtrate was measured by a UV visible spectrophotometer (Abilene 9400 – SCHOTT Instruments, EU) at 400, 470, and 500 nm for yellow, orange, and red pigments, respectively (Carels and Shepherd, 1977; Lin et al., 1992 and Orozco and Kilikian, 2008). If necessary, the filtrate was further diluted with distilled water to ensure that the absorbance reading was in the range between (0.3~0.7). The un-inoculated medium was used as blank.

For measuring the intracellular pigment, the freshly harvested washed mycelia (0.1g) were transferred to a 50 mL Erlenmeyer flask and 10 mL of ethanol 95% was added. The suspension was allowed to stand at 30°C for 12 hr (Lin & Demain, 1991 and Tseng et al., 2000). The supernatant was then recovered by centrifugation (using Himac CR 22GII, Hitachi Koki Company Limited, Japan) at 10 000 g, for 10 min. The absorbance of the clear solution was measured at 500, 470, and 400 nm using a UV visible spectrophotometer to determine red, orange, and yellow pigments, respectively (Chen & Johns, 1993; Lin & Iizuka, 1982 and Lin & Demain, 1991). If necessary, the supernatant was diluted with ethanol 95% to ensure that the absorbance reading was in the range between (0.3~0.7).

Extra and Intracellular Pigments yield in absorbance unit (AU) / ml were calculated by using following formula (Mekhael and Yousif, 2009):

$$AU = AU_{\text{Total extra}} + AU_{\text{Total intra}}$$

$$\text{Which: } AU_{\text{Total extra}} = AU_{\text{extra}} \times df$$

$$AU_{\text{Total intra}} = (AU_{\text{intra}} \times df) / (\text{weight of sample (g)})$$

$$\times \text{"total weight of biomass"}(\text{g})$$

Where, df is the dilution factor and AU is the total absorbance unit of extra and intracellular pigments.

Separation of pigments

The red, orange and yellow pigments were separated and purified individually from submerged culture, according to the procedures that described by Abdel-Raheem (2016). Separated water soluble red, orange and yellow pigments were used individually in the preparation of red, orange and yellow flavored yogurts.

Application of the separated pigments for the coloring of flavored yoghurts

Yoghurt was manufactured from Buffalo milk by the traditional methods and Red, orange and yellow pigments were added to yoghurts flavored with strawberry, orange and banana, respectively. Few drops of each concentrated pigment extract were added to give a color degree similar to that of the commercial corresponding products. All products were kept at 4°C for 3 days and then subjected to sensory evaluation. Attributes of color, taste, odor, texture and overall acceptability were tested using slandered score card.

Sensory evaluation

Sensory evaluation was carried out by ten panelists. The panelists were asked to evaluate taste, color, texture, odor and overall acceptability for prepared Red, orange and yellow flavored yoghurts according to the method described by Reitmeier and Nonnecke (1991).

Results and Discussion

Production of *Monascus ruber* pigments

As shown in Fig. 1 the use of submerged culture technique resulted in successful growth of the fungus *Monascus ruber* Went AUMC 5705 accompanied with successful production of fungal biomass and concentrated fungal pigments.

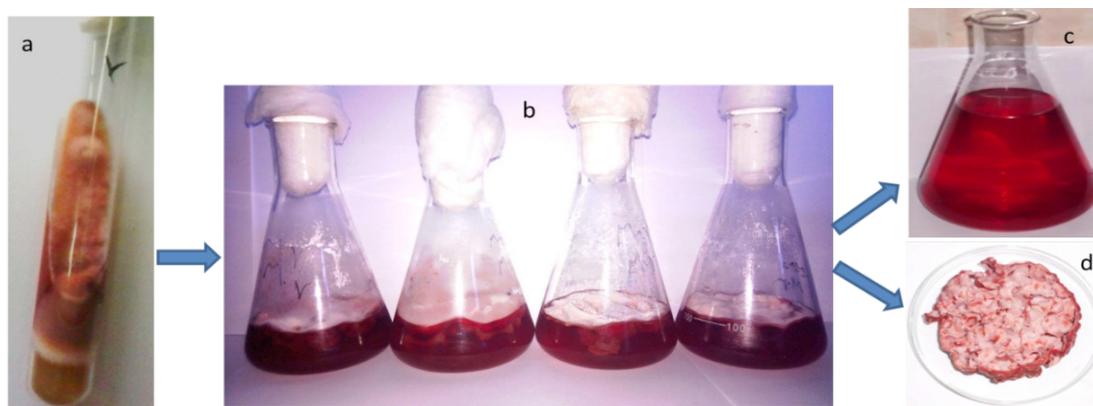


Fig. 1. Production of *Monascus ruber* Went AUMC 5705 pigments by submerged culture technique: (a) fungal slant, (b) Fermented broth culture, (c) Filtrated culture and (d) Separated biomass.

