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RED AND YELLOW *MONASCUS* PIGMENTS AS POTENTIAL NATURAL ANTIOXIDANTS FOR FATTY FOODS

Amany, M. Basuny[1] and Hossam E. F. Abdel-Raheam[2]
[1]Biochemistry Department; Faculty of Agriculture, Beni –Suef University, Egypt.
[2]Food Science Department, Faculty of Agriculture, Beni –Suef University, Egypt.
Corresponding author: dramany_basuny@yahoo.com

Abstract

To the best of our knowledge, this is the first report on application of red and yellow Monascus pigments as antioxidants in fatty foods system individually. Additionally, this application can be used commercially. The aim of the current investigation was to investigate the effect of red and yellow Monascus pigments compared to BHT as synthetic antioxidant on lipid oxidation and quality of ground beef during refrigerated storage at 0.00 ± 0.50°C for up to two weeks. DPPH and Rancimat methods were used for determination of the antioxidant activity of red and yellow Monascus pigments. Results indicated that the highest antioxidant was shown by the pigments, therefore 0.5, 0.75 and 1.00 % of either red and yellow Monascus pigments and BHT were added to minced meat and evaluate its effects on the lipid peroxidation of ground beef during storage process. TBA test as quality assurance test was conducted at the beginning of the experiment and after one and two weeks of storage. The results of this study showed that the red and yellow Monascus pigments had significantly antioxidant activity. Also, the obtained results indicated that red and yellow Monascus pigments had high antioxidative effect in reducing the formation of hydroperoxides during storage.

***Keywords*:** *Monascus* species, pigments, antioxidants, lipid oxidation.

Introduction

Lipid oxidation not only reduces nutritional quality, flavor and taste of food, but also produces something harmful on human health causing aging, heart diseases, emphysema, mutagenesis, and carcinogenesis by generating free radicals (Bera & Nag, 2006; Guo *et al*., 2016 & Rehman & Salariya, 2006). Therefore, it is essential to take measures to inhibit oxidation for edible oil. Currently, the addition of antioxidants was chosen as the preferred measures for controlling the oxidation of lipids.

Antioxidants can prevent free radical-induced cell and biological target damage by preventing the formation of radicals, scavenging them or by promoting their decomposition (Young & Woodside, 2001). At present, there were mainly two kinds of antioxidants to prevent lipid oxidation, namely synthetic antioxidants and natural antioxidants. Synthetic antioxidants such as tertbutyl hydroquinone (TBHQ), butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) and propyl gallate (PG) have a strong antioxidant capacity. However, considerable researches have shown that synthetic antioxidants may have potential toxicity that promotes DNA damages (Dolatabadi & Kashanian, 2010; Hou, 2003; Shahidi & Zhong, 2010). Due to health concern from consumers, considerable researches focus on natural antioxidants (Kathirvel & Rupasinghe, 2011; Basuny *et al*., 2012 & 2013).

It is well known that natural antioxidants mostly come from variety of plants, animals and micro-organisms. Among all the stated sources, microbes have vast potential to produce natural antioxidants agent, because of its natural character, medicinal properties, nutritive value, flexibility in production (i.e. production being independent of season, geographical to use) and easy down streaming process.

Antioxidants from microbial source were identified as early as in 1980s; however the relationship between microorganisms and antioxidants was established only in the beginning of this century (Forbes *et al*., 2007 & Meisinger *et al*., 2005). Lately, the potential for obtaining natural antioxidants from microbes to be used as naturally antioxidants are being investigated.

Among microbes capable of antioxidants production *Monascus* species Filamentous fungi are known to produce wide range of secondary metabolites, pivotal compounds, bio-pigments, anti-biotic, antioxidant, antihypertension, anti-cholesterol, anti-cancer, anti-inflamatory and anti-diabetic activities etc. (Higashikawa *et al*., 2012; Lee *et al*., 2006; Li *et al*., 1998; Wei *et al*., 2003; Yang and Mousa, 2012; Kongbangkerd *et al*., 2014 & Sen *et al*., 2019).

Aniya *et al.* (1999) & Koli *et al. (*2019) have initiated the investigation on the antioxidant activity of *Monascus* fermented product through the screening of 40 fungi species and showed 13 species with a high DPPH-scavenging activities. Since then, large number of *Monascus* species has been identified as the source of antioxidants. Keeping these factors in account unexplored *M. purpureus* Went NRRL 1992 pigments were investigated for their efficiency as antioxidant.

