Octopus Ink as a Source of Melanin: Preparation, Evaluation, and Utilization

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ABSTRACT

Octopus ink, as fish waste, is a good source of melanin pigment powder was prepared from the ink sac, Gross chemical composition, mineral content, total phenolic content, amino acid content, protein pattern, UV-vis spectra, microbiological examination, and antioxidant activity were determined. Protein, ash, fat, total phenolicand antioxidant activity were 36.59%, 8.90%, 0.08%, 218.06 mg/kg and 46.40%, respectively. Magnesium and copper were 116.53 and 15.15 mg/100g, respectively. Serine content was 56.4%.The powder was free from Salmonella spp/25g. Melanin powder was added to colorize pasta and olive paste, and the results of the sensory evaluation indicated that pasta cooked in 1% melanin powder were highly accepted by panelists. Melanin pigment powder from the octopus ink sac can be used as a natural black coloring agent in certain food product.

Key words: Octopus vulgaris, octopus ink, melanin pigments, melanin extraction.

INTRODUCTION

Fish wastes were defined as by-catch products having low commercial value, unconsumed body parts, undersized or damaged commercial species, as well as species of commercial value but having insufficient amounts to warrant sale. Fish waste involves skin, bones, entrails, shells, or ink secreted by some species of fish. Octopus ink, as fish waste is non-edible and has low commercial value. In many countries, they're thrown away or disposed of, which incurs unnecessary costs (Arvanitoyannis & Kassaveti, 2008). The consideration stresses the importance of finding good ways of managing and utilizing fish wastes, taking into account the possibility of utilizing them not only for fish feeds but also as potential sources of bioactive compounds (Kim & Mendis, 2006).

Despite the low value traditionally assigned to fish wastes, from the huge mass of unconsumed or underutilized resources, a significant amount of bioactive compounds can be applied to pharmaceutical and biotechnological purposes, including protein hydrolysates, lipids, astaxanthin, and chitin. Such utilization represents an added value from an economic point of view (Rochet &Trenkel, 2005, Davies *et al.,* 2009).

The octopus is a soft-bodied marine organism that belongs to the Order Octopoda; Class: Cephalopoda; Phylum: Mollusca and kingdom animals contain glands and sacs that contain ink, which is powerfully toxic to their prey but not harmful to humans (Rochet &Trenkel, 2005). The ink contains a high amount of melanin (Nauen, 2003); the name melanin originates from the ancient Greek *melaα nos*, meaning "dark". The term was first applied and used by the Swedish chemist Berzelius in 1840 to describe a dark pigment extracted from eye membranes (Berzelius, 1840).

Melanin is a natural pigment that is mostly found in organisms, including animals called animal melanin, plants such carrot, fungi, and bacteria such as *Asparagus bisporus* (Mourad *et al.,*2015). The major functions of melanin are the coloration of human skin, hair, and eyes. Harki *et al. (1997*) reported that dark-skinned people have more melanin in their skin than white-skinned people. Melanin pigments synthesised through a multiple-stage chemical process known as melanogenesis were generated by the oxidation of the amino acid tyrosine followed by polymerization, and these processes to produce melanin pigments occurr in specialized cells called melanocytes. Melanin pigments from fungi and bacteria are called microbial melanin (Rosas *et al*., 2000, Wang *et al*., 2006).

Melanin pigments vary in terms of structure and function (Zhang & Kearns, 2010). It's synthe-

sized from amino acids but it is not a protein. The chemical structure of melanin is made up of chains of carbon, hydrogen, oxygen, and nitrogen. The chemical formula of melanin is $C_{18}H_{10}N_2O_4$ and a molecular weight of 318 grams per mole. There are three basic types of melanin: eumelanin, pheomelanin, and neuromelanin. Eumelanin contains two groups, which are black eumelanin and brown eumelanin. Eumelanin is the most common melanin pigment. Pheomelanin is the type of melanin that contains polybenzothiazine parts, which are responsible for red-colored hair. Pheomelanin is a cysteine-derivative melanin. Another type of melanin is neuromelanin, which is found in the brain. Melanin pigments are made by biopolymer complex compounds that are found in two forms: eumelanin and pheomelanin. Each form differs in the molecular structure and weight of the precursors (Glass *et al*., 2012, Mourad *et al.,* 2015).