Optimum incubation temperature

The results illustrated in Fig. 2 show that production of red, orange and yellow pigments were increased gradually with increasing the incubation temperature from 20 to 30 °C and then turned to gradually decrease with increasing the incubation temperature up to 30 ~ 40 °C. So 30 °C was considered as optimal temperature, which gave maximal red, orange and yellow pigments productivity from *Monascus ruber* Went AUMC 5705.

This finding agrees with the reports of Rashmi and Padmavathi (2013 and 2012); Padmavathi and Prabhudessai (2013); Sunet al. (2004); Park et al. (2005); Jeon et al. (2006) and Juzlova et al. (1996). These observations were closely consistent with previous investigators (Sumathy et al., 2007; Lin, 1991; Perth, 1985). Dikshit and Tallapragada (2011) also found that, the pigment amount decreases at 37 °C and a drastic reduction in red pigment beyond 40 °C was reported by Babitha (2007a). Hence, temperature plays a pivotal role in cell metabolism, thus influencing pigment yield. On the other hand, Minoru et al., (1975) found that, the optimal temperature for red pigment production by *Monascus* sp. No.2 (AJ7744) fungus strain was 25 °C, and at the temperature lower than 25 °C and higher than 28 °C, markedly decrease in pigment production was observed.

Optimum inoculum size

The results in Fig. 3 show that, Production of red, orange and yellow pigments were increased as the inoculum size increased from up

to 4%. Therefore, 4% was selected as the best economical inoculum size for the tested fungus strain. Ji et al. (2012) and Babitha et al. (2007a) mentioned that high inoculum sizes may increase biomass, but decrease pigment production, the inhibition of pigment formation was due to the lack of some substances in culture medium which were consumed by the high bacterial biomass. Santos-Ebinuma et al. (2013b) found that, the highest production of yellow, orange, and red colorants was achieved with 4×10^8 (spores/ml)/100 ml medium while using submerged culture fermentation for production of pigments by *Penicillium purpurogenum* DPUA1275.

It is advantageous to achieve high levels of the product by small inoculum size 14×10^5 spores/100 ml broth medium as obtained from our results. This may reflect a high potentiality of the studied fungal strain.

Optimum initial pH value

It is well known that the initial pH of the medium, in which the organism is grown, has a great influence on enzymes and pigments production. Therefore, an experiment was designed to investigate the effect of different pH values on production of intra and extra cellular red, orange and yellow pigments by *Monascus ruber* Went AUMC 5705. The results illustrated in Fig. 4 indicated that the optimum pH for the production of red, orange and yellow pigments was 6.5 for the tested fungus strain. The change of the pH value above or below 6.5 resulted in decreasing the production of intra and extracellular red, orange and yellow pigments.

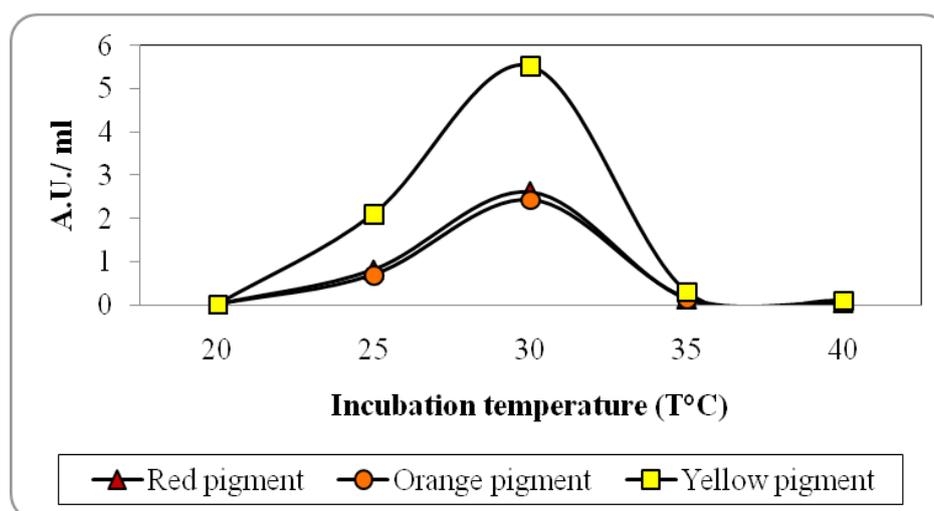


Fig. 2. Effect of incubation temperature on red, orange and yellow pigments production by submerged culture of *M. ruber* Went AUMC 5705.

