

Fermentation of *Pleurotus ostreatus* and *Ganoderma lucidum* mushrooms and their extracts by gut microbiota of healthy and osteopenic women: potential prebiotic effect and impact on human osteoblasts

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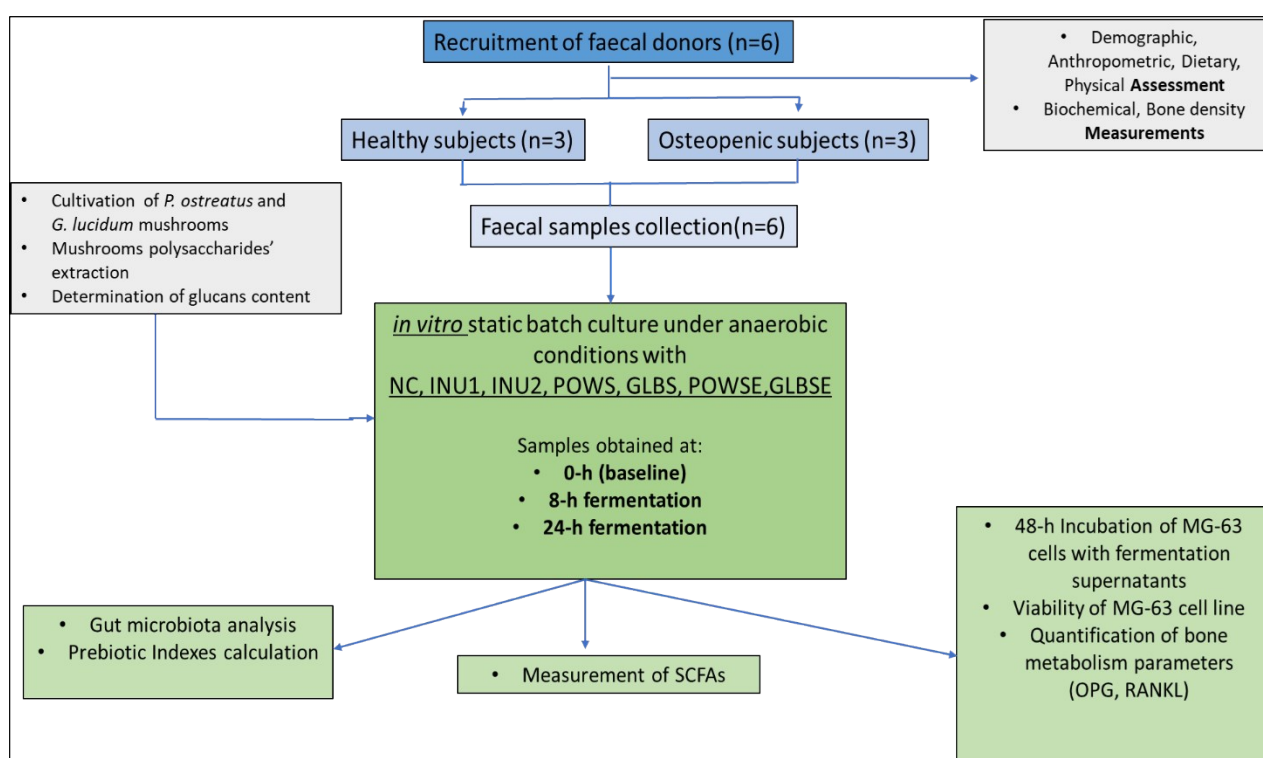


Figure S1: Methodology flowchart; NC: Negative control; INU1: Inulin 1% (w/v); INU2: Inulin 2% (w/v); POWS: *P.ostreatus* untreated mushroom powder; POWSE: *P.ostreatus* mushroom-extract; GLBS: *G.lucidum* untreated mushroom powder; GLBSE: *G.lucidum* mushroom-extract;

Table S1. Sequence of primers used to analyze fecal bacterial populations by quantitative PCR analysis.

Bacterial Targets	Primer name	Sequence (5'-3')	Annealing (°C)	Reference
Total Bacteria	Forward	TCCTACGGGAGGCAGCAGT	60°C	1
	Reverse	GGACTACCAGGGTATCTAATCCTGTT		
<i>Bifidobacterium spp.</i>	Forward	TCGCGTC(C/T)GGTGTGAAAG	58°C	2
	Reverse	CCACATCCAGC(A/G)TCCAC		
<i>Lactobacillus group</i>	Forward	AGCAGTAGGGAATCTTCCA	58°C	2
	Reverse	CACCGCTACACATGGAG		
<i>Clostridium perfringens group</i>	CPF	ATGCAAGTCGAGCGATG	55°C	3
	CPR	TATGCGGTATTAATCTCCCTTT		
<i>Bacteroides spp.</i>	Bac303F Bfr-Fmrev	GAAGGTCCCCACATTG CGCKACTGGCTGGTTCAG	60°C	4
<i>Clostridium leptum group</i>	Clep866mF Clept1240mR	TTAACACAATAAGTWATCCACCTGG ACCTTCTCCGTTTTGTCAAC	55°C	4
<i>Faecalibacterium prausnitzii</i>	FPR-2F Fprau645R	GGAGGAAGAAGGTCTTCGG AATTCGCCTACCTCTGCACT	60°C	5,6,7
<i>Roseburia spp.- Eubacterium rectale</i>	RrecF Rrec630mR	GCGGTRCGGCAAGTCTGA CCTCCGACTCTAGTMCAGC	60°C	8

