Supplementary Information

Isolation of Hydroxyapatite from Atlantic Salmon Processing Waste Using a Protease and Lipase Mixture

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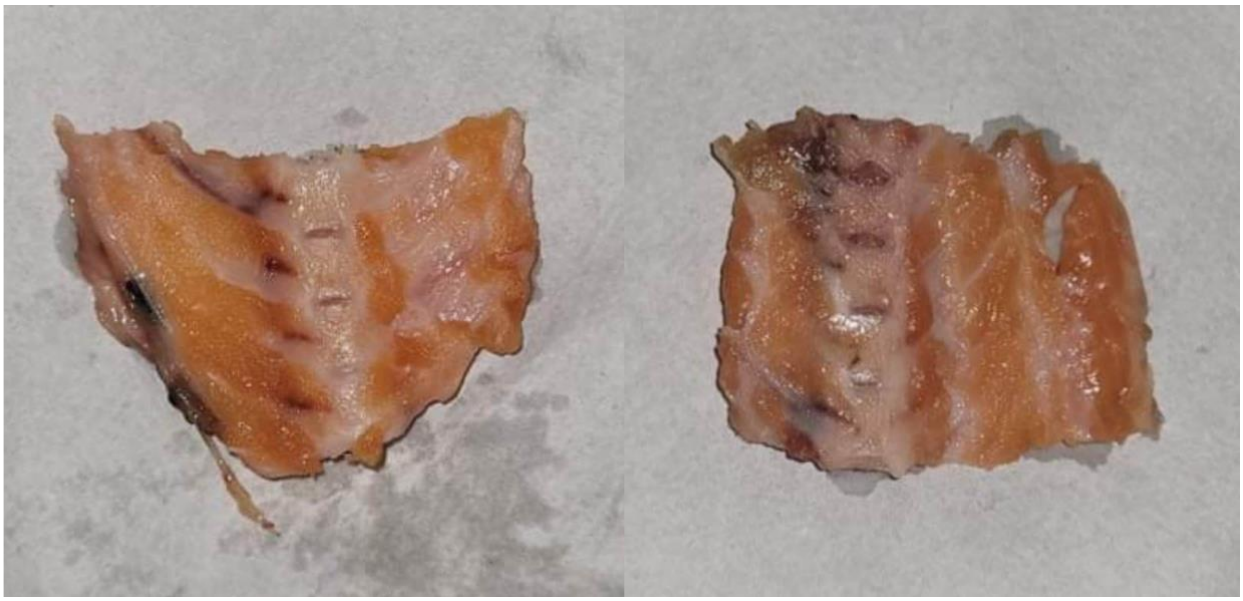

Figure S1. Images of 15 – 50 g samples used for Neutrase-only experiments (~ 5 × 7 cm).

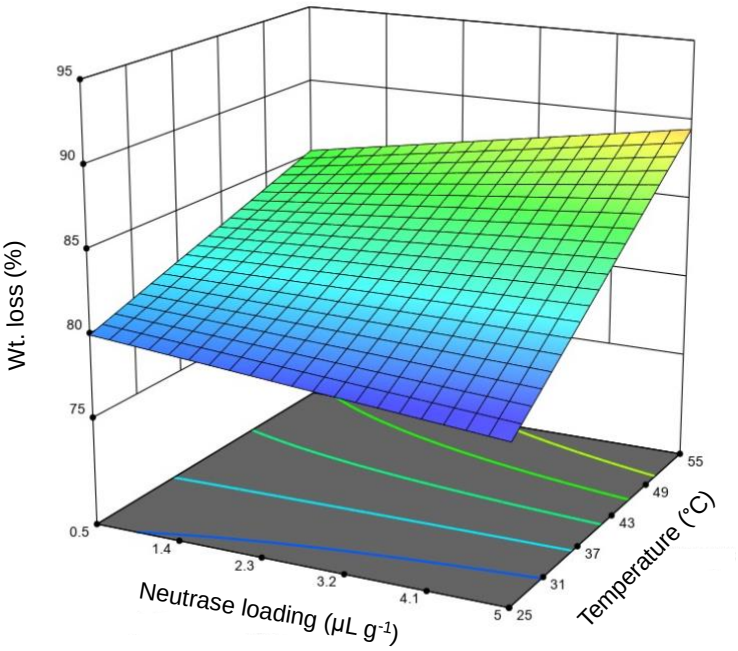

Figure S2. Response surface of wt. loss from enzymatically treating salmon frames based on Neutrase loading and the temperature of the reaction. Wt. loss increases as the temperature and Neutrase loading increase.

