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Application of *Monascus purpureus* Pigments Produced Using Some Food Industry Wastes in Beef Sausage Manufacture

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PHYSICOCHEMICAL properties and sensory evaluation of beef sausage samples containing *Monascus* pigments were followed during 90 days of storage at −18°C. The cooking yield of control beef sausage samples (containing 3% starch) was the lowest initial cooking and achieved a level of 82.9% and almost the highest value of cooking loss (17.11%). While, the beef sausage samples containing 0.25% dry red rice showed the highest initial cooking yield (91.11%) and the lowest cooking loss (8.87%). The water holding capacity (WHC) of all samples decreased progressively, throughout the storage period. Control samples had the lowest WHC after 90 days of storage at -18°C when compared with other samples containing *Monascus* pigments and all samples tended to decrease in plasticity. The chemical constituents of beef sausage samples were affected by freezing and storage period. The moisture, protein and ash contents were gradually decreased with increasing the storage period and *vice versa* for fat content. The results indicated that, thiobarbituric acid (TBA) values increased with increasing the storage period, and the control samples recorded the highest TBA values after 90 days of frozen storage. For the total volatile nitrogen (TVN) values, all samples had a slight increase in TVN values during frozen storage. Storage of different samples at −18 °C for 90 days considerably reduced the counts of the initial total bacterial, total *Coliform* and *Staphylococcus* sp. of beef sausage samples. The sensory evaluation of cooked beef sausage samples indicated that, the samples containing 0.3% and 0.45% dry biomass and 0.5% and 0.75% dry red rice gave a higher rating than the other samples. Results indicated that, high scores for the parameters of appearance, juiciness, tenderness, colour, aroma and overall acceptability for samples containing 0.3% and 0.45% of dry biomass, 0.25, 0.5 and 0.75% of dry red rice, 0.5% of red corn bran and 0.75% of dry red bagasse & proteilan mixture.

Keywords: *Monascus purpureus*, Pigments, Physicochemical properties, Microbial analysis, Sensory evaluation, Beef sausage.

Fink-Gremmels *et al.* (1991) used an extract of rice cultures of the mould *Monascus purpureus* DSMZ 1379 as an alternative to nitrite in frankfurter-type sausages. They found that, colour stability was better for sausages made with *M. purpureus* extract than for those made with nitrite. No adverse effect of *M.*

purpureus extract on flavour or aroma of the sausages was detected. Colour of sausages made with combinations of nitrite and *M. purpureus* extract was good, and colour stability was better than for products made with nitrite alone.

Patáková – Juzlová *et al.* (1994) reported that, the pigments produced from *Monascus* fungus have been used as colorants in the Far East for centuries. They are considered to be possible substitute for synthetic food dyes. In addition red pigments produced using solid state cultures by several species of the genus *Monascus* have been traditionally used in fermented foods.

Martinková *et al.* (1999) reported that, the tests of *M. purpureus* extract from the fungal mycelium in mice *in vivo* indicated its non- toxicity (mean lethal dose (LD_{50}) >10 g/kg body weight) on oral administration. Similarly, oral doses of up to 18 g red rice per kg body weight caused no toxic effect in mice.

Madkour *et al.* (2000) used *Monascus* pigments as natural red colourant in beef burger. Results indicated that, the burger samples produced using crude pigments from the biomass and from the red rice origin at the concentration of 0.32% dry biomass and 0.54% dry red rice were better sensory evaluated.

Some other strains primarily produced different hydroxyl- methyl- glutaryl coenzyme (HMG-Co A) reductase inhibitors, so called Monacolins which help to regulate blood lipids, decrease of total cholesterol, triglycerides, LDL-cholesterol, and increase of HDL- cholesterol (Mandt & Ziegler, 2002). They also found that *Monascus* fermented pigments are harmless and untoxic. Other exciting applications for red yeast rice are suggested by recent discoveries (Erdogrul & Azirak, 2004) that lovastatin and other statin drugs may be useful for treating or preventing cancer, osteoporosis, stroke, Alzheimer's disease and other dementias and macular degeneration.

The aim of this study was to utilize the crude red pigments produced from *Monascus purpureus* DSMZ 1379 as an alternative food colourant in beef sausage and also to study its effect on the WHC, plasticity, cooking yield and chemical, microbial and sensory qualities.

Material and Methods

Tested organism

The test organism in this investigation was *Monascus purpureus* DSMZ 1379 (red mold rice), which was used for the production of pigments, was provided from DSMZ (Deutsche Sammlung von Mikroorganismen und ZellKulturen, Braunschweig; Germany).

Wastes used for solid cultivation and pigment production

Broken rice and sugar cane bagasse were purchased from local markets, Cairo. Starch and corn starch wastes (corn bran and proteilan) were obtained from the Egyptian Starch and Glucose Company, Mustorod, Cairo, Egypt.

Fermentation process

The pigments were obtained by cultivation of *Monascus purpureus* in the liquid fermentation medium described by Lin (1973) and on the specified wastes as solid fermentation media descried by Martinkovà *et al.* (1995). All samples were oven dried at 80°C for 24 hr according to Broder & Koehler (1980).

Beef sausage manufacture

Meat and fat tissues were cut into pieces of about egg-size and frozen at -18°C for 24 hr. The frozen meat and fat were ground to particles of about a rice size. Sausage was prepared by blending (1.2 %) of spices mixture as mentioned by Zaika *et al.* (1978) with the following ingredients:- lean meat (70.0g), fat tissues (12.0g), sodium chloride (2.3g), water as ice (9.295g), starch (3.0g), spices mixture (1.2g), sodium nitrite (0.005g), garlic (1.0g) and onion (1.2g).

The sausage mixtures were stuffed by hand into mutton casings. The casings were then sealed and chipped (Shehata, 1989). The natural mutton casings were obtained from slaughtered animals and prepared according to El-Deep (1987).

