

1 **Green fractionation and hydrolysis of fish meal to improve**
2 **their techno-functional properties**

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16 **Supplementary material**

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18 **Table S1.** Amino acid profile of the fish meal (FM) and of the water-soluble protein (WSP) and non-water-soluble protein (NSP) fractions. Free amino acid
 19 profile of the hydrolysates obtained by subcritical water hydrolysis (SWH) and enzymatic hydrolysis with Alcalase® (Alc) of FM, WSP, and NSP substrates.

Amino acid	Fish meal, mg aa/g _{protein}	WSP, mg aa/g _{protein}	NSP, mg aa/g _{protein}	Free amino acids generated during hydrolysis, mg aa/g _{protein}					
				FM		WSP		NSP	
				SWH	Alc	SWH	Alc	SWH	Alc
ALA	89 ± 3	88 ± 7	64 ± 2	15 ± 2	0.9 ± 0.1	72.8 ± 0.4	9.7 ± 0.1	11.6 ± 0.1	0.38 ± 0.01
GLY	86 ± 2	199 ± 9	63 ± 1	16 ± 3	0.5 ± 0.05	130.4 ± 0.3	5.4 ± 0.1	9.7 ± 0.1	0.12 ± 0.01
VAL	43 ± 3	29 ± 5	45.8 ± 0.9	3.8 ± 0.5	0.73 ± 0.05	13.3 ± 0.1	5.03 ± 0.07	3.9 ± 0.1	0.13 ± 0.01
LEU	63 ± 4	36 ± 2	83 ± 1	4 ± 1	1.04 ± 0.05	13.4 ± 0.1	5.9 ± 0.1	3.6 ± 0.1	0.27 ± 0.02
ILE	36 ± 2	18 ± 2	36.2 ± 0.6	2.1 ± 0.4	0.54 ± 0.05	3.8 ± 0.1	3.4 ± 0.1	2.6 ± 0.1	0.05 ± 0.01
THR	43 ± 2	31 ± 2	48 ± 1	1.1 ± 0.1	0.53 ± 0.05	5.0 ± 0.1	2.5 ± 0.4	1.10 ± 0.03	0.10 ± 0.02
SER	44 ± 3	36 ± 5	38 ± 2	2.1 ± 0.5	0.56 ± 0.05	0.8 ± 0.1	2.44 ± 0.04	1.83 ± 0.05	0.13 ± 0.01
PRO	58 ± 3	111 ± 5	48.9 ± 0.8	5.6 ± 0.9	0.62 ± 0.05	52 ± 1	3.6 ± 0.1	4.3 ± 0.1	0.09 ± 0.02
ASP	94 ± 2	70 ± 7	130 ± 4	9.7 ± 0.2	2.47 ± 0.05	2.4 ± 0.1	3.0 ± 0.1	8.0 ± 0.1	0.55 ± 0.07
MET	27 ± 3	13 ± 2	35 ± 0.7	2.4 ± 0.5	0.66 ± 0.03	6.0 ± 0.3	2.00 ± 0.03	2.0 ± 0.1	0.23 ± 0.03
HYP	24 ± 3	58 ± 9	1.9 ± 0.6	3.2 ± 0.04	0.13 ± 0.01	12 ± 1	0.36 ± 0.01	4.63 ± 0.03	0.19 ± 0.05
GLU	110 ± 6	103 ± 14	127 ± 8	--	--	9.2 ± 0.2	1.93 ± 0.02	0.47 ± 0.03	--
PHE	34 ± 2	21 ± 2	47 ± 3	5.1 ± 0.1	1.1 ± 0.1	6.3 ± 0.1	3.1 ± 0.1	4.8 ± 0.1	0.37 ± 0.07
LYS	57 ± 4	37 ± 6	53 ± 5	5.0 ± 0.1	1.8 ± 0.07	6.2 ± 0.2	3.5 ± 0.1	4.9 ± 0.1	0.7 ± 0.1
HYS	22 ± 2	26 ± 2	22.6 ± 0.8	3.4 ± 0.1	3.8 ± 0.1	6.9 ± 0.1	19.0 ± 0.7	2.9 ± 0.1	0.8 ± 0.1
HLY	1.9 ± 0.4	53 ± 18	2.3 ± 0.2	--	0.82 ± 0.01	--	0.25 ± 0.02	0.78 ± 0.05	0.8 ± 0.2
TYR	26 ± 2	15 ± 3	36 ± 1	2.3 ± 0.5	0.6 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	2.3 ± 0.1	0.31 ± 0.05
TRP	8 ± 1	54 ± 12	11 ± 1	0.7 ± 0.3	0.2 ± 0.1	0.81 ± 0.05	0.72 ± 0.03	0.7 ± 0.1	0.40 ± 0.02
CYS	2.3 ± 0.6	25 ± 9	3.0 ± 0.3	0.67 ± 0.01	0.60 ± 0.01	0.26 ± 0.03	0.11 ± 0.01	0.64 ± 0.03	0.40 ± 0.1
Total	868 ± 48	1021 ± 121	896 ± 36	84 ± 4	18 ± 1	344 ± 5	75 ± 1	71 ± 2	6.5 ± 0.9
Very large, large, non-polar (%)*	22.3	15.2	27.7	19.8	23.0	9.7	23.8	22.5	25.1

ALA: alanine, GLY: glycine, VAL: valine, LEU: leucine, ILE: isoleucine, THR: threonine, SER: serine, PRO: proline, ASP: aspartic acid, MET: methionine, HYP: hydroxyproline, GLU: glutamic acid, PHE: phenylalanine, LYS: lysine, HYS: histidine, HLY: hydroxylysine, TYR: tyrosine, TRP: triptophan, CYS: cysteine.

