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Stability and Components Identification of *Monascus ruber´s pigments*

Nanis H. Gomah[1]and H. E. F. Abdel-Raheam[2]
[1]Dairy Science Dept. Faculty of Agriculture, Assiut Univ., Egypt.
[2]Food Science and Technology Dept. Faculty of Agric., Bene-swaif Univ., Egypt.

ABSTRACT

The aim of this study is to evaluate the stability of red, orange and yellow pigments produced from *Monascus ruber* Went AUMC5705 under the conditions of both solid state fermentation (SSF) and liquid state fermentation (LSF). Pigments extract of both fermentation techniques were exposed to different processing and storage conditions commonly employed in the production of dairy and food products such as thermal treatments (sterilization, pasteurization and hot air oven), different pH s (pH 4, 7 and10), light exposure (sunlight, fluorescent light and UV light). Storage temperatures (25,4 and -18°C) and the presence of organic acids (ascorbic, citric and lactic acids). In addition, SSF extract was analyzed by HPLC /MS to identify the main components of red, orange and yellow pigments. The results indicated that red, orange and yellow pigments were quite stable when exposed to sterilization, pasteurization, fluorescent light and UV light, refrigeration and freezing storage. It also exhibited high stability in the presence of organic acids and at all the tested pH values, but showed lower stability when subjected to hot air oven (105°C /15 min) sun light and room temperature storage. Pigments derivatives produced by SSF exhibited greater stability when compared with that produced by LSF. These data indicated *that M. ruber´s* pigment produced by SSF has potential to be applied as bio-colorants to all processed dairy and food products.

Keywords: *Monascus ruber* – stability- red pigmint – orange pigmint –yellow pigment.

INTRODUCTION

Natural pigments are receiving growing interest from both food manufacturers and consumers in the continuing replacement of synthetic dyes. However, replacing synthetic dyes with natural colorants offers a challenge because the color and stability of natural pigments are dependent on several factors, including structure and concentration of the pigment, pH, temperature, light intensity, presence of metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products, among others (Tang and Norziah, 2007). Stability evaluations are essential when assessing the commercial /industrial potential of microbial metabolites (Sant'Anna *et al.* 2010). Particularly, knowledge on pigment degradation at different processing conditions is of importance from a technological viewpoint aiming food applications. Although Monascus pigments are usually produced through solid-state processes, submerged cultivations are attractive alternatives that can benefit the production of many secondary metabolites (Panda *et al*. 2008). In submerged cultivations, the control of the process is simpler and easier, resulting in reduction of both cultivation time and production costs (Domínguez-Espinosa and Webb, 2003). On the other side, the use of agro industrial residues is an increasing trend in biotechnological processes since the utilization of these low-cost wastes as substrates for production of microbial metabolites, besides reflection on final product costs, represents a way of waste management (Daroit *et al*. 2007and Silveira *et al.* 2008).

Contradictory reports were showed in the literature concerning the Monascus pigments stability. Baranová *et al*., (2004) reported that, *Monascus* pigments remain very stable under high temperature and thus they can be applied to pork, poultry, fish, and tofu to improve the flavor and preservation of these products. Ochaikul *et al*., (2009) reported that *Monascus* pigments are heat stable and can be autoclaved. These properties, together with a color range from yellow to red, should make them good candidates for food colorants. On the other hand, Mapari, *et al.* (2006) and Jung, *et al.* (2011) reported that monascus pigments are sensitive to pH, UV, and temperature. Since the tendency of degradation to light, temperature, pH, solvents and the concentration of oxygen are concerned, economical users have to monitor and control these parameters properly in order to achieve the successful application. There are large differences in the properties of various pigments derived from Monascus strains. Many of the pigments are not hydro soluble and can discolor under light. However, they are stable in a pH range of 2 - 10 and are stable to autoclaving. Their low water solubility and discoloring under light has limited the use of Monascus pigments in the food industry (Wong and Koehler, 1983; Mortensen, 2006).

Therefore, the present study aimed to assess the stability of red, orange and yellow *M. ruber´s* pigments under the influence of various physical and chemical treatments (heat treatments, light, PH, storage conditions and organic acids preservatives) which usually apply in food industries. Also, the chemical components of these pigments were fractionated and identified to elucidate their chemical profile pattern.