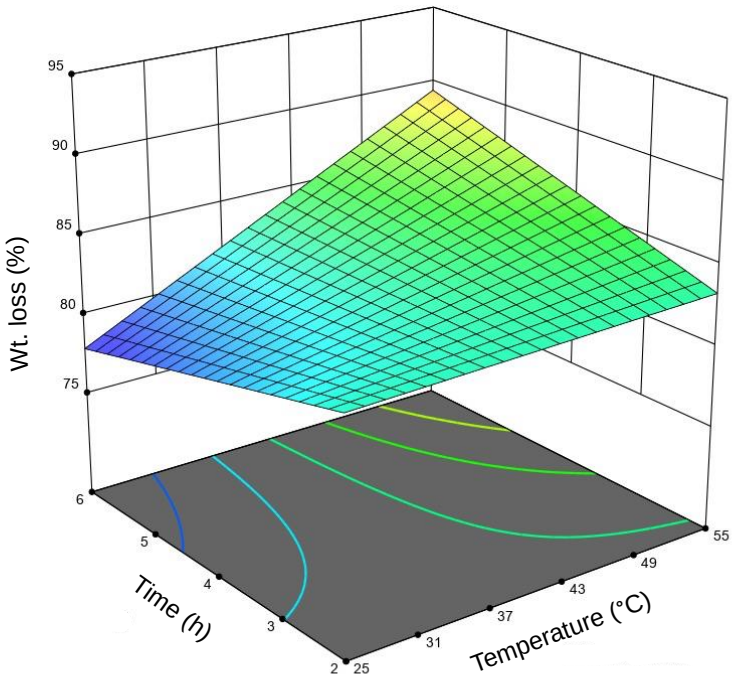

Figure S3. Response surface of wt. loss from enzymatically treating salmon frames based on the length and the temperature of the reaction. The wt. loss increases as the loading of both enzymes and the time increase.

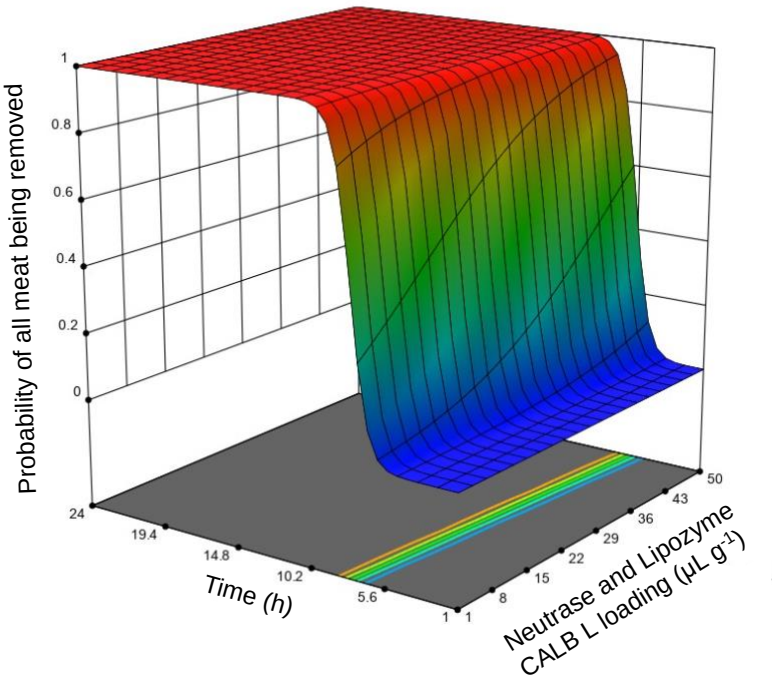

Figure S4. Response surface of whether an enzyme treatment is successful to hydrolyze all meat from the salmon frames based on enzyme loading and the length of the reaction. The probability of all meat being hydrolyzed from the salmon frame increases as the enzyme loading and the length of the reaction increase.

Table S1. Wt. loss from Neutrase optimization experiments to isolate sHAP including pass/fail response.