	Total (n=6)	Normal bone health (n=3)	Osteopenia n=3	p- value
Behavioral parameters				
Sitting or resting time (h wk ⁻¹)	29.17 ± 19.85	23.33 ± 23.63	35.00 ± 18.03	0.534
Moderate physical activity (MET min wk ⁻¹)	240.00 (0.00-560.00)	480.00 (240.00-4440.00)	0.00 (0.00-280.00)	0.487
Walking (MET min wk ⁻¹)	2937.00 ± 2428.76	4620.00 ± 2117.15	1254.00 ± 1329.45	0.080
Total Physical Activity (MET min wk ⁻¹)	2772.00 (1253.00-4638.00)	4638.00 (3705.00-9984.00)	1253.00 (775.00-2012.50)	0.255
Biochemical Measurements				
Total cholesterol (mg dL ⁻¹)	205.50 (183.50-232.00)	198.00 (197.50-242.00)	213.00 (178.00-213.50)	0.827
HDL cholesterol (mg dL ⁻¹)	76.00 ± 20.43	71.00 ± 15.62	81.00 ± 26.91	0.607
LDL cholesterol (mg dL ⁻¹)	79.67 ± 23.53	85.67 ± 18.01	73.67 ± 30.86	0.592
TGs (mg dL ⁻¹)	116.50 ± 43.57	138.67 ± 43.88	94.33 ± 36.69	0.251
FBG (mg dL ⁻¹)	100.00 ± 15.68	104.00 ± 24.46	96.67 ± 10.50	0.608
Insulin (mg dL ⁻¹)	7.70 ± 3.44	5.03 ± 0.31	10.37 ± 2.35	0.032 *
HOMA - IR	1.75 (1.10-2.53)	1.10 (1.10-1.40)	2.20 (2.00-2.85)	0.046 *

Table S2. Descriptive characteristics of faecal donors

MET: Metabolic equivalent of task; Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein Cholesterol (HDL-C), Triglycerides (TG); FBG: Fasting Blood Glucose; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; Values are expressed as mean and standard deviation (SD) for parametric or median and interquartile range (Q1-Q3) for nonparametric data; * significantly different (p<0.05).

Table S3a. Faecal microbial quantification (qPCR; log₁₀ copies of 16S rRNA gene mL⁻¹ of sample) at baseline and after 24-h fermentation of *Pleurotus ostreatus* IK1123 and *Ganoderma lucidum* LGAM 9720 in healthy subjects

	HEALTHY GROUP (n=3)														P overall
	Baseline (t=0-h)							After fermentation (t=24-h)							
	NC	INU1	INU2	POWS	GLBS	POWSE	GLBSE	NC	INU1	INU2	POWS	GLBS	POWSE	GLBSE	
Total bacteria	9.96 ±0.18	10.02 ±0.18	9.98 ±0.16	9.89 ±0.11	9.90 ±0.11	10.01 ±0.12	9.91 ±0.21	9.97 ±0.53 †	10.22± 0.38 *	10.26 ±0.40	10.31 ±0.22	9.78 ±0.49	10.10 ±0.18	9.92 ±0.26	0.163
<i>Bifidobacterium</i> spp.	7.64 ±0.80	7.64 ±0.81	7.6 ±0.80	7.60 ±0.88	7.56 ±0.83	7.66 ±0.71	7.56 ±0.78	7.83 ±0.94 †	8.76 ± 1.89 *	8.97 ±2.00	8.44 ±1.54	7.90 ±1.37	7.98 ±1.22	8.29 ±1.53	0.994
<i>Lactobacillus</i> group	6.20 ±0.54	6.23 ±0.45	6.16 ±0.52	6.12 ±0.52	5.86 ±0.47	6.33 ±0.42	6.14 ±0.49	6.3 ±0.68	7.92 ±0.67 a	8.04 ±1.29	7.11 ±1.47	6.10 ±1.40	6.53 ±1.36	6.76 ±1.38	0.630
<i>Clostridium perfringens</i> group	5.76 (5.72- 6.38)	5.83 (5.80- 6.41)	5.83 (5.83- 6.42)	6.06 (6.04- 6.52)	5.03 (4.83- 5.80)	5.88 (5.87- 6.47)	6.00 (5.83- 6.52)	5.92 (5.79- 6.53)	5.68 (5.37- 6.51)	5.88 (5.47- 6.83)	5.97 (5.88- 6.52)	5.42 (5.21- 6.32)	5.70 (5.65- 6.56)	5.48 (5.34- 6.44)	0.891
<i>Bacteroides</i> spp.	9.48 (9.31- 9.48)	9.42 ±0.19	9.38 ±0.21	9.39 ±0.27	9.31 ±0.23	9.51 ±0.25	9.36 ±0.30	9.29 ±0.20 †	9.68 ±0.35 *	9.73 ±0.48	9.00 ±0.31 †, ‡	9.41 ±0.38	9.65 ±0.14	8.96 ±0.34	0.306
<i>Clostridium leptum</i> group	8.78 (8.75- 9.01)	8.85 (8.81- 9.05)	8.82 (8.78- 9.05)	8.82 (8.73- 9.04)	8.67 (8.61- 8.97)	8.89 (8.89- 9.13)	8.77 (8.76- 9.36)	8.92 (8.88- 9.16)	9.10 (8.70- 9.22)	9.09 (8.73- 9.33)	8.90 (8.79- 9.18)	8.61 (8.60- 8.92)	9.11 (8.89- 9.16)	8.87 (8.75- 8.89)	0.917
<i>Faecalibacterium prausnitzii</i>	8.01 (7.31- 8.14)	8.14 (7.47- 8.23)	8.20 (7.49- 8.26)	8.19 (7.43- 8.26)	8.02 (7.19- 8.15)	8.22 (7.50- 8.25)	8.11 (7.46- 8.15)	8.11 (7.38- 8.12)	8.78 (7.75- 8.83)	8.84 (7.73- 8.91)	8.70 (7.72- 8.74) †	7.67 (6.97- 8.21)	8.40 (7.31- 8.65)	8.10 (7.51- 8.41)	0.947
<i>Roseburia</i> spp.- <i>Eubacterium rectale</i>	8.06 ±0.59	8.06 ±0.59	8.1 ±0.65	8.04 ±0.51	8.09 ±0.62	8.13 ±0.57	8.06 ±0.60	7.51 ±0.40 a	7.98 ±0.66	7.99 ±0.96	7.76 ±0.96	7.41 ±0.38	7.65 ±0.48	7.72 ±1.01	0.997

