

Production, characterization and bioactivity of fish protein hydrolysates from aquaculture turbot (*Scophthalmus maximus*) wastes.

SUPPLEMENTARY MATERIAL

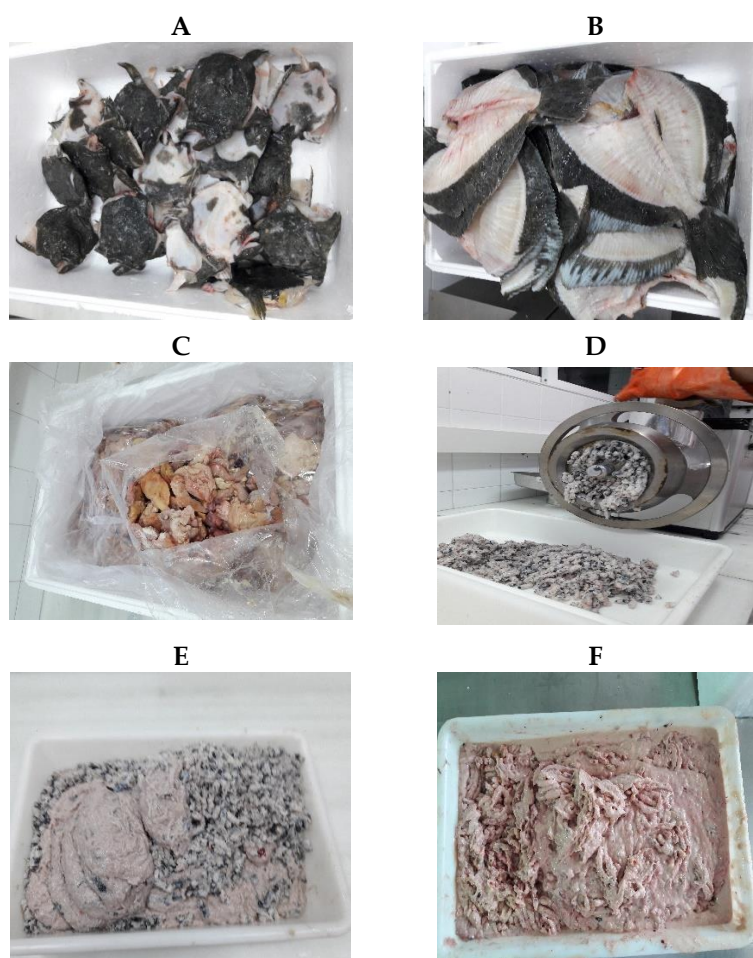


Figure S1. Pictures of initial and crushed turbot by-products: A) heads, B) trimmings and frames, C) viscera, D) minced heads. E) minced trimmings and frames and F) minced viscera.

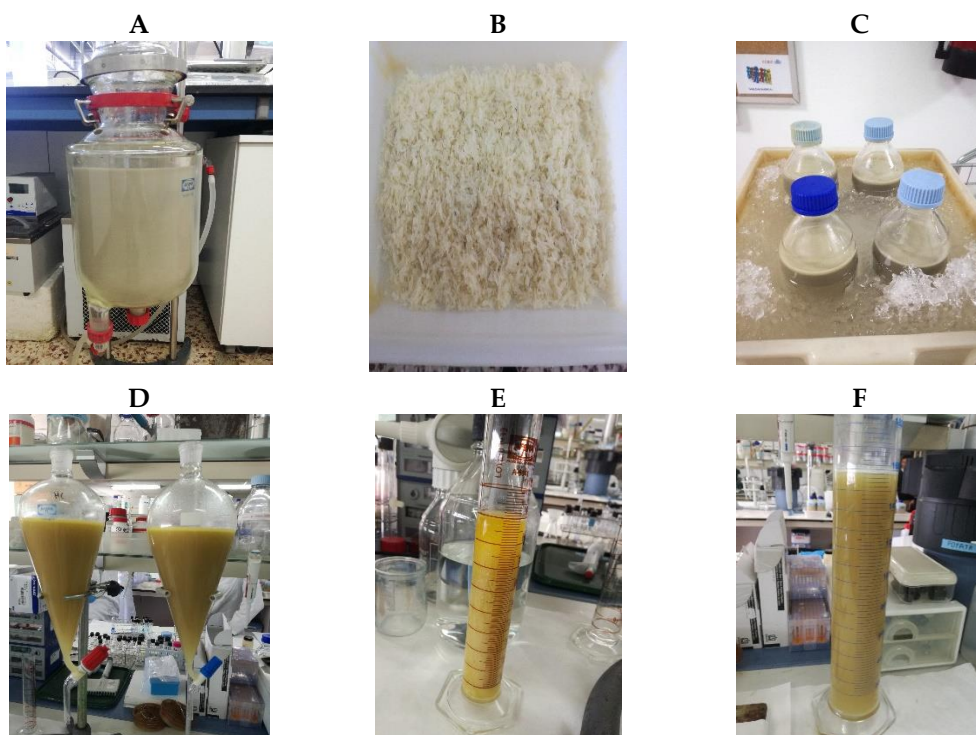


Figure S2. Different sequences of enzymatic hydrolysis of turbot wastes in a 5L-pH-stat reactor (A) with the differential recovery of clean bones (B) and fish oils (E) together with the production of liquid hydrolysate (F). The rest of the images show the cooling of hydrolysates prior centrifugation (C) and decantation of FPH to separate fish oil (D).

