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

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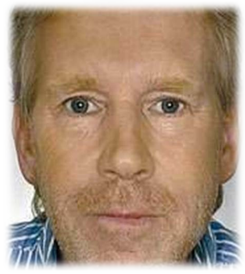
The Context

Follow the arrow

Before

Blue shark by-products

Collagen



After

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).

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

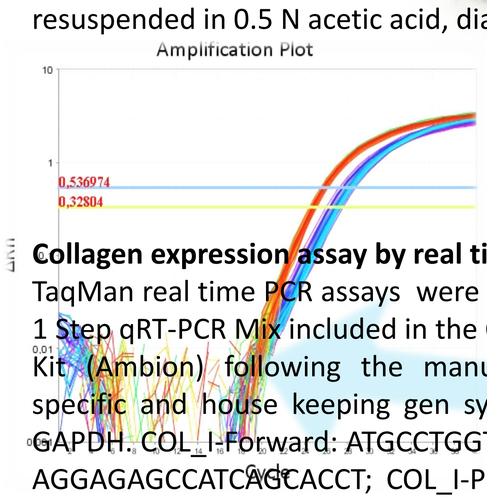
The aim of present work was to test the effect of collagen hydrolysates, from skin of *P. glauca* species, on the collagen expression in human fibroblast cell culture with the aim of using them as ingredient in cosmetic products.

Material and Methods

Extraction of Pepsin Soluble Collagen (PSC) from skin of *Prionacea 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.

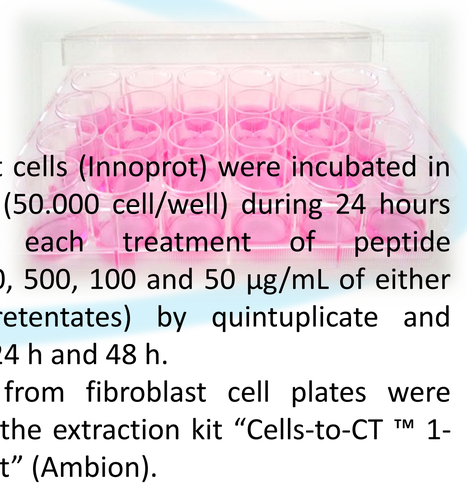


Collagen expression assay by real time PCR

TaqMan real time PCR assays were performed with the TaqMan® 1 Step qRT-PCR Mix included in the Cells-to-CT ™ 1 Step TaqMan® Kit (Ambion) following the manufacturer´s instructions. The specific and house keeping gen systems used were COL_I and GAPDH. COL_I-Forward: ATGCCTGGTGAACGTGGT; COL_I-Reverse: AGGAGAGCCATCAGCACCT; COL_I-Probe: 6-FAM-ACCAGCATCACC TCTGTC-MGB; GAPDH-Forward: GGAAGCTCACTGGCATGGC; GAPDH-Reverse: TAGACGGCAGGTCAGGTCCA and GAPDH-Probe: VIC-CCCCACTGC CAACGTGTC-MGB.

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 pH 8 and 55 ºC 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).

Results

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 hours 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.

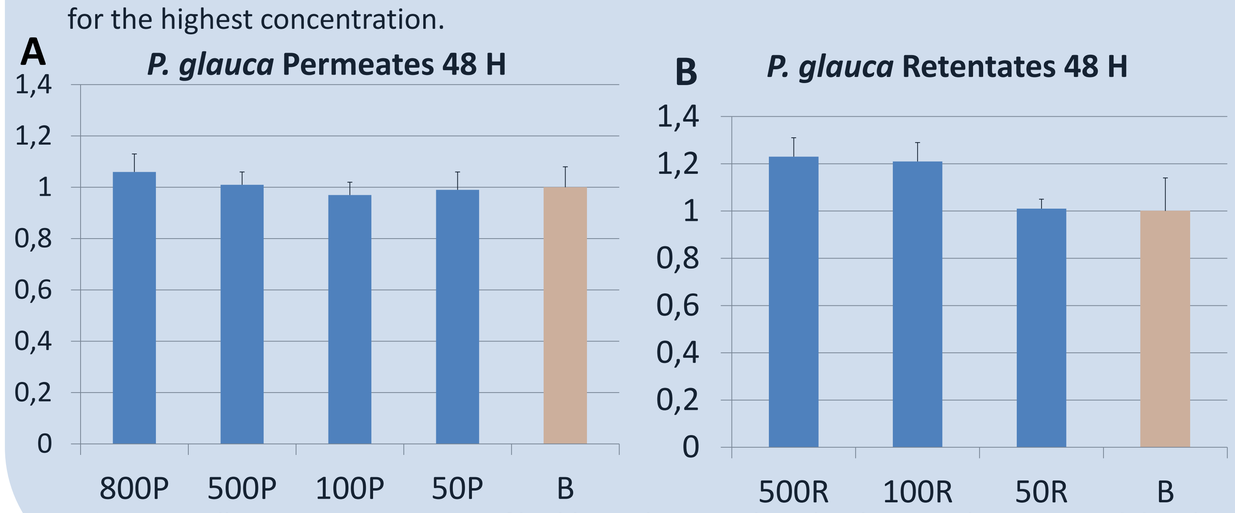


Figure 1. Collagen RNA expression in fibroblast culture incubated 48 hours with permeates (A) and retentates (B) of PSC hydrolysates.

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.

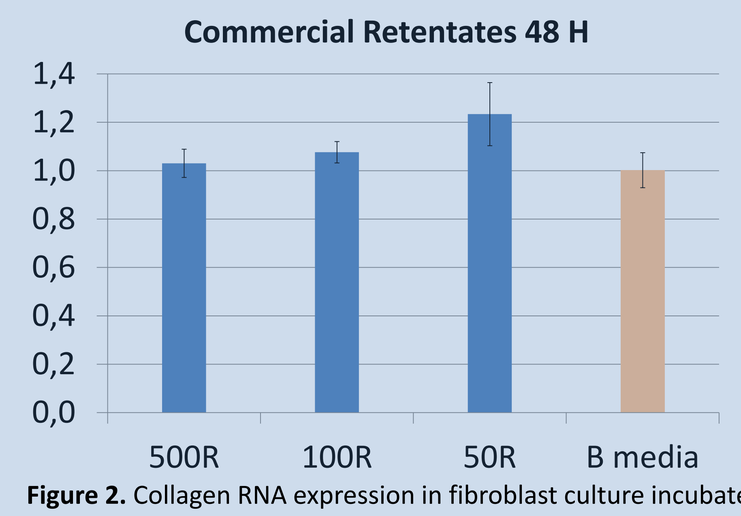


Figure 2. Collagen RNA expression in fibroblast culture incubated 48 hours with retentates of commercial hydrolysates.

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

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