Bioactive compounds derived from marine alien species in the Mediterranean for cosmeceutical applications.

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Introduction:

In the present study, the exploitation of marine alien species Lagocephalus sceleratus, Pterois miles and Fistularia commersonii was examined through the recovery and valorization of added value bioactive compounds with potential application on cosmetic products, with the aim of contributing to the control of their population and consolidation of Mediterranean basin. In particular, polyunsaturated fatty acids, collagen and naturally occurring fish toxins (i.e. tetradotoxin) were extracted from the flesh, skin, bones and internal organs of the three studied species. The efficient recovery of the pre-mentioned marine derived components was achieved through the optimization of proper protocols and application of state of the art extraction techniques. Moreover, analytical techniques were used in order to fully characterize the produced extracts. Finally, in order to cover the unpleasant odor of fish origin bioactive compounds and to protect them from adverse environmental conditions, their encapsulation in polymeric matrices is necessary. For the encapsulation the innovative electrohydrodynamic process was used and specifically electrospraying.

Materials & Methods:

Extraction of Tetradotoxin (TTX): Samples of L.sceleratus were dissected and the visceral organs were removed and separated from the muscle and skin. The intestines and muscle were separately crushed and 1% acetic acid in methanol was added in order to be homogenized using a BagMixer. The homogenized sample was then put in an ultrasonic bath for 10 min and freezed at -18°C for additional 10mins. The solution was centrifuged at room temperature at 4000 rpm for 20 min and the supernatant was freezed until further analysis.
In order to quantify TTX in LC-MS / MS, the sample was purified by solid phase extraction (SPE). For the elution of TTX and its analogues from the microcillars aqueous solution of 0.1N HCl was used. The samples were then diluted with water in a ratio of 1:20 and analyzed using liquid chromatography-twin mass spectrometry (LC-MS / MS, Agilent 1260 Infinity binary HPLC in combination with Agilent 6460C Triple Quadrupolr Mass Spectrometer).
The identification and quantification of TTX compounds was performed with external standard method using the Certified Reference Material (CRM-03-TTXs) of the company CIFGA.
Extraction of Lipid Content: According to Bligh and Dyer method, fish samples of muscle and skin from F.commersonii were homogenized using Cloroform/Methanol (1:2 v/v) using a bagmixer and magnetic stirrer. The chloroform phase of the filtrate was separated using a separation funnel and evaporated using a BUCHI Vacuum V-800. The sample was dried at 42°C for 2hrs and weighed. Total lipid content (TL) was gravimetrically determined according to the equation:

$$\%TL=W(g)oil/W(g)biomass*100$$

Following the determination of TL, preparation of fatty acid methyl esters (FAMEs) was followed by the redissolution of lipids into chloroform in order to be analyzed. FAMEs were identified using a GC-MS (GAS) system with Varian 450 analytical instrument equipped with a column DB5. The carrier gas was helium and its flow rate was equal to 1mL / min. The injection temperature was adjusted to 270 °C, the carrier gas flow was 1 mL / min and its column temperature is increased from 125 °C to 300 °C within 35 min at a rate of 5 °C / min. The PUFAs were identified by comparison with external standards and quantified using calibration curves.
Extraction of Collagen: Collagen content from P.miles was determined using the pepsin soluble collagen (PSC) method. For the extraction, samples were treated with NaOH 0.1M in a magnetic stirrer for 5hrs with the replacement of NaOH every 2hrs, in order to remove non-collagen proteins. After the removal of NaOH and washing of the biomass, 0.5 M acetic acid at a ratio of 1:5 w/v and 1.5% (w/v) pepsin were added and the sample was continuously stirred for 20Hrs. The extracts were then centrifuged and the supernatants were separated. The samples were spectrophotometrically determined according to collagen calibration curve at 650 nm.
Encapsulation of bioactive compounds: A cyclodextrin solution was used in order to dilute collagen and fatty acids using continuous stirring before the sample was put at the electrospraying nozzle. The encapsulation experiments were carried out in a Fluidnatek LE-10 (Bioinicia, Spain) electrospraying apparatus at 25^{O}C. The parameters of distance (cm) between the target and the capillary tip, the feed rate (μL/h) of the syringe pump delivering the solution and the voltage (kV) in order to form a stable Taylor cone.

Aknowledgments:

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Results & Discussion:

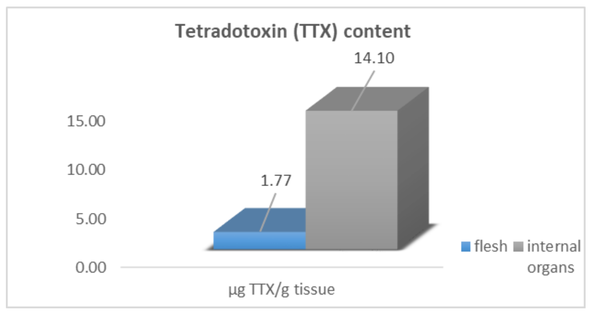


Figure 1 Comparison of TTX concentration of flesh and visceral organs of L.sceleratus

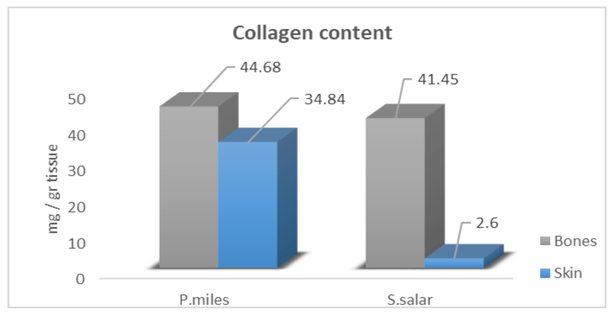
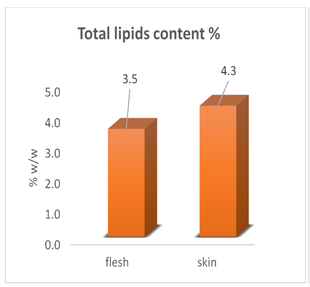


