

Fermented Brewers' Spent Grain Containing Dextran and Oligosaccharides as Ingredient for Composite Wheat Bread and Its Impact on Gut Metabolome In Vitro

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Supplementary Table S1. Technological parameters and texture analysis of BSG breads. WB: control wheat bread; BB: native BSG bread; EPS-BB: EPS- fermented BSG breads; EPS+BB: EPS+ fermented BSG breads. Baking loss was calculated as the decrease of weight between dough and loaf ($\%bake\ loss = ((dough\ weight - bread\ weight) \div dough\ weight) \times 100$). Staling rate was calculated as hardness difference in 4 days of storage ($Staling\ rate = (hardness\ in\ day\ 4 - hardness\ in\ day\ 1) \div days\ of\ storage$).

Parameters	Types of bread			
	WB	BB	EPS-BB	EPS+BB
Baking loss (%)	13.7 ± 0.5 ^a	10.4 ± 0.6 ^b	10.3 ± 0.6 ^b	11.1 ± 0.6 ^b
Specific volume (ml/g)	5.1 ± 0.1 ^a	3.0 ± 0.0 ^c	2.9 ± 0.0 ^d	3.4 ± 0.1 ^b
Hardness (g/day 1)	149.5 ± 17.3 ^a	455.3 ± 94.9 ^c	592.3 ± 87.5 ^d	281.8 ± 31.1 ^b
Hardness (g/day 4)	286.6 ± 43.1 ^a	778.9 ± 116.2 ^c	951.4 ± 127.0 ^d	497.3 ± 59.9 ^b
Staling rate (g/d)	34.3 ^a	80.9 ^c	89.8 ^d	53.9 ^b
Springiness (day 1)	0.97 ± 0.02 ^a	0.93 ± 0.01 ^b	0.92 ± 0.02 ^b	0.90 ± 0.02 ^c
Cohesiveness (day 1)	0.73 ± 0.02 ^a	0.67 ± 0.02 ^b	0.65 ± 0.01 ^c	0.65 ± 0.01 ^c
Chewiness (day 1)	105.9 ± 10.7 ^a	284.1 ± 53.7 ^c	350.4 ± 45.8 ^d	163.8 ± 15.8 ^b
Resilience (day 1)	0.38 ± 0.02 ^a	0.33 ± 0.01 ^b	0.31 ± 0.01 ^c	0.30 ± 0.01 ^d

*a–d Values with different letters in same row are significantly different (Tukey's test. P<0.05)

According to EU regulation ((EC) No 1924/2006), these enriched breads can be considered as source of fibre (up to 3.9 g per 100 g bread) and protein.

Supplementary Table S2. Gut microbiota composition of selected donor at genus level.

Kingdom	Phylum	Class	Order	Family	Genus	Relative abundance
Bacteria	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Faecalibacterium	0,209257934
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia	0,170365068
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Anaerostipes	0,128370317
Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	0,11095204
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Fusicatenibacter	0,066332618
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	[Ruminococcus] gauvreauii group	0,042949177
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	NA*	0,031257456
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Dorea	0,030541637
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	[Eubacterium] hallii group	0,020042949
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Roseburia	0,018372703
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae NK4A136 group	0,014077786
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium sensu stricto 1	0,011930327
Bacteria	Firmicutes	Clostridia	NA*	NA*	NA*	0,010737294
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae UCG-003	0,010260081
Bacteria	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Subdoligranulum	0,010021475
Bacteria	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	Erysipelotrichaceae UCG-003	0,009305655
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	[Eubacterium] eligens group	0,009305655
Bacteria	Firmicutes	Clostridia	Clostridia UCG-014	NA*	NA*	0,007874016
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	[Ruminococcus] torques group	0,007874016
Bacteria	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Holdemanella	0,007635409
Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Paraprevotella	0,007158196
Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Barnesiellaceae	Barnesiella	0,00691959
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	CAG-56	0,00691959
Bacteria	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	Monoglobus	0,005726557
Bacteria	Actinobacteriota	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Collinsella	0,00548795
Bacteria	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-002	0,00548795
Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	0,004533524
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	[Eubacterium] xylanophilum group	0,004056311
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0,003340492

