VERSCHUREN CENTRE

For Sustainability in Energy and the Environment

Assessment of the impact of extraction technologies on prebiotic saccharides

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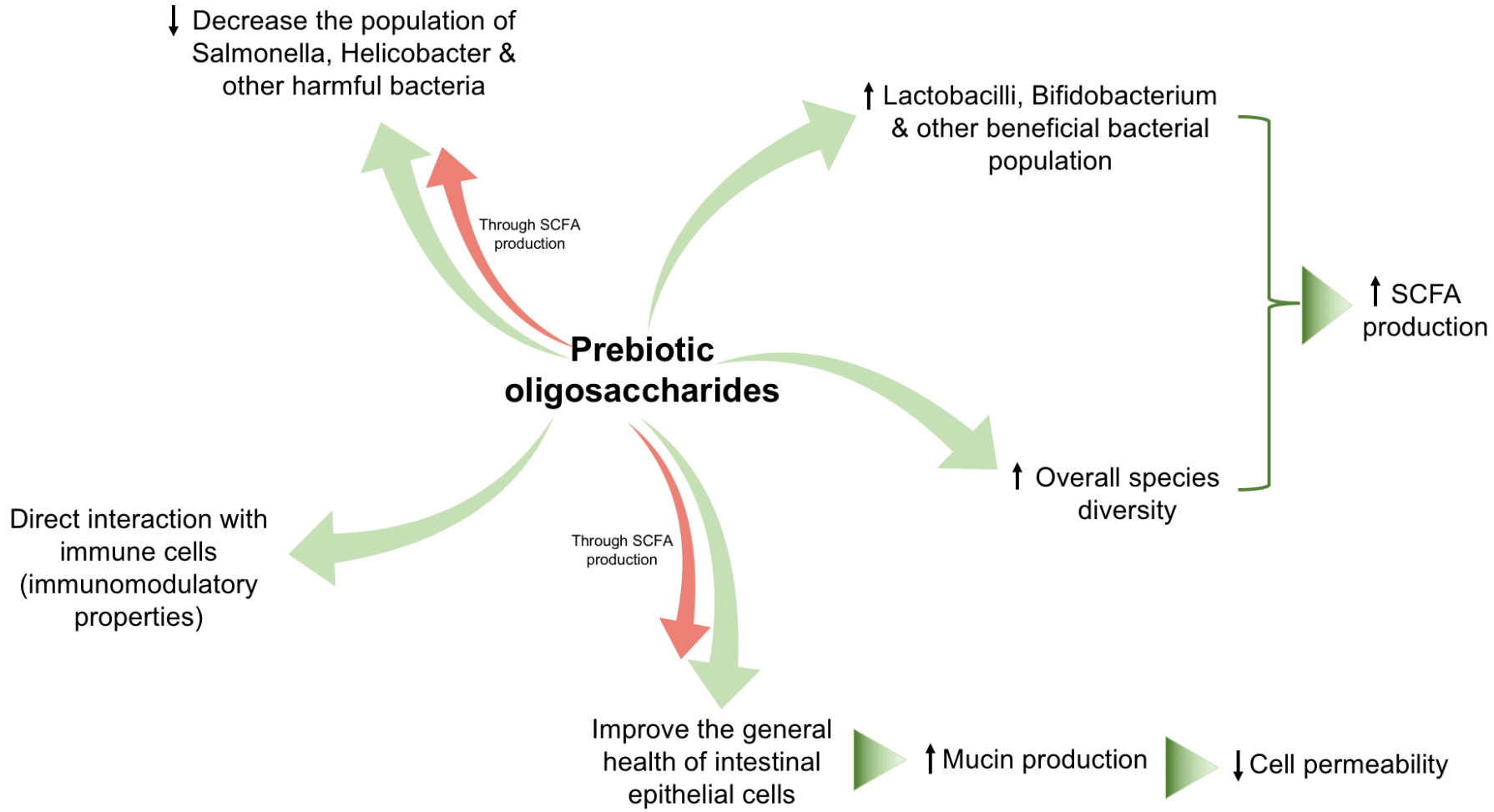
The Marine Ecosystem

- The ocean makes up about 70 % of the earth's surface and is home to a diverse range of marine life
- Underutilized marine macroalgae contain several active compounds that can be converted into value added products
- Food, feed, pharmaceutical and other industrial applications
- One group of such active components are polysaccharides from seaweeds with health-promoting prospects

Why Seaweeds

- Large group of marine multicellular algae: 1500–2000 species
- Abundant on the Atlantic (east) coast of Canada, aswell as the coast of Ireland
- Brown seaweeds contain a number of polysaccharides – laminarin, fucoidan, and alginate
- Prospective prebiotic for application as functional food ingredient/dietary supplement
- *Ascophyllum nodosum* – Rich source of fucoidan and alginate and are commercially viable

Prebiotics and Gut Health



Rajendran et al., 2017

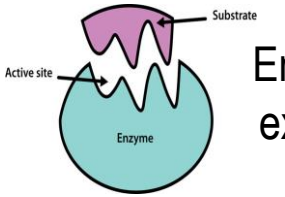
Extraction and Characterizations of Prebiotics

Extraction

Conventional extraction (CE)

Microwave-assisted extraction (MAE)

Ultrasound-assisted extraction (UAE)



Enzyme-assisted extraction (EAE)

Characterization

Monosaccharide composition (HPLC)

Molecular weight (GPC)

Sulphate levels (CHNS Analyser)

Sodium, potassium, and calcium (AAS)

in vitro prebiotic activity

Growth rate/doubling time –turbidometry (incubator, microplate reader)

Short chain fatty acid (HPLC)

pH measurement (pH meter)

Structure-function relationship between fucoidan extracts from *Ascophyllum nodosum* and *in vitro* prebiotic activity: Assessment of the impact of extraction technologies

Abbreviations of Fucoidan Extracts

Fuc-CCE	Fucoidan extracted from conventional chemical extraction
Fuc-MAE	Fucoidan extracted from microwave-assisted extraction
Fuc-UAE	Fucoidan extracted from ultasonication-assisted extraction
Fuc-EAE	Fucoidan extracted from enzyme-assisted extraction