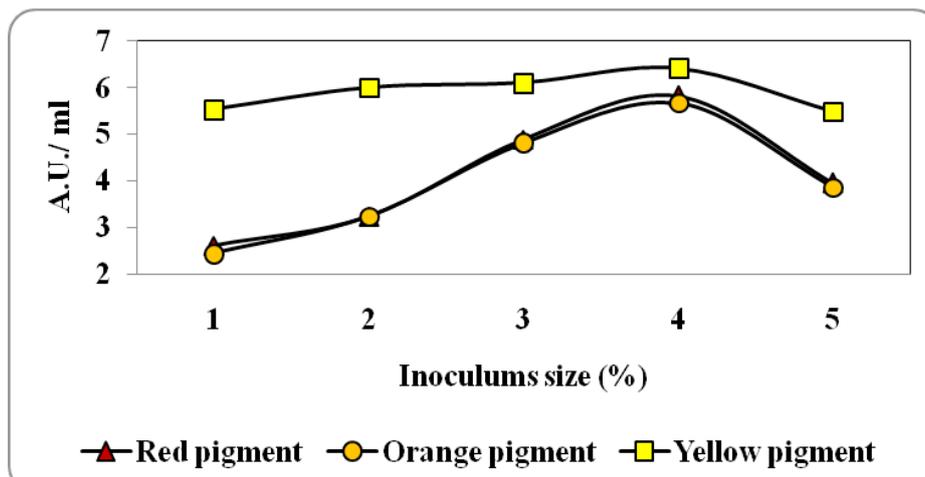


Fig. 3. Effect of inoculum size (%) on red, orange and yellow pigments production by submerged culture of *M. ruber* Went AUMC 5705.

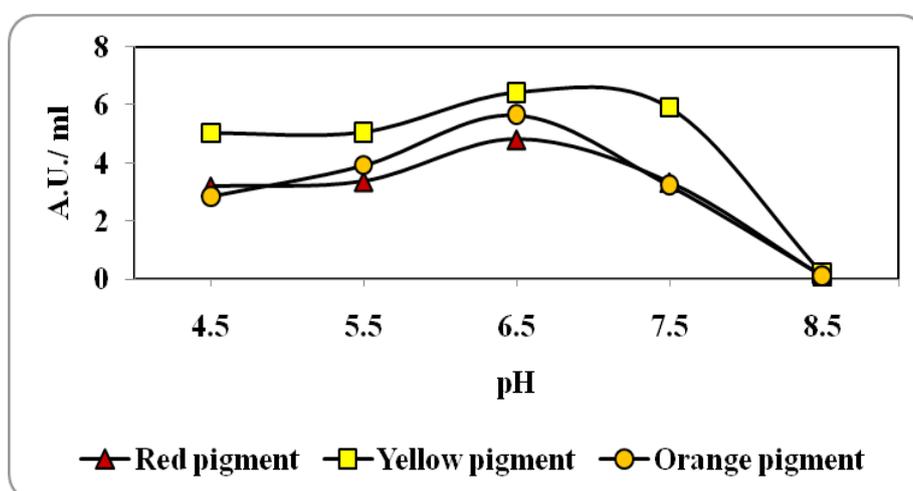


Fig. 4. Effect of initial pH on red, orange and yellow pigments production by submerged culture of *M. ruber* Went AUMC 5705.

These results agree with those obtained by Rashmi and Padmavathi (2013); Jeon et al. (2006); Suh et al. (2004) and Minoru et al. (1975).

However, the present results have rather differed from those described by Park et al. (2005) and Rashmi & Padmavathi (2012) who found that pigment production by *M. purpureus* MTCC410 and *M. purpureus* P-57 were highest in a medium with an initial pH of 5.5 and 5.0, respectively.

Optimum incubation period

In industrial fermentation, it is of great economic importance to harvest the product in short time. Therefore, an experiment was conducted to investigate the rate of the pigment production during different fermentation periods and to determine the suitable time at which the product could be maximized.

Data illustrated by Fig. 5 showed that the rate of pigment production increased gradually as the incubation period increased, reaching the maximum value after 11 days, and then dropped until it reached the minimum value after 19 days for the tested fungal strain.