Values are expressed as mean value and SD for parametric and median and interquartile range (Q1–Q3) for non parametric data; *: significantly different compared to [NC] at t=24-h (p <0.05); †: significantly different compared to [INU1] at t=24-h (p <0.05); ‡: significantly different compared to [INU2] at t=24-h (p <0.05); a: significantly different compared to baseline (p <0.05) (paired-samples T-test or Wilcoxon signed-rank test); p-overall refers to the Tests of Between-Subjects Effects and symbols *, †, ‡ to parameter estimates at t=0-h and 24-h (Repeated-Measures ANOVA or Friedman test; NC: Negative control; INU1: Inulin 1% (w/v); INU2: Inulin 2% (w/v); POWS: *P. ostreatus* untreated mushroom powder; POWSE: *P. ostreatus* mushroom-extract; GLBS: *G. lucidum* untreated mushroom powder; GLBSE: *G. lucidum* mushroom-extract;

Table S3b. Faecal microbial quantification (qPCR; log₁₀ copies of 16S rRNA gene mL⁻¹ of sample) at baseline and after 24-h fermentation of *Pleurotus ostreatus* IK1123 and *Ganoderma*

	OSTEOPENIC GROUP (n=3)														P overall
	Baseline (t=0-h)							After fermentation (t=24-h)							
	NC	INU1	INU2	POWS	GLBS	POWSE	GLBSE	NC	INU1	INU2	POWS	GLBS	POWSE	GLBSE	
Total bacteria	10.24 ±0.22	10.23 ±0.23	10.21 ±0.24	10.20 ±0.20	10.18 ±0.24	10.31 ±0.20	10.26 ±0.23	10.03 ±0.22 †, ‡, a	10.37 ±0.20 *, a	10.33 ±0.11 *	10.46 ±0.13 *	10.21 ±0.10	10.30 ±0.12 *	10.23 ±0.03	0.803
<i>Bifidobacterium</i> spp.	9.08 ±0.05	9.12 ±0.09	9.01 ±0.08	9.00 ±0.15	9.16 ±0.15	9.13 ±0.11	9.17 ±0.10	9.12 ±0.15 †, ‡	9.82 ±0.13 *, a	9.97 ±0.21 *, a	9.86 ±0.25 *, a	9.43 ±0.31 ‡	9.40 ±0.22 ‡	9.59 ±0.45 *	0.096
<i>Lactobacillus</i> group	6.36 ±1.43	6.30 ±1.31	6.29 ±1.43	6.89 ±1.34	6.20 ±1.31	6.39 ±1.36	6.35 ±1.40	6.38 ±1.31	6.79 ±0.56	7.16 ±0.68	6.70 ±1.13	6.23 ±1.35	6.74 ±0.80	7.37 ±0.62	0.994
<i>Clostridium</i> perfringens group	6.31 ±0.77	6.40 ±0.67	6.35 ±0.80	6.54 ±0.50	5.96 ±0.95	6.41 ±0.67	6.43 ±0.59	6.30 ±0.56	6.39 ±0.67	6.44 ±0.70	6.86 ±0.75	6.32 ±0.43	6.77 ±0.46	6.40 ±0.74	0.959
<i>Bacteroides</i> spp.	9.50 ±0.27	9.44 ±0.17	9.47 ±0.33	9.51 ±0.23	9.48 ±0.24	9.48 ±0.18	6.49 ±0.20	9.12 ±0.39 †, ‡	9.52 ±0.19 *	9.53 ±0.20 *	9.18 ±0.14	9.49 ±1.84	9.85 ±0.10 *, a	9.58 ±0.17 *	0.459
<i>Clostridium</i> leptum group	9.50 ±0.35	9.48 ±0.28	9.46 ±0.45	9.45 ±0.25	9.46 ±0.34	9.58 ±0.32	9.57 ±0.32	9.18 ±0.59	9.15 ±0.63	9.25 ±0.54	9.61 ±0.33	9.41 ±0.29	9.44 ±0.42	9.27 ±0.44	0.989
<i>Faecalibacterium</i> prausnitzii	8.99 (8.88- 9.06)	8.81 (8.81- 8.97)	8.98 (8.83- 9.02)	8.92 (8.89- 9.01)	8.90 (8.89- 9.00)	8.94 (8.93- 9.01)	8.90 (8.89- 9.05)	8.33 (8.07- 8.46) a	8.84 (8.41- 8.85)	8.88 (8.60- 8.91)	9.24 (9.15- 9.25) *, †, ‡	8.67 (8.64- 8.70)	8.94 (8.82- 8.97) *	8.49 (8.48- 8.65)	0.468
<i>Roseburia</i> spp.- <i>Eubacterium</i> rectale	8.89 (8.57- 8.95)	8.90 (8.51- 8.92)	8.95 (8.53- 8.96)	8.83 (8.57- 8.84)	8.96 (8.65- 9.00)	8.93 (8.65- 8.95)	8.93 (8.57- 8.99)	7.49 (7.44- 7.89)	8.45 (7.74- 8.85)	8.43 (7.90- 8.62)	8.56 (8.09- 8.60)	8.00 (7.77- 8.14) a	7.97 (7.73- 8.15) a	8.18 (7.99- 8.25) a	0.995