Structural Properties of Fucoidan Extracts

	Extract yield (%w/w of pre-extracted A.nodosum)	Monosaccharide composition (%w/w of fucoidan extract)		Sulphate content (%w/w of fucoidan extract)
		Fucose	Galactose	
Fuc-CCE	11.9 ± 2.93	27.4 ± 3.27	6.56 ± 0.92	21.7 ± 1.71
Fuc-MAE	5.7 ± 1.01	37.0 ± 6.82	13.0 ± 4.08	18.8 ± 0.39
Fuc-UAE	4.56 ± 0.63	27.1 ± 2.04	8.53 ± 0.85	17.3 ± 2.18
Fuc-EAE	3.89 ± 0.55	29.1 ± 1.42	10.6 ± 1.06	15.4 ± 1.49

Fuc-CCE had significantly higher yield and sulphate than other extraction methods

Trend: Fuc-MAE had higher monosaccharide content (no statistical significance)

Structural Properties of Fucoidan

Extraction method	Component	Number average molecular weight, Mn (kDa)	Weight average molecular weight, Mw (kDa)	Polydispersity index (Đ)	Peak area
Fuc- CCE	Peak 1	40.2 ± 3.57	97.5 ± 7.80	2.47 ± 0.29	318.3 ± 144.6
	Peak 2	2.62 ± 0.12	2.79 ± 0.16	1.07 ± 0.01	12.05 ± 6.72
Fuc - MAE	Peak 1	30.8 ± 1.99	81.2 ± 8.07	2.64 ± 0.19	335.3 ± 116.2
	Peak 2	2.55 ± 0.08	2.86 ± 0.10	1.12 ± 0.007	40.3 ± 13.6
Fuc - UAE	Peak 1	121.1 ± 3.94	136.3 ± 4.39	1.13 ± 0.07	239.6 ± 65.6
	Peak 2	2.58 ± 0.07	2.70 ± 0.05	1.05 ± 0.008	57.5 ± 17.9
Fuc- EAE	Peak 1	100.1 ± 15.3	115.2 ± 8.87	1.16 ± 0.09	227.4 ± 108.7
	Peak 2	2.57 ± 0.02	2.73 ± 0.02	1.07 ± 0.002	27.62 ± 21.6

Mn provides information on the statistical average of all polymer chains within a sample, whereas Mw accounts for the molecular size of the sample

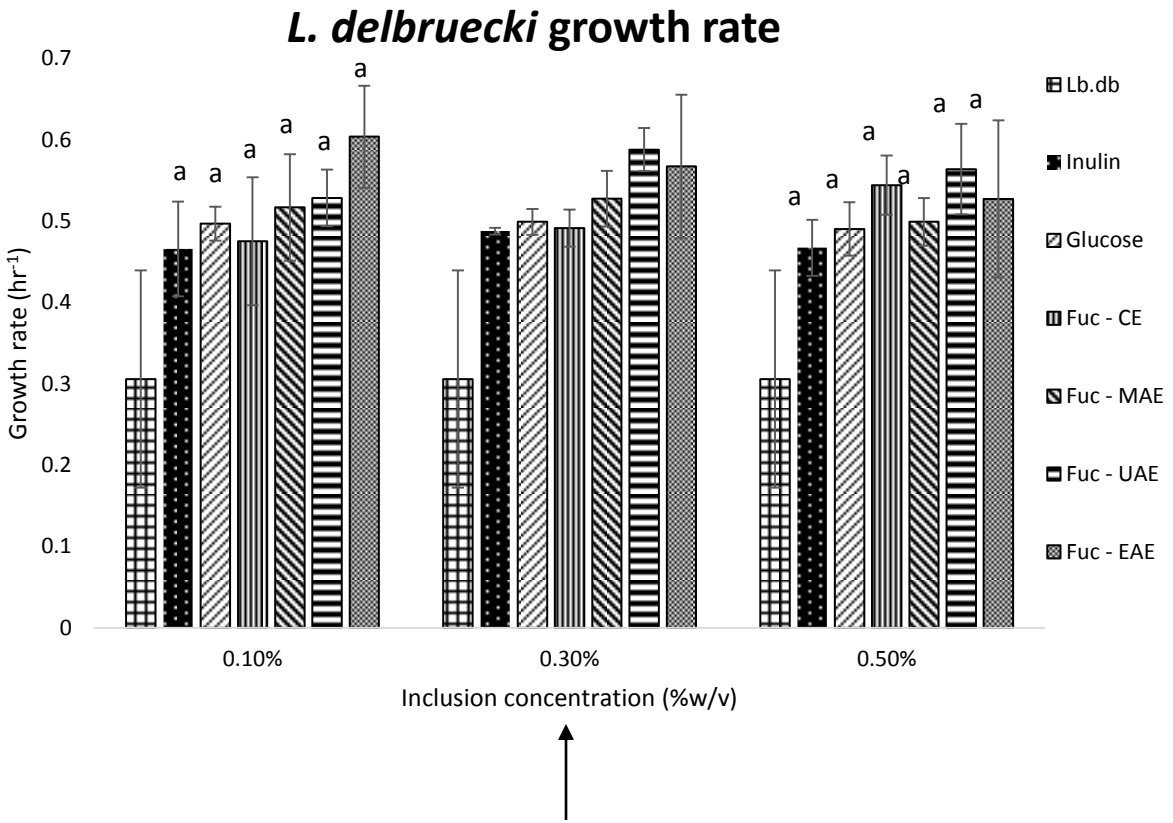
Fuc-MAE had the lowest molecular weight (both Mn and Mw), where as Fuc-UAE had highest molecular weight

Microwave extraction possibly resulted in partial hydrolysis generating lower molecular weight extracts