To evaluate the effects of the investigated *Monascus* pigments on sausage quality, substitution of starch in the basic formula of sausage with a suitable level of the selected concentrations of pigments was investigated according to the following treatments:

* Control: prepared with 3% starch.
* M1: prepared with 0.15% dry biomass.
* M2:prepared with 0.30% dry biomass.
* M3:prepared with 0.45% dry biomass.
* R1:prepared with 0.25% dry red rice.
* R2:prepared with 0.50% dry red rice.
* R3:prepared with 0.75% dry red rice.
* G1: prepared with 0.25% dry red corn bran.
* G2: prepared with 0.50% dry red corn bran.
* G3: prepared with 0.75% dry red corn bran.
* B1:prepared with 0.25% dry red bagasse, proteilan mixture.
* B2:prepared with 0.50% dry red bagasse, proteilan mixture.
* B3:prepared with 0.75% dry red bagasse, proteilan mixture.

The various sausage samples were packaged in polyethylene packages and stored frozen at -18 °C for 3 months until analysis. Samples in three replicates from each batch were subjected to chemical, physical and microbiological analysis initially and periodically every month during frozen storage. Sensory evaluation was carried out also every 30 days.

Physiochemical analysis

Cooking yield and cooking loss

Cooking yield was determined on cooked sausage by calculating the weight difference of sausage before and after cooking in boiling water for 10 min according to George & Berry (2000) as follows:

$$\text{\% Cooking yield} = \frac{\text{Cooked weight}}{\text{Raw weight}} \times 100$$

$$\text{\% Cooking loss} = \frac{\text{Raw weight- Cooked weight}}{\text{Raw weight}} \times 100$$

Change of sausage diameter and length by cooking

The change of sausage diameter and length was measured on cooked samples as mentioned by George & Berry (2000) using the following equations:-

$$\text{(\%) Change in sausage diameter} = \frac{\text{Raw sausage diameter (cm)} - \text{Cooked sausage diameter (cm)}}{\text{Raw sausage diameter (cm)}} \times 100$$

$$\text{(\%) Change in sausage length} = \frac{\text{Raw sausage length (cm)} - \text{Cooked sausage length (cm)}}{\text{Raw sausage length (cm)}} \times 100$$

Water holding capacity (WHC) and plasticity

The water holding capacity (WHC) and plasticity were measured by the filter press method according to the method described by Voloviskaya & Kelmen (1962).

After pressing, two zones were formed on the filter paper; the outer zones resulted from secretion of water from the samples and the internal zones were derived from the area of the pressed meat. The zones were then measured with a planimeter (KOIZUMI Digital Planimeter PLACOM KP-92) in cm^2. WHC and plasticity in the sausage samples were determined as area of released water in cm^2 / 0.3 g sample.

Hardness

Hardness of samples was determined according to the method described by Sanderson *et al.* (1988) by measuring tension compression using a precision tester (model Dillon Advanced, Force Gauge, AFG-500). A probe of 1 mm diameter was used to penetrate the sample at a head speed of 300 mm/min. The result was calculated as g/ cm^2.

Moisture, crude protein, lipid and ash contents

These parameters were determined for each sample according to the A.O.A.C (1995).

Thiobarabituric acid value (TBA)

The TBA values were colorimetercically determined in minced sausage samples as described by Harold *et al.* (1987).

Total volatile nitrogen (TVN)

Total volatile nitrogen values were determined according to the method of the Harold *et al.* (1987). The results were calculated as mg TVN/100g sample.

Microbial analysis

The various materials and ingredients used for sausage manufacture were first microbiologically examined under aseptic condition. Samples were taken immediately after processing and during storage.

Sausage samples were examined for total viable bacterial count as well as *Salmonella* sp. *Staphylococcus* sp. and *Coliform*.

Sensory evaluation

Cooked sausage samples were assessed for their quality attributes by ten panelists according to Klein & Bardy (1984).

Statistical analysis

Sausage samples were evaluated by a test panel of 10 panelists. The panelists rated appearance, juiciness, tenderness, colour, aroma and overall acceptability using a 1 to 10 rating scale for each attribute according to Larmond (1970). Analysis of variance was performed and followed by a multiple comparison using the least significant different (LSD) test at the 5% level of significance (SAS, 1996).

Results and Discussion

Physical properties evaluation of beef sausage during frozen storage

Cooking yield and cooking loss

The cooking yield of beef sausage samples as affected by frozen storage is listed in Table 1. The cooking yield of control samples (containing 3% starch) showed the lowest initial cooking yield and achieved a level of 82.90%. While, the beef sausage samples containing 0.25% dry red rice (R1) showed a higher initial cooking yield (91.11%). Such a behaviour could be explained by the damage in starch granules present in sausage and in pigments upon freezing and thawing. This caused a reduction in the capacity of starch and all *Monascus* pigment samples to absorb high amounts of water and to swell during cooking causing higher losses of moisture to the heating media. Such hypothesis could be confirmed by the results of Berry (1997). Results of the cooking loss of sausage sample are also given in Table 1. The cooking loss of samples was increased as the freezing storage period progressed.

TABLE 1. Percentage of cooking yield and cooking loss of beef sausage produced using some fermented food industry wastes by *M. purpureus* during frozen storage at -18 °C.

