"Antioxidant and hemolytic activity of melanin from *Octopus maya* ink"

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INTRODUCTION

Melanin is a pigment that has a wide field of biotechnological applications due to the chemical structure that possesses since it has shown to be useful for metal chelation, catalysis, as a biomaterial, biomedical and pharmacology applications. Among the pharmacological properties is the antioxidant activity and it has reported this compound does not cause cytotoxicity (Pralea *et al.*, 2019). A natural source of melanin is within the ink produced by cephalopods such as octopus, squid, and cuttlefish. The Yucatan Peninsula has a high catch of octopus (*Octopus maya* y *Octopus vulgaris*) of approximate 24,000 tons of year which only the muscular part (mantle and arms) is used, discarding the viscera without any integral management for the disposal of these residues, within them is the melanin contained in the mollusk's ink bag (Tello *et al* 2018). Considering the above, the objective of this work is to characterize the melanin obtained from the ink of the *Octopus maya* and its potential use as an antioxidant agent.

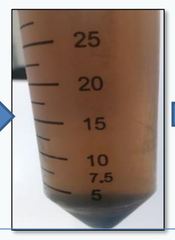
METODOLOGY

Extraction

O. maya

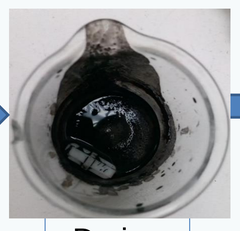
Ink bag

Acid wash



Water wash

Solvents wash



Drying

Dry melanin

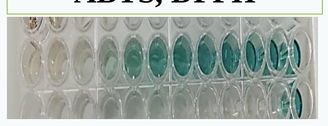
Characterization

- **Physical**: TGA, DSC
- **Morphological and elemental**: SEM-EDS
- **Structure**: FTIR, XRD

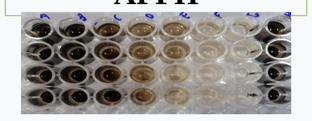
Biological Activity

Antioxidant activity

Colorimetric methods **ABTS, DPPH**

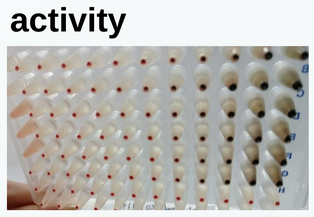


ORAC method **APPH**



Hemolityc activity

Test using human erythrocytes



RESULTS AND DISCUSSION

Physical-Chemical characterization

Table 1. Summary of the physicochemical analyzes of melanin´s *O. maya* and *S. Officinalis.*

Sample	TGA		DSC		XRD	Sizes particles
	Temperature (° C)	% total mass loss	$T_{\Delta H}$ (° C)	Enthalpy (J/g)	Minerals traces	(nm)
O. maya	40 -102	8.15	110	378		
	103 – 175	5.17	277	0.8	—	170 ± 50
	176 – 352	17.55	363	2.7		
	352 - 770	35.0	418	0.9		
S. officinalis	40 – 115	15.54	105	706	C_2CaO_4*2H_2O	
	116 – 163	2.88	226	72	NaCl	107 ± 23
	164 – 395	20.26	338	43.32		
	396 - 770	31.19				

Table 1 shows the thermal results by **TGA** that showing similarity in the temperatures ranges. **DSC** show different endothermic peaks because of variations of salts and metals bind to melanin that modifies thermal resistance (Pralea *et al.*, 2019).
Spherical structures were obtained from the **SEM** analysis, the smallest being the *S. officinalis* melanin compared to *O. maya* melanin.

Table 2. Elemental percentage from melanins samples by EDS

Element (% weight)	*O. maya*	*S. officinalis*
C	57.81± 0.75	46. 37± 1.38
N	11.52 ± 0.78	7.05 ± 1.08
O	29.98 ± 0.12	29.9 ± 0.70
Ca	0	1.57 ± 0.18
Mg	0	1.60 ± 0.25
Na	0	6.37 ± 0.74
S	0.68 ± 0.16	0.43
Cl	0	6.43 ± 0.43
K	0	0.18 ± 0.07
All data are expressed as means ± SD. n=2		

The **EDS** elemental analysis (table 2) shows that there is no presence of metals in *O. maya* melanin compared with *S. officinalis*, this is due to the elimination of marine salts by the washes carried out (Pralea *et al.*, 2019) and the same that is corroborated with **XRD** (table 1).