In the present study, we aimed to evaluate the antioxidant activity of red and yellow *Monascus* pigments, which separated from fermented substrate (solid state fermentation) of *Monascus purpureus* Went NRRL 1992, then it was applied in food system (ground beef), unlike the traditional methods which used a mixture of total pigments extract. Additionally, a comparison was made between microbial based antioxidant (red and yellow *Monascus* pigments) and synthetic antioxidant (BHT) to provide evidences for replacement of synthetic antioxidant.

Materials and Methods

1. Culture: A culture of *Monascus purpureus* Went NRRL 1992 obtained from Microbiological Resources Center (MIRCEN), Ain Shames Univ. Cairo, Egypt, was used in the present study. It was maintained on Yeast Extract-Peptone-

Dextrose agar (YEPD) medium, at 4°C and subcultured periodically every three weeks. This fungal strain was examined for its ability to produce the mycotoxin citrinin and found to be non-producing under our production conditions.

2. Inoculum preparation: *M. purpureus* Went NRRL 1992 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, 10 mL of sterile distilled water was added and the spores were scraped under strict aseptic conditions. The spores suspension obtained was used as inoculum (approximately 7×10^5 spores per mL). As described by Babitha *et al*. (2007)

3. Fermentation medium: Broken rice was obtained from a local rice mill at Assiut Governorate, Egypt. It was ground well to 2.0 mm particle size using an electrical mill, packed in polyethylene bags and stored at 4˚C until used.

4. Solid-state fermentation procedures: Fermentation procedures were carried out according to the optimum conditions adapted in our previous study (Abdel- Raheam, 2016) as follow, Ten grams of broken rice (with 2.0mm particle size) was placed in a 250 mL Erlenmeyer flask and 1mL zinc sulfate ($ZnS0_4.7H_2O$, 0.128 M) solution was added to the flask (Babitha *et al*., 2006 and Nimnoi and Lumyong, 2009). Initial moisture content was adjusted to 55% (V/W) by adding an appropriate amount of distilled water (with pH=6.5) and supplemented with 3.0% (W/W) Ammonium sulfate as nitrogen source. The broken rice was then allowed to soak at 30°C for 1h. Flask contents were mixed thoroughly, then covered with two layers of aluminum foil to prevent moisture loss and autoclaved at 121°C for 15 min. After cooling to room temperature, flask was inoculated with 105×10^4 spores /10gds (inoculation rate) and incubated at 30°C for 17 days in the dark.

5. Analysis of citrinin: Presence of the mycotoxin citrinin (if any) was estimated by Thin Layer Chromatography according to Rasheva *et al*. (2003). Authentic sample of Citrinin (Sigma) was used as a standard.

6. Extraction and Separation of Monascus red pigments from dried fermented rice: At the end of the incubation period, the content of flask were dispersed on aluminum foil sheet, dried at room temperature for 24h and ground to a fine powder using an electrical mill. red pigments was successfully and successively extracted and purified individually from dried fermented rice of SSF, according to the modified procedure described by Abdel-Raheam (2016) as shown in Figure (1).The separated red pigments concentrated on glass dishes in *vacuo*, then scraped to form paste of red pigment which ready to use in foods systems.

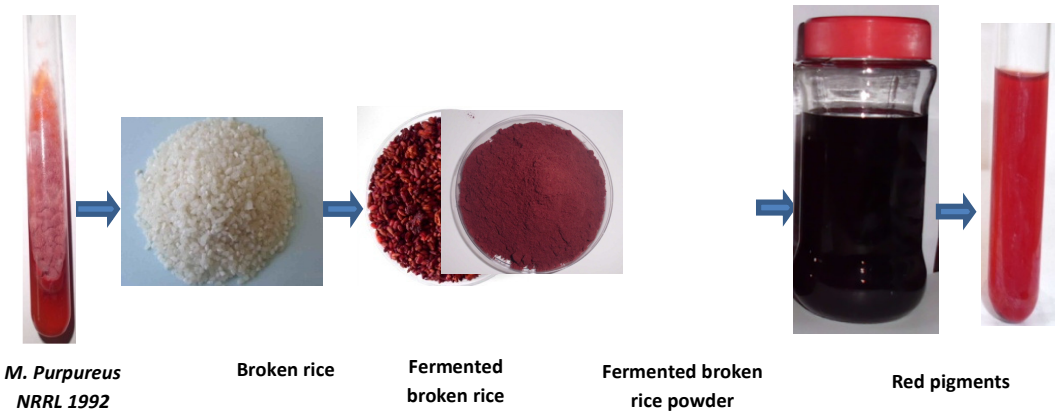

Fig. 1 : Separation of pigments from dried fermented rice culture.

