Characterization of glycosaminoglycans in gaping and intact connective tissue of farmed Atlantic salmon (*Salmo salar*) fillets by mass spectrometry

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Table S1. Details of HS disaccharides measured.

HS disaccharide	Ion species	m/z	Retention time (min)
D0A0	1-	378	108
	Dimer, 1-	757	108
	Adduct, 1-	468	108
D2A0/D0A2	1-	458	98
D2A6	1-	538	92
	2-	268.5	92
D0S0	1-	416	103
D2S0/D0S6	1-	496	96
	2-	247.5	96
	SO_3 loss, 1-	416	96
D2S6	1-	576	89
	2-	287.5	89
	SO_3 loss, 1-	496	89
	SO_3 loss, 2-	247.5	89
U0A0	1-	396	108
U2A0/U0A6	1-	476	98
U2A6	1-	556	92
	2-	277.5	92
U0S0	1-	434	103
U2S0/U0S6	1-	514	96
	2-	256.5	96
	SO_3 loss, 1-	434	96
U2S6	1-	594	89
	2-	296.5	89
	SO_3 loss, 1-	514	89
	SO_3 loss, 2-	256.5	89

Figure S1

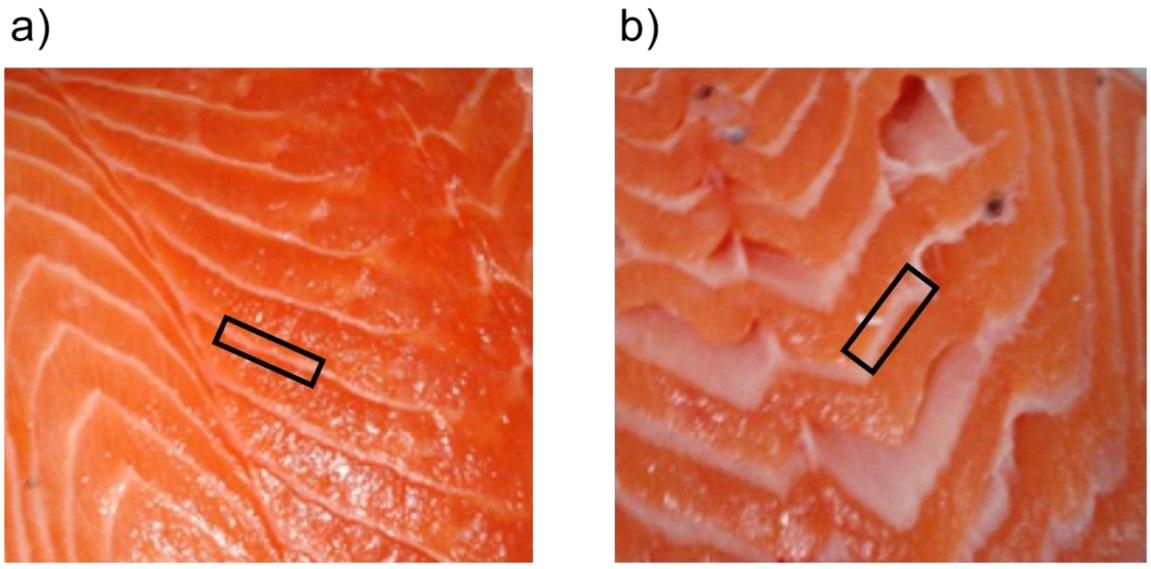

Figure S1. Fotographs of (a) intact and (b) gaping salmon fillets. Samples used in this study were obtained by cutting out the connective tissue and adjoining muscle as illustrated by the areas outlined in black. Photograph courtesy of Durita Nielsen. Copyright 2019.