The present results are rather different from those described by Rashmi and Padmavathi (2013) who reported that maximum pigment production were achieved by *Monascus sanguines* within 16 days, while using MGPB (malt glucosepeptone broth) and PDB (potato dextrose broth) in submerged fermentation. Santos-Ebinuma et al., (2013b) found that, the highest colorants (yellow, orange, and red) production by *Monascus purpureus* MTCC 410 and *Penicillium purpurogenum* DPUA1275 was achieved with

in 14 days and 12 days of incubation time, respectively. On the other hand Chatterjee et al., (2009) reported that, the yield of pigment production reached to its highest level at 10th day while using submerged culture fermentation for production of pigments by *Monascus purpureus* MTCC 1090.

Selection of the most efficient carbon sources

The results given in Fig. 6 indicated that dextrin was the best carbon source for red, orange and yellow pigment production followed by glucose for the studied fungus strain.

These results agree with those obtained by Joshi et al. (2003) who found that, the volumetric pigment formation by *Monascus sp.* is best on starch and dextrin, moderate on glucose and maltose but poor on fructose. They also reported that, maltose and glucose as carbon sources gave very dark liver pigment by *M. Purpureus*, where as sucrose produced a light and uneven red pigment. Also, Lin and Demain (1991) reported that,

glucose and its oligo- and polysaccharides were better carbohydrates than maltose and fructose for both growth and MPs production of *Monascus sp.* TTWMB 6042.

These results agree with those obtained by Lee et al. (1995) who investigated complex carbon sources such as starch for possible use in producing *Monascus* pigments in submerged culture. They found that the viscosity of starch solution was leading to the poor oxygen transfer, consequently, low biomass and pigments production.

Glucose was held by most authors (Chatterjee et al. (2009); Juzlova et al. (1994); Lin and Demain (1991); Panitz et al. (1991); Broder and Koehler (1980) and Yoshimura et al. (1975)) to be a superior substrate for pigment production by *Monascus* species. However, others (Miyake et al., 1982) found glucose to be less suitable for this purpose. This may be caused by strain differences or by other differences in medium composition (glucose concentration, type of nitrogen source).

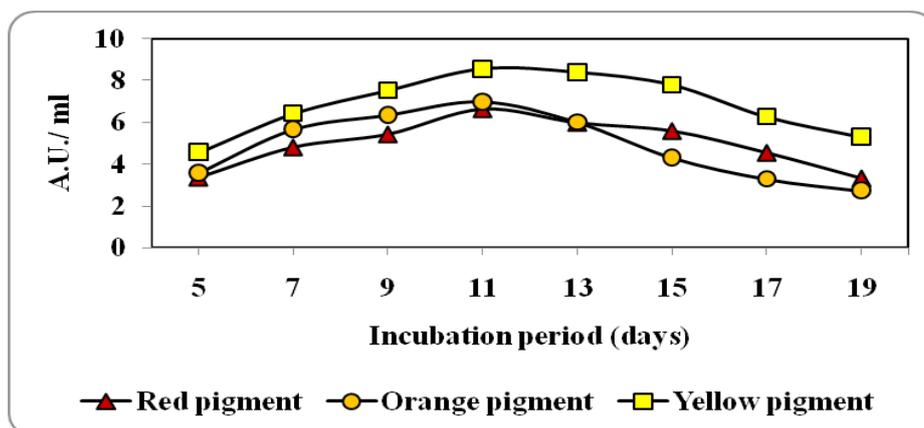


Fig. 5. Effect of incubation time (days) on red, orange and yellow pigments production by submerged culture of *M. ruber* Went AUMC 5705.

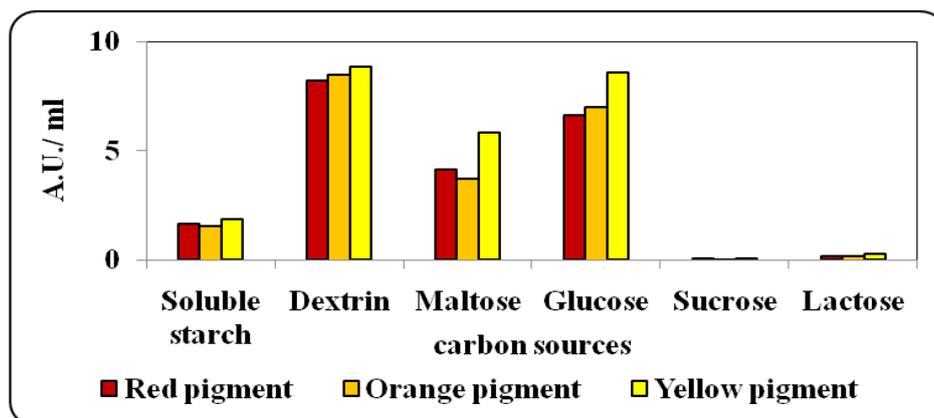


Fig. 6. Effect of carbon sources on red, orange and yellow pigments production by submerged culture of *M. ruber* Went AUMC 5705.

