## OP168
## Isolation and characterization of acid and pepsin-solubilized collagen from squid mantle (Loligo vulgaris)

N Cozza[1], W Jankangram[1], RI Perez-martin[2], W Bonani[1], A Motta[1] and C Migliaresi[1]
[1]*Department of Industrial Engineering, University of Trento, Italy;* [2]*Burapha University, Thailand*

*Introduction:* Collagen represents the principal structural protein in vertebrates, accounting nearly 30% of total proteins in the animal body [1]. Collagen has a wide range of applications. It's frequently utilized also in the field of tissue engineering due to its biocompatibility, biodegradability, low immunogenicity and cell-adhesive properties [2]. Traditional sources of collagen are bovine skin and tendon as well as porcine skin. However, collagen and collagen-derived products from land-based animals involve the risk of infection with diseases such as bovine spongiform encephalopathy. Additionally, religious constraints limits their use. Therefore, in recent years, there was an increasing attention to development of alternative collagen sources. For example, marine organisms provide potential suitable raw materials for the extraction. Properties of collagen extracted from different marine sources is strongly dependent on the habitat, the species and the part of the organism from which it is isolated [3]. For these reasons, a thorough characterization of the proteins is necessary. The aim of this work was to isolate and characterize Acid- (ASC) and Pepsin-Soluble Collagens (PSC) extracted from the mantle of European Squid (*Loligo Vulgaris*).
*Materials and methods:* Collagen extraction was performed as previously reported [4] with a minor modification. Extracted collagen was characterized using SDS-polyacrylamide gel electrophoresis (SDS-PAGE), Fourier Transform Infrared Spectroscopy (FTIR) and Gel Permeation Chromatography (GPC). Amino acid composition and solubility of collagen were also evaluated. Denaturation temperature was measured by viscosity change and confirmed with thermal analyses using Differential Scanning Calorimetry (DSC).
*Results:* ASC and PSC were isolated with a yield of 5.1% and 24.2% (dry weight basis), respectively. The SDS-PAGE showed that ASC is mostly comprised of α1- and α2-chains; while, PSC consisted of α1- and α2-chains (α1 to α2 ratio, 2 to 1), with β- and γ-components. The GPC analysis proved that both ASC and PSC contain fractions with different molecular weights and high molecular weight aggregates, that may be due to the marine source and the extraction method. For PSC denaturation temperature ($T_d$) was 21°C, while the ASC $T_d$ ranged from 22 to 24°C, according on organism body temperature and amino acid content of collagen.
*Discussion and conclusions:* This study investigated optimal collagen extraction conditions for processing squid mantle (*Loligo Vulgaris*) and characterized the chemical properties of the extracted collagen. The results of this study demonstrated that squid mantle has a potential as an alternative source of collagen.
*Disclosures:* The authors have nothing to disclose.
*References*
1. Foegeding EA et al. Food Chemistry. 902–906, 1996.
2. Glowacki J et al. Biopolymers. 89, 338–344, 2008.
3. Falguni P et al. Bioresource Technology. 101, 3737–3742, 2010.
4. Nagai T, Suzuki N. Food Chemistry. 68, 277–281, 2000.

## OP169
## Marine origin collagen membranes for drug delivery

ALP Marques[1], A Domingues[1], J Moreira-Silva[1], RI Perez-Martin[2], CG Sotelo[2], TH Silva[1] and RL Reis[1]
[1]*3B's Research Group, University of Minho, Caldas das Taipas, Portugal;* [2]*Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Cientificas, Vigo, Spain*

*Introduction:* Collagen is the most abundant protein of animal connective tissues, found in skins, bones or cartilages, which turn it into one of the key polymers to be considered for biomedical applications, namely tissue engineering and drug delivery.

Current industrial procedures to extract collagen involves bovine and porcine as main sources. However, due to religious factors and the risk of transmitting diseases to humans, the search for new sources has been growing. Marine origin is one of the alternatives that has been explored, particularly, through by-products of fish processing, such as skins, scales or spines, with both economic and environmental benefits [1].

In this work, collagen was extracted from shark *Scyliorhinus canicula* skin. The collagen was processed and further evaluated as alternative for dermal membranes, regarding sustained release of drugs.
*Materials and methods: Extraction of collagen:* Skins of shark (Scyliorhinus canicula) were treated with 0.1 M NaHO to remove non-collagenous proteins, cleaned with distilled water and then collagen was extracted with 0.5 M acetic acid, overnight. After centrifugation, the supernatant was purified by dialysis and the resultant collagen solution was freeze-dried. The produced collagen was characterized by FTIR, SDS-PAGE, aminoacid composition and μ-DSC.

*Preparation of membranes:* Collagen was dissolved in 0.5 M acetic acid to obtain 1% (w/v) solution. Then, a 5% (v/v) hexamethylene diisocyanate (HMDI) was added at a ratio of 1% and 5% with respect to collagen, and allowed to react for 24 h. The mixture was cast in Petri dish, and let to dry at room temperature. Non-crosslinked membranes were prepared as reference. In order to prepare membranes for drug delivery assessment, dexamethasone was added to the collagen solutions and membranes were prepared as described. Collagen membranes were characterized by determining water contact angle, mechanical properties and stability in PBS. Furthermore, the release profile of dexamethasone was also determined.
*Results:* SDS-Page analysis indicates that the extracted collagen from shark skin is mainly of type I. μ-DSC analysis indicates a denaturation temperature of about 33°C, lower than mammalian collagen. The aminoacid analysis confirmed the presence of hydroxyproline and the high quantity of glycine, characteristic of collagen.

Collagen membranes showed more stability in PBS as long as the degree of crosslinking is higher, which also influences their mechanical properties. Moreover, the crosslinking degree also affects the hydrophobicity of the membranes. The release profile of dexamethasone was evaluated, with the drug being released in a progressive and sustained manner.
*Discussion and conclusions:* Collagen has been successfully extracted from shark skins and used on the preparation of membranes by solvent casting. The properties of the collagen membranes can be tunned, according to crosslinking degree, revealing a promising potential for application in biomedical field, namely as dermal membranes for controlled drug release.
*Acknowledgments:* Funding is acknowledged from projects IBEROMARE, MARMED and POLARIS. FCT Post-doc fellowship (JMS) is also acknowledged.
*Reference*
1. Silva TH, …, Reis RL, International Materials Reviews, 57 (2012), 276–306.