Prebiotic Activity of Fucoidan Extracts

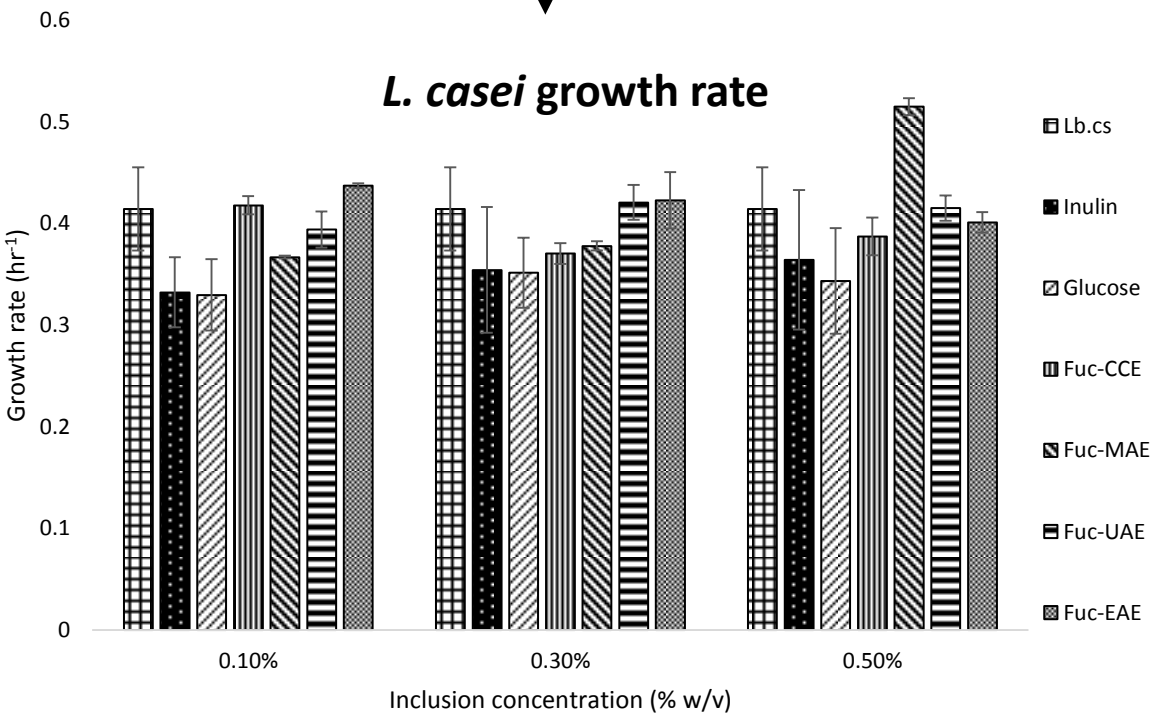
Extraction Method	*L. delbruecki* doubling time (hr)				*L. casei* doubling time (hr)			
	MRS+Lb.db	0.1%	0.3%	0.5%	MRS+Lb.cs	0.1%	0.3%	0.5%
Inulin	3.90 ± 2.19	3.031 ± 0.298	2.888 ± 0.558	2.806 ± 0.479	2.426 ± 0.239	2.171 ± 0.291	2.051 ± 0.017	2.149 ± 0.157
Glucose		3.057 ± 0.339	2.863 ± 0.267	2.960 ± 0.486		2.015 ± 0.087	2.005 ± 0.063	2.046 ± 0.140
Alg-CCE		2.03 ± 0.019	1.877 ± 0.187	1.597 ± 0.082		2.080 ± 0.036	1.927 ± 0.035	2.325 ± 0.064
Alg-MAE		1.870 ± 0.091	1.934 ± 0.103	1.312 ± 0.044		1.726 ± 0.129	1.807 ± 0.210	2.693 ± 0.457
Alg-UAE		2.135 ± 0.066	2.349 ± 0.109	1.762 ± 0.240		1.953 ± 0.066	1.830 ± 0.099	2.494 ± 0.023
Alg-EAE		2.04 ± 0.128	2.152 ± 0.033	1.686 ± 0.036		2.071 ± 0.095	1.928 ± 0.162	2.724 ± 0.363

Prebiotic Activity of Fucoidan Extracts



L. delbruecki growth rate improved at 0.1% and 0.5% w/v but no significant difference in activity amongst the extraction methods