Entry	Enzyme loading (μL g^{-1})	Temperature (°C)	Time (h)	Wt. loss (%)	Pass/fail
1A	5.0	55.0	6	92.1	Fail
1B	5.0	55.0	2	87.1	Fail
1C	0.50	55.0	6	88.1	Fail
1D	0.50	55.0	2	82.2	Fail
1E	5.0	25.0	6	79.4	Fail
1F	5.0	25.0	2	82.0	Fail
1G	0.50	25.0	6	78.6	Fail
1H	0.50	25.0	2	89.2	Fail
1I	2.8	40.0	6	79.8	Fail
1J	0.50	40.0	4	86.8	Fail
1K	5.0	40.0	4	81.6	Fail
1L	2.8	40.0	2	82.8	Fail
1M	2.8	55.0	4	79.5	Fail
1N	2.8	25.0	4	84.4	Fail
1O	1.6	47.5	5	84.3	Fail
1P	1.6	47.5	3	87.0	Fail
1Q	3.9	47.5	5	87.4	Fail
1R	5.0	55.0	4	84.0	Fail
1S	5.0	55.0	2	84.3	Fail
1T	0.50	55.0	4	83.9	Fail
1U	3.5	55.0	6	87.3	Fail

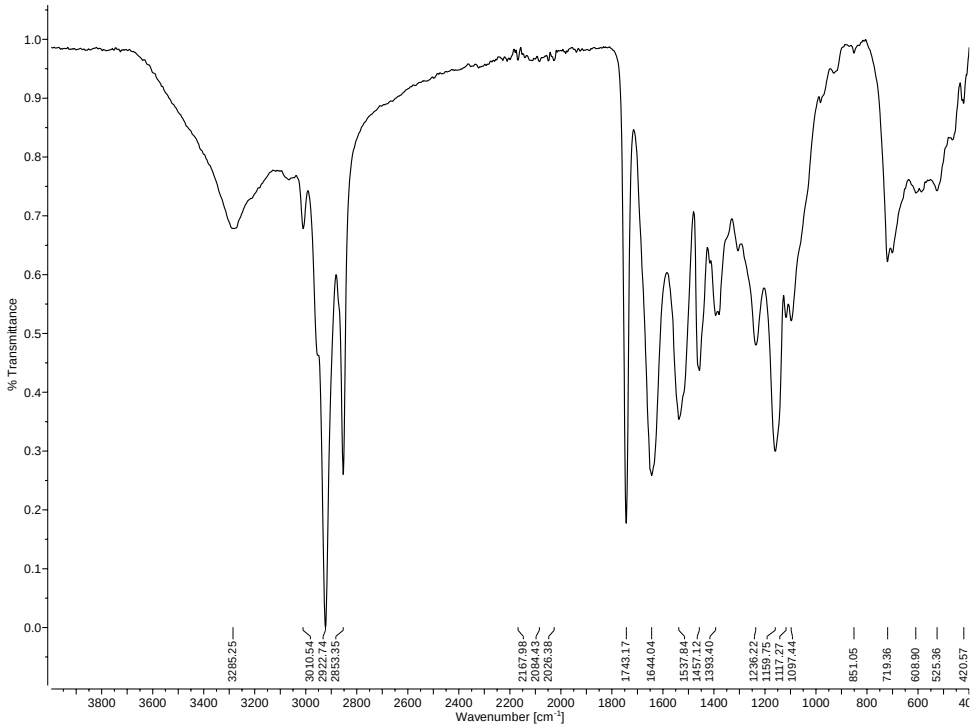

Figure S5. FTIR spectrum of freeze-dried, raw salmon meat.

Table S2. Peak list and assignment of FTIR spectrum of freeze-dried, raw salmon meat.

Functional group	Assignment	Wavenumber (cm^{-1})
-OH	Water/proteins/lipids	3285
-C-H	Proteins/lipids	3010, 2923, 2853, 1457, 1393
-C=O	Collagen/lipids	1743
$-CONH_2$	Collagen	1644, 1538, 1236
-COOC-	Lipids	1305, 1160, 1097
PO_4^{3-}	Lipids	1117, 1097, 609, 525
C=C	Lipids	851