MATERIALS AND METHODS

Fungal strain:

A culture of *Monascus ruber* Went AUMC 5705 obtained from Assuit University Mycological center (AUMC), Assuit, Egypt, was used in the present study. It was maintained on yeast extract- peptone- dextrose (YEPD) agar medium at 4°C and sub cultured periodically every three weeks. The culture was tested for production of the mycotoxin citrinin and proved to be non-producing.

Solid-state fermentation:

Fermentation procedures were carried out according to the optimum conditions adapted in our previous study (Abdel- Raheam, 2016).Twenty grams sample of broken rice was placed in a 250 ml Erlenmeyer flask and its initial moisture content was adjusted to 39% by adding an appropriate amount of

distilled water. Monosodium glutamate (0.4 g) and zinc sulfate (1.0 ml, 0.128 M) solution were added to each flask (Babitha *et al.*, 2006 and Nimnoi and Lumyong, 2009). The flasks were covered with two layers of aluminum foil and autoclaved at 121°C for 15 min. After cooling to room temperature, each flask was inoculated with 2 ml spores suspension (126×10^4spores / ml) and incubated at 30°C for 15 days.

Liquid- state fermentation:

One hundred milliliters of broth fermentation medium (consists of glucose, 20; monosodium glutamate, 5; K_2HPO_4, 5; KH_2PO_4, 5; $CaCl_2$, 0.1; $MgSO_4$. $7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.01; $ZnSO_4.7H_2O$, 0.01 and $MnSO_4.H_2O$, 0.03 g / L) were dispensed into a 250 ml Erlenmeyer flask, adjusted to pH 6.5 and autoclaved at 121°C for 15 min. After cooling, medium was inoculated with 2 ml spores suspension (126×10^4spores / ml) and incubated at 30°C for 13 days (Abdel- Raheam, 2016).

Extraction of pigments from SSF:

At the end of the incubation period, the contents of each flask were dispersed on aluminum foil sheet, dried at room temperature for 24h and ground to a fine powder using an electrical mill. Two grams of fermented rice powder were extracted with 200 ml of 95% ethanol in an Erlenmeyer flask for 30 min using a magnetic stirrer, then centrifuged at 10 000 g for 10 min to remove the suspended solids. The ethanolic pigments extract was sterile filtered (0.45 µm; Minisart, Sartorius stedim Biotech, Germany). Pigments concentration was measured using a double beam spectrophotometer (UV iline 9400 – Schott Instruments, EU) at 400, 470 and 500 nm for yellow, orange and red pigments, respectively. The results were expressed as absorbance unit (AU) per gram of dried solids (Carvalho *et al.*, 2003).

$$\text{Absorbanceunit(AUg}^1\text{)} = \frac{(\text{OD} \times \text{Total volume of Solvent} \times \text{Dilution factor})}{\text{Dried sample (g)}}$$

OD= Optical Density.

Extraction of pigments from LSF:

Culture broth was filtered using Whatman No. 1 filter paper and the mycelium was washed twice with distilled water (2 x 100mL). The pigments extract was made up to 500 ml with distilled water and sterile filtered (0.45 µm; Minisart, Sartorius stedim Biotech, Germany). Pigments concentration was measured as previously mentioned in SSF. 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 uninoculated medium was used as blank. The pigment absorbance was then calculated by multiplying the measured absorbance by the dilution factor (df). The pigments concentration was estimated using the equation:

$$\text{Concentration} = \text{AU} \times \left(\frac{500\ \text{mL}}{100\ \text{mL}}\right) \times \text{df}$$

Where:

AU is the absorbance units and df is the dilution factor (Orozco and Kilikian, 2008).

Pigments stability

The effects of various physical and chemical processing conditions such as thermal treatments, pH values, lightning sources, organic acids preservatives and storage temperature on the stability of red, orange and yellow pigments were determined as the method of Srivastava *et al.* (1999) and Perumal *et al.* (2009) with some modifications as following:

To assess the pigments stability toward storage under ambient temperature (25-35°C), under refrigerating temperature (4±1°C) and under freezing temperature (-18±1°C) for 7 days; nine screw capped sterile Pyrex tubes were individually filled with 10 ml of the pigments extract. Three tubes set were stored under each of the aforementioned conditions. The residual concentration of all pigments was measured after the storage period. Absorbance was recorded for each tube against 95% ethanol blank (Chiu and Poon, 1993).