Derby (2014) showed that the chemical contents of the *Loligo duvauceli* ink had a moisture content of 79.25%, protein (13.67%), lipid (0.91%), ash (2.5%), and carbohydrate content of 5.59%. The *Sepia officinalis* ink had moisture content (78%), ash (5.24%), protein (18.71%), carbohydrate (0.8%), and lipid content (3.33%) (Thanonkaew *et al.,* 2006; Lopez-Gonzalez *et al*., 2014,Ganesan *et al*., 2017).

The method of melanin extraction depends on the sources from which melanin is extracted and the form of melanin required, either in suspension or liquid form. The melanin can be extracted either by using acetone or under vacuum (Saini &Melo, 2015, Mourad *et al*., 2015). Also, the method of extraction depends on the nature and type of melanin, such as homopolymer melanin, which is a type of aromatic compound that is unstable at a temperature above 31 °C and loss a unit of indole-5,6-quinone with a release of heat of 120.2 J g^{-1}. When heat is applied to it, broad exotherm occurs with a heat evolution of 42.56 J g^{-1} and a heat absorption of 40.39 J g^{-1}, and the residual organic mass is converted into gases (Fabo, 2005).

Melanin from various sources exhibits significant antioxidant activity; the tendency and antioxidant power of melanin depend on its source. The role of melanin is to act as a scavenger for superoxide anions and singlet oxygen species. Melanin pigment interacts with reactive oxygen species, which are produced through physiological reactions (Luo & Liu, 2013). Melanin extracted from the tea leaves was found to inhibit the oxidation of low-density lipoproteins, which supports the idea of an inhibitory effect of melanin against peroxyl radicals and other free radicals (Nofsinger *et al.,* 2002). In addition, reduced tea melanin were found to be more effective as anticarcinogenic agents than non-reduced melanin (Fabo, 2005, Wu *et al.,* 2008).

Application of melanin in food packaging used in coated nanostructures that have antimicrobial activity against food pathogens can be considered suitable for many applications of food packaging because they are more effective for inhibiting microbial growth, especially pathogens, resulting in extending the shelf life of packed food products (Horneck *et al*., 2010).

MATERIALS AND METHODS

Materials

Octopus Ink

Ten litters of octopus ink were collected from a fish market in Dar es Salaam, Tanzania.

Sample packaging and transportation

The sample of octopus ink was put into a plastic bottle and then transported in a frozen state in an ice box to the Laboratory of Food Analysis, Faculty of Agriculture, University of Alexandria, Egypt. All chemicals, reagents and other materials used in this study were purchased from El-Gomhoria Company and local markets in Alexandria City, Egypt.

Methods

Technological methods

Preparation of melanin powder

About 750 milliliters of octopus ink were treated with one drop of concentrated HCl and filtered by sieve to remove any impurities, then 5g of dimethyl oxalate (DMO) added to obtain a complete dissolve of melanin. Then, 100 ml of acetone added to precipitate melanin. Precipitated melanin was separated from the solution by decantation. The decanted melanin was purified three times by washing with distilled water and precipitating with acetone. There after, the melanin was placed in a circulated hot air fan oven (Model PS0-451, India), at 25 ^{o}C overnight to get melanin slag. The slag was weighed then milled into powder and used for the chemical and physical analysis as described by LI Xing-wang *et. al.,* (2009).

Utilization of melanin powder

Melanin powder of octopus was used as a colouring agent in black pasta and olive paste as following: -

Preparation of black pasta

One kilogram of wheat pasta was divided into three equal parts. Each part was cooked in water after adding 1, 5, and 10% (w/v) of melanin powder separately. Then, pasta ingredients, i.e., 2 ml of vinegar, 20g of sugar, and 5 g of salt, were added. The mixture was left for a few minutes until the pasta was cooked. Cooked pasta was put into the sieve for draining the boiled solution. The final product of pasta was put into a dish meal to be ready for sensory evaluation.

Preparation of olive paste

One kilogram of pickled olives was divided into three groups. Each group was boiled for 15 minutes with a melanin powder solution of 1%, 5%, and 10% (w/v), and 2 ml of vinegar and 5g of salt were added separately. The cooked olives mixture was put into a sieve for draining the boiled solution. Finally, olive products were ground by mortars and pestles to get a paste which packed in a dish meal to be ready for sensory evaluation.

Analytical methods

Chemical composition

Crude protein (NX6.25), crude fat and ash content were determined according to AOAC (2000). Carbohydrate was calculated by the difference (%Carbohydrate = 100 – (%Moisture + %Protein + %Fat+ %Ash)).