Fuc-MAE improved the growth rate of L. casei by 24.5% at 0.5% w/v

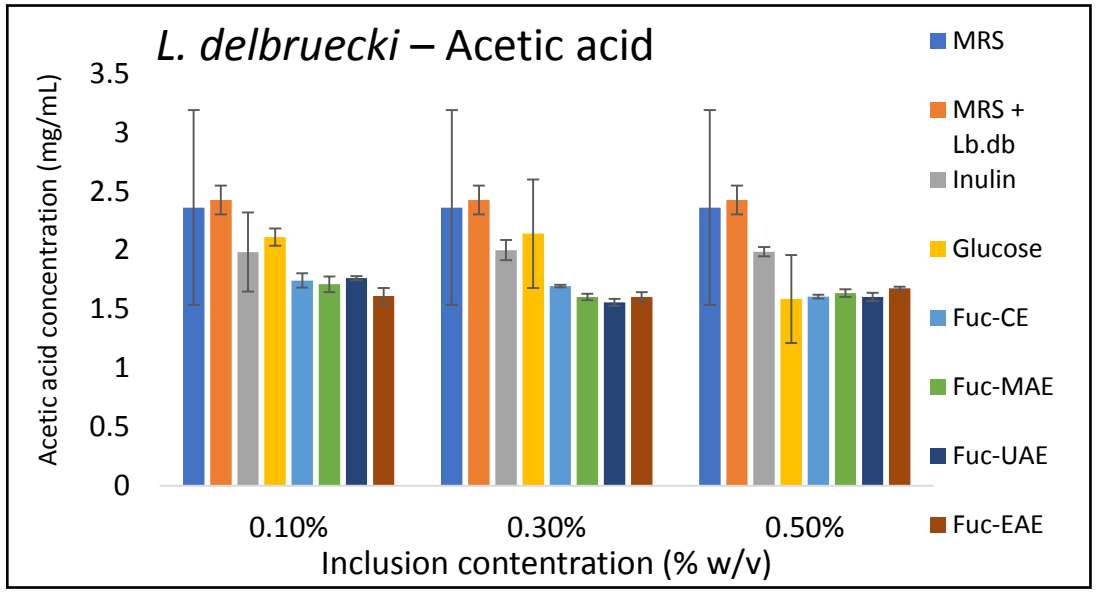


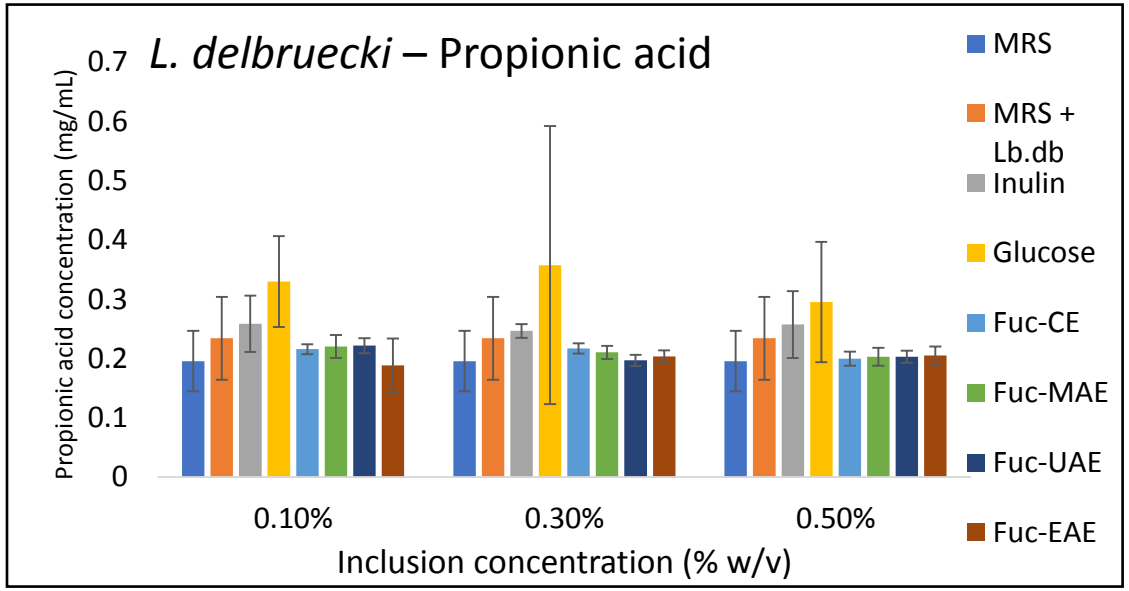
Prebiotic Activity of Fucoidan Extracts

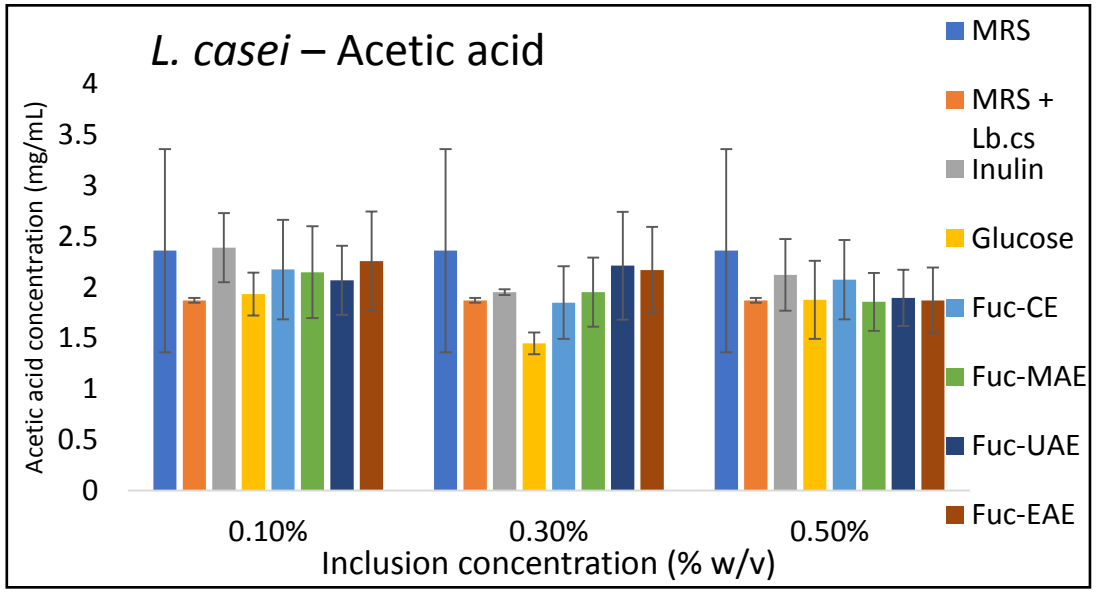
Extraction Method	Inclusion concentration	Change in pH of *L. delbruecki* media	Change in pH of *L. casei* media
MRS	-	-0.036	-0.036
L. delbruecki/L. casei	-	-1.275	-1.839
Inulin	0.1	-2.014	-1.876
	0.3	-1.984	-1.872
	0.5	-2.003	-1.927
Glucose	0.1	-2.010	-1.854
	0.3	-1.990	-1.877
	0.5	-1.996	-1.868
Fuc-CCE	0.1	-1.110	-2.314
	0.3	-0.987	-2.208
	0.5	-1.080	-2.216
Fuc-MAE	0.1	-1.419	-2.257
	0.3	-1.015	-2.143
	0.5	-1.150	-2.280
Fuc-UAE	0.1	-1.455	-2.277
	0.3	-1.103	-2.129
	0.5	-1.204	-2.171
Fuc-EAE	0.1	-1.261	-2.095
	0.3	-1.247	-2.114
	0.5	-1.097	-2.268