Storage period (days)	Cooking yield												
	Treatments												
	Control	M1	M2	M3	R1	R2	R3	G1	G2	G3	B1	B2	B3
Zero	82.9 ±0.80	87.51 ±0.36	85.05 ±0.13	84.67 ±0.10	91.11 ±0.10	90.05 ±0.29	87.99 ±0.28	84.82 ±0.23	83.17 ±0.97	82.78 ±0.67	83.79 ±0.50	83.1 ±0.22	82.27 ±0.24
30	81.13 ±0.44	86.46 ±0.49	84.04 ±0.15	83.17 ±0.15	88.81 ±0.51	88.06 ±0.95	86.27 ±0.17	83.37 ±0.21	82.3 ±0.80	81.71 ±0.33	82.24 ±0.42	81.52 ±0.20	80.98 ±0.34
60	78.92 ±0.50	85.04 ±0.19	83.14 ±0.11	82.37 ±0.24	86.77 ±0.19	85.79 ±0.10	84.61 ±0.11	81.77 ±0.25	81.34 ±0.11	80.18 ±0.16	81.34 ±0.11	79.69 ±0.24	79.32 ±0.16
90	76.63 ±0.39	83.82 ±0.14	81.66 ±0.01	80.67 ±0.16	85.09 ±0.25	84.31 ±0.19	83.22 ±0.10	80.11 ±0.24	79.61 ±0.06	78.42 ±0.54	78.13 ±0.32	77.64 ±0.52	77.28 ±0.41
	Cooking loss												
Zero	17.11 ±0.20	12.49 ±0.36	14.95 ±0.17	15.33 ±0.29	8.87 ±0.21	9.94 ±0.28	12.01 ±0.51	15.17 ±0.56	16.83 ±0.69	17.21 ±0.37	16.21 ±0.19	16.90 ±0.42	17.73 ±0.17
30	18.87 ±0.25	13.54 ±0.48	15.96 ±0.50	16.83 ±0.49	11.18 ±0.44	11.93 ±0.58	13.73 ±0.61	16.63 ±0.48	17.70 ±0.25	18.29 ±0.26	17.75 ±0.44	18.47 ±0.36	19.02 ±0.61
60	21.08 ±0.70	14.96 ±0.60	16.86 ±0.45	17.63 ±0.20	13.23 ±0.19	14.21 ±0.52	15.39 ±0.28	18.23 ±0.49	18.66 ±0.46	19.82 ±0.56	18.66 ±0.36	20.31 ±0.71	20.68 ±0.49
90	23.37 ±0.50	16.18 ±0.40	18.35 ±0.55	19.35 ±0.59	14.93 ±0.42	15.65 ±0.31	16.77 ±0.50	19.92 ±0.52	20.38 ±0.65	21.61 ±0.45	21.88 ±0.55	22.33 ±0.54	22.70 ±0.41

Such increases were 17.11% for the control sample at zero time, and reached to 23.37% after freezing at -18°C for 90 days. The beef sausage samples containing 0.25% dry red rice (R1) showed the lowest initial cooking loss, and the highest initial cooking loss was observed in samples containing 0.75% dry red bagasse (B3). All sausage samples showed a slight increase in cooking loss during frozen storage at -18°C. These results are in agreement with those obtained by Salama *et al.* (1994).

Change in diameter and length of cooked beef sausage samples

Reduction percentage of diameter and length of cooked beef sausage samples compared to the raw sample is given in Table 2. Reduction in both length and diameter was observed as a result of cooking of different beef sausage. Reduction in diameter and length is an ultimate result of the losses in cooking yield. As expected, sausage samples with low cooking yield showed the highest reduction in diameter and length after 90 days of frozen storage. These results are in agreement with Hanenian *et al.* (1989).

TABLE 2. Percentage change in diameter and length of cooking beef sausage produced using some fermented food industry wastes by *M. purpureus* during frozen storage at -18°C.

Storage period (days)	% Change in diameter												
	Treatments												
	Control	M1	M2	M3	R1	R2	R3	G1	G2	G3	B1	B2	B3
Zero	6.82 ±0.31	4.93 ±0.20	5.95 ±0.21	6.53 ±0.15	5.63 ±0.14	5.88 ±0.33	6.26 ±0.32	5.88 ±0.45	6.53 ±0.12	7.84 ±0.40	4.65 ±0.09	6.38 ±0.13	8.21 ±0.35
30	10.32 ±0.15	5.74 ±0.29	7.18 ±0.29	8.68 ±0.21	6.17 ±0.24	7.85 ±0.38	9.39 ±0.48	7.64 ±0.27	8.56 ±0.23	9.58 ±0.27	7.59 ±0.47	8.82 ±0.51	9.6 ±0.22
60	11.67 ±0.45	5.93 ±0.41	7.66 ±0.19	9.11 ±0.27	6.81 ±0.32	8.32 ±0.48	9.81 ±0.39	8.28 ±0.33	8.89 ±0.24	10.13 ±0.70	8.11 ±0.45	9.33 ±0.55	10.81 ±0.52
90	11.93 ±0.40	6.43 ±0.30	8.13 ±0.31	9.72 ±0.52	7.47 ±0.36	9.26 ±0.25	10.32 ±0.31	8.86 ±0.31	9.62 ±0.49	10.86 ±0.50	9.22 ±0.51	10.17 ±0.38	11.25 ±0.40
	% Change in length												
Zero	8.87 ±0.25	5.46 ±0.28	5.87 ±0.21	7.94 ±0.29	6.33 ±0.30	7.52 ±0.24	8.59 ±0.31	5.60 ±0.17	6.34 ±0.31	8.84 ±0.38	5.34 ±0.31	7.27 ±0.45	8.84 ±0.29
30	11.67 ±0.80	8.34 ±0.33	9.47 ±0.30	10.24 ±0.07	9.03 ±0.31	11.36 ±0.35	12.49 ±0.50	8.48 ±0.45	9.56 ±0.33	10.53 ±0.20	8.49 ±0.38	10.86 ±0.52	12.30 ±0.50
60	13.41 ±0.53	10.81 ±0.53	11.31 ±0.47	11.66 ±0.53	10.71 ±0.36	11.86 ±0.59	12.81 ±0.19	10.31 ±0.27	10.91 ±0.53	11.44 ±0.33	10.62 ±0.41	11.33 ±0.29	12.86 ±0.39
90	15.22 ±0.27	13.61 ±0.15	14.26 ±0.59	14.92 ±0.20	12.61 ±0.45	13.23 ±0.41	14.52 ±0.35	13.61 ±0.42	14.51 ±0.38	14.83 ±0.33	13.27 ±0.38	14.26 ±0.23	14.76 ±0.45

The diameter and length of the cooked sausage samples are important parameters for consumer acceptance. Therefore, replacement of 3% starch with dry biomass or with dry red rice with different ratios in beef sausage production is recommended to keep these reductions at a minimum level especially during frozen storage.