Determination of mineral content

Minerals (calcium, copper, manganese, magnesium, zinc and iron) were determined by Atomic Absorption Spectrophotometer (9 Series - AA Spectrometers, PerkinElmer) as described by A.O.A.C. (2000).

Amino acid analysis

Quantification and identification of amino acids were analyzed and determined by High-Performance Liquid Chromatography using an LC-300 HPLC system (Sykam, Chroma Tech, Germany) that equipped with two detectors set at 570 and 440 nm (λmax). 0.1 ml of sample was separated on a cation exchanger resin column (50 mm x 4 mm x 5 μmi.d No.2619 resin). The column (300mm-length and 7.8mm-dimeter) operated at 17°C using citrate buffer at pH 2.2 with a flow rate of 1.0 mL/min as described bySchmidt *et al.,* (2006).

Determination of total phenolic content

Total phenolic was determined by Folin – Ciocalteu reagent as described by AOAC (2000).

Protein pattern by electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used for separation and identification of proteins as described by Pennington & Dunn (2001) and Marion *et al.*, (2010). The apparatus used was a gel casting system, 10 cm x 10 cm, FB-GC10-1 (model 422 Electro-Eluter), and the electrical field was set at 200 volts for 2 hours at 15^{O}C. The stacking gel used was 4%, and the resolving gels were 7.5% and 12%. Following electrophoresis, the gel was stained with 0.05% Coomassie Brilliant Blue in 15% methanol and 5% acetic acid and distained with 30% methanol and 10% acetic acid. The separated bands were identified by comparison with a high molecular weight standard protein marker (Bio-Rad Laboratory, USA).

Determination of the pH value.

The pH value was measured by pH meter (AD1020,Adwa - Professional pH-ORP-Temp Bench Meter with GLP, Hungary) at 20^{o}C as described by De-Silva (2003).

Spectroscopic scanning analysis of melanin pigments

Half gram of purified melanin pigments and 0.05 ml of ink of octopus were dissolved in 10 ml of 1 mol/L KOH. The ultraviolet and visible absorption spectrum of the octopus melanin pigments scanned in the wavelength range from 300 – 760 nm with a UV–visible Spectrophotometer (BOEGO spectrophotometer model S-220 UV/VIS, Germany). 1 mol/L KOH solution was used as a reference(Blank **)** and absorbance (λ_{max}) were recorded as described by Bothma *et al*(2008) and Kim *et al*(2013).

Determination of antioxidant activity

The antioxidant activity of ink and purified octopus melanin powder was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH), as explained by Bothma *et al.* (2008). The diluted working solutions of the test samples were prepared in

methanol. One ml of DPPH (2% in methanol) was mixed with 1 ml of a tested sample in methanol. The mixture was shaken well and left to settle in the dark chamber for 30 minutes. Absorbance was measured at 517 nm using a Lambda 25 UV-visible spectrophotometer (Perkin-Elmer). Ascorbic acid was used as a positive control.

The radical scavenging activity was measured as a decrease in the absorbance of DPPH. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. DPPH radical scavenging activity is calculated by using the following formula:

The absorbance of DPPH was plotted against the tested sample concentrations as a standard curve to calculate the radical scavenging activity of the samples.

Microbiological examination

All microbiological examinations were carried out in the quality control lab of the factory for gelatin manufacturing factor, Alexandria, Egypt.

Total bacterial count

Ten grams of sample were dissolved in 90 ml of distilled water in a water bath at 45^{o}C. One ml of solution was added to a 9 cm-diameter Petrie dish that contains nutrient agar (N.A.) medium and incubated at 32^{0}C for 48 hours. The total count of bacterial colonies was recorded (CFU/g) as the number of colonies per gram of sample (Anderson & Shi, 2006).

Salmonella sp.

One ml of solution which prepared for the total count was added to a 9 cm-diametric Petrie dish that contains *Salmonella Shigella* Agar (SSA) medium and incubated at 32^{0}C for 48 hours. The total count of bacterial colonies was recorded and reported as the number of colonies per gram (CFU/g) (Banerjee *et al.,* 2014).

Coliform bacteria

Ten ml of sample were dissolved into 90 ml of double distilled water in a water bath at 45^{o}C. One ml of solution was added to a 9 cm-diameter Petrie dish, which contains solidified MacConkey medium, and incubated at 32^{0}C for 48 hours. The total count of bacterial colonies was recorded and reported as the number of colonies per gram (CFU/g) (Casadevall *et al*., 2000).