In another tubes set (10 ml each), pigments extracts were adjusted to different pH values: acidic (pH 4), neutral (pH 7) and alkaline (pH 10) by adding a few drops of either 0.5 M HCl or 0.5 M NaOH (Cesar *et al.*, 2005). All tubes were covered with aluminum foil and kept at room temperature (approximately 25°C) for 8 h. The residual concentration of the three pigments was estimated as previously mentioned.

To investigate the effect of different thermal treatments on the color stability. Another set of tubes containing 10 ml of the pigments extract were subjected separately to hot air oven (105°C / 15 min); boiling water bath (85°C/1 min); and autoclaving (121°C /15 min).

To assess pigments stability in the presence of organic acids, ascorbic acid, citric acid and lactic acids were added separately at 0.1, 0.1 and 0.15% w/v, respectively to pigments solutions in sealed tubes and kept at 25°C for 1hour.

For measuring the color stability against lightning, tubes of pigments solution were exposed to different lighting sources such as sunlight (natural light), fluorescent light (400-500 lux, at 30 cm distance) and UV light (356 nm) for 4 hours of each treatment. Three tubes were directly subjected to each treatment and another three tubes were similarly treated after covering with aluminum foil for a comparison. In all cases, the color intensity of red, orange and yellow pigments were measured at 500, 470 and 400 nm, respectively and expressed as percentage of that recorded for the control samples.

Identification of pigment constituents:

The Identification of *Monascus* pigment constituents was performed by Liquid chromatography–photodiode array detector –mass spectrometry (HPLC–PAD – MS) method as described by Zheng, *et al.*, (2009).

Sample preparation

After solid state fermentation, the obtained fermented rice powder (1.0 g) was extracted with n-hexane, benzene and ethyl alcohol to extract yellow, orange and red pigments, respectively. After 10 min sonication and a subsequent 10 min centrifugation at 12,000 rpm, the supernatant was filtered through a 0.45µm syringe filter and then degassed for the HPLC analysis.

HPLC Analysis

The chromatographic system consisted of a Waters 1525 Binary Pump (Waters, USA), a Waters 2996 Photodiode Array Detector (Waters, USA). The photodiode array detector (PAD) was set at 200–600 nm and the chromatogram speed at 390 nm. A column of Waters Cosmosil C18 (250 mm × 4.6 mm i.d., 5µm) was used and the column temperature was set at 30°C. The injection volume was 20 µL for each experiment.

Liquid chromatography–mass spectrometry

An Agilent 1100 Series Ion Trap LC/MS Systems (Agilent Technologies, USA) was used to determine molecular weight of *Monascus* pigments. The mass spectrometer was equipped with an electrospray ionization (ESI) source. The ESI conditions were: capillary voltage, 3.5 kV; nebulizer pressure, 40 psi; drying gas flow, 10 ml min^{-1}; temperature, 350°C. The mass range was from 100 to 1200 *m/z*.

RESULTS AND DISCUSSION

1 - Effect of thermal treatments on the stability of pigments:

Samples of pigments extract obtained from both SSF and LSF were subjected separately to autoclaving (121°C /15 min), pasteurization (85ºC/1min) and hot air oven (105°C / 15 min) and the remaining intensity of red, orange and yellow colors were recorded in Table (1). The results indicated that the stability of red orange and yellow pigments obtained from SSF not affected by autoclaving and pasteurization treatments but reduced to about 93.6, 92.5 and 87.1% of their initial values, respectively when subjected to hot air oven at105°C/ 15min. At the same time, pigments produced by LSF showed lower thermal stability than that produced by SSF. The remaining stability of red, orange and yellow colors were reduced to about 66.32, 84.57 and 87.19%; 59.54, 82.10 and 85.57% after treating with autoclaving and hot air oven, respectively. However, all pigments were highly stable against pasteurization treatment. These results are in agreement with that reported by Said (2010) who found that red pigment produced by *M. ruber* was more degradable when exposure to autoclaving at 121°C /15 min than to water bath heating at100°C for 1 min. He added that pigment produced by solid state culture was more sensitive to heat than that produced by submerged culture. The same observation was also reported by Lee and Chen, (2000) and Kaur *et al*., (2009).