lucidum LGAM 9720 in osteopenic subjects

Values are expressed as mean value and SD for parametric and median and interquartile range (Q1–Q3) for non parametric data; *: significantly different compared to [NC] at t=24-h (p < 0.05); †: significantly different compared to [INU1] at t=24-h (p < 0.05); ‡: significantly different compared to [INU2] at t=24-h (p < 0.05); a: significantly different compared to baseline (p < 0.05) (paired-samples T-test or Wilcoxon signed-rank test); p-overall refers to the Tests of Between-Subjects Effects and symbols *, †, ‡ to parameter estimates at t=0-h and 24-h (Repeated-Measures ANOVA or Friedman test); NC: Negative control; INU1: Inulin 1% (w/v); INU2: Inulin 2% (w/v); POWS: *P. ostreatus* untreated mushroom powder; POWSE: *P. ostreatus* mushroom-extract; GLBS: *G. lucidum* untreated mushroom powder; GLBSE: *G. lucidum* mushroom-extract;

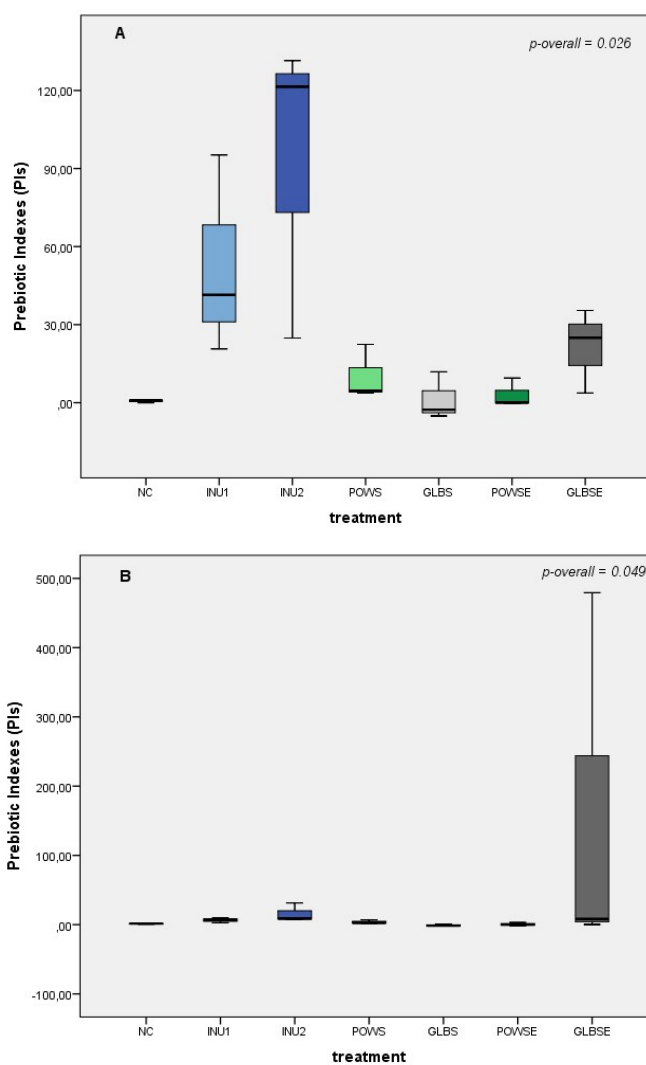


Figure S2A-B: Prebiotic indexes (PIs) for healthy (1A) and osteopenic subjects (1B); NC: Negative control; INU1: Inulin 1% (w/v); INU2: Inulin 2% (w/v); POWS: *P.ostreatus* untreated mushroom powder; POWSE: *P.ostreatus* mushroom-extract; GLBS: *G.lucidum* untreated mushroom powder; GLBSE: *G.lucidum* mushroom-extract; Data are presented as Boxplots, with the horizontal line representing the median and the whiskers the minimum and maximum values.