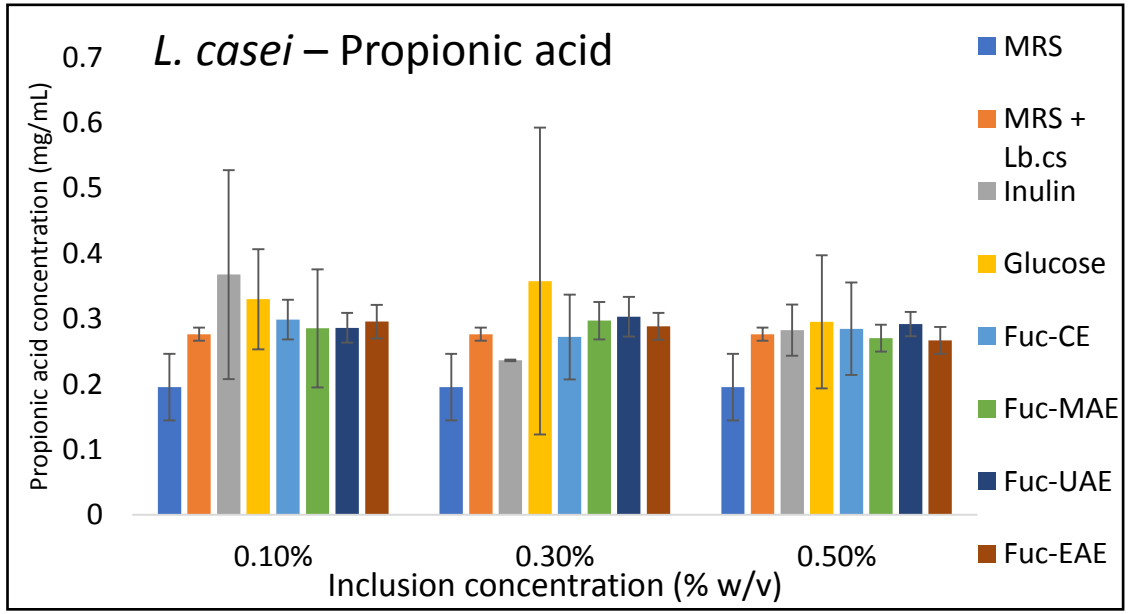
Higher reduction in the pH of extract supplemented media than blank control

Short Chain Fatty Acid Production









The impact of extraction technologies on the structure-function relationship between sodium alginate extracts and their in vitro prebiotic activity

Abbreviations of Fucoidan Extracts

Alg-CCE	Alginate extracted from conventional chemical extraction
Alg-MAE	Alginate extracted from microwave-assisted extraction
Alg-UAE	Alginate extracted from ultasonication-assisted extraction
Alg-EAE/CCE	Alginate extracted from enzyme-assisted extraction

Structural Properties of Alginate

Alg - CCE	71.61 ± 4.263
Alg - MAE	56.35 ± 1.344
Alg - UAE	70.15 ± 3.953
Alg – EAE/CCE	90.32 ± 5.198

Alg-EAE/CCE had significantly higher yield than other extraction methods, whereas Alg-MAE had lower yield

Sodium alginate extracts	Guluronic acid equivalent % (w/w of sodium alginate extract)	Mannuronic acid equivalent % (w/w of sodium alginate extract)
Alg-CCE	0.772 ± 0.150†	1.945 ± 0.357
Alg-MAE	0.334 ± 0.077†	0.905 ± 0.183
Alg-UAE	4.099 ± 0.390*	9.841 ± 0.926ϟ
Alg-EAE/CCE	2.503 ± 0.651#	6.053 ± 1.546ϟ
Alg - STD.*	25.78 ± 3.227	61.32 ± 7.661

Low uronic acid equivalent values were observed compared to the commercial alginate

Structural Properties of Alginate

Extraction method	Component	Number average molecular weight, Mn (kDa)	Weight average molecular weight, Mw (kDa)	Polydispersity index (Đ)	Peak area
Alg-CCE	Peak 1	56.8 ± 4.17	103.4 ± 2.78	1.82 ± 0.09	80.92 ± 10.1
	Peak 2	4.19 ± 0.05	5.02 ± 0.1	1.20 ± 0.01	43.61 ± 24.1
Alg-MAE	Peak 1	46.9 ± 2.89	65.4 ± 9.29	1.40 ± 0.26	59.99 ± 23.55
	Peak 2	4.41 ± 0.36	5.03 ± 0.19	1.14 ± 0.08	32.15 ± 15.9
Alg-UAE	Peak 1	121.5 ± 17.9	215.3 ± 7.88	1.18 ± 0.07	88.77 ± 70.12
	Peak 2	3.55 ± 0.36	3.85 ± 0.68	1.08 ± 0.08	5.54 ± 4.91
Alg-EAE/CCE	Peak 1	121.9 ± 13.5	172.4 ± 14.1	1.42 ± 0.08	147.2 ± 52.54
	Peak 2	4.73 ± 0.04	5.70 ± 0.2	1.21 ± 0.03	52.04 ± 41.12

Alg-UAE and Alg-EAE/CCE had higher molecular weight than other extracts

Similar to Fuc-MAE, Alg-MAE also had lower molecular weight. Additionally the peak area was also lower (corresponding with lower yield)

Structural Properties of Alginate

Extraction method	M-OH wave number (cm^{-1})	G-OH wave number (cm^{-1})	M-OH peak intensity	G-OH peak intensity	M/G ratio
Alg-CCE	1032	1085	44.54	51.44	0.866
Alg-MAE	1034	1095	49.16	54.73	0.898
Alg-UAE	1034	1085	24.65	31.36	0.786
Alg-EAE/CCE	1034	1086	41.29	46.78	0.883
Alg-STD*	1031	1095	53.93	55.84	0.966