Sensory evaluation

Texture, taste, colour, odour and overall acceptability of the samples were evaluated by 25 panelists, a hedonic scale of 1 -9 was used according to Rustad (2003).

Statistical analysis

All data were statistically analyzed through analysis of variance (ANOVA) and Duncan's multiple comparison range tests at a 5% level of probability (Jones, 2004).

RESULTS AND DISCUSSION

Melanin powder

Chemical analysis of octopus ink and melanin powder

The moisture content of octopus ink was 82.36 ± 0.56%, as shown in Table (1). Derby (2014) found the moisture content of *L. duvauceli* ink was 79.25%, as well as the moisture content of octopus ink, was 80.09%, as reported by Lopez-Gonzalez *et al.* (2014). The *Sepia officinalis* ink contained 78% moisture (Thanonkaew *et al.*,2006) while the *L. vulgaris* ink contained 81.40% moisture content (Nadarajah *et al.,* 2017).The amount of protein in octopus ink was 47.17±4.04 % (Table 1) based on dry weight. The octopus's ink contains a higher amount of protein content than Sepia ink, ranging from 43.41± 0.72% (for female ink) to 40.12 ± 0.50% (for male ink) (Mai *et al.*, 2006). Derby (2014) and Ganesan *et al*. (2017) reported that the protein content of *L. duvauceli* ink was 45.62%, and cuttlefish ink contained 46.71%. Therefore, it may be considered that octopus ink is a good source of protein.

Ash content of octopus ink was 15.82 ± 0.014% on DWB (Table 1). Derby (2014) and Nadarajah *et al.* (2017) reported that, ash content of *Octopus vulgaris* ink were 18.3%, 14.5%, and 12.91%, respectively. The fat content of octopus ink was 0.201± 0.014% (Table 1). Lian *et al.*, (2005) showed that the male squid ink of cephalopods contains a higher amount of fat than the female squid ink. The *L. duvauceli* ink had 0.91% fat, as reported by Derby (2014). Meanwhile, Thanonkaew *et al.* (2006) showed that the *Sepia officinalis* ink had 3.33% fat, which is higher than *L. duvauceli*(1.07%), as reported by Nadarajah *et al.*, (2017). Carbohydrates in octopus ink and melanin powder

Table 1: Chemical composition of octopus ink and melanin powder*

Parameter	Octopus ink***		Melanin powder***	
	Wet basis	Dry basis	Wet basis	Dry basis
Moisture	82.36±0.566		14.90 ± 2.470	
Crude protein **	8.32 ± 0.042	47.166[a]	31.14 ± 0.671	36.593[b]
Ash	2.79 ± 0.014	15.816[a]	7.575 ± 0.177	8.901[b]
Fat	0.08 ± 0.007	0.201	0.07 ± 0.002	0.085
Carbohydrate	6.33 ± 0.306	35.884	46.315±2.104	54.437
pH value	7.1 ± 0.141		6.35 ± 0.145	

*Mean ± SD **Crude protein = %(N) x 6.25
***There's significant difference between rows at $P \leq 0.05$

contributed 35.88% and 54.437%, respectively, as shown in Table (1). The gross chemical composition of octopus melanin powder (Table 1) revealed that melanin powder contains 36.59 ± 0.67 % protein, 0.08 ± 0.0018% fat, and 8.90 ± 0.147% ash. Octopus melanin powder contains a higher amount of protein compared to the other cephalopod squid inks (Nair *et al.,* 2012).

The pH value of octopus ink and melanin powder

The pH of the octopus ink solution was 7.1 ± 0.1414 (Table .1). Nair *et al.* (2012) reported that the pH of cuttlefish ink was 7.26±0.02.Palumbo (2003) showed the pH of squid ink and cuttlefish ink powder was 6.48±0.01 and 7.18±0.02 respectively. The pH value is an intrinsic factor affecting the growth of microorganisms in squid and cuttlefish ink powders (Palumbo, 2003). The pH of the melanin powder was 6.35 ± 0.14, the pH of melanin powder depends on the methods of extraction and final purification. Nair *et al.*, (2011) reported that the pH of cuttlefish ink was 7.26.

Mineral contents of octopus ink and melanin powder

The minerals composition of octopus inkon DWB mg/100g sample are presented in Table 2.The concentrations of copper were 37.392 ± 0.265, zinc (22.196 ± 0.532), iron (5.628 ± 0.456 mg/100g), calcium (5.685 ± 0.350 mg/100g), manganese (5.685 ± 0.098), and magnesium (388.724 ± 12.784)**.** These minerals have significant importance to human body especially for development, regulatory and maintenance.