Optimum Dextrin concentrations

From the previous experiment, dextrin secured maximum yield of red, orange and yellow pigments when used as a sole carbon source for the studied fungus strain. Therefore, an experiment was carried out to study the effect of different concentrations of dextrin that could stimulate the highest pigment production. Results recorded in Fig. 7 clearly showed that, the accumulation of red, orange and yellow pigments were increased gradually as the dextrin concentration increased, reaching the maximum level at 4% concentration for the tested fungus strain (data not shown).

Selection of the most efficient nitrogen sources

This experiment was aimed to investigate

the best nitrogen source that could stimulate maximum pigment production by *Monascus ruber* Went AUMC 5705. Data represented by Figure 8 indicated that monosodium glutamate (MSG) was the most favorable nitrogen source for the stimulation of red, orange and yellow pigment production by the studied fungus strain. These results were consistent with the published reports for other *Monascus* strains by Babitha et al. (2007a); Carvalho et al. (2005) and Chatterjee et al. (2009). These workers reported that MSG proved to be the best nitrogen source for producing the red pigment in submerged culture. Also they reported that, the nitrogen sources that contained NH_4^+ proved to be quite unsatisfactory for development of the red pigment.

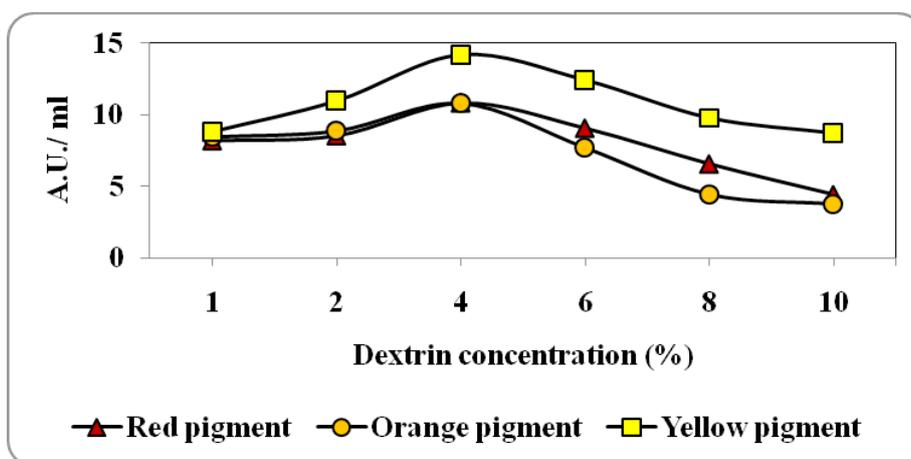


Fig. 7. Effect of Dextrin concentration (%) on red, orange and yellow pigments production by submerged culture of *M. ruber* Went AUMC 5705.

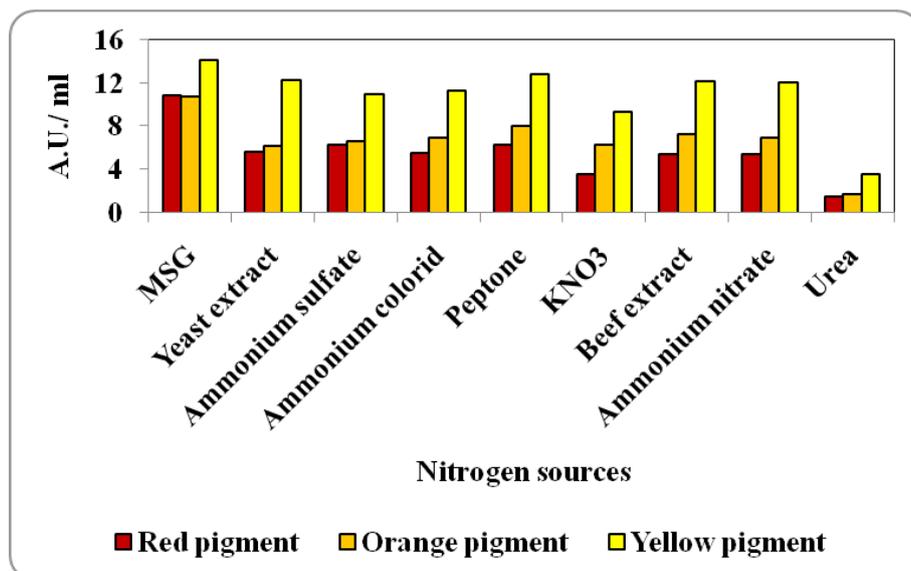


Fig. 8. Effect of nitrogen sources on red, orange and yellow pigments production by submerged culture *M. ruber* Went AUMC 5705.

