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Article

Supplemental Material

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Evaluation of the prebiotic potential of arabinoxylans extracted from wheat distillers' dried grains with solubles (DDGS) and in-process samples

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Supplemental Table S1: AXOS/XOS hydrolysates assimilation in pH-controlled batch cultures after inoculation at 0 h, 4 h and 8 h after fermentation. Values are expressed as data percentage of total peak area (nC*min) relative to time 0.

		Time 0 h	Time 4 h	Time 8 h
DDGS-Xyl	Xylose	47.07 (13.57)	35.66 (4.72)	7.38 (7.70)
	Xylobiose	1.13 (0.08)	2.67 (0.93)	0.84 (0.96)
	Xylotriose	24.21 (20.47)	7.42 (7.27)	1.92 (2.61)
	Xylotetraose	3.69 (3.06)	10.00 (4.87)	2.45 (0.82)
	Xylopentaose	3.50 (2.12)	3.99 (3.29)	2.10 (1.06)
	Xylohexaose	3.79 (3.98)	6.24 (7.13)	3.91 (4.35)
	XA3XX	2.35 (2.00)	4.60 (3.28)	1.23 (1.64)
DDGS-Xyl+FAE	Xylose	33.50 (4.70)	16.80 (10.81)	0.25 (0.43)
	Xylobiose	16.80 (1.24)	14.32 (0.43)	2.50 (4.33)
	Xylotriose	14.93 (7.83)	12.20 (4.74)	0.06 (0.10)
	Xylotetraose	9.75 (0.99)	7.65 (1.69)	1.47 (2.55)
	Xylopentaose	1.35 (0.21)	1.21 (2.20)	0.60 (1.03)
	Xylohexaose	2.95 (2.70)	0.68 (0.79)	0.60 (1.03)
	XA3XX	5.39 (0.71)	0.77 (0.75)	*ND
WS-Xyl	Xylose	29.75 (8.10)	32.12 (10.81))	2.24 (2.30)
	Xylobiose	3.56 (1.32)	5.08 (0.43)	2.98 (2.64)
	Xylotriose	12.78 (8.32)	14.09 (4.74)	7.26 (8.47)
	Xylotetraose	13.25 (1.85)	14.75 (1.69)	6.61 (6.68)
	Xylopentaose	7.58 (0.57)	8.10 (2.20)	5.36 (4.66)
	Xylohexaose	4.20 (0.96)	4.27(0.79)	4.46 (4.34)
	XA3XX	4.49 (0.92)	3.82 (2.32)	4.95 (4.80)
WS-Xyl+FAE	Xylose	31.16 (11.51)	40.17 (11.85)	6.79 (8.46)
	Xylobiose	3.09 (1.00)	4.92 (1.40)	3.85 (3.34)
	Xylotriose	5.61 (2.89)	8.16 (3.55)	2.03 (1.82)
	Xylotetraose	10.58 (0.59)	13.87 (4.37)	9.20 (13.64)
	Xylopentaose	7.39 (1.27)	5.49 (2.17)	5.48 (5.27)
	Xylohexaose	4.59 (1.33)	4.31 (0.83)	4.13 (3.62)
	XA3XX	3.03 (0.28)	2.10 (1.83)	2.44 (2.49)

*ND. Non-detected. Standard deviation is shown in parentheses ($n = 3$). Oligosaccharides obtained from DDGS and WS were totally depleted at 24 h of fermentation

Supplemental Table S2: Mean bacterial populations in pH-controlled batch cultures at 0, 4, 8 and 24 h