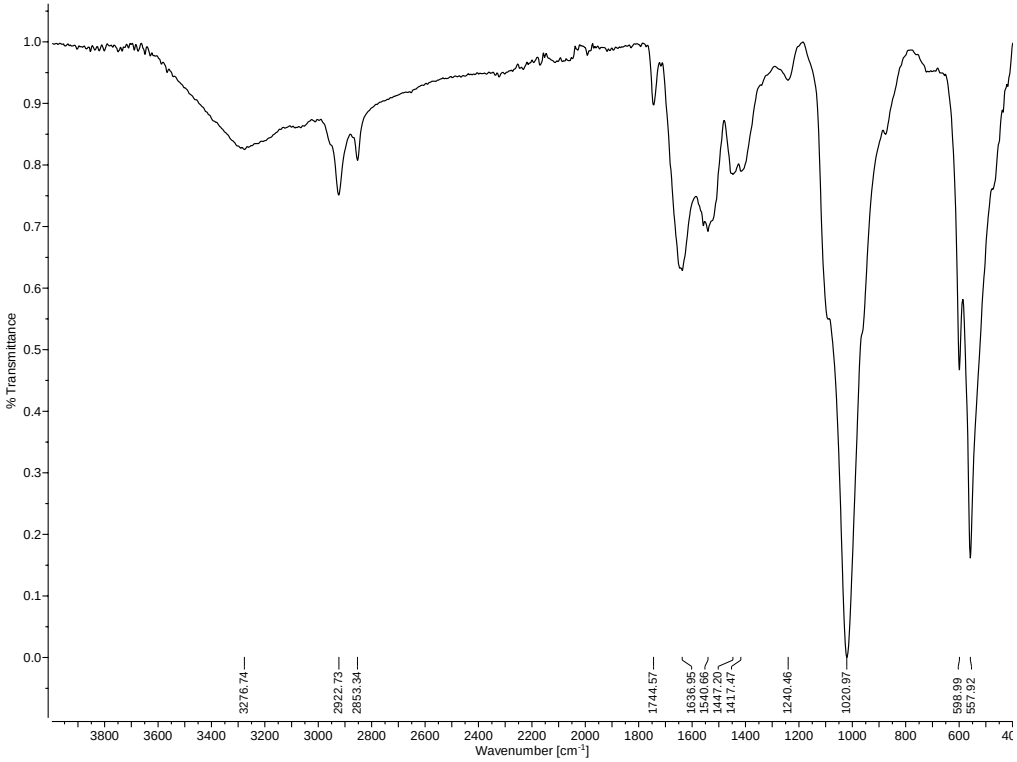

Figure S6. FTIR spectrum of crude sHAP (1A: 5 μL g^{-1} Neutrase, 6 h, 55 °C).

Table S3. Peak list and assignment of FTIR spectrum of crude sHAP (1A: 5 μL g^{-1} Neutrase, 6 h, 55 °C).

Functional group	Assignment	Wavenumber (cm^{-1})
-OH	Water	3277
-C-H	Proteins/lipids	2923, 2853
-C=O	Collagen/lipids	1745
$-CONH_2$	Collagen	1637, 1541, 1241
CO_3^{2-}	Carbonate ions	1447, 1417
PO_4^{3-}	HAP	1021, 599, 558

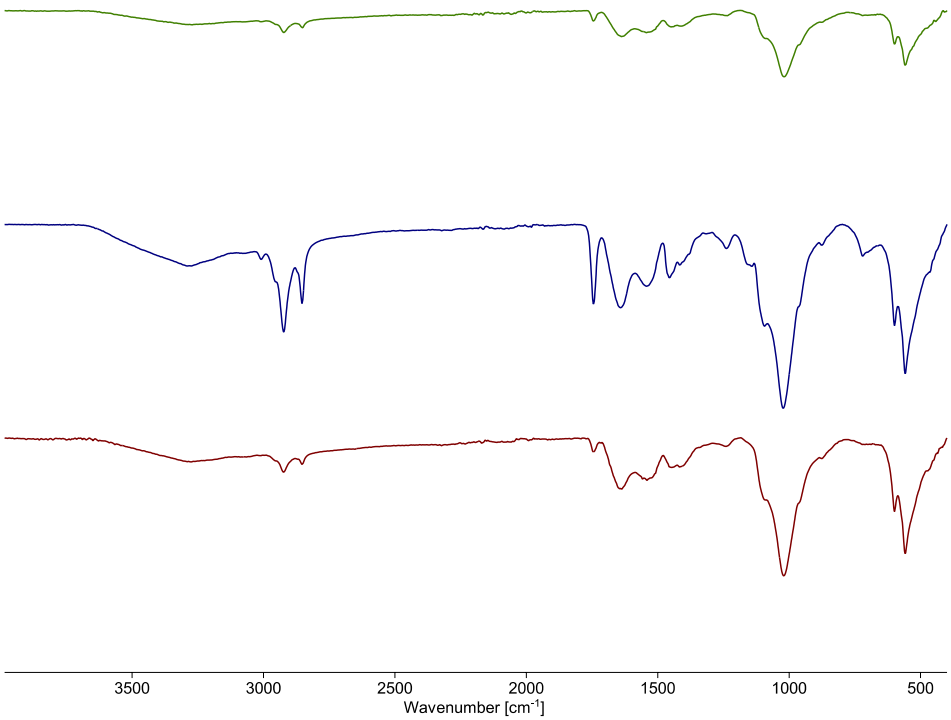

Figure S7. FTIR spectra of 3 samples treated with 5 μL g^{-1} Neutrase for 6 h at 55 °C. The relative intensity of the carbonyl stretch associated with lipid presence is inconsistent between spectra.

