Effect of soluble collagen hydrolysate from *Prionace glauca* skin in the expression of human fibroblast collagen

Sánchez A., Correa B., Pérez M., Blanco M., Ramos P., Pérez-Martín R.I., Sotelo C.G.

Instituto de Investigaciones Marinas (IIM-CSIC) Vigo, Spain

### The Context

Follow the arrow

Seafood discards and by-products represent a management and environmental problem for the fishery industry. In last European CFP (UE)1380/2013 regulation, stakeholders are encouraged to find alternative uses for these discards and subproducts different from direct human consumption. One potential for these materials is obtaining high value-added products such as proteins with technological properties (collagen and gelatins), peptides with functional properties (antimicrobial activities, antioxidant, antiproliferative and anti-hypertensive) or hemo-pigments (myoglobin).

### Material and Methods

**Extraction of Pepsin Soluble Collagen (PSC) from skin of *Prionace glauca***

Extraction of collagen from *P. glauca* skin was carried out according to the methodology of Liu et al. (2012) with slight modifications which comprised an alkaline treatment followed by an acid-pepsin extraction. The obtained extract was centrifuged and the supernatant treated with 2 M NaCl. Finally the precipitated collagen is resuspended in 0.5 N acetic acid, dialyzed and lyophilized.

### Results

Collagen overexpression was not observed in fibroblast treated with permeates of PSC hydrolysates at 24 h of incubation. Only a slight overexpression was detected with 800 µg/mL at 48 h (see figure 1A), while with retentates the effect was observed already after 24 h with 100 µg/mL of hydrolysate. At 48 h of incubation with retentates (figure 1B) the collagen overexpression become more evident and increases as concentration rises, reaching a 20% of overexpression for the highest concentration.

### Conclusions

Collagen overexpression in fibroblast was demonstrated after treatment with both *P. glauca* and commercial collagen hydrolysates. Although both hydrolysates retentates, have a molecular weight between 3,000 and 10,000 Da, the average peptide size of commercial retentates was higher than PGLA hydrolysates. This fact might influence overexpression of collagen that could explain the different expression pattern between *P. glauca* and commercial hydrolysates.

### References


### Acknowledgements

The authors are grateful to the “Programa Operativo FEDER, Cooperación Transfronteriza España-Portugal” for the financial support through the project 0687_NOVOMAR_1P. We also thank to Francisco Santacalla for his contribution to this work.

---

**Before**

**Blue shark by-products**

**Collagen**

**After**

The Skin of *Prionace glauca* species, a significant subproduct of the fishery industry in Spain, is a potential raw material for collagen that could be used in diverse fields including food, cosmetic and biomedical industries (Chen et al. 2015; Weng et al. 2014).

**Objective**

The aim of present work was to test the effect of collagen hydrolysates, from skin of *P. glauca* species, on the collagen expression in human fibroblast cell culture with the aim of using them as ingredient in cosmetic products.

**Hydrolysis of PSC.**

Freeze-dried PSC suspended in deionized water (1:10 w/v) was hydrolyzed in a stirred batch reactor with Alcalase (1:20 w/v) at 50°C and 150 rpm during 3 h. Hydrolyzed PSC was subjected to ultrafiltration to obtain two peptide fractions: Permeate (<3000 Da) and Retentate (≥3000 Da).

**Cell Culture:**

Human fibroblast cells (Innoprot) were incubated in a 24 well plates (50,000 cell/well) during 24 hours before adding each treatment of peptide hydrolysates (800, 500, 100 and 50 µg/mL of either permeates or retentates) by quintuplicate and incubated again 24 h and 48 h. RNA extraction from fibroblast cell plates were carried out with the extraction kit "Cells-to-CT ™ 1-Step TaqMan ® Kit" (Ambion).

These data were compared with commercial hydrolysate treatment (figure 2), obtaining similar results. However, for commercial hydrolysate, collagen expression increases with decreasing concentration of treatment, contrary to what occurs with *P. glauca* hydrolysate.