Table S1. Experimental domain and coding of the independent variables in the factorial design executed to study the joint effect of pH and temperature on the Alcalase hydrolysis of turbot heads (Tu_H).

Coded values	Natural values	
	pH	T (°C)
-1.41	6.0	30.0
-1	6.6	37.3
0	8.0	55.0
+1	9.4	72.7
+1.41	10.0	80.0

Codification: $V_c = (V_n - V_0) / \Delta V_n$
 Decodification: $V_n = V_0 + (\Delta V_n \times V_c)$
 V_n = natural value of the variable to codify
 ΔV_n = increment of V_n for unit of V_c
 V_0 = natural value in the centre of the domain
 V_c = codified value of the variable

Constant conditions
 Agitation= 200 rpm
 r (S:L)= 1:1
 [Alcalase]= 0.5% (v/w) or 12 AU/kg of heads
 time of hydrolysis= 3 h

Table S2. Proximate composition of turbot by-products in terms of moisture (Mo), organic matter (OM) and ashes (Ash). Total lipids (Lip), proteins (Pr-tN, as total nitrogen x 6.25) and proteins after degreasing samples (Pr-tN*) were determined using dried substrates. Error are the confidence intervals for n=3 (independent samples) and $\alpha=0.05$. Different letters in each column means significant difference between fish discards ($p < 0.05$).

	Mo (%)	OM (%)	Ash (%)	Lip (%)	Pr-tN (%)	Pr-tN* (%)
Tu_H	72.7±0.6 ^a	20.6±0.3 ^a	6.8±0.9 ^a	5.9±0.2 ^a	65.2±1.6 ^a	73.3±1.5 ^a
Tu_TF	64.1±3.2 ^b	26.9±1.2 ^b	8.9±2.5 ^a	26.0±0.5 ^b	43.9±3.6 ^b	69.0±9.2 ^a
Tu_V	71.6±0.9 ^a	27.2±0.9 ^b	1.2±0.1 ^b	40.0±0.2 ^c	49.6±1.8 ^c	81.5±2.5 ^b

Table S3. Kinetic parameters and associated error (as intervals of confidence) obtained from Weibull equation modeling the time course of the hydrolysis degree (H) of turbot by-products catalysed by Alcalase. Determinaton coefficients (R^2) of fittings and p-values are also shown. Different letters in each column means significant difference between fish discards ($p < 0.05$).

	H_m (%)	α (dimensionless)	τ (min)	v_m (% min ⁻¹)	R^2	p-values
Tu_H	37.46±0.32 ^a	0.696±0.010 ^a	28.83±0.56 ^a	0.313±0.007 ^a	0.999	<0.005
Tu_TF	37.81±0.34 ^a	0.717±0.012 ^a	25.37±0.50 ^b	0.370±0.009 ^b	0.998	<0.005
Tu_V	29.78±0.12 ^b	0.699±0.011 ^a	13.68±0.19 ^c	0.528±0.008 ^c	0.998	<0.005

Table S4. Fatty acids (as %) presents in the fish oils recovered from turbot wastes complementary to the production of FPHs. Error are the intervals of confidence for n=3 and $\alpha=0.05$.

Formula	Fatty acids	Tu_TF	Tu_H	Tu_V
C14:0	Myristic acid	5.88±0.55	5.52±0.30	5.71±0.35
C15:0	Pentadecanoic acid	0.56±0.15	0.64±0.12	0.64±0.10
C16:0	Palmitic acid	19.80±1.37	20.68±1.39	20.59±1.04
C16:1n7c	Palmitoleic acid	6.87±1.49	6.54±0.44	6.77±0.90
C18:0	Stearic acid	2.82±0.38	2.52±0.23	2.68±0.28
C18:1n9c,t	Oleic acid	34.14±5.60	33.54±1.68	33.22±2.92
C18:2n6c,t	Linoleic acid	12.89±2.25	12.15±0.97	12.51±1.20
C18:3n3	Linolenic acid	5.14±0.98	6.00±0.63	5.51±0.65
C20:1n9	Eicosenoic acid	3.15±2.16	2.56±0.38	3.25±1.15
C20:2n6	Eicosadienoic acid	1.57±0.30	1.53±0.23	1.61±0.21
C20:4n6	Arachidonic acid	1.36±0.44	1.32±0.15	1.38±0.18
C21:4n3	Heneicosatetraenoic acid	1.46±0.47	1.36±0.15	1.46±0.28
C20:5n3	Eicosapentaenoic acid (EPA)	0.46±0.17	0.45±0.06	0.48±0.10
C22:6n3	Docosahexaenoic acid (DHA)	3.87±2.27	5.19±0.47	4.19±1.38
	DHA+EPA (%)	4.34±2.12	5.65±0.43	4.67±1.30
	r: ω-3 / ω-6	0.71±0.18	0.87±0.11	0.77±0.14