Table 1. Effect of different thermal treatments on the stability of *M. ruber´*s pigments

	Stability	SSF			LSF		
Treatments		**Red**	**Orange**	**Yellow**	**Red**	**Orange**	**Yellow**
Thermal treatments	Control treatment (A.U. /g or ml.)	1424.80	1692.40	1442.40	7.44	7.82	7.00
Sterilization (121°C/ 15min.)	Residual absorption (A.U. /g or ml.)	1424.12	1692.35	1397.41	4.927	6.612	6.104
	Residual stability (%)	99.95 %	99.99%	96.88%	66.32 %	84.57 %	87.19 %
Pasteurization (85°C / 1min.)	Residual absorption (A.U. /g or ml.)	1424.59	1692.38	1436.03	7.33	7.50	6.79
	Residual stability (%)	99.99%	99.99%	99.56 %	98.52 %	95.94 %	97.00 %
Hot air oven (105°C/ 15min.)	Residual absorption (A.U. /g or ml.)	1333.71	1566.06	1256.17	4.430	6.42	6.00
	Residual stability (%)	93.61%	92.54 %	87.09 %	59.54 %	82.10 %	85.57 %

2 - Effect of lightning sources:

Pyrex tubes containing pigments extracted from solid state culture and submerged culture were held under sunlight, UV light and fluorescent light up to four hours. An identical set of tubes covered with aluminum foil were held under the same conditions for comparison. Data in Table (2) indicated that all pigments were more sensitive to sunlight, followed by fluorescent light and UV light. Exposure to sunlight reduced the stability percentages to 44.82, 47.21 and 69.95% for red, orange and yellow pigments produced by solid state fermentation and to 62.6, 62.8 and 77.7 % for red, orange and yellow pigments produced by submerged fermentation, respectively. All pigments were quite stable for up to 91.4% and 96.3% of the total stability when exposure to fluorescent light and UV light, respectively. No marked differences were observed between the stability of pigments produced by SSF and LSF. However, yellow color exhibited higher stability than orange and red colors. Similar results were found by Lee and Chung (2000), who reported that Monascus pigments were more stable in UV light compared to fluorescent light and sunlight.

Table 2. Effect of different lightning sources on the stability of *M. ruber´*s pigments

	Stability	SSF			LSF		
Treatments		**Red**	**Orange**	**Yellow**	**Red**	**Orange**	**Yellow**
Lightning source	Control treatment (A.U. /g or ml.)	1424.80	1692.40	1442.40	7.44	7.82	7.00
Sunlight	Residual absorption (A.U. /g or ml.)	638.62	798.9	1008.90	4.65	4.91	5.44
	Residual stability (%)	44.82 %	47.21 %	69.95 %	62.62 %	62.80 %	77.73 %
UVLight	Residual absorption (A.U. /g or ml.)	1385.52	1663.63	1418.08	7.20	7.53	6.85
	Residual stability (%)	97.24 %	98.30%	98.31 %	96.91%	96.29 %	97.85%
fluorescent light	Residual absorption (A.U. /g or ml.)	1373.82	1636.11	1420.87	6.80	7.22	6.56
	Residual stability (%)	96.42 %	96.67 %	98.51 %	91.40%	92.30 %	93.67 %

3 – Effect of pH Values:

It is well known that foods are differ greatly in their own pH values, fruits and its products possess an acidic nature, other foods such as meat and milk are nearly neutral but turned to acidic if processed to fermented products. Therefore, it is essential to evaluate the effect of different pH values on the pigments stability before applying it for coloring such foods. Data presented in Table (3) clearly showed that the derivatives of yellow, orange and red pigments obtained from both solid state and liquid state cultures were found to be quite stable at acidic and neutral pHs (pH 4

and 7). However, the absorption intensity recorded at pH 4 for the orange color was reduced by about 1.6% to 10.2% of the control treatment. On the other hand, all pigments were relatively more sensitive for the alkaline pH (pH 10). The stability percentages were reduced to about 98.59, 89.06 and 97.26% for red, orange and yellow pigments obtained from SSF and to 97.89, 92.97 and 95.76% for the same pigments produced from LSF, respectively. The obtaind results are in agreement with that of previous investigators. Baranová *et al*., (2004) reported that, *Monascus* pigments remain very stable under pH-changes, and thus they can be applied to pork, poultry, fish, and tofu to improve the flavor and preservation of these products. Also, Babitha *et al*. (2006) studied the impact of pH on the stability of *Monascus* pigment, and observed that, *Monascus* pigments in the range of pH 3-10 are relatively stable, but above or below this pH range the stability decreased significantly.

Table 3. Effect of different pH values on the stability of *M. ruber´*s pigments

Treatments	Stability	SSF Red	SSF Orange	SSF Yellow	LSF Red	LSF Orange	LSF Yellow
pH conditions	Control treatment (A.U. /g or ml.)	1424.80	1692.40	1442.40	7.44	7.82	7.00
Acidic (pH 4)	Residual absorption (A.U. /g or ml.)	1424.80	1665.40	1442.40	7.30	7.05	6.93
	Residual stability (%)	100 %	98.40 %	100 %	98.12 %	89.81 %	98.95 %
Neutral (pH 7)	Residual absorption (A.U. /g or ml.)	1420.43	1692.40	1442.40	7.40	7.24	6.91
	Residual stability (%)	99.69 %	100 %	100 %	99.46%	96.22 %	98.64 %
Alkaline (pH 10)	Residual absorption (A.U. /g or ml.)	1404.80	1507.30	1402.84	7.27	7.27	6.70
	Residual stability (%)	98.59 %	89.10 %	97.26 %	97.89 %	92.97 %	95.76%

4 - Effect of organic acids preservatives:

Ascorbic acid, citric acid and lactic acid were separately added at concentrations of 0.1%, 0.1% and 0.15% (w/v), respectively to the pigments extract of both solid state and liquid state cultures which were incubated at room temperature (25°C) before measuring the intensity of red, orange and yellow colors. It was inferred from data in Table (4) that pigment extracts of both SSF and LSF showed similar spectrum profiles. Red and yellow colors in both extracts retained more than 96% of their initial stability in the presence of all the studied acids. At the same time, the residual stability of orange pigment ranged between 81.33-86.71% and 74.36-86.78% in the presence of ascorbic and citric acid, respectively. Gunasekaran and Poorniammal (2008) examined the effect of ascorbic acid, citric acid and sodium bisulfate on the stability of natural pigments produced from *Penicillium* sp. They found that citric acid did not have any effect on the color intensity of the pigments but ascorbic acid and sodium bisulfate reduced the pigments stability to about 78 % and 88 %, respectively.

Table 4. Effect of organic acids preservatives on the stability of *M. ruber´*s pigments

Treatments	Stability	SSF Red	SSF Orange	SSF Yellow	LSF Red	LSF Orange	LSF Yellow
preservatives	Control treatment (A.U. /g or ml.)	1424.80	1692.40	1442.40	7.44	7.82	7.00
Ascorbic acid (0.1% w/v)	Residual absorption (A.U. /g or ml.)	1414.00	1467.39	1412.58	7.34	6.36	6.80
	Residual stability (%)	99.24 %	86.71 %	97.93 %	98.66 %	81.33 %	97.14 %
Citric acid (0.1% w/v)	Residual absorption (A.U. /g or ml.)	1403.11	1478.59	1388.49	7.25	5.82	6.81
	Residual stability (%)	98.41%	86.78%	96.26 %	97.45 %	74.36 %	97.29 %
Lactic acid (0.15% w/v)	Residual absorption (A.U. /g or ml.)	1396.19	1489.45	1312.56	7.09	5.77	6.71
	Residual stability (%)	97.99%	88.01%	90.99%	95.29%	73.79%	95.85%

5 – Effect of storage temperatures:

To investigate the effect of storage temperature on the stability of pigments produced from *M. ruber* by SSF and LSF, an immediately produced extract from each fermentation technique was put into sterilized Pyrex tubes (10 ml, each). All tubes were covered with aluminum foil to avoid the influence of light, and then divided into three groups which were separately kept for 7 days under room (ambient), refrigeration and freezing temperatures. The absorption intensities of red, orange and yellow colors were measured at the end of storage period and the stability of each color was calculated as a percentage of the control (freshly produced extract) measurement. Data presented in Table (5) revealed that all pigments were quite stable under freezing temperature (-18°C) but under refrigeration temperature, the stability of all colors was slightly reduced by about 2-5% for SSF pigments and11-15% for LSF pigments. On the other hand, higher discoloration of all pigments was observed at room temperature storage. The residual concentrations were estimated by about 82.13, 83.93 and 85.92%, respectively for red, orange and yellow pigments obtained from SSF and about 55.04, 63.93 and 66.16% for red, orange and yellow pigments obtained from LSF, respectively. From results of all experiments, it could be concluded that pigments produced by submerged fermentation were more sensitive and had lower stability than pigments produced by solid state fermentation. Similar results were recorded by Sheu *et al.* (2000) and Ochaikul *et al.* (2006) who examined the color stability of *Monascus*–bacterial cellulose and found that no appreciable color change was showed of any sample after exposure to freezing storage at -20 °C. Also Said (2010) studied the stability of red *M. ruber´*s pigments and found that red pigment extracted from submerged culture was always less stable than the pigments extracted from solid- state culture. He suggested that differences in stability of pigments from different types of fermentations may be due to differences in the solvents used for extraction.

Table 5.Effect of different storage conditions on the stability of *M. ruber´*s pigments

Treatments	Stability	SSF			LSF		
		Red	Orange	Yellow	Red	Orange	Yellow
Storage conditions	Control treatment (A.U. /g or ml.)	1424.80	1692.40	1442.40	7.44	7.82	7.00
Rome temp. (25°C)	Residual absorption (A.U. /g or ml.)	1170.17	1420.58	1239.36	4.09	5.00	4.63
	Residual stability (%)	82.13 %	83.93 %	85.92 %	55.04 %	63.93 %	66.17 %
Refrigeration (4°C)	Residual absorption (A.U. /g or ml.)	1350.43	1637.36	1410.90	6.26	6.97	6.26
	Residual stability (%)	94.78%	96.75 %	97.82 %	84.25 %	89.18 %	89.53 %
Freezing (-18°C)	Residual absorption (A.U. /g or ml.)	1424.80	1678.64	1432.18	7.39	7.82	6.94
	Residual stability (%)	100 %	99.19 %	99.29 %	99.33%	99.93 %	99.14 %

Identification of pigments components:

The pigments componewnts were determined and identified by means of HPLC–MS as shown in Table (6). The obtained results showed that seven red coloring components were detected in ethyl alcohol fermented rice extract and identified on the bases of their molecular weight as monascorubramine, N-glutarylrubropunctamine, N-glucosylrubropunctamine, derivatives of threonine from rubropunctatin, derivatives of cysteine from rubropunctatin, derivatives of aspartic acid from rubropunctatin and derivatives of glutamic acid from rubropunctatin (Jung *et al*., 2003 and Miyake *et al*., 2008). The orange pigment extracted with benzene contained only 5 components with molecular weight of 354.3, 382.4, 384.146,356.4 and 440.256. So, they could be identified as rubropunctatin, monascorubrin, monapilol A, monapilol B and monapilol C (Akihisa *et al*., 2005b and Hsu *et al*., 2011b). Data in Table (6) also revealed that 14 yellow compounds were detected in n-hexane fermented rice extract and identified based on their mass spectra by means of HPLC– MS as Monascin, Ankaflavin, Monascodilone, Monascopyridine A, Monascusone A, Monascusone B, Monarubin**,** Rubropunctin**,** Monaphilone A, Monaphilone C, Monascoflavin, Monapurone A, Monapurone B and Monapurone C (Akihisa *et al.*, 2005b; Hsu *et al*., 2010 and Li *et al*., 2010a).

Table 6. Identification of red, orange and yellow pigment components by means of HPLC / MS.