Change in water holding capacity and plasticity of raw beef sausage samples

The water holding capacity (WHC) in the sausage samples was determined as the area of released water in cm^2 / 0.3 g sample (Table 3). It could be noticed that, the WHC of all samples progressively decreased with the increase of outer zones, resulted from secretion of water from samples, throughout the storage period. Control sample of beef sausage which contained 3% starch had the lowest WHC after 90 days of storage at -18°C when compared to other samples containing *Monascus* pigments.

TABLE 3. Changes in water holding capacity (WHC) and plasticity of raw beef sausage produced using some fermented food industry wastes by *M.purpureus* during frozen storage at -18°C.

Storage period (days)	Water holding capacity (WHC)*												
	Treatments												
	Control	M1	M2	M3	R1	R2	R3	G1	G2	G3	B1	B2	B3
Zero	7.62 ±0.27	6.81 ±0.30	6.50 ±0.47	6.32 ±0.21	5.30 ±0.38	5.20 ±0.36	5.00 ±0.82	6.62 ±0.25	6.32 ±0.30	6.10 ±0.47	6.21 ±0.50	6.10 ±0.16	5.95 ±0.28
30	7.83 ±0.21	7.20 ±0.37	7.12 ±0.40	6.56 ±0.25	5.62 ±0.43	5.44 ±0.29	5.31 ±0.56	6.83 ±0.35	6.45 ±0.38	6.15 ±0.47	6.40 ±0.30	6.21 ±0.21	6.14 ±0.28
60	8.41 ±0.34	7.31 ±0.31	7.20 ±0.58	6.90 ±0.51	5.83 ±0.31	5.81 ±0.61	5.70 ±0.55	7.41 ±0.35	6.90 ±0.48	6.70 ±0.44	6.81 ±0.26	6.45 ±0.33	6.37 ±0.39
90	8.90 ±0.33	8.10 ±0.55	7.86 ±0.26	7.20 ±0.56	6.35 ±0.38	6.10 ±0.33	5.95 ±0.77	7.80 ±0.18	7.35 ±0.51	7.10 ±0.45	7.10 ±0.42	6.81 ±0.48	6.65 ±0.39
	Plasticity**												
Zero	5.30 ±0.27	5.45 ±0.22	5.73 ±0.20	6.10 ±0.29	4.62 ±0.28	4.70 ±0.36	4.85 ±0.48	4.40 ±0.36	4.65 ±0.31	4.95 ±0.33	4.31 ±0.28	4.80 ±0.38	4.96 ±0.30
30	5.15 ±0.58	5.30 ±0.47	5.44 ±0.42	5.81 ±0.26	4.33 ±0.45	4.50 ±0.25	4.66 ±0.33	4.25 ±0.31	4.40 ±0.20	4.53 ±0.35	4.20 ±0.25	4.30 ±0.17	4.50 ±0.25
60	4.85 ±0.41	4.90 ±0.70	5.21 ±0.47	5.45 ±0.38	4.15 ±0.53	4.21 ±0.44	4.40 ±0.35	4.06 ±0.41	4.15 ±0.41	4.20 ±0.37	4.00 ±0.45	4.15 ±0.40	4.18 ±0.31
90	4.30 ±0.28	5.20 ±0.34	5.40 ±0.27	5.65 ±0.24	4.53 ±0.35	4.60 ±0.27	4.65 ±0.24	3.85 ±0.27	4.00 ±0.55	4.10 ±0.29	3.80 ±0.02	3.90 ±0.34	3.97 ±0.29

*WHC of raw meat = 8.45 cm^2 / 0.3g sample.

**Plasticity of raw meat = 5.10 cm^2 / 0.3g sample.

However, sausage samples containing dry red rice showed the highest stability of WHC during storage. This progressive decrease occurred with all samples and may be due to the protein denaturation or aggregation, or to the biochemical changes associated with freezing of meat products, as reported and suggested by Fox *et al.* (1990). The reduction of WHC values at the end of frozen storage could be ascribed to the loss of water by evaporation, rather than to any improvement of water holding capacity (Hashem *et al.*, 1978).

Plasticity (cm^2/ 0.3g. sample) of all sausage samples under investigation tendeds to decrease progressively during freezing at -18°C until the termination of studied storage period (90 days). This might be explained on the basis of denaturation and / or aggregation of protein during frozen storage, as well as to the decrease in WHC of the studied frozen meat products. This reduction might be due to the tightening of sausage structure because of evaporation of water. The decrease of plasticity was clearly pronounced in the samples prepared with red corn bran and sugar cane bagasse followed by control samples at the end of the storage period. These results agree with the results of Fox *et al.* (1990) and Madkour *et al.* (2000).

Change in hardness of raw beef sausage samples

Hardness is an important physical and organoleptic characteristic of processed meat products such as sausage and patties. It is an indicator of the force needed by the teeth and jaws to chew meat products. Table 4 represents the values of hardness obtained for the sausage samples. Control sample of the raw sausage showed a hardness value of 250 g/cm^2 at zero time, the maximum values were achieved by addition of red sugar cane bagasse followed by red corn bran (ranged from 260 to 310g / cm^2).

Frozen storage has generally increased the hardness values of raw sausage samples. However, sausage samples prepared with 0.75 % red sugar cane bagasse (B3) had the highest value of hardness (360g / cm^2 sample) after 90 days of frozen storage at -18°C. These samples which were prepared with dry biomass and dry red rice had the lowest hardness values at the end of storage (90 days). These results are in agreement with those results obtained for cooking yield and WHC of sausage samples (Tables 1 and 3).

TABLE 4. Degree of hardness (g/cm^2) of raw beef sausage produced using some fermented food industry wastes by *M. purpureus* during frozen storage at -18 °C.

Storage period (days)	Treatments												
	Con. trol	M1	M2	M3	R1	R2	R3	G1	G2	G3	B1	B2	B3
Zero	250	230	250	260	240	260	270	260	280	300	270	280	310
30	270	250	260	270	250	270	280	280	300	320	280	310	340
60	280	260	270	280	250	270	290	280	300	330	280	320	350
90	290	260	270	290	250	270	300	290	310	350	300	330	360

Chemical changes of beef sausage samples

Proximate chemical analysis of raw beef sausage containing different kinds and concentrations of *Monascus* pigment sources are listed in Table 5.