Probe	Time (h)	Bacterial population (\log_{10} cells mL^{-1} batch culture fluid) with substrate:				
		Negative control	FOS	DDGS-Xyl	DDGS-Xyl+FAE	WS-Xyl
BIF 164	0	6.85 (0.25)a	6.88 (0.25)a	7.14 (0.03)a	7.00 (0.17)a	6.97 (0.10)a
	4	6.91 (0.30)a	7.54 (0.36)a	8.28 (0.42)a*	8.01 (1.06)a	7.65 (0.51)a
	8	6.77 (0.16)a	7.63 (0.49)ab	8.47 (0.54)b*	8.27 (0.79)ab	7.16 (0.70)ab
	24	6.76 (0.18)a	8.20 (0.33)b*	8.70 (0.17)b*	8.33 (0.82)b*	8.66 (0.77)b*
LAB 158	0	6.16 (0.78)a	5.92 (0.92)a	6.27 (0.59)a	5.99 (0.63)a	5.85 (0.65)a
	4	5.68 (0.46)a	6.10 (0.70)a	6.14 (0.08)a	5.95 (0.35)a	5.99 (0.61)a
	8	5.76 (0.11)a	5.65 (0.70)a	6.07 (0.33)a	6.13 (0.36)a	6.24 (1.03)a
	24	5.77 (0.23)a	6.15 (0.35)a	6.86 (0.29)a	6.47 (0.38)a	7.30 (0.38)a
BAC 303	0	6.87 (0.07)a	6.54 (0.80)a	6.79 (0.37)a	6.58 (0.43)a	6.49 (0.25)a
	4	6.58 (0.21)a	7.07 (0.50)a	6.78 (0.90)a	6.56 (1.33)a	6.78 (0.36)a
	8	7.07 (0.63)a	6.58 (0.78)a	6.80 (0.73)a	6.48 (1.58)a	6.50 (1.14)a
	24	6.75 (0.25)a	6.12 (0.49)a	8.07 (0.36)a	7.16 (1.20)a	8.22 (0.89)a
EREC 482	0	8.08 (0.35)a	8.09 (0.26)a	8.28 (0.10)a	8.08 (0.11)a	8.14 (0.17)a
	4	7.79 (0.00)a	8.25 (0.18)a	8.36 (0.17)a	8.14 (0.75)a	8.38 (0.29)a
	8	7.91 (0.23)a	7.33 (0.65)a	8.40 (0.20)a	8.47 (0.37)a	8.02 (0.99)a
	24	7.74 (0.23)a	7.56 (0.35)a	8.64 (0.62)a	8.62 (0.50)a	8.34 (0.64)a
RREC 584	0	6.84 (0.31)a	6.83 (0.25)a	7.16 (0.42)a	6.89 (0.31)a	6.98 (0.42)a
	4	6.16 (1.13)a	6.82 (0.52)a	6.49 (0.63)a	6.48 (0.75)a	6.90 (1.03)a
	8	6.60 (0.24)a	6.18 (0.97)a	6.76 (0.73)a	6.90 (1.16)a	6.69 (1.14)a
	24	6.26 (0.17)a	5.74 (0.49)a	7.30 (1.10)a	6.96 (0.91)a	7.16 (1.17)a
ATO 291	0	6.18 (1.52)a	5.92 (1.20)a	6.15 (1.01)a	5.76 (1.27)a	5.83 (1.23)a
	4	6.70 (1.18)a	6.63 (0.59)a	6.60 (1.43)a	6.79 (0.55)a	6.64 (1.40)a
	8	6.77 (0.79)a	6.02 (1.26)a	6.81 (1.18)a	6.35 (1.31)a	6.10 (1.45)a
	24	6.52 (1.13)a	6.08 (1.44)a	6.99 (1.23)a	6.87 (0.77)a	6.55 (1.29)a
PRO 853	0	6.66 (0.04)a	6.47 (0.75)a	6.75 (0.14)a	6.60 (0.49)a	6.58 (0.30)a
	4	6.28 (0.68)a	7.02 (0.86)a	6.83 (1.06)a	6.50(1.21)a	6.49 (0.75)a
	8	7.18 (0.75)a	6.70 (0.80)a	7.10 (0.76)a	6.81 (1.29)a	6.69 (0.89)a
	24	6.62 (0.16)a	6.55 (0.27)a	7.99 (0.37)a	7.28 (0.90)a	8.16 (0.83)a
FPRAUTZ 655	0	7.58 (0.48)a	7.64 (0.34)a	7.88 (0.15)a	7.68 (0.15)a	7.75 (0.18)a
	4	7.18 (0.72)a	7.71 (0.33)a	7.57 (0.54)a	7.22 (0.45)a	7.88 (0.61)a
	8	7.38 (0.11)a	6.77 (0.90)*	6.92 (1.16)a	6.95 (0.71)a	7.34 (0.84)a
	24	6.89 (0.25)ab	6.45 (0.15)a*	7.99 (0.36)b	7.47 (0.17)ab	7.93 (0.62)b
DSV 687	0	7.38 (0.23)a	7.37 (0.12)a	7.65 (0.15)a	7.42 (0.10)a	7.50 (0.10)a
	4	7.02 (0.81)a	7.28 (0.33)a	7.27 (0.91)a	6.77 (0.61)a	7.50 (0.60)a
	8	6.76 (0.19)a	6.33 (1.10)a	6.31 (0.98)a	6.12 (1.09)a	6.88 (0.53)a
	24	6.45 (0.57)a	6.19 (0.71)a	7.11 (0.49)a	7.01 (0.57)a	7.05 (0.67)a
CHIS 150	0	6.16 (0.53)a	6.17 (0.37)a	6.42 (0.18)a	6.30 (0.28)a	6.30 (0.38)a
	4	6.15 (0.96)a	6.14 (0.41)a	6.37 (0.74)a	6.05 (1.03)a	6.26 (0.90)a
	8	6.24 (0.20)a	5.70 (0.62)a	6.17 (0.48)a	6.42 (0.81)a	5.73 (0.55)a
	24	5.84 (0.05)a	6.23 (0.63)a	6.77 (0.11)a	6.78 (0.34)a	6.83 (0.09)a