Chen and Johns (1993) reported that ammonium chloride, peptone also yielded superior growth and pigment amounts by *M. purpureus* UQM 192F (FRR 2190) when compared with sodium nitrate. These results agree with those obtained with Chatterjee et al. (2009) who observed that maximum red pigment yield by *Monascus purpureus* MTCC 1090 was observed when the production broth medium was supplemented with 0.3% monosodium glutamate (MSG). Also, Lee et al. (2001) found that maximum red pigment yield by *Monascus purpureus* (ATCC 16365) was observed when the production broth medium was supplemented with (1.5g/L) 0.15% MSG.

Optimum MSG concentration

The results illustrated in Fig. 9 showed that the optimum concentration of MSG that secured the highest pigment production were 1.5% for the studied fungus strain. These results agree with those obtained by Joshi et al. (2003) who reported that in *Monascus purpureus*, 1.5% MSG medium produced an appealing red colour, whereas other nitrogen sources produced faint or foggy red pigment.

The present results have rather differed from those described by Lee et al., (2001) who found that, maximum red pigment yield by *Monascus purpureus* (ATCC 16365) was observed when the production broth medium was supplemented with (1.5g/L) 0.15% MSG. While, Chatterjee et al., (2009) observed that, maximum red pigment yield by *Monascus purpureus* MTCC 1090 was observed when the production broth medium was supplemented with 0.3% MSG in liquid fermentation.

Qualitative analysis of the mycotoxin, citrinin

Studies were carried out to identify the presence of Citrinin toxin in the submerged fermented culture by *Monascus ruber* Went AUMC 5705 using thin-layer chromatography (TLC). As shown in Fig. 10 TLC analysis showed that no Citrinin was detected in submerged culture extract. Hence the *Monascus ruber* Went AUMC 5705 used in this study was found to be safe for food use.

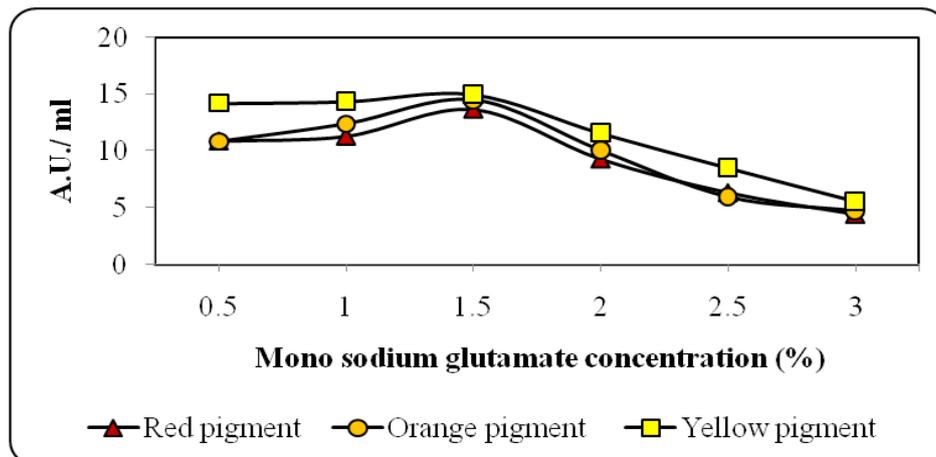


Fig. 9. Effect of MSG concentration (%) on red, orange and yellow pigments production by *M. ruber* Went AUMC 5705.

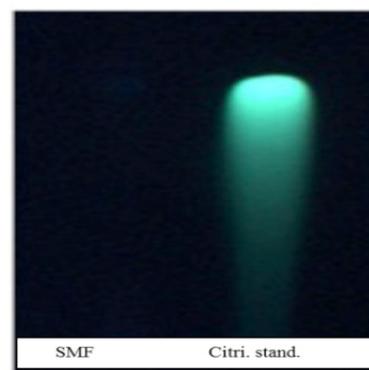


Fig. 10. Detection of Citrinin by TLC under UV detector on extract of submerged culture.