TABLE 5. Proximate chemical analysis of raw beef sausage produced using some fermented food industry wastes by *M. purpureus* during frozen storage at -18 °C.

Constituents	Storage period (days)	Treatments												
		Control	M1	M2	M3	R1	R2	R3	G1	G2	G3	B1	B2	B3
Moisture	Zero	62.62 ±0.43	63.31 ±0.45	63.94 ±0.49	64.61 ±0.48	63.11 ±0.51	64.22 ±0.51	64.35 ±0.12	62.68 ±0.45	63.77 ±0.44	65.11 ±0.46	62.68 ±0.56	62.71 ±0.37	63.92 ±0.70
	90	59.21 ±0.16	59.43 ±0.30	59.66 ±0.49	60.44 ±0.36	59.76 ±0.31	60.22 ±0.43	60.67 ±0.56	59.41 ±0.20	59.83 ±0.29	60.11 ±0.47	59.42 ±0.38	59.71 ±0.40	59.86 ±0.39
Protein	Zero	15.13 ±0.39	15.33 ±0.54	15.44 ±0.34	15.52 ±0.31	15.18 ±0.43	15.31 ±0.30	15.50 ±0.36	15.15 ±0.57	15.23 ±0.58	15.34 ±0.56	15.14 ±0.79	15.28 ±0.65	15.35 ±0.68
	90	13.41 ±0.40	13.91 ±0.44	14.07 ±0.65	14.62 ±0.51	13.66 ±0.38	13.68 ±0.41	14.18 ±0.64	13.54 ±0.49	13.52 ±0.36	13.72 ±0.61	13.30 ±0.27	13.82 ±0.40	13.82 ±0.46
Fat	Zero	13.31 ±0.30	13.65 ±0.20	13.83 ±0.35	14.08 ±0.81	13.43 ±0.10	13.63 ±0.51	13.82 ±0.39	13.41 ±0.37	13.62 ±0.21	13.71 ±0.35	13.37 ±0.34	13.44 ±0.33	13.54 ±0.22
	90	13.52 ±0.31	14.11 ±0.36	14.26 ±0.42	14.71 ±0.34	13.88 ±0.67	14.07 ±0.24	14.36 ±0.22	13.75 ±0.48	14.16 ±0.52	14.19 ±0.35	13.62 ±0.55	13.92 ±0.34	13.96 ±0.32
Ash	Zero	2.19 ±0.40	2.23 ±0.44	2.37 ±0.19	2.46 ±0.30	2.24 ±0.32	2.38 ±0.17	2.53 ±0.32	2.20 ±0.52	2.40 ±0.28	2.60 ±0.39	2.20 ±0.22	2.26 ±0.42	2.36 ±0.47
	90	1.98 ±0.36	1.99 ±0.31	2.05 ±0.40	2.12 ±0.29	1.99 ±0.20	2.09 ±0.14	2.16 ±0.38	1.98 ±0.24	2.10 ±0.35	2.23 ±0.31	1.97 ±0.16	2.03 ±0.21	2.06 ±0.41

Changes in moisture content of raw beef sausage samples

Control samples (3% starch) contained less moisture content (62.62%) than other samples, which contained moisture in the range of 62.68 to 64.61%.

In conclusion, raw beef sausage prepared with dry *Monascus* biomass and dry red rice showed higher moisture content than the control samples after 90 days of frozen storage. These results agree with results obtained for WHC and cooking yield of sausage samples, and are in harmony with Cheng & Ockerman (1998).

Change in protein content of raw beef sausage samples

The protein content of control samples (15.13%) was close to the protein levels of other beef sausage samples (from 15.14 to 15.52%). The data showed that a continuous decrease in protein content in all sausage samples throughout the storage period. This decrease in protein content may be due to protein hydrolysis by natural meat enzymes (cathapsins) and bacterial enzymes that are produced before freezing as well as the loss of water soluble protein with separated drip. These results are similar to that obtained by Wagner (1976) and Madkour *et al.* (2000).

Change in fat content of raw beef sausage samples

The fat content of raw control sausage was 13.31% and those containing *Monascus* pigments ranged from 13.37 to 14.08%. The fat content increased during storage due probably to water loss. This increase of fat content was observed in samples prepared with (0.45%) dry *Monascus* biomass (from 14.08 to 14.71%), which had a higher fat content from the start to the end of frozen storage period. This finding was observed by Hunt *et al.* (1990).

Change in ash content of raw beef sausage samples

Results indicated that, samples prepared by (0.75%) red rice contained the highest value (2.16%), followed by the sample prepared with (0.45%) dry biomass (2.12%) after 90 days storage. The ash content was slightly decreased in all samples. The loss in ash content affected by frozen storage may be due to the decrease of mineral content, which are soluble in water and lost in separated drip. These results are in agreement with the results obtained by Barbut *et al.* (1984).

Thiobarabituric acid (TBA)

The thiobarabituric acid (TBA) test has been widely used to estimate the extent of lipid oxidation in meat and meat products (Wu *et al.*, 2000). TBA values (expressed as O.D) of beef sausage containing different *Monascus* pigments were measured during frozen storage of different samples and the results are given in Table 6. All sausage samples had similar TBA value at zero time of storage. During storage, TBA values tended to increase and the control samples recorded the highest TBA values after 90 days of storage. The TBA values increased as a function of storage time, indicating some fat oxidation during storage. These results are in agreement with Cheng & Ockerman (1998) as well as Madkour *et al.* (2000).

Total volatile nitrogen (TVN)

Total volatile nitrogen values were used as an indicator for protein hydrolysis, the results are given in Table 6 and showed that, all sausage samples had similar TVN values at zero storage (12.56 - 12.58 mg TVN /100g sample). A slight increase in TVN was observed during storage of different samples (13.92 to 16.64 mg TVN /100g sample). The increase in TVN during frozen storage of sausage samples might be attributed to the break-down of nitrogenous substances by microbial activity. These results are in agreement with those of Madkour *et al.* (2000).