Figure 2 Comparison of collagen concentration of bones and skin in lionfish P.miles versus salmon S.salar



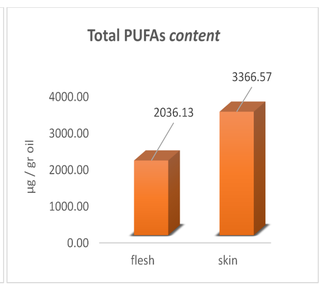


Figure 3 A) Total lipids content (%) of F. commersonii of flesh and skin samples B) Total concentration of FAMEs (μg/gr fishoil) in F.commersonii samples of flesh and skin

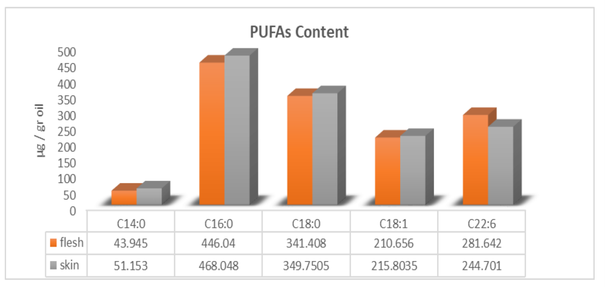


Figure 4.FAMEs quantification of flesh and skin in F. commersonii identified as Myristic acid C14.0, Palmitic acid C16.0, Stearic acid C18.0, Oleic acid C18.1 and Eicosidexaenoic acid (DHA) C22.6

Table 1 Quantification of identified FAMEs in F.commersonii using GC-MS

C:D	Common name	Elution time (min)	% w/w in *F. commersonii* Flesh	% w/w in *F. commersonii* Skin
C14:0	Myristic acid	11,86	3,32	3,85
C16:0	Palmitic acid	15,79	33,70	35,21
C18:0	Stearic acid	19,52	25,79	26,31
C18:1	Oleic acid	19,12	15,91	16,23
C22:6	Eicosidexaenoic acid (DHA)	25,71	21,28	18,41
Total			100	100

Changes in the levels of TTX in L.scleratus have been shown to be a function of season and sex, where most toxic fishes were found to be female in the summer season [1,2]. According to the same research the highest TTX level in internal organs of female fish reached an amount of 52.1 while muscle tissue was reported at 2.83μg/g during the winter but was otherwise below the toxic limit. In accordance to these results, the TTX level of internal organs and muscle tissue in our study reached 14.10 μg/g and 1.77 μg/g respectively.

For the cosmetic industry, marine collagen has been previously obtained from coldwater fish skins, such as cod, haddock and salmon [3]. According to literature salmon collagen shows skin antiageing and systemic redox effects [4], therefore it was selected as reference material to be compared with the recovery yields of collagen from P. miles skin and bones. Based on our research, both species show similar amount of collagen content in bones, whereas P. miles contained a significantly higher amount of collagen in skin samples. Specifically, the collagen content in P. miles skin samples were found to be thirteen times higher than those of S. salar (Figure 2).

The lipid content of F. commersonii in both flesh and skin tissue samples was found to be 3.5 and 4.3% respectively. Although, the total lipid content of skin tissue samples was 22% higher than those of flesh, the PUFA content in lipid fraction of skin was found to be 65% higher. At this point it is essential to mention that in cosmetic industry there has been increasing interest in the relationship of fish oil with skin protection and homeostasis, especially with respect to the omega-3 polyunsaturated fatty acids (PUFAs) and particularly docosahexaenoic acid (DHA) [5]. According to our study, DHA was found to be in escess in the lipid fraction of both flesh and skin tissue samples of F. commersonii with 21,28 and 18,41% of PUFAs content respectively. This fact posing F. commersonii as an ideal source of lipids for application in cosmetic industry.
In order to sufficiently incorporate in cosmetic products, the fish collagen and omega-3 fatty acids isolated in this study, the encapsulation electrospraying process was evaluated aiming at the efficient odour covering, enhancement of bioactive compounds' protection and bioavailability. The process functional parameters were optimized based on the uniformity of the final encapsulated powder product. **The results obtained from the investigation, showed that the best operating conditions of electrospraying are achieved for feed rate equal to 1000μL / h, voltage equal to 27kV and distance of the collection surface from the nozzle equal to 13cm, for cyclodextrin solution with a content of 7% omega-3 fatty acids and 3% collagen.**

Conclusions:

The present study can turn the current problem of invasive alien species into a "Win-Win" solution for both Mediterranean fisheries and cosmetology companies. In addition to the innovation brought to the cosmetics sector due to the opening of a new market of innovative formulations based on unique products origin, the invasive species' exploitation is expected to create a new type of fishery that will bring economic benefits to professional fishermen themselves. In addition, it will contribute to the reduction of invasive fish stocks in the Mediterranean and consequently in the control of the ecological and economic impact on marine life, fisheries, human well-being and health.
According to our research, new cosmetic markets will open up based on natural and sustainable ingredients of unique origin. In addition, the integration of new technologies and know-how in the Mediterranean cosmetic industry will give a significant advantage over its competitors in the international market. The utilization of bioactive ingredients from marine alien species will have a significant financial impact since easily applicable to industry and cost-effective processes are proposed for the valorization of an unexploited and in excess source. The use of bioactive ingredients obtained in the context of this study will lead to the development and production of innovative end products for cosmetology, enriched with genuine natural substances that will have significant social and health benefits.

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