Georgantelis (2001) and Nauen (2003) found that *L. duvauceli* contains the highest level of copper (20.33 mg/kg), while a low level was found in *S. lessoniana* (0.59 mg/kg). Zinc is always present in seafood, but higher concentrations are found in mollusks. In general, *L. duvauceli* recorded a higher concentration (26.53 mg/kg) while *S. lessoniana* recorded a lower concentration (17.65 mg/kg), as reported by Prafulla *et al.*, (2001).*L. duvauceli* is rich in iron content (3.32 mg/kg). Normally, squid liver has a high content of iron, which was found to range from 26.18 to 214.20 mg/kg.

The data given in Table (2) reveal the following minerals in octopus melanin powder: copper (15.152 ± 0.524) zinc (7.334 ± 0.309), iron (8.457 ± 0.194), calcium (2.119 ± 0.272), manganese (1.970 ± 0.056) and magnesium (116.527 ± 1.776). The mineral contents of melanin powder contained lower amounts of copper, zinc, calcium, and magnesium than ink. This indicated that during melanin extraction, large amounts of minerals were leached out and lost.

Table 2:Mineral contents of octopus ink and melanin powder* (mg/100g) on DBW

Element	Octopus ink	Melanin powder
Copper (Cu)	37.392 ± 0.265[a]	15.152 ± 0.524[b]
Zinc (Zn)	22.196 ± 0.532[a]	7.334 ± 0.309[b]
Iron (Fe)	5.628 ± 0.456[b]	8.457 ± 0.194[a]
Calcium (Ca)	5.685 ± 0.350[a]	2.119 ± 0.272[b]
Manganese (Mn)	5.685 ± 0.098[a]	1.970 ± 0.056[b]
Magnesium (Mg)	388.724± 12.784[a]	116.527± 1.776[b]

*Mean ± SD

**Different letters in rows indicate significantly different values at $P \leq 0.05$

Amino acid composition of octopus melanin powder

The amino acid content of melanin powder are given in Table (3). Octopus melanin powder contained a high amount of serine (56.4%), followed by aspartic acid (6.3%) and isoleucine (5.0%), while lower amounts were observed for phenylalanine (1.3%), leucine (1.0%), and tyrosine (0.4%).

Table 3: Amino acid composition of octopus melanin powder

Amino acid	Amount (mg/100g)	Total acids (%)
Aspartic acid	110.827	6.3
Threonine	57.549	3.3
Serine	991.854	56.4
Glutamic acid	73.204	4.2
Glycine	74.991	4.3
Alanine	63.336	3.6
Cystine	52.560	3.0
Valine	56.865	3.2
Methaionine	47.126	2.7
Isoleucine	87.144	5.0
Leucine	17.668	1.0
Tyrosine	6.363	0.4
Phenylalanine	22.008	1.3
Histidine	66.932	3.8
Lysine	29.138	1.7
Total amino acids	1757.564	100.0
TEAA %*	925.3	52.6
TNEAA %**	173.27	47.4

*TEAA: Total essential amino acids

**TNEAA: Total non-essential amino acids

Yield of melanin powder

One liter of octopus ink yields 150.03g of melanin powder, meaning that, 1 gram of melanin powder was produced from 6.67 milliliters of octopus ink. This approach has the potential to generate quantity of melanin with a relatively low cost and high yield. Diverse applications and products derived from melanin are dependent on the production cost and quantity of melanin extracted (Saini & Melo, 2015, Patil *et al.,* 2018).

Total phenolic contents of octopus ink and melanin powder

The total phenolic contents of octopusink and melanin powder were 287.5 ± 1.96 mg/g, and 218.06±1.96 mg/g, respectively (Table 4).The octopus ink contained higher total phenolic compounds than melanin powder**.** This may be due to the loss of phenolic compounds during the extraction and purification of melanin powder.

Fig. 1: Photograph of dried octopus melanin

Table 4: Total phenolic contents of octopus ink and melanin powder

Sample	Totalphenoliccontents (mg/g)*
Octopus ink	287.50 ± 1.96[a]
Octopus melanin powder	217.06±1.96[b]

*Mean ± SD

**Different letters in columns indicate significantly different values at P≤0.05

Antioxidant activity of octopus ink and melanin powder

The antioxidant activity of octopus ink and melanin powder was examined based on the free radical scavenging effect of the stability of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The antioxidant activity of octopus ink was 32.843 ± 0.231%, while it was 46.404±0.461% for octopus melanin powder (Table 5).