TABLE 6. Thiobarbituric acid (TBA) and total volatile nitrogen (TVN) of raw beef sausage produced using some fermented food industry wastes by *M.purpureus* during frozen storage at -18 °C.

Storage period (days)	Thiobarbituric acid (TBA)												
	Treatments												
	Control	M1	M2	M3	R1	R2	R3	G1	G2	G3	B1	B2	B3
Zero	0.037	0.032	0.033	0.035	0.034	0.034	0.035	0.036	0.037	0.037	0.033	0.035	0.036
	±0.005	±0.001	±0.002	±0.002	±0.001	±0.002	±0.003	±0.002	±0.001	±0.002	±0.004	±0.003	±0.003
30	0.081	0.051	0.052	0.071	0.066	0.071	0.086	0.076	0.081	0.092	0.066	0.076	0.082
	±0.004	±0.005	±0.005	±0.004	±0.002	±0.004	±0.002	±0.001	±0.004	±0.003	±0.002	±0.001	±0.002
60	0.316	0.243	0.296	0.307	0.289	0.306	0.362	0.296	0.301	0.316	0.299	0.301	0.316
	±0.007	±0.006	±0.006	±0.001	±0.005	±0.003	±0.010	±0.003	±0.003	±0.003	±0.004	±0.003	±0.004
90	0.385	0.309	0.326	0.341	0.322	0.341	0.376	0.306	0.317	0.357	0.302	0.311	0.326
	±0.006	±0.004	±0.004	±0.004	±0.004	±0.005	±0.012	±0.006	±0.006	±0.005	±0.009	±0.005	±0.007
	Total volatile nitrogen (TVN)												
Zero	12.56	12.57	12.57	12.58	12.57	12.57	12.58	12.57	12.58	12.58	12.58	12.58	12.58
	±0.15	±0.20	±0.38	±0.13	±0.26	±0.30	±0.20	±0.35	±0.31	±0.41	±0.25	±0.25	±0.32
30	13.61	12.97	13.06	13.11	12.96	12.97	12.99	12.96	13.08	13.11	12.81	12.96	13.09
	±0.17	±0.37	±0.48	±0.66	±0.41	±0.46	±0.65	±0.36	±0.20	±0.59	±0.15	±0.33	±0.30
60	15.89	13.42	14.81	15.31	13.26	13.31	13.61	13.98	14.03	14.06	13.69	13.86	14.21
	±0.57	±0.46	±0.41	±0.51	±0.41	±0.25	±0.21	±0.31	±0.29	±0.39	±0.21	±0.55	±0.18
90	16.64	14.52	15.09	15.62	13.92	14.09	14.23	14.52	14.67	14.83	14.60	14.81	15.06
	±0.39	±0.36	±0.26	±0.26	±0.31	±0.36	±0.42	±0.29	±0.22	±0.40	±0.48	±0.58	±0.61

Microbial analysis of beef sausage during storage

The various beef sausage samples were microbiologically evaluated for total viable bacterial count, total viable *Coliform* count, and viable count of *Staphylococcus* sp., also detection of *Salmonella* immediately after processing as well as during storage for 90 days at -18°C, and the results are given in Table 7. The initial total viable bacterial count, total *Coliform* count and *Staphylococcus* sp. count at zero time of storage period ranged from (9 x 10^5 to 3 x 10^4), (5 x 10^3 to 2 x 10), and (2 x 10^2 to 2 x 10) cfu / g of tested samples, respectively.

Detection of *Salmonella* was determined in all sausage sample and the results were negative for *Salmonella*. These results agree with the results of Rao *et al.* (1984).

TABLE 7. Total viable bacterial count (cfu /g), total *Coliform* count (cfu /g) and viable count of *Staphylococcus* sp. (cfu/g) of raw beef sausage produced using some fermented food industry wastes by *M. purpureus* during frozen storage at -18 °C.

Storage period (days)	Total viable bacterial count (cfu /g)												
	Treatments												
	Control	M1	M2	M3	R1	R2	R3	G1	G2	G3	B1	B2	B3
Zero	9×10^5	7×10^5	4×10^5	1×10^5	5×10^5	7×10^4	3×10^4	8.5×10^5	2×10^5	7×10^4	3×10^6	9×10^5	5×10^5
30	3×10^5	1.4×10^5	8×10^4	6.7×10^4	9×10^4	3.6×10^4	9.2×10^3	6×10^5	8.6×10^4	2.3×10^4	8.4×10^5	4.6×10^5	9×10^4
60	6.8×10^4	6×10^4	3.2×10^4	9×10^3	2.4×10^4	8×10^3	3×10^3	1.2×10^5	2×10^4	8×10^3	4.3×10^5	9.6×10^4	5×10^4
90	7.6×10^3	5×10^4	8.3×10^3	1.3×10^3	6.3×10^3	1.4×10^3	8×10^2	7.6×10^4	5.5×10^3	1.2×10^3	8.7×10^4	3×10^4	2×10^4
	Total *Coliform* count (cfu /g)												
Zero	3×10^3	2×10^3	5×10^2	9×10	2×10^2	8×10	2×10	3×10^3	3×10^2	2×10^2	5×10^3	3×10^3	7×10^2
30	2.6×10^3	9.2×10^2	1.4×10^2	6×10	7.6×10	4×10	8×10^0	8.3×10^2	1.6×10^2	6.7×10	1.3×10^3	8.4×10^2	2×10^2
60	1.8×10^3	6.2×10^2	8.6×10	3.2×10	3.6×10	1.8×10	3×10^0	4.4×10^2	7.2×10	3.1×10	8×10^2	2.8×10^2	9.3×10
90	4×10^2	2×10^2	4×10	9×10^0	7×10^0	ND	ND	8×10	3×10	6×10^0	4×10^2	7×10	2×10
	Viable count of *Staphylococcus* sp. (cfu/g)												
Zero	2×10^2	7×10	2×10	ND	2×10	ND	ND	2×10^2	6×10	ND	1×10^2	6×10	ND
30	$7.3 X 10$	$3 X 10$	$6 X 10^0$	ND	$7 X 10^0$	ND	ND	9.3×10	$2.4 X 10$	ND	7.6×10	2.1×10	ND
60	2.6×10	1.6×10	ND	ND	ND	ND	ND	4.4×10	9×10^0	ND	3.3×10	1.1×10	ND
90	7×10^0	4×10^0	ND	ND	ND	ND	ND	7×10^0	3×10^0	ND	6.8×10^0	4×10^0	ND

Sensory evaluation of beef sausage during frozen storage

The sausage samples prepared with various *Monascus* pigment sources were conducted to sensory evaluation of appearance, juiciness, tenderness, colour, aroma and overall acceptability.

