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which contains phycobiliproteins, mainly phycoerythrins, as accessory pigments. The extraction of phycoerythrins from *Gracilaria gracilis* was optimized, using several extraction methods, namely maceration, ultrasounds, sonicator, and freeze/thaw. Different extraction conditions such as the concentration of phosphate buffer (C), solvent/biomass ratio (R), homogenization time (T1) and extraction time (T2) were optimized using a Response Surface Methodology. Maceration was the most efficient method (optimal conditions T1 = T2 = 10 min.; C = 0.1 M; R = 1:50) reaching values of 3.6 mg of phycoerythrin/g of algae, giving yields ~45% higher than with the other methods tested. As regards the conditions, T2 was the most influential variable in the yield while the increase of C resulted in lower values. Moreover, a stability study was performed at different storage temperatures (C = 0.0); 19 and 40 °C) and pH's (4; 6.9 and 8) for 10 days showing greater stability at C = 0.00 and 5 °C and at pH 6.9. Ultimately, the phycoerythrins were semi-purified by precipitation with ammonium sulfate followed by dialysis and used as colorant in pancakes. This study has shown that C = 0.01 may be a good source of phycoerythrins for food applications.

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Comparing the Detection of Histamine by Using GC-MS and ELISA Methods: Clues for a Faster and Direct Detection of Biogenic Amines in Seafood Products

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The presence of biogenic amines (e.g., histamine, spermidine, putrescine) in seafood is of great concern to the food industry and consumers since it can present some risks to human health [1]. Therefore, an efficient and fast evaluation of the quality of food products is fundamental to strength the confidence of consumers. Accordingly, there is an increasing demand to decrease the allowed limits of biogenic amines in food. In addition, there is a need to develop new methodologies to detect biogenic amines allowing faster and easy detection of biogenic amines [2]. The development of new methods and new technology approaches for the assessment of seafood quality in addition to the conventional methodologies have been encouraged in the most developed countries and by the European Union [2]. In this study we present, for the first time a method for the analysis of biogenic amines (histamine and spermidine) by gas chromatography-ion mobility spectrometer (GC-IMS) device which analyzes the VOCs pattern these amines [3].

Finally, a conventional technique based on an immunoassay (ELISA) was also used to survey histamine and spermidine in seafood samples and compared with reference standards. The results are compared with results from GC-IMS and later can be uturnoversed to optimize this technique.

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References

- 1. Pierina, V. Biogenic amines in raw and processed seafood. Front. Microbiol. 2012, 3, 188.
- 2. Alasalvar, C.; Miyashita, K.; Shahidi, F.; Wanasundara, U. (Eds.) *Handbook of Seafood Quality, Safety, and Health Applications*; Blackwell Publishing Ltd.: Hoboken, NJ, USA, 2011; ISBN 978-1-4051-8070-2.
- Ulanowska, A.; Ligor, M.; Amann, A.; Buszewski, B. Determination of Volatile Organic Compounds in Exhaled Breath by Ion Mobility Spectrometry. Chem. Anal. 2008, 53, 953–965.

Porphyrin Pigments in Polychaeta: Explorations on the Evolution of Haem Metabolism in Marine Eumetazoans

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Porphyrins are haem-derived tetrapyrrole heterocyclic compounds that are synthetized by all major animal groups, among which biliverdin and bilirubin are well-known representatives. Porphyrins are prized targets for biotechnology as anti-oxidants and photosensitisers, depending on numerous modifications to the basic tetrapyrrole structure (such as alkylation). Naturally, coastal marine animals, owing to their colourful patterns, abundance and diversity, are promising, albeit neglected, subjects for the discovery of novel porphyrins. Our case study, the intertidal Polychaeta Eulalia, has been found to produce a wide variety of porphryrinoids that, altogether, contribute to its uncanny bright-green colour. Making use of RNASeq and pathway analysis, we identified five major genes that putatively code for proteins genes involved in haem metabolism: protoporphyrinogen oxidase (PPOX), uroporphyrinogen decarboxylase (UROD), uroporphyrinogen synthase (UROS), ferrochelatase (FECH) and δ-aminolevulinic acid dehydratase (ALAD). Multi-sequence Bayesian phylogenetics showed that haem metabolism is well-conserved among eumetazoans, even though the evolution of the metabolic pathway does not entirely match the animal tree-of-life across representative taxa. While the Polychaeta were more closely related to molluscs, a higher-order clade grouped the two taxa with cnidarians, echinoderms and, surprisingly, with Limulus. In their turn, vertebrates (humans included) and crustaceans were clustered apart, with the latter being set farthest. These relationships do not necessarily reflect those of each individual gene, meaning different rates of divergence, which could explain the wide variability of porphyrinoids in marine organisms like Eulalia. Our findings demonstrate that the evolution of haem metabolism reflects marine diversity, leading to a higher multiplicity of porphyrinoid pigments than anticipated.

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