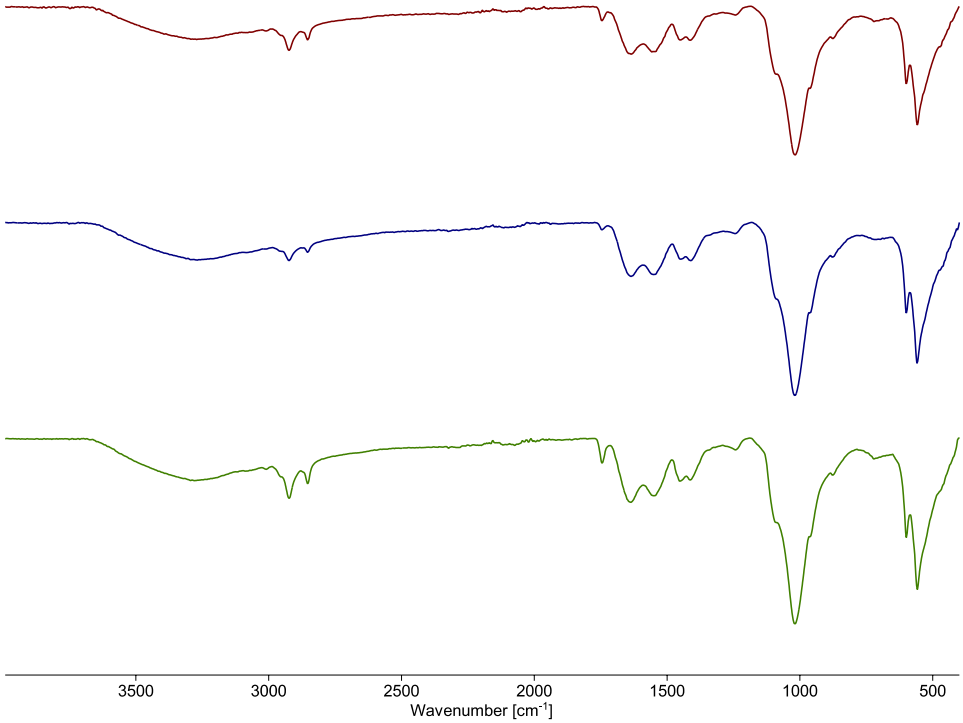

Figure S8. FTIR spectra of 3 samples treated with 25 μL g^{-1} Neutrase and 25 μL g^{-1} Lipozyme CALB L for 24 h at 55 °C. The relative intensities of all stretches are consistent.

Figure S9. Image of sample used for Neutrase and Lipozyme CALB L optimization experiments.

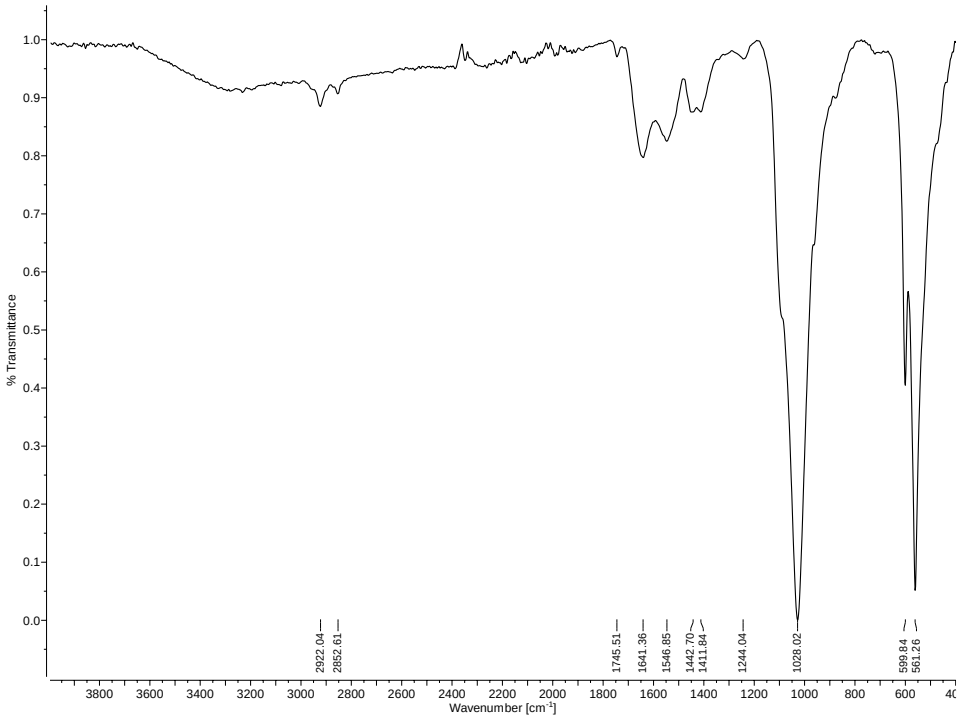

Figure S10. FTIR spectrum of pure sHAP (2Q: 15 μL g^{-1} Neutrase, 7.5 μL g^{-1} Lipozyme CALB L, 6 h, 40 °C).