Appearance

Means of appearance of sausage samples prepared by using different sources of *Monascus* pigments stored at -18°C for 90 days are shown in Table 8. The mean appearance score values of sausage control samples were 7.2 at zero time and 6.6 after 90 days of frozen storage. In contrast, the sausage samples prepared with dry biomass (M1, M2 and M3) had mean appearance scores of 8.4, 9.1 and 9.1, respectively before storage, whereas the samples prepared using red rice (R1, R2 and R3) the mean score values slightly increased to 9.1, 9.4 and 8.8, respectively.

Control beef sausage which contained 3 % starch had the lowest mean values of appearance from the start to the end of frozen storage period, and the samples containing 0.25% and 0.5% dry red rice (R1 and R2), 0.3% and 0.45% dry biomass (M2 and M3) had a higher scores.

TABLE 8. Means values for appearance, juiciness, tenderness, colour, aroma and overall acceptability scores of cooked beef sausage produced using some fermented food industry wastes by *M. purpureus* during frozen storage at -18 °C.

Storage period (days)	Control	M1	M2	M3	R1	R2	R3	G1	G2	G3	B1	B2	B3	L.S.D**
	Means * of appearance scores													
Zero	7.2 c	8.4 abc	9.1 ab	9.1 ab	9.1 a	9.4 a	8.8 ab	8.1 abc	8.1 abc	8.0 abc	8.0 abc	7.9 bc	8.1 abc	1.49
90	6.6 b	7.5 ab	7.9 ab	7.9 ab	8.0 ab	8.7 a	7.9 ab	6.8 ab	8.7 a	7.3 ab	7.3 ab	7.8 ab	7.8 ab	2.03
	Means * of juiciness scores													
Zero	7.6 b	8.3 ab	8.7 ab	9.1 a	9.0 ab	9.1 a	9.1 a	8.2 ab	8.4 ab	8.0 ab	8.1 ab	8.1 ab	7.8 ab	1.47
90	6.4 d	7.9 abcd	8.7 ab	8.2 abc	7.9 abcd	9.0 a	8.6 ab	6.9 cd	8.7 ab	7.9 abcd	7.4 bcd	8.1 abc	8.5 ab	1.55
	Means * of tenderness scores													
Zero	7.3 b	8.4 *ab*	8.7 ab	9.2 a	9.0 a	9.1 a	9.0 a	8.2 ab	8.3 ab	8.0 ab	8.1 ab	8.1 ab	7.8 ab	1.45
90	6.3 c	7.6 abc	8.3 a	8.2 ab	7.8 abc	8.7 a	8.3 a	6.5 bc	8.6 a	7.9 abc	7.5 abc	7.8 abc	8.3 a	1.70
	Means * of color scores													
Zero	6.9 c	8.1 bcde	8.8 abc	9.3 ab	9.1 abc	9.5 a	9.1 abc	7.4 de	7.8 cde	8.5 abcd	8.2 abcde	8.2 abcde	8.2 abcde	1.33
90	6.3 c	7.9 ab	8.4 ab	8.5 ab	8.3 ab	9.0 a	8.4 ab	7.3 bc	8.4 ab	7.6 abc	7.4 bc	8.3 ab	8.2 ab	1.48
	Means * of aroma scores													
Zero	7.8 d	8.4 abcd	8.8 abcd	9.2 ab	9.1 abc	9.5 a	9.0 abcd	7.9 cd	7.9 cd	7.9 cd	8.1 bcd	8.4 abcd	8.3 abcd	1.28
90	6.2 d	7.5 bc	8.4 abc	8.7 ab	8.4 abc	8.9 a	8.3 abc	7.2 cd	8.5 ab	7.8 abc	7.5 bc	8.5 ab	8.0 abc	1.27
	Means * of overall acceptability scores													
Zero	7.4 c	8.4 abc	9.0 ab	9.3 a	9.15 ab	9.4 a	9.2 ab	8.15 abc	8.0 bc	8.0 bc	8.25 abc	8.4 abc	8.15 abc	1.26
90	6.2 d	7.8 bc	8.3 abc	8.65 ab	8.2 abc	9.2 a	8.5 ab	7.1 cd	8.7 ab	7.8 bc	7.5 bc	8.3 abc	8.1 abc	1.27

*Means in the same row showing the small letters described the effect of treatments are not significantly different (P > 0.05).

** L.S.D: least significant different.

Juiciness

There were no significant differences ($P > 0.05$) observed in the panel scores of juiciness (Table 8) between sausage samples prepared by different sources of *Monascus* pigment, but the control sample showed significant differences compared with (M3, R2 and R3) samples at zero time and had the lowest scores from the start to the end of storage period. Sample prepared by 0.5% red rice (R2) had higher means of scores after 90 days storage.

Analysis of variance indicated that, the sensory juiciness scores were insignificant decreased ($P > 0.05$) by time as would be expected. Cheng & Ockerman (1998) found the similar results, indicated that the sensory juiciness evaluation decreases with increased storage time.