7- Extraction and Separation of Monascus yellow pigments from dried fermented rice: At the end of the incubation period, the content of flask were dispersed on aluminum foil sheet, dried at room temperature for 24h and ground to a fine powder using an electrical mill. yellow pigments was successfully and successively extracted and purified individually from dried fermented rice of SSF, according to the modified procedure described by Abdel-Raheam (2016) as shown in Figure (2).The separated yellow pigments concentrated on glass dishes in *vacuo*, then scraped to form paste of yellow pigment which ready to use in foods systems.

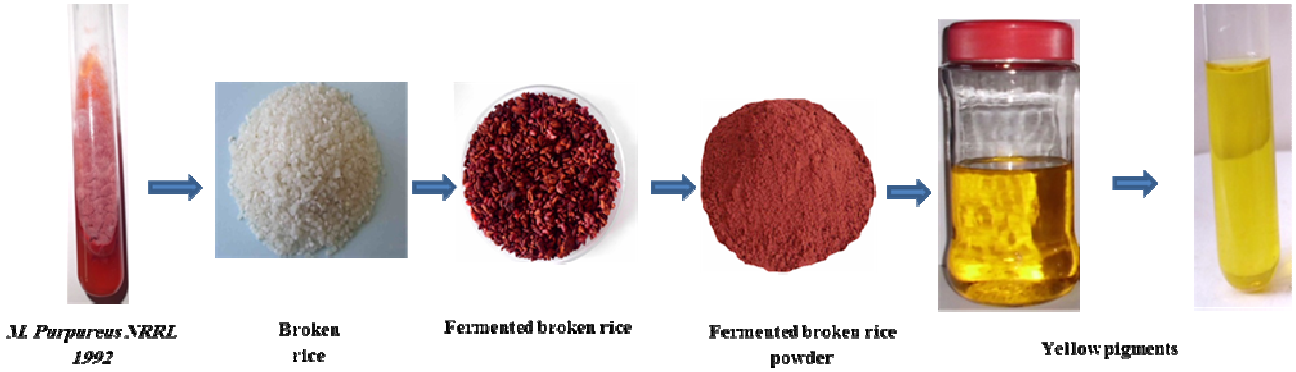

Fig. 2 : Separation of pigments from dried fermented rice culture.

8. DPPH free radical-scavenging activity: The DPPH free radical scavenging assay was cassied out, as previously reported by Lee *et al.* (2009) with some modification. The extract from red and yellow Monascus pigments at (200 ppm) were added to a 0.06nm DPPH solution in ethanol and reaction mixture was shaken vigorously. After incubation for 30 min at room temperature, the absorbance at 517nm was recorded spectorphotometrically. BHT at 200ppm was used as a reference as the test compounds. A control solution, without the tested compound, was prepared in the same manner as the assay mixture. All the analysis was done in triplicate. The degree of disclorisation indicates the free radical scavenging efficiency of the substances. The antioxidant activity was calculated as an inhibitory effect (IE%) of the DPPH radical formation as follows:

$$IE\% = 100 \text{ X } (A_{517cntrol}-A_{517sample} /A_{517cntrol}).$$

9. Antioxidant activity by Rancimat: Concentration of red and yellow Monascus pigments (200ppm) and BHT (200ppm) were individually added to sunflower oil to study their antioxidant behavior. The designation of an induction period, measured by using a Rancimat instrument (679 Metrohom Ltd, CH-9100 Herisau, Switzerland), was taken as a tool to compare the effectiveness of the phenolic on sunflower oil stability (Mendez *et al.,* 1996).

10. Preparation of ground beef: Beef meat (5kg) was cut into small pieces and homogenized in stainless steel blender. Ground beef were mixed by latex gloved hands with 400 ppm of either the highest antioxidant efficiency of red and yellow Monascus pigments and BHT (200 ppm). Minced beef without additives was run as control. The abovementioned samples were packed in polyethylene bags, each bag contain 250 g and stored at 0 ± 0.5˚C in refrigerator for two weeks.

11. Thiobarbituric acid-reactive substances (TBARS): The TBARS values were determined in triplicate samples by the extraction method of Mielnik *et al*. (2006). For extraction 10g meat was homogenized with 30 ml of a 7.50 % aqueous solution of trichloroacetic acid (TCA). After filtration, 5.00 L^{-1} of 0.02 L^{-1} aqueous thiobarbituric acid (TBA) in a stoppered test tube. The samples were incubated at 100˚C for 35 min in cold water-bath and subsequently cooled for 10 min in cold water. Absorbance was measured at 532 nm by using UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). Against a blank containing 5 ml distilled water and 5 ml TBA reagent. Results expressed as milligrams malondialdehyde kg^{-1} meat.