Octopus melanin powder had higher antioxidant activity than octopus ink. The reason behind this is that melanin powder contains higher concentrations of purified melanin pigments as compared to ink, which contains a low concentration of melanin pigments (Kim *et al.*, 2013).Melanin pigments are natural antioxidant compounds that are capable of inhibiting oxidation by scavenging free radicals. Melanin and other antioxidants react with oxygen species or act through several chemical mechanisms: hydrogen atom transfer (HAT), single electron transfer (SET), and the ability to

chelate transition metals. Thereby, preventing the cell or tissues from being damaged is necessary to maintain normal cell activity (Bothma*et al*., 2008).

Table 5: Antioxidants activity of octopus ink and melanin powder

Sample	Antioxidant activity (%) *
Octopus ink	32.843±0.231[b]
Octopus melanin powder	45.404±0.461[a]

*Mean± SD

**Different letters in rows indicate significantly different values at P≤0.05

Spectrophotometric spectra of octopus ink and melanin powder

A spectrophotometric curve explains how much light is passing through a sample (transmittance) or how much light is absorbed. The absorbance was recorded at the wavelength range of 300nm to 760 nm (Figure 2).The highest absorbance of light foroctopus ink occurs at a wavelength of nearly 360 – 380 nm. When increasing the wavelength, the intensity of absorbance decreases and is almost linear. The lowest value of the light intensity absorbance appeared at wavelengths above 700nm.

For octopus melanin powder, the highest absorbance of light intensity occurred at wavelengths between 360 nm and 380nm. The maximum peak of absorbance and light intensity occurred at a wavelength of 380 nm. Furthermore, an increase in the wavelength decreases the intensity of absorbance. On the other hand, the lowest value of light absorbance was at a wavelength above 700nm (Brenner & Hearing, 2008, Guo *et al*., 2014).

Protein patterns of octopus ink and melanin powder

The protein pattern of octopus ink in comparison with the melanin powder is shown in Figure (3). Both proteins consisted of a mixture of bands with average molecular weight ranges from 5KDa to 155KDa which was almost identical. Octopus ink protein had dense bands at 11kDa, 13kDa, 50 KDa and 55KDa, while those in melanin powder were 20 at KDa, 26KDa, 55KDa and 62 KDa.Melanin powder had a high concentration of a higher molecular weight than octopus ink. This can be attributed to the loss of some low molecular protein weight during the extraction process and thereby increase the protein in melanin powder. The protein pattern and molecular weight of protein subunits depend on the methods of extractions, conditions of extraction and nature of materials used(Nair, 2012).