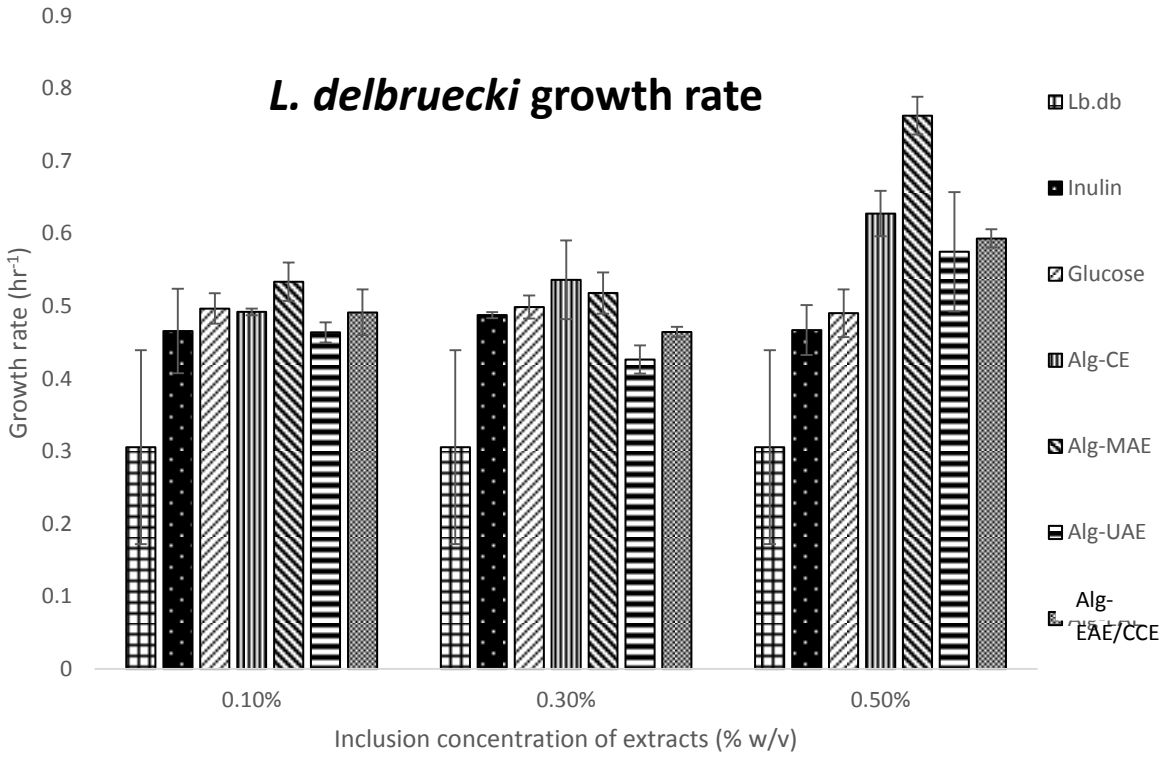
M/G ratio indicates mannuronic (M) and guluronic acid (G) variations in the Alginate. Particularly important in determining the gelling properties

M/G ratio of the extracts showed higher guluronic acid intensity. Among the extracts, no significant difference was observed

Structural Properties of Alginate

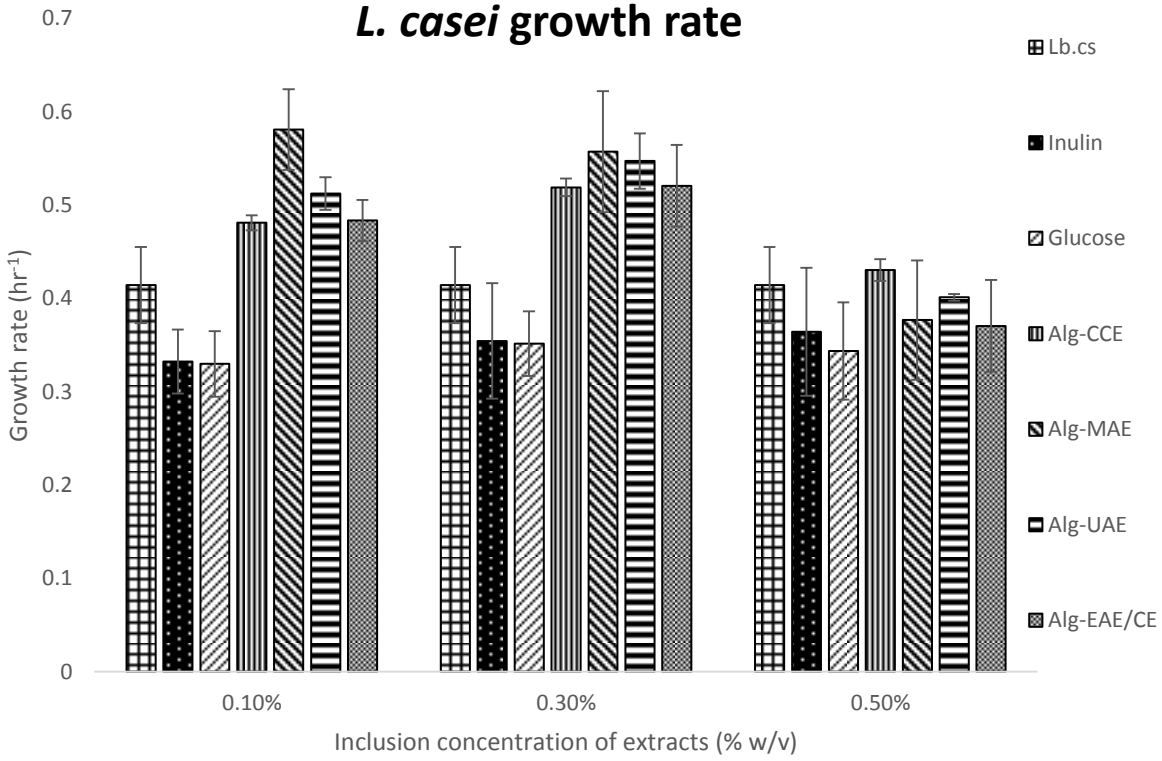
Extraction Method	*L. casei* doubling time (hr)				*L. delbruecki* doubling time (hr)			
	MRS+Lb.db	0.1%	0.3%	0.5%	MRS+Lb.cs	0.1%	0.3%	0.5%
Inulin	3.90 ± 2.19	3.031 ± 0.298	2.888 ± 0.558	2.806 ± 0.479	2.426 ± 0.239	2.171 ± 0.291	2.051 ± 0.017	2.149 ± 0.157
Glucose		3.057 ± 0.339	2.863 ± 0.267	2.960 ± 0.486		2.015 ± 0.087	2.005 ± 0.063	2.046 ± 0.140
Fuc-CCE		1.597 ± 0.082	1.726 ± 0.240	1.843± 0.124		2.393 ± 0.051	2.701 ± 0.075	2.586 ± 0.124
Fuc-MAE		1.956 ± 0.249	1.901 ± 0.119	2.008 ± 0.114		2.723 ± 0.011	2.646 ± 0.031	1.942 ± 0.032
Fuc-UAE		1.898 ± 0.127	1.704 ± 0.076	1.785 ± 0.167		2.540 ± 0.115	2.379 ± 0.097	2.410 ± 0.072
Fuc_EAE		1.668 ± 0.165	1.790 ± 0.263	1.937 ± 0.327		2.287 ± 0.012	2.369 ± 0.155	2.494 ± 0.062