Tenderness

Data in Table 8 show that the tenderness scores followed similar trends as juiciness. The juicy samples were found to be more tender and those with lower juiciness scores were more tough and dry. There were no significant differences between samples with *Monascus* pigments, but the control sample had significant differences ($P < 0.05$) compared to M3, R1, R2 and R3 treatments and had the lowest scores. Analysis of variance indicated that, the sensory tenderness scores of all samples were insignificantly decreased with increasing storage period to 90 days at -18°C. In conclusion, the higher or lower juiciness and tenderness scores might be due to the closely parallel percent of fat indicating the relationship between juiciness and fat content. These results are in harmony with Cheng & Ockerman (1998).

Colour

Colour is one of the most important aspects of sausage because colour is one criterion a consumer uses to select sausage from the grocer's shelf. The colour of sausage samples is primary provided by pigments. The mean data in Table 8 indicates that control samples had the lowest scores from the start to the end of storage, and that samples prepared with 0.3, 0.45% dry biomass and 0.25, 0.5 and 0.75 dry red rice had higher scores. The data showed no significant differences for colour with increasing storage period, but all samples slightly decreased with the increasing of storage periods. Fabre *et al.* (1993) indicated that *Monascus* pigments remained stable when stored for three months at 4°C under vacuum for sausage and decrease of hue value during the first 45 days was due to dehydration of the product.

Aroma

Data of Table 8 indicate that control samples had lowest scores from the start to the end of storage. In the same time at zero storage time, samples of sausage prepared with (M3, R1 and R2) have the highest scores. At the of storage period 90 days all sausage samples, except (G1) have highest scores compared to control samples.

Overall acceptability

Control samples had the lowest scores from the start to the end of storage (Table 8). At zero storage time, samples of sausage prepared with (M3, R1 and R2) had the highest scores. At the end of storage period (90 days) all sausage samples, except (G1), had higher scores compared to control samples. On contrary, the mean scores (6.2) of control sample were lower at the end of time of frozen storage at -18°C. In contrast, the R2 and M3 had higher mean scores (9.2 and 8.65, respectively) compared with the other treatments.

In conclusion it is to be noted that, the samples prepared with dry biomass and dry red rice had the overall acceptability of beef sausage samples in concentrations of 0.45% and 0.5%, respectively, followed by the samples prepared with dry red rice and dry red corn bran in concentrations of 0.75% and 0.5%, respectively.

Conclusion

The use of dry biomass in concentrations of 0.3 and 0.45%; dry red rice in concentrations of 0.25, 0.5 and 0.75% in preparation of beef sausage samples improved the water holding capacity, plasticity, cooking yield, hardness and also sensory properties of the produced samples especially during frozen storage.

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إســتخدام صــبغات فطــر *Monascus purpureus* المنتجــة باستخدام بعض مخلفات تصنيع الأغذية فــي تصــنيع الســجق البقري

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تم تتبع الخواص الطبيعية والكيميائية والتقييم الحسي لعينــات الســجق البقــرى المحتوية على مصادر مختلفة لصبغة الـ *Monascus* خلال ٩٠ يــوم مــن التخزين تحت ظروف تجميد على – ١٨ °م وقد أوضــحت النتــائج المتحصــل عليها ما يلي:–

- حصلت عينات الكنترول (الضابطة) المحتوية على ٣٪ نشا على اقل نسبة مئوية لقيمة ناتج الطهي ٨٢,٩٪ وتقريبا أعلى نسبة لفقد الطهي (١٧,١١٪) في بداية التخزين بالتجميد ، في حين أن عينات السجق المحتوية على ٠,٢٥٪ أرز أحمر مجفف أعطت أعلى نسبة مئوية لناتج الطهي (٩١,١١٪) وأقل قيمة لفقد الطهي (٨,٨٧٪).
- وجد أن هناك انخفاض متقدم في قيم المقدرة على الإرتباط بالماء (WHC) في جميع العينات خلال فترات التخزين وكانت العينة الكنترول أقل العينات في القدرة على الإرتباط بالماء مقارنة بالعينات الأخرى المحتوية على المصادر المختلفة لصبغة الــ *Monascus* كما وجد أن هناك انخفاض في القيم البلاستيكية في جميع عينات السجق مع التخزين بالتجميد على– ١٨ °م وحتى مرور ٩٠ يوم من التخزين.
- وجد أن المكونات الكيميائية الموجودة في عينات السجق تتأثر خلال فترة التخزين بالتجميد وأن قيم كلا من الرطوبة والبروتين والرماد يحدث لها إنخفاض مع زيادة مدة الحفظ بالتجميد على العكس يحدث زيادة بسيطة في المحتوى الدهني للعينات.
- دلت النتائج على أن أعلى زيادة في قيمة الــ TBA وجدت في عينة المقارنة ولوحظ أن هناك زيادة بسيطة لجميع العينات في قيم الــ TVN بعد التخزين على درجة حرارة – ١٨ °م لمدة ٩٠ يوم.
- وجد أن تخزين عينات السجق المختلفة يسبب انخفاضا في العدد الابتدائي الكلى للبكتريا ومجموعة الكوليفورم وكذلك العدد الكلى لميكروبات *Staphylococcus* sp.
- وجد من نتائج التحكيم الحسي المتحصل عليها أن عينات السجق المحتوية على ٠,٣٪ ، ٠,٤٥٪ ميسليوم الفطر الجاف وكذلك العينات المحتوية على ٠,٥٪ ، ٠,٧٥٪ أرز أحمر جاف أعطت أعلى معدلات للتقييم الحسي مقارنة بباقى العينات ، وأوضحت النتائج أن أعلى درجات التقييم الحسي للعينات لصفات المظهر العام والعصيرية والطراوة واللون والطعم والرائحة والقبول العام كانت ملحوظة مع العينات المحتوية على ٠,٣٪ و٠,٤٥٪ ميسليوم الفطر الجاف وكذلك مع ٠,٢٥٪ ، ٠,٥٠٪ ، ٠,٧٥٪ ارز أحمر جاف وكذلك ٠,٥٪ ردة الذرة الحمراء، ٠,٧٥٪ مخلوط مصاصة القصب مع البروتيلان الحمراء.