Table S2: *Cont.*

Kingdom	Phylum	Class	Order	Family	Genus	Relative abundance
Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	NA [*]	0,003340492
Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	0,003101885
Bacteria	Firmicutes	Clostridia	Oscillospirales	[Eubacterium] coprostanoligenes group	NA [*] NA [*]	0,002624672
Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Tannerellaceae	Parabacteroides	0,001908852
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospira	0,001908852
Bacteria	Actinobacteriota	Coriobacteriia	Coriobacteriales	Coriobacteriales Incertae Sedis	NA [*]	0,001670246
Bacteria	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcus	0,001431639
Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Romboutsia	0,001431639
Bacteria	Actinobacteriota	Coriobacteriia	Coriobacteriales	Eggerthellaceae	Senegalimassilia	0,001193033
Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	NA [*]	NA [*]	0,000954426
Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae UCG-004	0,000954426
Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	NA [*]	NA [*]	0,00071582
Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Sutterellaceae	Sutterella	0,00071582
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Ligilactobacillus	0,000477213
Bacteria	NA [*]	NA [*]	NA [*]	NA [*]	NA [*]	0,000477213

^{*}NA means not applicable

Supplementary Table S3. Pearson correlation among bread EPS+BB composition and SHIME metabolites in luminal samples after one week of treatment. Values are different from 0 with a significance level $\alpha = 0.05$. * < 0.01.

Proximal Colon (PC)			Distal Colon (DC)		
SHIME® Metabolites	EPS+BB variables	Pearson correlation	SHIME® Metabolites	EPS+BB variables	Pearson correlation
GABA	Fructose	0.9999804*	Asp	Oligo, DP6-DP7	0.99986908
Pro	Fructose	-0.998426	Thr	Oligo, DP3-DP4	-0.9970823
GABA	Dextran	-0.998111	Thr	Oligo, DP5	-0.9971446
Thr	Oligo, DP3-DP4	-0.997082	Ser	Oligo, DP6-DP7	0.99927674
Thr	Oligo, DP5	-0.997145	Ala	Oligo, DP6-DP7	0.99979295
Butyric acid	Oligo, DP5	0.9979189	Val	Oligo, DP6-DP7	0.99960577
GABA	Fiber	0.9999264*	Cys	Oligo, DP6-DP7	0.99827202
Pro	Fiber	-0.999289	Phe	Oligo, DP6-DP7	0.99996378*
Lys	Fat	0.9999993*	GABA	Glucose	-0.9993081
Cys	Fat	0.9999502*	Amm	Fructose	0.99985969
Arg	Fat	0.9998715	Amm	Dextran	-0.9992606
Met	Fat	0.9998629	Amm	Fiber	0.99938254
Orn	Fat	0.9996952	Lys	Oligo, DP6-DP7	0.99770572
Ser	Fat	0.9995196	Arg	Oligo, DP6-DP7	0.99859048
Ala	Fat	0.9988279	Total AA	Total carbohydrates	0.99912942
Ile	Fat	0.9987423	Total AA	Maltose	0.99871669
Phe	Fat	0.9981856	Total AA	Dextran	-0.9987998
Trp	Acetic acid	0.9979543	Asp	TTA	-0.9983843
Asp	pH	-0.998382	Glu	TTA	0.99917676
Glu	pH	0.9991769	Val	TTA	-0.9989898
Val	pH	-0.998991			

Supplementary Table S4. Pearson correlation among bread EPS-BB composition and SHIME metabolites in luminal samples after one week of treatment. Values are different from 0 with a significance level $\alpha = 0.05$. * < 0.01 .

Proximal Colon (PC)			Distal Colon (DC)		
SHIME® Metabolites	EPS-BB variables	Pearson correlation	SHIME® Metabolites	EPS-BB variables	Pearson correlation
His	Fructose	0.993429905	Leu	Maltose	0.99983734
His	Sucrose	0.997803687	Tyr	Fructose	0.99849293
His	Maltose	0.999165168	Leu	Fructose	0.99757812
Tyr	Lactic Acid	-0.99745827	Tyr	Protein	0.99977263
Leu	Lactic Acid	-0.99999896*	Trp	Fat	-0.9980155
Thr	pH	1*	Leu	Protein	0.99866799
			Propionic acid	Lactic Acid	-0.99712572
			His	Lactic Acid	-0.998811792
			Trp	Acetic acid	-0.998851736