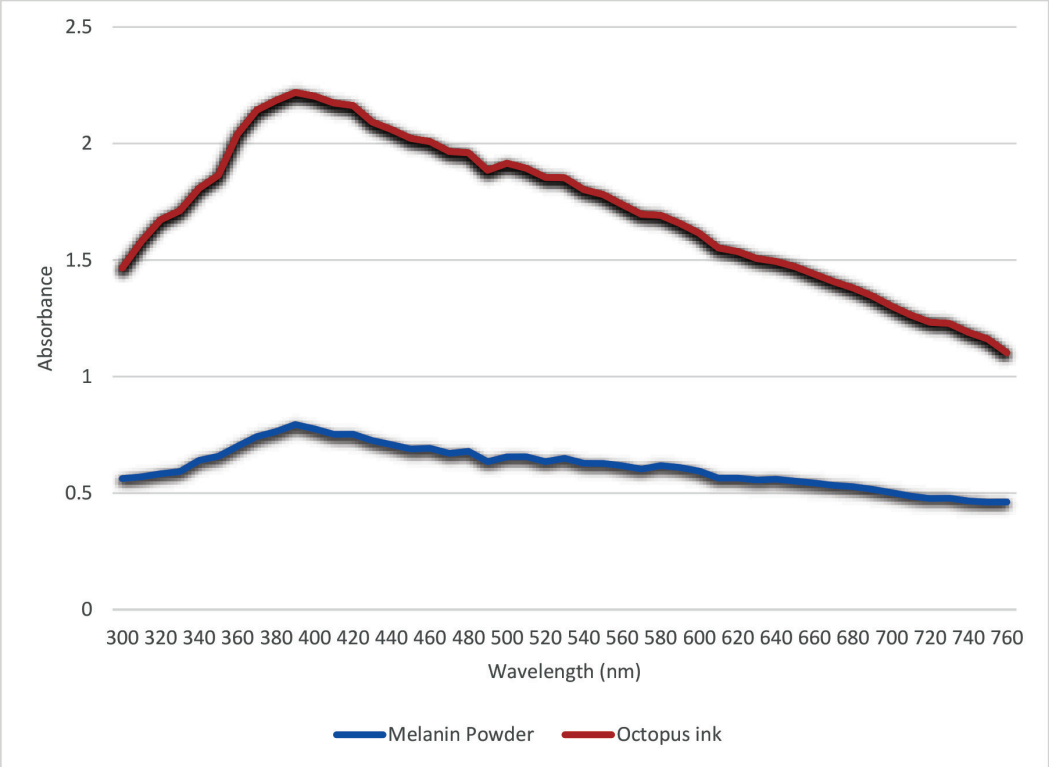

Fig. 2: Spectrophotometric spectra of octopus ink and melanin powder

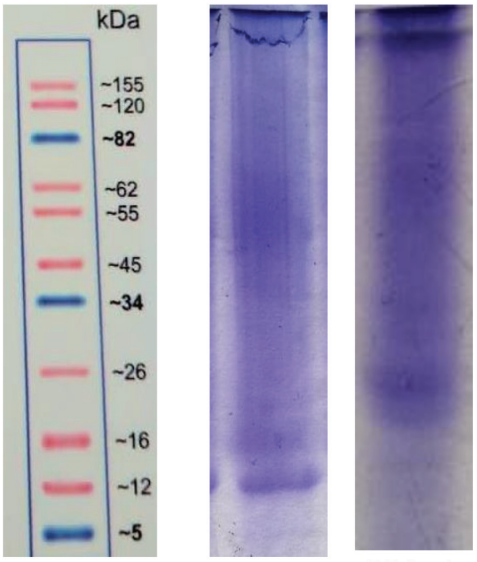

Fig. 3: SDS-PAGE of protein from octopus ink and its melanin powder

Microbiological examination of octopus ink and melanin powder.

Microbiological analysis (Table 6) of octopus ink and melanin powder revealed that all samples were free from *Salmonella* spp /25g. While the total count of bacteria was 2.5 x 10^2 and 1.2 x 10^2 cfu/g, respectively. Coliforms were not detected in melanin powder, while octopus ink contains 2.4 x 10^2cfu/g (Table 6).

All tested samples could be considered acceptable from a food manufacturer's point of view. Anderson & Shi (2006) reported that, there is an antibacterial potential of squid ink extract against some pathogens, which can be employed as a novel therapeutic anti-cancer agent in the near future.Melanin exerts antimicrobial activity against bacteria, fungi, and parasites (Casadevall *et al*., 2000, Banerjee *et al.*, 2014).

Table 6: Microbiological analysis of octopus ink and melanin powder

Test	Octopus ink	Octopus melanin
Total plate count, cfu/g	2.5 x 10^2	1.2x 10^2
Coliforms, cfu/g	2.4 x 10^2	Not detected
Salmonella spp/25g	Not detected	Not detected

Application of octopus melanin

In this study, prepared melanin was utilized as a food colouring agent in the preparation of black pasta and black olive paste. Different concentrations (1%, 5%, and 10% (w/v)) of melanin were used.

Sensory evaluation of black pasta

Black pastas were prepared at a ratio of 1%, 5%, and 10% (w/v) of melanin powder as colouringagent. The organo leptic properties were evaluated by 25 trained panelists from the Food Science and Technology Department. The comparisons (*at P≤0.05*) were made among the prepared black pasta (Figure 4).

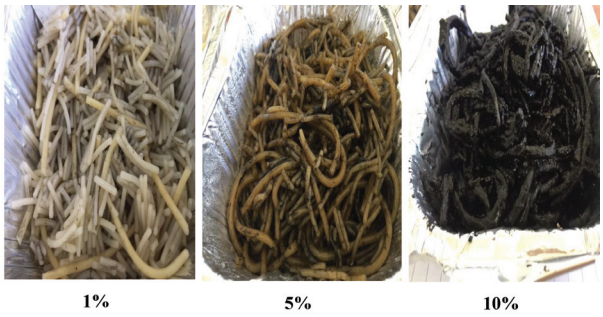

Fig. 4: Black pasta prepared in different concentrations (1%, 5% and 10%) of melanin powder