Separation of the produced pigments

The method used for pigments separation successfully resulted in fractionation of the crude *Monascus ruber* pigments into three individual colors, red, orange and yellow ready to use as food colorants (Fig. 11).

Sensory evaluations of the produced pigments as colorant additives for yogurts

Concentrated pigments which extracted and separated from submerged *Monascus ruber* Went AUMC 5705 culture were utilized as colorant additives for yogurts to enhance its appearance and acceptability. The red, orange and yellow flavored yogurts were developed by adding the separated *Monascus ruber* pigments individually. In the current study, *M. ruber* pigments directly mixed with the food products during their preparation (as described by Abdel-Raheem, (2016)) to impart red, orange and yellow pigments individually to these products (Fig. 12) and improving the aesthetic value.

The prepared yogurts using *Monascus ruber* pigments as colorants were sensory evaluated for taste, color, texture, odor and overall acceptability by ten panelists. Data in Table 1 shows the average sensory analysis scorecard and total scores for the separated *M. ruber* pigments as natural colors for the butterscotch flavored yogurt. The overall average score for the red, orange and yellow

flavored yogurts samples were 48.8, 46.5 and 46.4, respectively.

In general, all prepared food product samples colored with *Monascus ruber* Went AUMC 5705 pigments were recorded highly scores in all sensory evaluated tested parameters as shown in Table 1. The average score for taste, color, texture, odor and overall acceptability were between 8.9 to 9.9 scored to be as 'like extremely' for all tested food products samples as described by Wang and Zhao (2008). The result shows that the incorporation of *Monascus ruber* Went AUMC 5705 pigments for coloring prepared food product has proved to be excellent. The pigment distributed evenly in the food product giving a pleasing appearance.

These results were similar to that reported by previous investigators such as Blanc et al., (1995) who reported that, food products gain more intense and stable red color and improved organoleptic characteristics when *Monascus purpureus* pigment was used. Moreover, application of the natural pigment promotes consumers health protection by decreasing the intake of salt and allows manufacturing fully natural food without any synthetic additives (Su et al., 2005). Traditionally, red rice, red wine, sausages, fish sauces, meat products, soybean curd were prepared with these pigments (Anonymous, 1999).

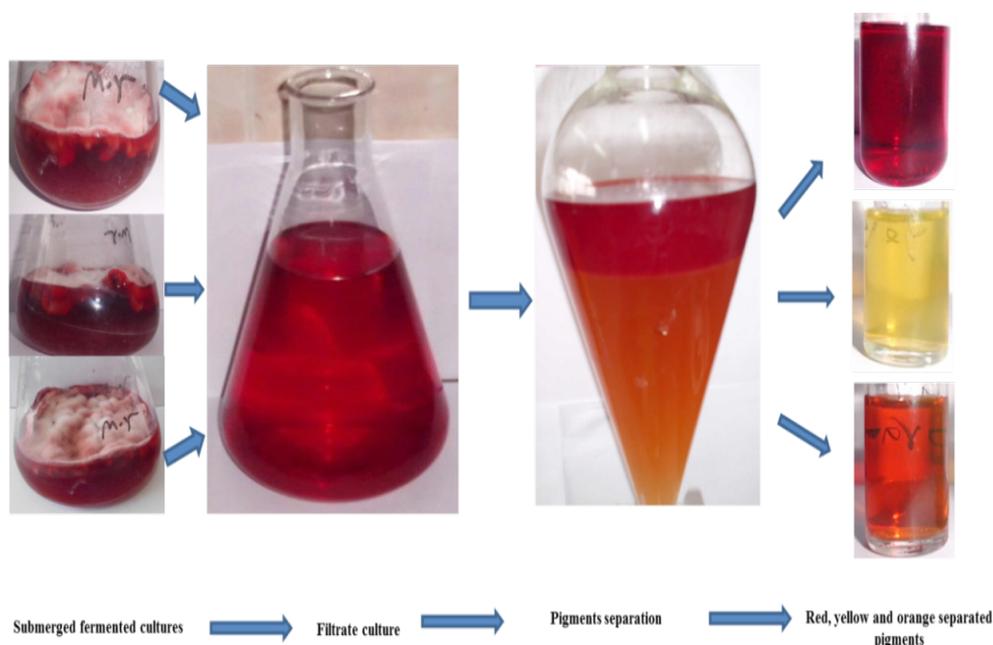


Fig. 11. Separation of *Monascus ruber* Went AUMC 5705 pigments from submerged fermented culture.