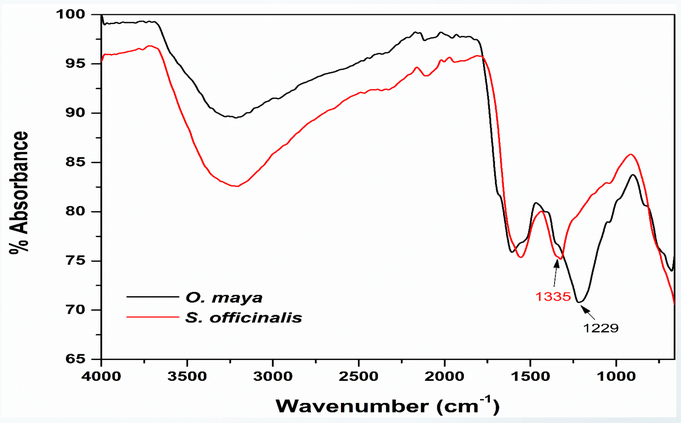


Figure 1. IR spectrum of the *O. maya* and *S. officinalis.*

In fig. 1 the similarity of the **FTIR** spectra of melanin of *O. maya* and *S. officinalis* is observed, however the -COOH band of *O. maya* shifts to lower wavenumber (1229 cm^{-1}) compared to the *S. officinalis* melanin (1335 cm^{-1}) which is bound to metal cations (Madkhali *et al.*, 2019).

Hemolytic activity

The results of the hemolytic activity of the *O. maya* (Fig. 2) show that a concentration of 1.4 mg/mL only causes 43 % hemolysis. a lower percentage of hemolysis than other cephalopods (Mimura *et al.*,1982).

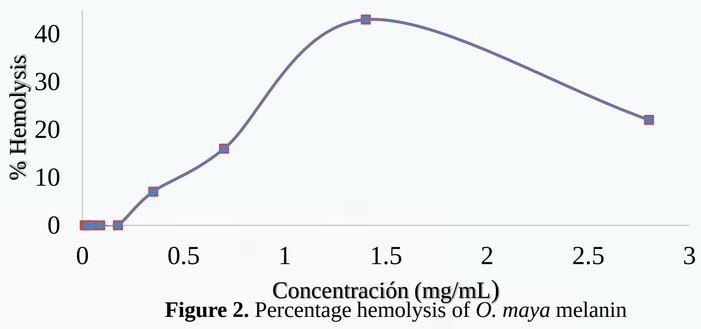


Figure 2. Percentage hemolysis of *O. maya* melanin

Antioxidant activity

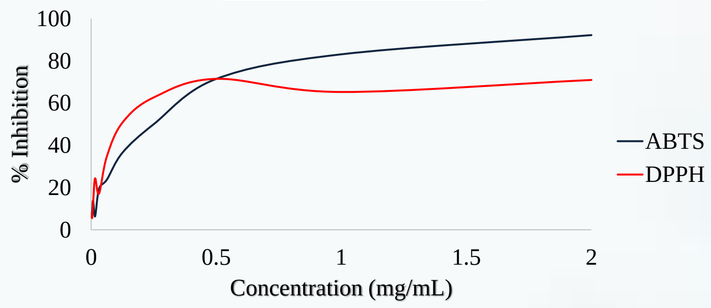


Figure 3. Percentage inhibition of melanin against radicals.

IC_{50} ABTS= 202 ± 3.75 µg/ml
IC_{50} DPPH = 237 ± 23.1 µg/l.

O. maya melanin inhibited 92% of the ABTS radical having a higher percentage of inhibition than for melanin from marine bacteria. While only inhibited 71% for DPPH radical an activity higher than *S. officinalis* melanin but less activity than melanin from marine bacteria.

The antioxidant capacity could be related to the presence of hydroxyl (OH) groups in the form of catechol DHI and DHICA in the chemical structure (Wang & Rhim, 2019).

O. maya melanin had an **ORAC activity** of 483±72.73 µM TE/g sample, not any previous report of an ORAC assay of melanins but it has higher antioxidant activity than the protein found in cephalopod ink (Vate & Benjakul, 2013).

CONCLUSIONS

A decreased of inorganic impurities was observed in *O. maya* melanin with respect to that *of S. officinalis* as a result of the purification methodology used.
Eliminating salts does not affect the thermal resistance of *O. maya* melanin up to temperatures of 400 °C.
The antioxidant activity of the *O. maya* melanin varies between the different radicals and the IC_{50} is in low concentrations. Despite melanin having slight hemolysis, the maximum percentage is above the IC50 of antioxidant activity, so melanin can be considered as a possible food additive with antioxidant properties.

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