Table S4a. Total Volatile Fatty Acids (VFAs) concentrations ($\mu\text{mol mL}^{-1}$ of sample) and Molar ratios (%) of SCFAs at baseline and after 8-h and 24-h fermentation in healthy subjects.

HEALTHY (n=3)												
Baseline (t=0-h)												
	Total VFAs ($\mu\text{mol mL}^{-1}$ of sample)	Molar ratios of SCFA			Molar ratios of Branched-chain SCFA (%) †				Molar ratios of Other SCFA (%) ‡			
		Acetate (%)	Propionate (%)	Butyrate (%)	Branched-chain SCFA (%) §	Iso-butyrate (%)	Iso-valerate (%)	Iso-caproic acid (%)	Other SCFA (%) ¶	Valerate (%)	Caproic acid (%)	Heptanoic acid (%)
NC	4.64 † (4.45-4.89)	35.26 (28.84-42.05)	12.79 (10.74-14.98)	24.93 (22.50-34.07)	5.99 (5.36-12.14)	2.15 ± 1.06	2.20 (2.16-4.06)	4.11 ± 4.56	4.01 (3.53-17.27)	3.26 (2.71-4.91)	0.48 (0.43-4.83)	0.43 (0.39-7.61)
INU1	2.50 *, † (2.27-2.75)	36.68 ‡ (36.04-39.45)	13.10 (12.60-14.95)	26.95 (26.38-34.00)	9.42 (7.63-9.52)	2.03 ± 0.36	3.31 (3.02-3.89)	2.76 ± 1.59	5.62 (5.02-10.30)	3.84 (3.34-3.84)	0.93 (0.88-2.55)	0.82 (0.80-3.86)
INU2	2.74 * (2.39-2.93)	30.73 † (30.50-31.30)	10.73 (8.96-14.80)	43.09 (39.12-47.44)	6.29 (5.35-8.78)	1.80 ± 0.58	2.39 (2.31-4.06)	2.07 ± 1.10	4.73 (4.59-6.95)	3.01 (2.96-3.04)	0.95 (0.83-1.62)	0.87 (0.76-2.37)
POWS	2.93 (2.79-3.84)	32.48 (30.78-34.53)	8.36 (5.85-11.77)	46.53 *, † (46.48-47.17)	5.25 (5.25-5.41)	1.44 ± 0.56	2.75 (2.52-3.19)	1.03 ± 0.59	3.91 (3.57-4.95)	2.32 *, † (2.18-2.39)	0.75 (0.69-1.19)	0.69 (0.62-1.37)
GLBS	2.79 (2.65-4.09)	35.07 (31.72-35.87)	10.65 (10.36-12.13)	45.58 *, † (44.76-47.57)	4.82 (4.56-5.60)	1.81 ± 0.45	2.08 (1.90-2.82)	0.91 ± 0.34	3.64 † (3.27-4.03)	1.72 *, † (1.67-1.95)	0.76 (0.71-1.06)	0.69 (0.65-1.02)
POWSE	2.61 (2.53-3.02)	36.67 (33.92-37.85)	10.55 (10.50-12.18)	44.09 (41.40-45.58)	4.10 (3.98-5.69)	1.86 ± 0.91	2.05 (1.99-2.84)	0.69 ± 0.21 *	4.43 (4.27-4.56)	2.55 (2.54-2.73)	0.79 (0.62-0.81)	0.04 (0.03-0.34)
GLBSE	2.17 * (2.15-2.43)	36.22 (35.84-36.42)	11.72 (11.42-12.77)	44.02 (40.64-44.08)	4.07 (3.83-5.55)	1.42 ± 0.20	1.92 (1.75-3.26)	0.78 ± 0.19 *	4.65 (4.64-5.17)	3.45 (3.14-3.80)	0.81 (0.69-0.88)	0.74 (0.68-0.80)
After fermentation (t=8-h)												
	Total VFAs ($\mu\text{mol mL}^{-1}$ of sample)	Molar ratios of SCFA			Molar ratios of Branched-chain SCFA (%) †				Molar ratios of Other SCFA (%) ‡			
		Acetate (%)	Propionate (%)	Butyrate (%)	Branched-chain SCFA (%) §	Iso-butyrate (%)	Iso-valerate (%)	Iso-caproic acid (%)	Other SCFA (%) ¶	Valerate (%)	Caproic acid (%)	Heptanoic acid (%)
NC	16.31 †, ‡, a (14.56-16.59)	48.01 (45.18-48.07)	15.77 a (14.65-17.84)	24.61 (24.18-25.86)	9.30 †, ‡, a (6.98-9.42)	2.17 ± 0.95	5.71 †, ‡ (4.57-6.11)	0.44 ± 0.51 †, ‡	4.31 †, ‡ (3.94-4.89)	3.99 ‡ (3.42-4.14)	0.59 (0.38-0.78)	0.15 †, ‡ (0.14-0.19)