Statistical analysis (*at P ≤ 0.05*) of panel testing reveals highly significant differences among treatments (Table 7). Black pasta cooked in 1% melanin powder was highly accepted by panelists, followed by 5% and 10% in terms of colour, odour, and taste. Increased concentrations of melanin powder resulted in an increase in the unacceptable black colour,

Table 7: Sensory evaluation of black pasta

Treatment	Sensory attributes			
	Texture	Taste	Odour	Colour
1%	6.10 ± 0.978^{a**}	6.93 ± 1.861^{a}	6.13 ± 1.648^{a}	7.2 ± 0.414^{a}
5%	5.81± 0.872^{ab}	5.39 ± 1.256^{ab}	5.42 ± 1.207^{ab}	6.6 ± 0.828^{ab}
10%	5.10 ± 1.921^{b}	3.67 ± 1.904^{b}	4.6 ± 2.414^{b}	5.0 ± 1.732^{b}

*Mean ± Standard Deviation
**Different letters in a column indicate significantly different values at P≤0.05

odour, and taste. It can be concluded that the addition of 1% melanin powder is optimum concentration to be used in the preparation of black pasta.

Evaluation of black olive paste

Statistical analysis (at $P \leq 0.05$) of panel testing reveals highly significant differences among treatments (Table 8). The olive paste cooked in 1% melanin powder was highly accepted by panelists, followed by 5% and 10% in terms of texture, colour, odour, and taste. Increased concentrations of melanin powder resulted in an increase in the unacceptable black color, odor, and taste. It can be concluded that the addition of 1% melanin powder is optimum concentration to be used in the preparation of black olive paste.

CONCLUSIONS

Octopus ink as waste, is a good source for melanin pigment, and this study succeeded to extract melanin powder from ink. Melanin powder utilized as natural black colouring agent for pasta and olive, and recommended to use melanin at concentration (1% w/v) as colour ingagent. The result may be valuable for the efficient production of melanin pigment powder and its application as a natural food colouring agent and antioxidant. Therefore, recycling octopus ink into marketable products can reduce amount of waste in our environment.

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Table 8: Sensory evaluation of black olive paste*

Treatment	Property**			
	Texture	Color	Taste	Odor
1%	8.2 ± 0.414^{a}	8.4 ± 0.507^{a}	8.6 ± 0.507^{a}	8.2 ± 0.414^{a}
5%	7.8 ± 0.775^{ab}	7.6 ± 1.056^{ab}	8.2 ± 1.207^{b}	6.6 ± 0.828^{ab}
10%	6.2 ± 1.656^{b}	6.4 ± 1.242^{b}	7.6 ± 2.414^{c}	5.0 ± 1.732^{b}

*Mean ± Standard Deviation

**Different letters in columns indicate significantly different values at P≤0.05

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حبر الأخطبوط كمصدر للميلانين الإعداد والتقييم والاستخدام

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حبر الأخطبوط هو مخلف سمكى و يعتبر مصدرا جيدا لصبغة الميلانين . تم تحضير مسحوق الميلانين من هذا الحبر وتم تقدير التركيب الكيميائى الإجمالى له علاوة على محتواه من المعادن والمواد الفينولية والأحماض الأمينية وطيف الأشعة فوق البنفسجيه والفحص الميكروبيولوجى والنشاط المضاد للأكسدة .

تبين أن محتوى الحبر من كل من البروتين ،الرماد ،الدهن ، المواد الفينولية الكلية كانت: ٥٩ , ٦ ٪، ٩٠ , ٨ ٪، ٨, ٠ ٪، ٠٦ , ٢١٨ ملجم/كجم على الترتيب. وكان محتواه من الماغنسيم والنحاس ٥٣ , ١١٦ ، ١٥ , ١٥ ملجم/١٠٠ جم على الترتيب . و كان محتوى الحبر من السيرين ٤ , ٥٦ ٪ أما النشاط المضاد للأكسده فكان ٤٠ , ٤٦ ٪، كما تبين خلو مسحوق الحبر من السالمونيلا /٢٥ جم .

تم إضافة مسحوق الميلانين لتلوين المكرونة و عجينة الزيتون ، و أوضحت نتائج التقييم الحسى أن كلا من المكرونة التى تم طهيها فى وجود ١ ٪ من مسحوق صبغة الميلانين و عجينة الزيتون كانت مقبولة بدرجة عالية من قبل المحكمين ، مما يدل على إمكانية استخدام هذه الصبغة الطبيعية وذات النشاط المضاد للأكسدة لتلوين الأغذية.