Table S4. Peak list and assignment of FTIR spectrum of pure sHAP (2Q: 15 µL g^{-1} Neutrase, 7.5 µL g^{-1} Lipozyme CALB L, 6 h, 40 °C).

Functional group	Assignment	Wavenumber (cm^{-1})
-C-H	Collagen	2922, 2853
-C=O	Collagen/amino acid residues	1746
$-CONH_2$	Collagen	1641, 1547, 1244
CO_3^{2-}	Carbonate ions	1443, 1412
PO_4^{3-}	HAP	1021, 599, 561

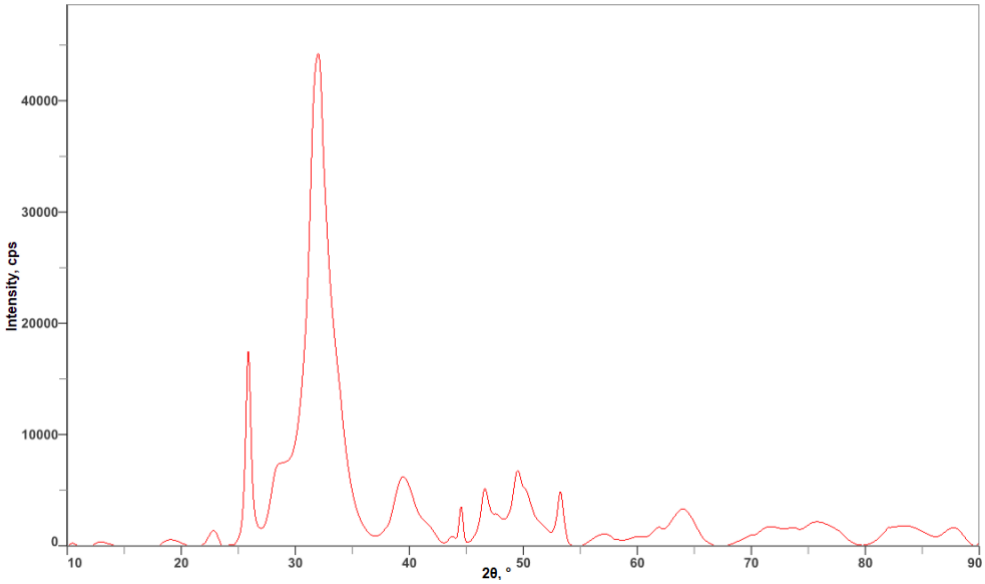

Figure S11. XRD diffractogram of crude sHAP (1A: 5 µL g^{-1} Neutrase, 6 h, 55 °C).

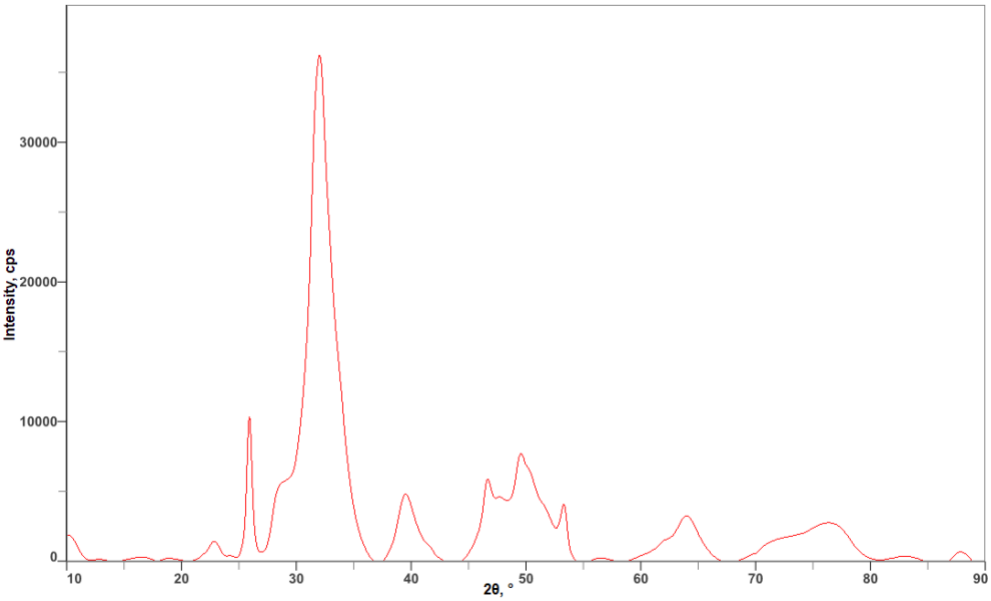

Figure S12. XRD diffractogram of pure sHAP (2Q: 15 µL g^{-1} Neutrase, 7.5 µL g^{-1} Lipozyme CALB L, 6 h, 40 °C).