Standard deviation is shown in parentheses (n = 3). 2 way ANOVA with Tukey's post hoc tests were used for the statistical analysis. Significant differences ($P < 0.05$) among treatment at the same time point are indicated with different letters. (*) Significant different from 0 h value, $P < 0.05$.

Supplemental Table S3: Mean SCFAs and lactic acid concentrations in pH-controlled batch cultures at 0, 4, 8, and 24 h.

	Lactate	Formic	Acetic	Propionic	Butyric	Total SCFAa
No treatment						
0 h	0.32 (0.47)a	0.06 (0.06)a	7.34 (3.74)a	2.61 (3.98)a	2.58 (1.13)a	12.91 (5.66)a
4 h	1.08 (1.49)a	0.03 (0.06)a	7.30 (6.27)a	17.67 (27.66)a	1.83 (1.16)a	27.92 (28.86)a
8 h	0.22 (0.33)a	0.06 (0.11)a	10.23 (8.46)a	10.55 (14.26)a	1.31 (1.64)a	22.38 (17.77)a
24 h	0.03 (0.06)a	0.05 (0.09)a	12.90 (10.90)a	3.03 (1.27)a	1.55 (2.21)a	17.56 (12.27)a
FOS						
0 h	0.65 (0.32)a	0.37 (0.23)a	8.13 (3.81)a	4.71 (7.87)a	1.79 (0.80)a	15.64 (6.39)a
4 h	3.50 (1.21)a	1.17 (1.77)a	18.86 (15.21)a	4.09 (3.97)a	1.06 (1.43)a	28.67 (15.44)a
8 h	19.20 (8.94)b*	10.97 (12.62)a	43.11 (28.10)ab	15.60 (10.61)a	3.14 (3.94)a	92.02 (43.02)ab*
24 h	4.75 (8.01)a	8.92 (10.79)a	65.26 (20.41)b*	22.67 (15.13)a	2.61 (3.57)a	104.21 (35.04)bc*
DGGS-Xyl						
0 h	1.16 (0.79)a	0.57 (0.64)a	10.21 (5.60)a	2.70 (4.51)a	1.76 (1.85)a	16.40 (5.72)a
4 h	2.83 (2.50)a	9.52 (5.77)a	32.98 (13.92)a	13.53 (16.87)a	1.57 (0.86)a	60.44 (21.63)a
8 h	5.79 (2.33)a*	9.53 (6.41)a	51.98 (8.04)ab**	14.56 (5.04)a	3.17 (2.80)a	85.03 (10.18)ab**
24 h	0.14 (0.23)a±	3.78 (6.55)a	58.99 (13.80)b**	18.42 (2.44)ab	10.38 (6.63)a	91.70 (26.10)bc**
DGGS-Xyl + FAE						
0 h	1.44 (1.37)a	1.77 (1.55)a	18.01 (5.61)a	4.46 (2.07)a	2.49 (2.28)a	28.15 (6.00)a
4 h	4.03 (2.38)a	8.41 (7.17)a	31.21 (19.02)a	4.37 (3.44)a	2.24 (3.18)a	50.25 (33.89)a
8 h	8.31 (2.90)ab*	8.08 (1.27)a	49.04 (21.30)ab	11.45 (9.91)a	5.80 (3.56)a	82.69 (35.18)ab
24 h	0.06 (0.10)a±	0.65 (1.12)a	55.30 (4.51)b	14.12 (4.24)ab	5.45 (5.05)a	75.58 (12.37)ab
WS-Xyl						
0	1.97 (0.55)a	0.89 (0.71)a	10.24 (1.35)a	4.03 (6.73)a	1.91 (0.47)a	19.04 (8.36)a
4	5.90 (3.34)a	3.91 (1.16)a	17.73 (6.34)a	23.51 (20.14)a	0.97 (0.66)a	52.02 (19.98)a
8	3.07 (3.74)a	13.59 (8.41)a	63.86 (2.55)b***	22.51 (11.65)a	6.45 (10.22)a	109.48 (16.97)b**
24	3.40 (5.72)a	4.43 (6.83)a	87.88 (3.12)b***	44.37 (20.75)b	7.37 (8.82)a	147.45 (28.53)c***
WS-Xyl + FAE						
0	0.60 (0.26)a	0.17 (0.29)a	7.34 (2.57)a	6.35 (5.53)a	1.47 (0.70)a	15.93 (4.11)a
4	3.12 (0.60)a	4.68 (0.78)a	23.59 (2.85)a	13.83 (10.98)a	0.97 (0.60)a	46.18 (9.78)a*
8	1.91 (1.49)a*	9.48 (6.53)a	58.52 (12.62)b***	15.92 (7.23)a	1.16 (0.72)a	86.99 (11.26)ab**
24	0.24 (0.06)a±	3.82 (6.56)a	58.52 (11.84)b***	19.76 (4.35)ab	3.37 (1.15)a	85.71 (9.54)bc**