Statistical analysis: A one-way ANOVA followed by Duncan's multiple range test (DMRT) were performed using SPSS 11.00 (SPSS Inc., Chicago, IL, USA) to analyze and compare the data. Results were presented as mean ± SD and P- values ≤ 0.05 were regarded as statistical significance.

Results and Discussion

1. Production of Monascus pigments by solid state fermentation technique: Production of Monascus pigments by solid state fermentation were carried out according to the optimum conditions adapted in our previous study (Abdel-Raheam, 2016), which highly pigments were production to rich 1282.0000, 1604.0032 and 1328.4017 AU ⁄ gds for red, orange and yellow pigments respectively.

Fig. 3 : Production of *Monascus purpureus* NRRL 1992 pigments by solid state fermentation culture technique in broken rice as solid-state fermentation medium.

2. Qualitative analysis of Citrinin using thin-layer chromatography (TLC): Under used fermentation production conditions, TLC analysis showed that no Citrinin was detected in solid state culture extract. Hence the *Monascus purpureus* used in this study was found to be safe for food use.

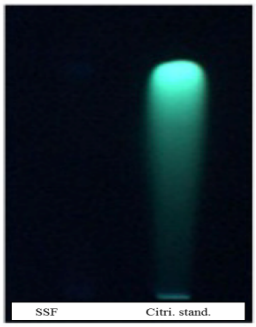

Fig. 4 : Detection of Citrinin by TLC under UV detector on extracts of *Monascus purpureus* NRRL 1992 solid-state culture.

The antioxidant activity by Rancimat method: The antioxidant activities of red and yellow Monascus pigments were assessed by the Rancimat method. This method assigned the induction period for the onset of oxidative rancidity in sunflower oil at 100ºC. In the present study, simple model systems comprising sunflower oil with various concentrations 200 and 400 ppm of red and yellow Monascus pigments were used to assess oxidation behavior. An experiment was performed with sunflower oil and BHT (200ppm) to compare the antioxidant efficiency of red and yellow Monascus pigments with the most commonly used synthetic antioxidant material. Figure (5) shows the effect of red and yellow Monascus pigments on the oxidative rancidity of sunflower oil. The results illustrate that the concentration 200 ppm of red and yellow Monascus pigments, exhibited antioxidant activity. It is worth noting that red and yellow Monascus pigments at 200ppm level superior to that of BHT in retarding sunflower oil oxidative rancidity.

DPPH radical-scavenging activity: There are different methods for estimation of antioxidant activity but the most widely methods are those that involve generation of free radical species which are then neutralized by antioxidant compounds. DPPH radical is commonly used as substrate to evaluate antioxidant activity; it is useful and stable free radical that can accept on electron or hydrogen radical to become a stable molecule. The reduction of DPPH free radical was determined by the decrease in its absorbance at 517 nm induced by different antioxidants. DPPH free radical reacts with antioxidant, consequentially, absorbance decreases and the DPPH free radical is converted into the DPPH form. The degree of discoloration indicates the scavenging potential of antioxidant compounds of extracts in terms of hydrogen donating ability (Jiao *et al.,* 2012). Figure (6) shows the effect of 200 and 400 ppm concentrations of red and yellow Monascus pigments compared with BHT (200ppm) were used with a very high scavenging capacity of 40.00 after only 10 min. in all cases the scavenging capacity did not increase after the first 10 min of incubation. The reactions of BHT with DPPH were similar to red and yellow Monascus pigments with DPPH; the scavenging capacities were similar.

Thiobarbituric acid-reactive substances (TBARS) value of ground beef during refrigerated storage: Ground meat tends to become rancid and brown more rapidly, due to pigment and lipid oxidation. An oxidative reaction in muscle foods leads to degradation of lipid and proteins, resulting in deterioration of flavour, texture and nutritive value and is considered as one of the major problems in the development of new convenient meat products and processes (Gray & Pearson, 1987). In the present investigation based on the antioxidant activities results, the highest antioxidant activity was shown by the red and yellow Monascus pigments and BHT were added to minced meat to evaluate its effects on the lipid peroxidation of ground beef during storage process. Generally, the TBA values are increased gradually and significantly ($P \geq 0.05$) during storage period. The phospholipids in muscle membrane provide an ideal substrate for lipid peroxidation. Iron bound to negatively charged phospholipids promotes lipid peroxidation, resulting in generation of warmed-over flavor (Empson *et al*., 1991). However, mixing minced beef with red and yellow Monascus pigments caused a significant ($P \geq 0.05$) reduction of TBA values compared to control sample. Control samples had significantly the highest TBA value was 2.00 mg malondialdehyde kg^{-1} at the end of the storage period, the highest TBA values of the control sample might be due to an interaction between the natural substances (for example, polyunsaturated fatty acids) and catalysts (for example, iron ion) from the meat tissue during storage (Kim *et al.,* 2000), while beef samples mixed with 200 ppm red and yellow Monascus pigments had significantly ($P \geq 0.05$) the lowest TBA values was 0.69 mg malondialdehyde kg^{-1} at the end of the storage period. No significant differences were observed in TBA values of ground beef mixed with red and yellow Monascus pigments and that samples treated with 200 ppm of synthetic antioxidant BHT. The obtained results indicate high antioxidative effect in reducing the formation of hydroperoxides during storage process (Figure 7). These results are in good agreement with those obtained by (El-Rayes, 2009& Basuny *et al*., 2012). In this respect the results of Abd El-Hamied *et al.* (2009) observed that the addition of rosemary, sage and their combination showed high antioxidative effects during refrigerated and frozen storage of minced meat. Also Basuny (2004) show that mixing oil with various levels of grape seed phenolic compounds caused significant decrease of the formation of secondary products during frying process.