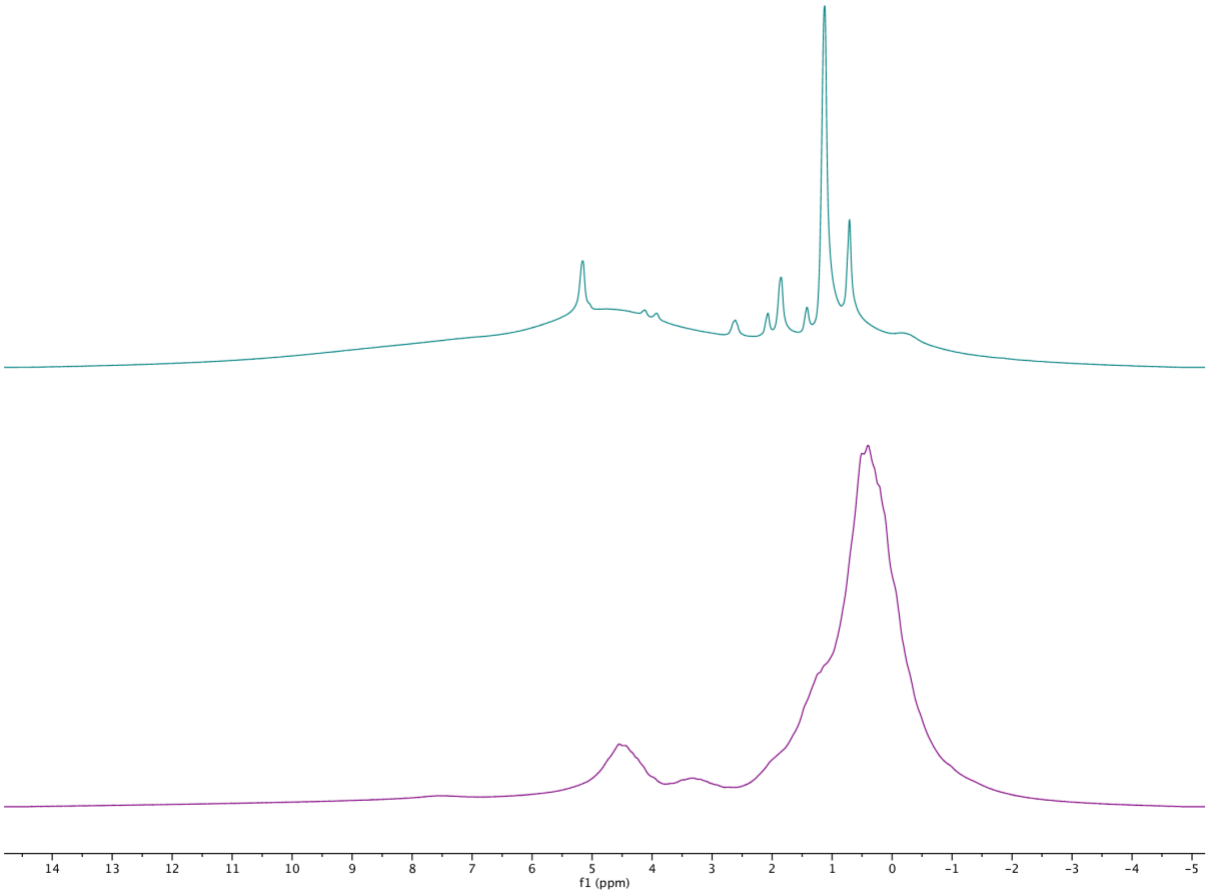

Figure S13. ^{1}H MAS NMR spectra of pure sHAP (2Q: 15 µL g^{-1} Neutrase, 7.5 µL g^{-1} Lipozyme CALB L, 6 h, 40 °C) (top, blue) and freeze-dried, raw salmon meat (bottom, purple).