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INU1	40.72 *,a (36.91-44.63)	48.62 (44.56-49.43)	13.96 (11.94-28.80)	34.55 (23.47-36.47)	1.98 * (1.40-2.23)	1.34 ±0.81	0.40 *,a (0.36-0.40)	0.05 ±0.01 *	0.89 * (0.75-0.94)	0.70 *,a (0.62-0.75)	0.05 (0.04-0.14)	0.05 * (0.04-0.05)
INU2	41.17 *,a (37.28-45.83)	45.75 a (42.98-47.10)	13.73 (11.57-27.65)	34.53 (24.90-38.78)	2.20 * (1.68-2.32)	1.57 ±0.64	0.30 * (0.29-0.34)	0.05 ±0.01 *	0.73 * (0.68-0.80)	0.55 *,a (0.50-0.66)	0.05 (0.04-0.14)	0.04 * (0.04-0.04)
POWS	48.90 *,†,a (47.80-51.23)	40.40 a (38.50-40.86)	20.69 (18.07-31.27)	33.85 (24.03-40.24)	1.96 * (1.41-3.02)	1.78 ±1.50	0.37 *,a (0.34-0.48)	0.09 ±0.08	0.67 * (0.57-0.83)	0.59 *,a (0.49-0.75)	0.04 * (0.04-0.04)	0.04 * (0.04-0.04)
GLBS	32.73 *,‡,a (29.61-35.92)	41.34 a (41.07-43.38)	22.85 (20.15-27.62)	26.32 a (24.30-29.27)	3.17 (2.85-4.47)	1.41 ±0.55	2.31 †,‡ (1.63-3.01)	0.10 ±0.07	1.99 †,‡ (1.86-2.85)	1.64 ‡ (1.62-2.51)	0.18 (0.11-0.25)	0.06 ‡ (0.05-0.11)
POWSE	43.63 *,a (42.66-43.97)	47.02 (42.54-49.28)	22.71 (20.46-28.07)	24.57 a (23.57-26.03)	2.38 (2.08-4.02)	2.34 ±1.98	0.79 *,†,‡ (0.67-0.91)	0.14 ±0.09	1.56 *,‡,a (1.28-1.81)	1.29 ‡ (1.11-1.62)	0.05 a (0.05-0.14)	0.04 * (0.04-0.05)
GLBSE	34.28 *,a (35.63-39.93)	46.87 (46.87-48.56)	21.22 (17.23-21.97)	26.39 (26.23-32.21)	1.59 * (1.36-2.15)	1.09 ±0.80	0.46 *,‡ (0.44-0.57)	0.20 ±0.13	0.87 * (0.80-1.09)	0.75 *,a (0.70-0.98)	0.06 a (0.05-0.06)	0.05 a (0.05-0.06)
After fermentation (t=24-h)												
		Molar ratios of SCFA			Molar ratios of Branched-chain SCFA (%) †				Molar ratios of Other SCFA (%) ‡			
	Total VFAs (μmol mL ⁻¹ of sample)	Acetate (%)	Propionate (%)	Butyrate (%)	Branched-chain SCFA (%) ×	Iso-butyrate (%)	Iso-valerate (%)	Iso-caproic acid (%)	Other SCFA (%) ¥	Valerate (%)	Caproic acid (%)	Heptanoic acid (%)
NC	23.68 †,‡ (18.8-23.68)	40.14 (37.63-43.59)	13.46 (12.73-15.07)	25.88 (25.35-26.04)	10.13 †,‡,c (13.13-11.36)	3.57 ±0.22 †,‡	6.31 †,‡ (6.24-7.04)	0.63 ±0.31 †,‡	6.83 †,‡ (5.17-11.16)	4.15 ‡ (3.76-5.21)	1.97 (1.03-4.09)	0.71 (0.39-1.85)
INU1	40.69 * (40.48-55.79)	49.28 (43.93-49.56)	10.93 (10.75-28.80)	37.07 (24.27-37.30)	9.42 * (7.63-9.52)	1.16 ±0.60 *	0.46 *,‡,c (0.42-0.48)	0.23 ±0.17 *	0.78 * (0.75-0.92)	0.66 *,c (0.63-0.68)	0.04 (0.04-0.16)	0.04 (0.04-0.11)
INU2	36.00 * (33.80-55.72)	48.66 C (45.54-52.17)	11.62 (10.77-25.13)	30.70 (23.23-35.33)	1.36 * (1.12-1.75)	0.93 ±0.60 *	0.33 *,† (0.29-0.34)	0.22 ±0.17 *	0.61 * (0.63-0.84)	0.58 *,c (0.53-0.61)	0.06 (0.04-0.20)	0.05 (0.04-0.06)