Conclusion

The results of the paper recommend the use of red and yellow Monascus pigments as natural antioxidants for increase stability of oils and fats or fatty foods that contain a high percentage of fats. Also, these pigments are very safe and cheap compared with synthetic antioxidants which have harmful effects.

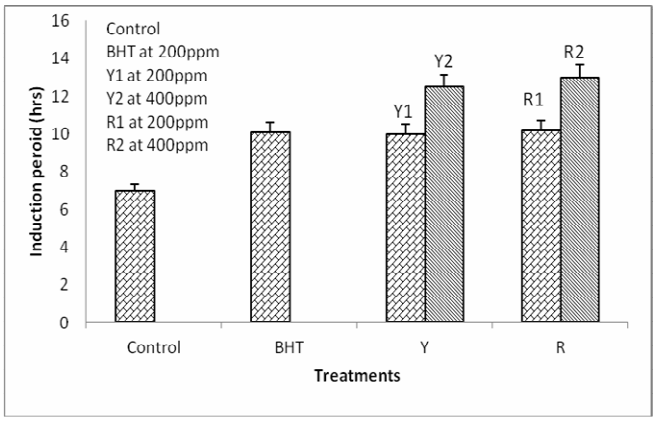

Fig. 5 : Antioxidant activity of red and yellow *Monascus* pigments by Rancimat method. Data are expressed as mean ± SE. Each sample was analyzed three times.

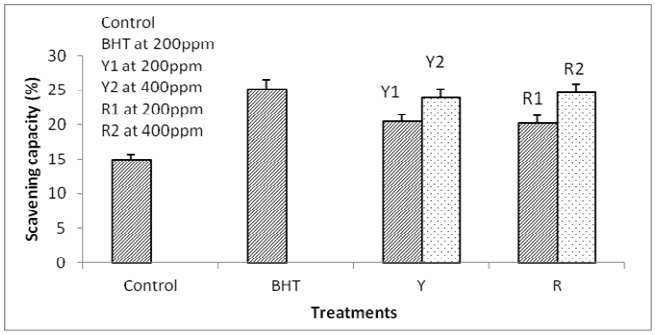

Fig. 6 : Antioxidant activity of red and yellow *Monascus* pigments DPPH free radical-scavenging activity. Data are expressed as mean ± SE. Each sample was analyzed three times.

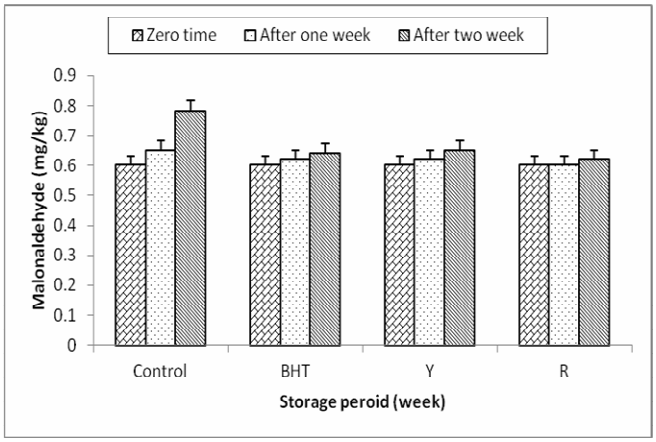

Fig. 7: Effect of red and yellow *Monascus* pigments and BHT on TBA number (mg malondialdehyde kg^{-1} meat) of minced beef during storage at 0˚C. Data are expressed as mean ± SE. Each sample was analyzed three times.

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