Equation S1. Dynamic light scattering PDI:

$$\mathrm{PDI} = \left(\frac{\text{st. dev.}}{\text{mean}}\right)^2$$

For generally nanoparticle or colloidal suspensions with little aggregation, a PDI of 0.1 – 0.4 would be expected. Anything above 0.4 indicates that the particles tend to aggregate and the solution is not monodisperse.

Life Cycle Analysis of Fish Processing Waste Treatments

Assumed data for all compounds

Volume air	= 1.00×10^{10} m^3
Volume water	= 7.00×10^{6} m^3
Volume soil	= 9.00×10^{3} m^3
Volume sediment	= 2.00×10^{4} m^3
Density soil	= 1.5 tonnes m^{-3}
Density sediment	= 1.5 tonnes m^{-3}
Organic soil fraction	= 0.02
Organic fraction sediment	= 0.04
Energy consumption	= 0.042 g CO_2 kJ^{-1}

Equations used to calculate potentials

Acidification potential (I_{AP}) = $\sum AP_i \times m_i$

If not a gas, $AP_i = 0$

Smog formation (I_{SF}) = $\sum SFP_i \times m_i$

SFP_i = MIR_i / MIR_{ROG}

Global warming (I_{GW}) = $\sum_i (GWP_i \times m_i) + (X \text{ g } CO_2 \text{ kJ}^{-1} \times Y \text{ kJ})$

Heating liquid = $m \times C_p \times (T_f - 293.15 \text{ K}) \times n$

n = time (h) x 0.5

Oven/furnace = $(T_f - 293.15 \text{ K}) \times n$

n = time (h) x 0.5

Toxic inhalation (I_{INHT}) = $\sum_i INHTP_i \times m_i$

$INHTP_i$ = $(C_{i,a} / LC_{50,\,i}) / (C_{tol,\,a} / LC_{50,\,tol})$

Toxic ingestion (I_{INGT}) = $\sum_i INGTP_i \times m_i$

$INHTP_i$ = $(C_{i,w} / LD_{50,\,w}) / (C_{tol,\,w} / LD_{50,\,w})$

Persistence (PER)

If PER < weeks = LOW

If PER > weeks, < months = MOD

If PER > months = HIGH

Bioaccumulation (ACCU) = $\log(K_{ow})$

If $\log(K_{ow}) < 3.5$ = LOW

If $\log(K_{ow})$ 3.5 – 4.3 = MOD

If $\log(K_{ow}) > 4.3$ = HIGH

Abiotic depletion (I_{AD}) $= \sum_i ADP_i \times m_i$

ADP_i $= ((\text{depletion rate})_i / (\text{reserves})_i) / ((\text{depletion rate})_{ref} / (\text{reserves})_{ref})$

Potentials omitted from Table 5 (LCA):

I_{AP} and I_{AD} were 0 for all treatments discussed, therefore they have been omitted from the LCA table.

Assumptions for enzymes

K_{oc} value of enzymes: This is not studied. Enzymes are soluble in water, therefore it should be close to 1. Methanol has a K_{ow} of 0.18 and butanol has a K_{ow} of 6.3. Therefore, we have assigned the K_{ow} of enzymes to be 3 and its log(K_{ow}) would be 0.48. To determine K_{oc}, we multiplied K_{ow} by 0.41 as suggested by Philip Jessop, therefore the value of K_{oc} is 1.23.

H value of enzymes: This is not studied. However, because we assume they have zero volatility, its value has been assigned to $H = 1 \times 10^{-50}$ as suggested by Jessop.

Molecular weight of enzymes: MW of proteases has been determined to be in the range of 15 to 30 kDa while the MW of lipases has been determined to be in the range of 19 to 60 kDa, therefore we assigned a MW of 22.5 kDa (2.25×10^4 g mol^{-1}) to Neutrase and Alcalase and a MW of 39.5 kDa (3.95×10^4 g mol^{-1}) to Lipozyme CALB L.

Assumptions for NaOH and H_2O_2 treatments

H value of NaOH: NaOH has zero volatility, its value has been assigned to $H = 1 \times 10^{-50}$ as suggested by Jessop.

"Highly alkaline solution": assume this means 50% NaOH.

For Ahamed et al.: assume 250 g NaOH since not indicated.[1]

For Yamamura et al.: assume 200 g NaOH and 150 g H_2O_2 since not indicated.[2]

References

1. A. F. Ahamed, M. Manimohan and N. Kalaivasan, *J. Inorg. Organomet. Polym.*, 2022, **32**, 3902–3922.

2. H. Yamamura, V. H. P. da Silva, P. L. M. Ruiz, V. Ussui, D. R. R. Lazar, A. C. M. Renno and D. A. Ribeiro, *J. Mech. Behav. Biomed. Mater.*, 2018, **80**, 137–142.