Prebiotic Activity of Alginate



Alg-MAE improved growth rate of *L. delbruecki* by 75% (at 0.1% w/v), 150% (at 0.3 % w/v) and by 40% (at 0.5% w/v)

Alg-MAE also improved the growth rate of *L. casei* by 40% (at 0.1% w/v) and 34% (at 0.3% w/v)



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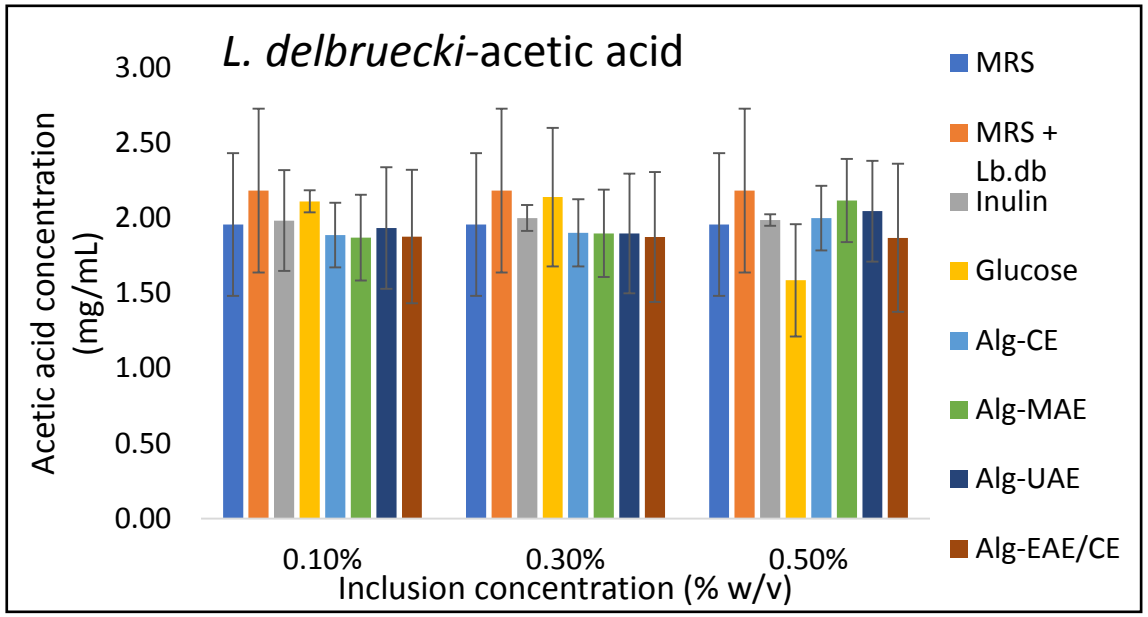
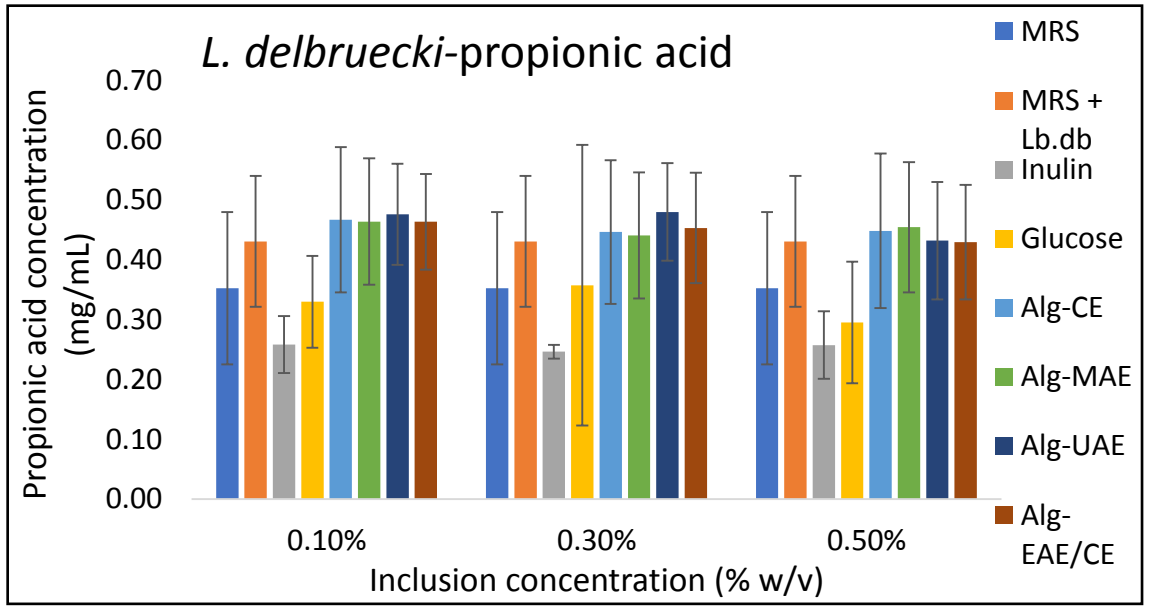
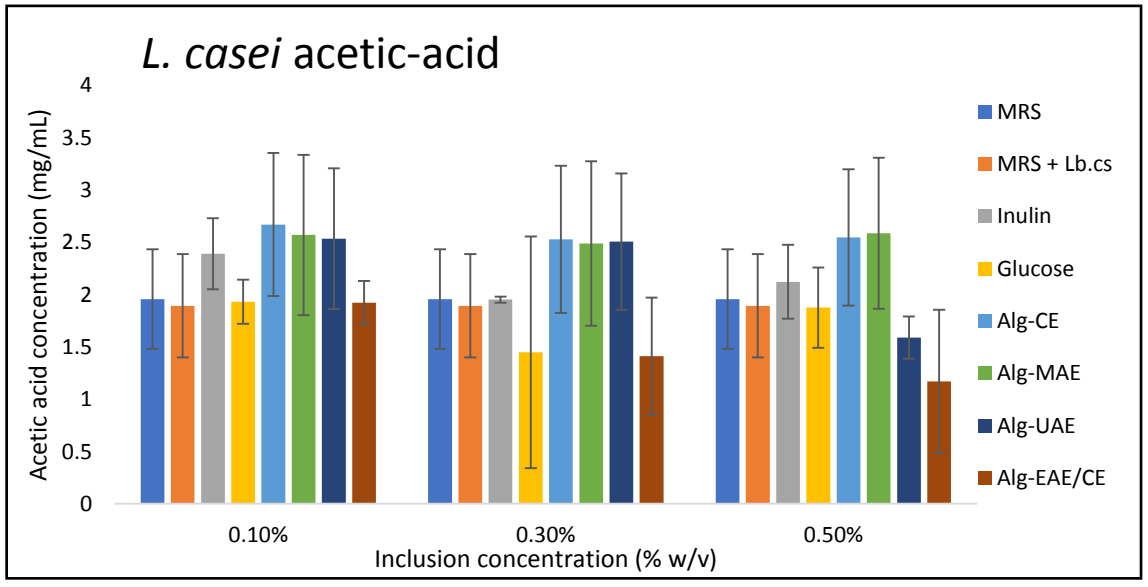
Glucose	0.1	-2.010	-1.854
	0.3	-1.990	-1.877
	0.5	-1.996	-1.868
Alg-CCE	0.1	-1.593	-2.629
	0.3	-2.318	-3.384
	0.5	-2.929	-3.469
Alg-MAE	0.1	-1.730	-2.710
	0.3	-2.448	-3.475
	0.5	-3.178	-3.451
Alg-UAE	0.1	-1.560	-2.613
	0.3	-2.172	-3.294
	0.5	-3.029	-3.405
Alg-EAE/CCE	0.1	-1.410	-2.637
	0.3	-2.143	-3.175
	0.5	-3.037	-3.452