* Includes LEU, ILE, MET, PHE, TYR and TRP, according to IMGT amino acid classes(Pommić et al., 2004).

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22 **Table S2.** Stern-Volmer parameters of the FDHs obtained from fish meal (FM), its water-soluble
 23 fraction (WSP), and its non-water-soluble fraction (NSP) by subcritical water hydrolysis (SWH)
 24 and enzymatic hydrolysis with Alcalase[®] (Alc). Free amino acid concentration in the synthetic
 25 solution as in Table S1 for WSP [SWH].

	K_{SV} * (M⁻¹)	intercept	R²
SWH			
FM	12.3 ± 0.2	1.31 ± 0.06	0.9963
WSP	3.9 ± 0.4	1.23 ± 0.07	0.9510
NSP	12.9 ± 0.2	1.07 ± 0.05	0.9970
Alc			
FM	8.3 ± 0.3	1.04 ± 0.06	0.9902
WSP	3.16 ± 0.08	1.12 ± 0.02	0.9949
NSP	7.82 ± 0.08	1.08 ± 0.02	0.9990
Free aa solution	16.7 ± 0.3	0.88 ± 0.07	0.9970

* K_{SV}: Stern-Volmer constant.

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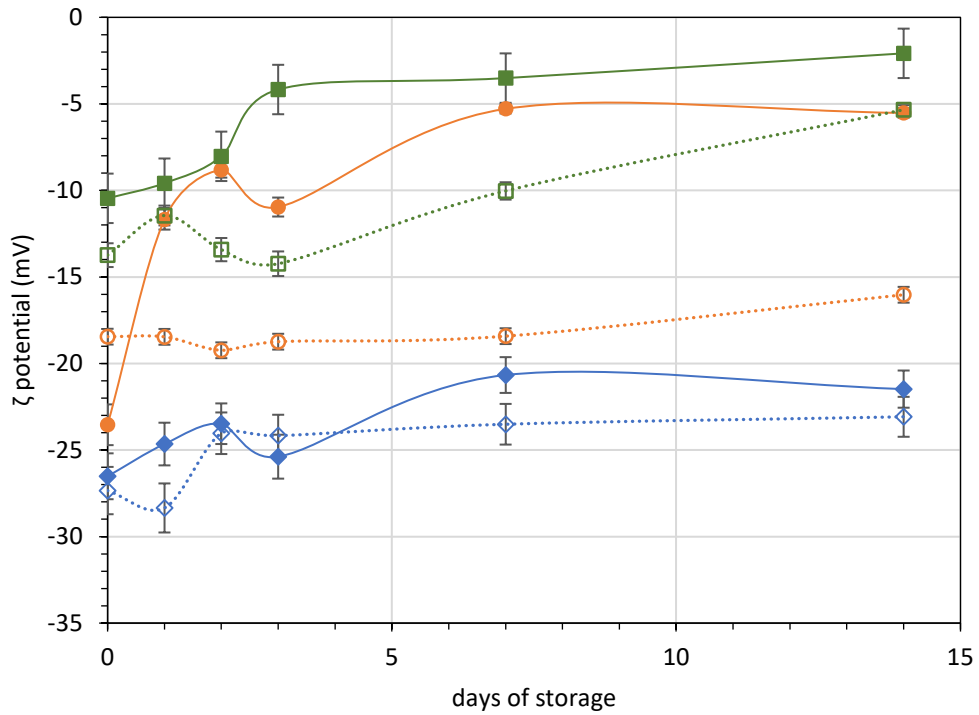
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29 **Table S3.** Evolution of the volume weighted mean droplet size (D[4,3]) of emulsions stabilised
30 with freeze-dried hydrolysates (FDHs) during storage. FDHs obtained from fish meal (FM), its
31 water-soluble fraction (WSP), and its non-water-soluble fraction (NSP) by subcritical water
32 hydrolysis (SWH) and enzymatic hydrolysis with Alcalase® (Alc).

D [4, 3] - Volume weighted mean (µm)						
days	0	1	3	7	14	14 + SDS
SWH						
FM	0.224	0.582	1.289	9.206	15.468	2.045
WSP	11.733	25.555	28.035	37.399	73.092	18.144
NSP	0.155	0.184	0.190	0.220	6.041	0.323
Alc						
FM	7.939	7.678	11.678	12.316	11.609	2.382
WSP	9.096	11.306	14.537	27.628	58.150	10.089
NSP	4.586	4.803	4.669	4.816	4.901	4.234

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37 **Figure S1.** Evolution of the electrokinetic potential (ζ potential) of emulsions stabilised with
 38 freeze-dried hydrolysates (FDHs) during storage. FDHs obtained from fish meal (FM, ● ○), its
 39 water-soluble fraction (WSP □ ■), and its non-water-soluble fraction (NSP, ◆ ◇) by
 40 subcritical water hydrolysis (SWH, full symbols) and enzymatic hydrolysis with Alcalase®
 41 (Alc, hollow symbols). Lines are drawn to guide the eye.

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43 **References**

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