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POWS	74.08 *, †, ‡, b,c (72.50- 76.06)	36.56 †, ‡ (35.26- 36.99)	19.45 (17.18- 28.98)	40.02 (32.85- 43.28)	1.23 * (0.99- 2.12)	1.15 ±0.99 *	0.34 *,c (0.27- 0.54)	0.08 ±0.05 *	0.61 * (0.49- 0.79)	0.47 *,c (0.46- 0.70)	0.03 * (0.03- 0.07)	0.02 *, †, ‡, b (0.02- 0.02)
GLBS	52.57 *,b,c (48.09- 55.48)	36.71 (35.57- 42.23)	21.18 (19.48- 23.13)	26.29 c (25.75- 27.63)	8.11 †, ‡, c (7.52- 8.15)	2.71 ±0.42 †, ‡, b,c	4.83 †, ‡ (4.69- 5.02)	0.18 ±0.14 *	5.02 †, ‡ (3.68- 5.51)	3.56 ‡ (2.92- 3.92)	1.19 (0.61- 1.27)	0.27 (0.15- 0.33)
POWSE	61.36 *,b,c (59.14- 63.03)	41.70 b (37.92- 43.98)	28.24 c (24.40- 28.67)	29.65 (26.80- 30.31)	3.22 * (2.80- 3.37)	1.32 ±0.82 *	1.42 *, †, ‡ (1.32- 1.66)	0.20 ±0.17 *	1.72 *, †, ‡ (1.49- 2.58)	1.66 ‡ (1.38- 2.15)	0.04 (0.03- 0.34)	0.03 * (0.03- 0.09)
GLBSE	46.15 *,c (45.80- 47.39)	44.58 c (43.09- 46.65)	20.51 (17.22- 21.66)	30.80 (29.77- 35.28)	1.04 * (1.02- 2.26)	1.03 ±1.08 *	0.46 *, ‡ (0.42- 0.82)	0.15 ±0.09 *,c	1.00 * (0.87- 1.15)	0.72 *, †, c (0.70- 0.96)	0.05 c (0.04- 0.14)	0.04 c (0.04- 0.04)
P overall	0.003	0.076	0.964	0.516	0.018	0.189	0.001	0.109	<0.001	<0.001	0.132	0.004

Values are expressed as mean value and SD for parametric; median and interquartile range (Q1 –Q3) for non-parametric data; * Sum of iso-butyrate, iso-valerate and iso-caproic acid; † Sum of valerate, caproic acid and heptanoic acid; *: significantly different compared to [NC] at t=0-h, 8-h or 24-h (p <0.05); †: significantly different compared to [INU1] at t=0-h, 8-h or 24-h (p <0.05); ‡: significantly different compared to [INU2] at t=0-h, 8-h or 24-h (p <0.05); a: significantly different from baseline compared to after 8-h of fermentation (p <0.05) (Paired Samples t-test or Wilcoxon for non-parametric); b: significantly different from after 8-h of fermentation compared to after 24-h of fermentation (p <0.05) (Paired Samples t-test or Wilcoxon for non-parametric); c: significantly different from baseline compared to after 24-h of fermentation (p <0.05) (Paired Samples t-test or Wilcoxon for non-parametric); p-overall refers to the Tests of Between-Subjects Effects and symbols *, †, ‡ to parameter estimates at t=0-h, 8-h and 24-h (Repeated-Measures ANOVA or Friedman test); NC: Negative control; INU1: Inulin 1% (w/v); INU2: Inulin 2% (w/v); POWS: *P. ostreatus* untreated mushroom powder; POWSE: *P. ostreatus* mushroom-extract; GLBS: *G. lucidum* untreated mushroom powder; GLBSE: *G. lucidum* mushroom-extract;

Table S4b. Total Volatile Fatty Acids (VFAs) concentrations ($\mu\text{mol mL}^{-1}$ of sample) and Molar ratios (%) of SCFAs at baseline and after 8-h and 24-h fermentation in osteopenic subjects.