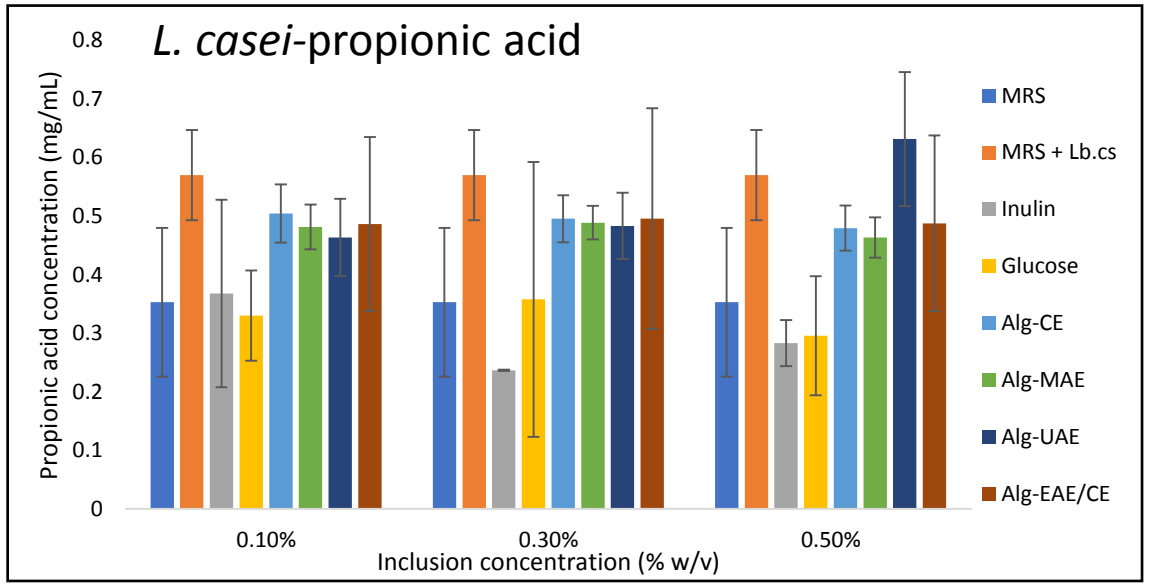
Higher reduction in the pH of extract supplemented media than blank control

Short Chain Fatty Acid Production









Summary

- Microwave-assisted extractions resulted in extracts with desirable structural properties including lower molecular weight, higher monosaccharide content and higher sulphate content – particularly for fucoidan

- Both fucoidan extracts showed prebiotic potential on *L. delbruecki* and alginate extracts showed prebiotic potential on both *L. delbruecki* and *L. casei*

- The extraction techniques did not have an impact on the prebiotic property of the fucoidan and alginate extracts under the tested conditions

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Thank You