NO.	Red Pigment		Orange Pigment		Yellow Pigment	
	Molecular Weight. (m/z)	components	Molecular Weight. (m/z)	components	Molecular Weight. (m/z)	Components
1	381.205	monascorubramine	354.3	Rubropunctatin	382.4	Monascin
2	484.392	N-glutarylrubropunctamine	382.4	Monascorubrin	386.4	Ankaflavin
3	516.101	N-glucosylrubropunctamine]	384.146	monapilol A	256.117	Monascodilone
4	456.253	red derivatives of threonine from rubropunctatin	356.4	monapilol B	355.084	Monascopyridine A
5	458.376	red derivatives of cysteine from rubropunctatin	440.256	monapilol C	254.297	Monascusone A
6	492.563	red derivatives of aspartic acid from rubropunctatin			302.4	Monascusone B
7	506.579	red derivatives of glutamic acid from rubropunctatin			331	Monarubin
8					359	Rubropunctin
9					360	MonaphiloneA
10					336.9	MonaphiloneC
11					358.1	Monascoflavin
12					330.1	Monapurone A
13					344.102	Monapurone B
14					344.102	Monapurone C

CONCLUSION

From the obtained results, it could be concluded that red, orange and yellow pigments produced from *M. ruber* Went AUMC5705 by solid state fermentation were shown to display surprising stability upon the common processing and storage treatments. Therefore, its application as natural food colorants appears to be promising.

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القدرة على الثبات وتعريف المكونات الخاصة بصبغات *Monascus ruber*

نانيس حسنين جمعة[1] و حسام الدين فرغلى عبد الرحيم[2]

[1]قسم علوم الألبان – كلية الزراعة – جامعة أسيوط - ج.م.ع.

[2]قسم علوم وتكنولوجيا الأغذية – كلية الزراعة – جامعة بنى سويف - ج.م.ع.

الهدف من هذه الدراسة هو تقييم درجة ثبات الصبغات الحمراء والبرتقاليه والصفراء المنتجة من فطر *Monascus ruber* Went AUMC 5705 تحت ظروف كلا من التخمر الصلب والتخمر المغمور. تم تعريض الصبغات المستخلصة من بيئات التخمر لمختلف ظروف التصنيع والتخزين الشائع استخدامها فى انتاج منتجات الاغذية والالبان وتشمل المعاملات الحرارية (تعقيم و بسترة وتسخين بأفران الهواء الساخن) وأوساط pH مختلفة (4pH و 7 و 10) والتعرض لمصادر إضاءة مختلفة (ضوء الشمس و الضوء الفلوروسنتى والاشعة فوق البنفسجية) وكذلك درجات حرارة التخزين المختلفة (درجة حرارة الغرفة 25°م وحرارة التبريد 4°م وحرارة التجميد -18°م) وكذلك تعريضها لبعض الاحماض العضوية (الاسكوربيك و الستريك واللاكتيك) . بالاضافة الى ذلك فقد تم تحليل مستخلص الصبغات المنتجة بطريقة التخمر الصلب بواسطة كروماتوجرافيا الغاز السائل والتحليل الطيفى HPLC/MS للتعرف على المركبات المكونة للصبغات الحمراء والبرتقالية والصفراء. وقد اشارت النتائج الى ان الصبغات الثلاثه كانت ثابته تماما عند تعرضها للتعقيم والبسترة والضوء الفلوروسنتى والاشعة الفوق بنفسجية ودرجة حرارة الثلاجة ودرجة التجميد وكذلك اعطت نتائج ثبات جيدة فى حالة التعرض للاحماض العضوية ودرجات ال pHالمختلفة ، ولكنها أظهرت درجات ثبات اقل فى حالة تعرضها لدرجه حرارة فرن الهواء الساخن (105°م / 15 دقيقة) وضوء الشمس ودرجة حرارة الغرفة اثناء التخزين. وقد أظهرت الصبغات المنتجه بطريقة التخمر الصلب درجة ثبات اعلى مقارنة بالصبغات المفرزة تحت ظروف التخمر المغمور. وهذه النتائج تدعم استخدام تلك الصبغات فى تلوين المنتجات اللبنية والغذائية كبديل للالوان الصناعيه الضاره.