Figure S2

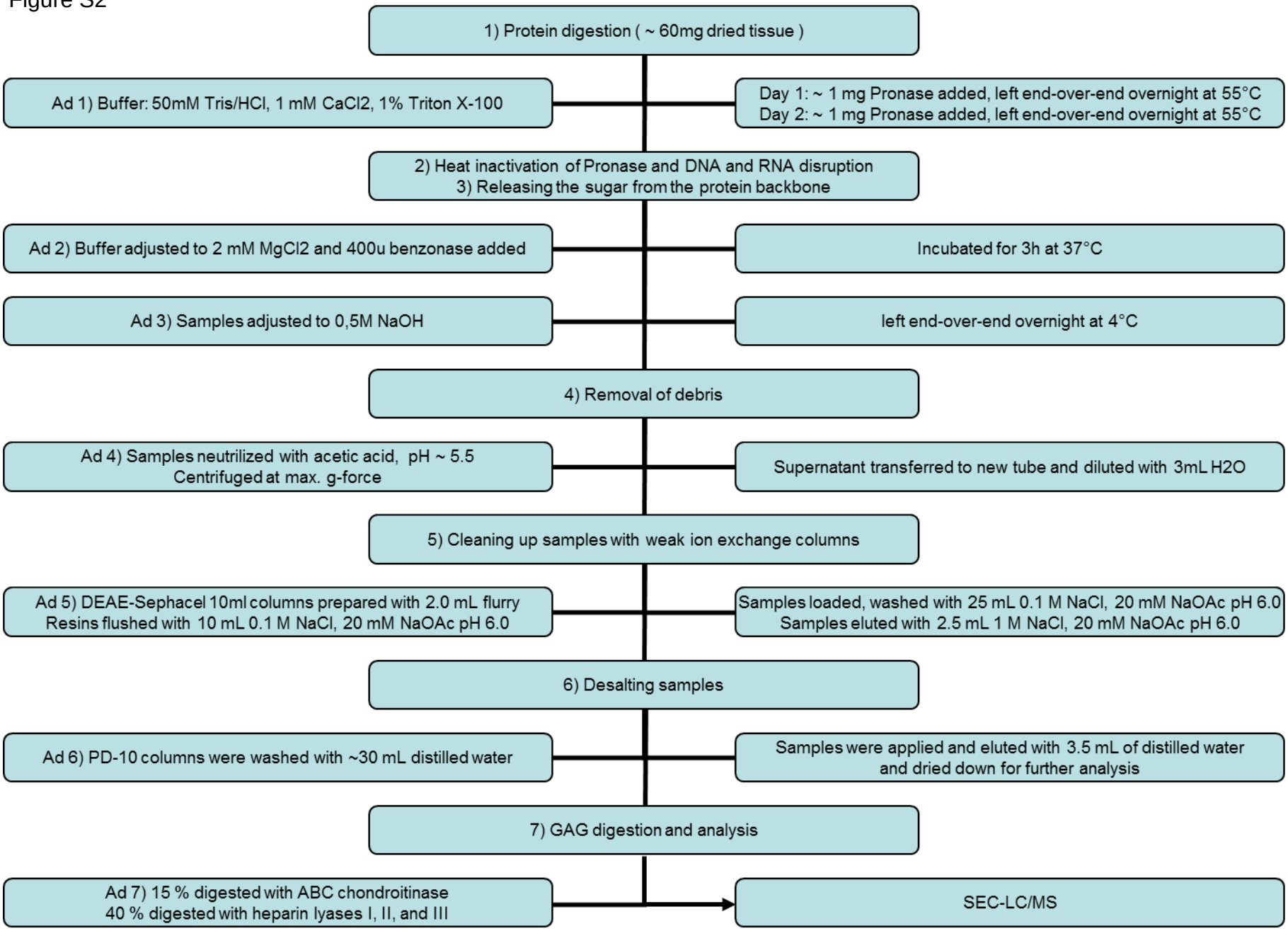
1) Protein digestion (~ 60mg dried tissue)
Ad 1) Buffer: 50mM Tris/HCl, 1 mM CaCl2, 1% Triton X-100
Day 1: ~ 1 mg Pronase added, left end-over-end overnight at 55°C
Day 2: ~ 1 mg Pronase added, left end-over-end overnight at 55°C
2) Heat inactivation of Pronase and DNA and RNA disruption
3) Releasing the sugar from the protein backbone
Ad 2) Buffer adjusted to 2 mM MgCl2 and 400u benzonase added
Incubated for 3h at 37°C
Ad 3) Samples adjusted to 0,5M NaOH
left end-over-end overnight at 4°C
4) Removal of debris
Ad 4) Samples neutrilized with acetic acid, pH ~ 5.5
Centrifuged at max. g-force
Supernatant transferred to new tube and diluted with 3mL H2O
5) Cleaning up samples with weak ion exchange columns
Ad 5) DEAE-Sephacel 10ml columns prepared with 2.0 mL flurry
Resins flushed with 10 mL 0.1 M NaCl, 20 mM NaOAc pH 6.0
Samples loaded, washed with 25 mL 0.1 M NaCl, 20 mM NaOAc pH 6.0
Samples eluted with 2.5 mL 1 M NaCl, 20 mM NaOAc pH 6.0
6) Desalting samples
Ad 6) PD-10 columns were washed with ~30 mL distilled water
Samples were applied and eluted with 3.5 mL of distilled water
and dried down for further analysis
7) GAG digestion and analysis
Ad 7) 15 % digested with ABC chondroitinase
40 % digested with heparin lyases I, II, and III
SEC-LC/MS

Figure S2. Outline of the GAG extraction and digestion procedures performed in the study