Standard deviation is shown in parentheses ($n = 3$). 2-way ANOVA with Tukey's post hoc tests were used for the statistical analysis. Significant differences ($P < 0.05$) among treatment at the same time point are indicated with different letters. (*) Significant difference from 0 h value, $P < 0.05$.

Supplemental Table S4: Mean bacterial populations in pH –controlled mini batch cultures using FOS, XOS or AXOS/XOS DP \geq 3 as substrates at 0,8 and 24 h of fermentation.

Probe	Time (h)	Bacterial population (\log_{10} cells ml $^{-1}$ batch culture fluid) with substrate		
		Negative control	FOS	XOS
EUB	0	7.77 (0.33)	7.68 (0.24)	7.67 (0.26)
	8	7.53 (0.59)	8.43 (0.47)	8.41 (0.36)
	24	7.65 (0.42)	8.28 (0.33)	8.39 (0.33)
BIF	0	7.06 (0.45)	6.79 (0.27)	6.80 (0.47)
	8	6.88 (0.67)a	8.29 (0.49)b	8.29 (0.39)b
	24	6.72 (0.35)a	8.14 (0.42)b	8.28 (0.33)b
LAB	0	5.90 (0.48)	5.79 (0.39)	5.73 (0.32)
	8	5.93 (0.65)	6.23 (0.50)	5.88 (0.66)
	24	6.02 (0.32)	5.24 (0.29)	5.50 (0.54)
BAC	0	6.20 (0.55)	6.16 (0.56)	6.12 (0.45)
	8	6.21 (0.47)	6.56 (0.21)	6.33 (0.07)
	24	6.29 (0.28)	5.80 (0.24)	5.79 (0.59)
EREC	0	7.27 (0.26)	7.20 (0.18)	7.17 (0.20)
	8	6.70 (0.54)	7.52 (0.51)	7.38 (0.44)
	24	6.59 (0.4)	7.48 (0.23)	7.48 (0.41)
RREC	0	6.42 (0.41)	6.44 (0.34)	6.33 (0.45)
	8	5.69 (0.45)	6.41 (0.62)	5.98 (0.36)
	24	6.01 (0.3)	5.78 (0.63)	5.52 (0.46)
ATO	0	5.63 (0.92)	5.43 (1.00)	5.62 (0.60)
	8	5.57 (0.92)	6.06 (0.81)	5.54 (0.80)
	24	6.29 (0.7)	5.38 (0.33)	5.76 (0.75)
PRO	0	6.22 (0.54)	6.22 (0.57)	6.15 (0.47)
	8	6.36 (0.35)	6.58 (0.13)	6.66 (0.26)
	24	6.30 (0.3)	5.93 (0.10)	5.98 (0.40)
FPRAUTZ	0	6.92 (0.19)	6.96 (0.22)	6.93 (0.20)
	8	6.63 (0.57)	6.85 (0.41)	6.92 (0.29)
	24	6.23 (0.21)	6.47 (0.68)	6.34 (0.56)
DSV	0	6.61 (0.22)	6.77 (0.33)	6.62 (0.30)
	8	6.33 (0.55)	6.64 (0.41)	6.39 (0.29)
	24	6.14 (0.43)	6.04 (0.54)	5.96 (0.34)
CHIS	0	6.14 (0.24)	5.96 (0.15)	5.98 (0.17)
	8	5.73 (0.29)	6.36 (0.25)	6.15 (0.42)
	24	6.19 (0.33)	5.48 (0.29)	5.51 (0.28)

Standard deviation is shown in parentheses (n = 3). 2 way ANOVA with Tukey's post hoc tests were used for the statistical analysis. Significant differences ($P < 0.05$) among treatment at the same time point are indicated with different letters. (*) Significant different from 0 h value, $P < 0.05$).