TABLE 1. Mean sensory scores of food products samples coloured with *Monascus ruber* Went AUMC 5705 pigments.

Total score (50)	Overall Acceptability (10)	Texture 10	Odour 10	Colour 10	Taste 10	Name of product
48.8	9.9	9.7	9.4	9.9	9.9	Red yogurt
46.5	9.2	9.1	9.5	9.0	9.7	Orange yogurt
46.4	9.1	9.6	9.3	8.9	9.5	Yellow yogurt

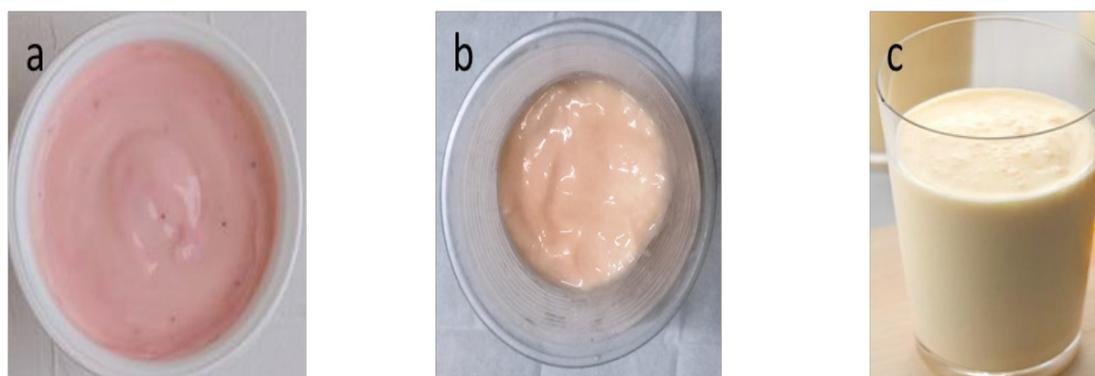


Fig. 12. Red (a), orange (b) and yellow (c) yogurts prepared by separated *Monascus* pigments.

Also, Vidyalakshmi et al. (2009) reported that *Monascus ruber* fermented rice (MFR) used as colorants in the preparation of food products (Kesari), and showed very good color and appearance. They also studied the incorporation of MFR for coloring flavored milk, which showed that pigment distributed evenly giving an appealing color and pleasing appearance with better acceptability.

Also, this finding agrees with the reports of Mamucod and Dizon (2014); Pattanagul et al. (2007), Kraboun et al. (2009) and El-Kholie et al. (2012).

Conclusion

Considering the results of this study, the highest yield of yellow, orange and red pigments were 14.97, 14.46 and 13.65 AU/ml, respectively, revealed that optimization of the environmental conditions has maximized *Monascus ruber* Went AUMC 5705 pigment production in 2.70, 5.94 and 5.23 times more than basal medium (5.53, 2.44 and 2.61 AU / ml) for yellow, orange and red, respectively in SMF. The produced pigments were applied as coloring agents for yogurt only (red, orange and yellow flavored yogurts). On the sensory evaluation, applications of this natural colorant in a preparation of these products were found to be highly acceptable.

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الإنتاج الأمثل لصبغيات *Monascus ruber* على البيئة السائلة وتطبيقه في الزبادي المنكه

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كان الهدف من هذه الدراسة هو تحسين ظروف إنتاج الأصباغ الصفراء والبرتقالية والحمراء من الفطر *Monascus ruber* Went AUMC 5705 من خلال التخمير المغمور (SMF)، ثم فصل وتطبيق الأصباغ المنتجة بشكل فردي في الزبادي المنكه. تم تحقيق أقصى إنتاجية للأصباغ عند درجة حرارة التحضين البالغة 30 درجة مئوية، وحجم اللقاح بنسبة 4 % (V / V) مع (36 × 10⁴ جراثيم / مل من اللقاح)، و pH = 6.5 وفترة حضانة 11 يوماً. كانت مصادر الكربون والنيتروجين المواتية هي الدكستريز والجلوتامات أحادية الصوديوم بنسبة 4% (W / V) و 1.5% (W / V). كشفت الدراسة الحالية أيضاً أن منتج الزبادي المكمل بهذه الأصباغ حصلت على قبول كبير. على حد علمنا، هذا هو التقرير الأول عن تطبيق الأصباغ الصفراء و البرتقالية والحمراء بشكل فردي في الزبادي المنكه. ومن المأمول بشدة أن تحصل هذه الأصباغ الفطرية في المستقبل على اهتمام أكبر في مجال الصناعات الغذائية.