OSTEOPENIC (n=3)												
Baseline (t=0-h)												
		Molar ratios of SCFA			Molar ratios of Branched-chain SCFA (%) †				Molar ratios of Other SCFA (%) ‡			
	Total VFAs ($\mu\text{mol mL}^{-1}$ of sample)	Acetate (%)	Propionate (%)	Butyrate (%)	Branched-chain SCFA (%) †	Iso-butyrate (%)	Iso-valerate (%)	Iso-caproic acid (%)	Other SCFA (%) ‡	Valerate (%)	Caproic acid (%)	Heptanoic acid (%)
NC	3.96 (2.53-4.23)	34.39 (34.18-41.38)	12.56 ± 0.97	35.13 ‡ (27.96-39.14)	8.62 ± 2.37	1.85 (1.42-2.58)	3.83 (2.88-5.18)	2.97 (2.21-2.99)	7.87 (5.68-8.60)	4.19 ± 1.32	1.44 (0.93-2.11)	1.32 (0.85-1.54)
INU1	2.32 (1.89-2.46)	38.20 (34.13-42.62)	14.44 ± 3.23	35.63 ‡ (29.05-37.29)	8.46 ± 2.89	1.71 (1.55-2.35)	2.18 (1.71-2.62)	4.23 (2.67-5.85)	7.37 (5.57-7.61)	3.94 ± 1.52	1.10 (0.94-2.05)	0.71 (0.66-0.85)
INU2	2.42 (2.34-2.82)	31.93 (27.96-34.97)	12.25 ± 2.37	47.59 *, † (43.36-47.61)	6.10 ± 0.61	1.02 (0.96-1.71)	2.18 (1.57-2.42)	2.81 (1.81-3.68)	4.71 (4.78-6.22)	3.58 ± 1.10	0.81 (0.79-1.60)	0.06 (0.06-0.18)
POWS	2.79 (2.71-4.03)	33.71 (31.59-35.34)	5.86 ± 1.26 *, †, ‡	52.07 *, †, ‡ (50.03-53.69)	4.81 ± 1.18 *, †	0.92 (0.88-1.03)	2.50 (2.43-2.64)	0.62 *, †, ‡ (0.57-1.70)	3.67 (3.67-4.41)	2.44 ± 0.04 *	0.65 (0.64-1.46)	0.57 (0.52-0.58)
GLBS	2.69 (2.60-3.24)	30.78 (27.64-33.28)	7.55 ± 2.05 *, †, ‡	56.65 *, †, ‡ (52.39-56.88)	4.28 ± 0.83 *, †	1.82 (1.36-1.98)	2.11 (1.95-2.18)	0.66 *, †, ‡ (0.56-0.68)	3.64 * (3.59-4.01)	2.19 ± 0.16 *, †	0.71 (0.68-1.33)	0.60 (0.51-0.62)
POWSE	2.68 (2.48-2.81)	34.03 (31.77-35.38)	9.76 ± 2.99 †	48.39 *, † (44.91-49.60)	4.86 ± 1.00 *, †	1.60 (1.33-1.64)	2.97 (2.40-3.12)	0.72 *, †, ‡ (0.66-0.77)	5.14 (4.56-5.63)	3.10 ± 0.67	0.82 (0.77-1.64)	0.66 (0.60-0.70)
GLBSE	2.15 (2.09-2.44)	35.68 (32.34-37.33)	10.90 ± 2.64	46.67 (43.80-46.81)	4.51 ± 1.35 *, †	1.98 (1.63-2.01)	1.44 (1.28-2.30)	0.87 † (0.77-0.92)	4.98 (4.80-5.48)	2.89 ± 0.39	0.98 (0.92-1.87)	0.79 (0.70-0.84)
After fermentation (t=8-h)												
		Molar ratios of SCFA			Molar ratios of Branched-chain SCFA (%) †				Molar ratios of Other SCFA (%) ‡			
	Total VFAs ($\mu\text{mol mL}^{-1}$ of sample)	Acetate (%)	Propionate (%)	Butyrate (%)	Branched-chain SCFA (%) †	Iso-butyrate (%)	Iso-valerate (%)	Iso-caproic acid (%)	Other SCFA (%) ‡	Valerate (%)	Caproic acid (%)	Heptanoic acid (%)
NC	16.89 (11.90-17.75)	47.30 ‡ (43.42-48.50)	19.45 ± 6.01	35.13 (27.96-39.14)	4.79 ± 2.37 †, ‡	1.32 (1.22-1.47)	4.05 †, ‡ (2.82-4.12)	0.13 (0.11-0.19)	4.31 †, ‡ (3.94-4.89)	2.93 ± 1.52 †, ‡	0.13 (0.11-0.62)	0.12 † (0.10-0.18)

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INU1	35.49 a (28.37-39.38)	52.24 (51.93-57.13)	17.12 ±7.68	31.95 (20.97-33.23)	1.18 ±0.49 *	0.80 (0.50-0.94)	0.39 * (0.39-0.47)	0.05 (0.05-0.06)	0.83 *,a (0.82-0.97)	0.72 ±0.32 *	0.05 (0.05-0.19)	0.04 *,a (0.04-0.06)
INU2	26.07 a (24.79-33.65)	69.04 * (60.39-69.76)	14.06 ±2.73	17.40 (14.88-23.71)	1.48 ±0.34 *,a	1.12 (0.94-1.19)	0.40 * (0.34-0.41)	0.06 (0.06-0.07)	0.80 * (0.75-0.83)	0.59 ±0.22 *	0.06 (0.06-0.18)	0.05 a (0.05-0.06)
POWS	35.76 * (34.45-49.11)	57.04 (49.58-60.07)	17.78 ±5.74	21.49 (18.67-30.95)	1.52 ±0.66 *	0.74 (0.67-1.21)	0.41 *,a (0.37-0.43)	0.05 (0.04-0.16)	0.61 * (0.55-0.80)	0.56 ±0.35 *,a	0.05 (0.04-0.13)	0.04 *,a (0.04-0.05)
GLBS	27.67 a (26.52-33.81)	43.65 †, ‡, a (42.55-46.06)	22.23 ±1.71 a	28.05 a (25.42-30.57)	3.00 ±1.80	0.82 (0.70-1.18)	1.88 †, ‡ (1.25-2.64)	0.05 a (0.05-0.06)	2.94 †, ‡ (1.86-3.00)	2.08 ±1.43	0.05 (0.05-0.18)	0.05 a (0.05-0.06)
POWSE	37.30 * (30.53-49.54)	45.95 †, ‡, a (42.44-47.33)	25.54 ±8.07 ‡	26.01 a (24.85-27.57)	2.06 ±0.73 *,a	0.93 (0.81-1.17)	0.84 †, ‡, a (0.77-1.15)	0.04 *,a (0.04-0.06)	1.73 * (1.29-1.96)	1.40 ±0.87	0.04 (0.04-0.20)	0.04 *,a (0.04-0.06)
GLBSE	41.86 *,a (33.84-42.70)	49.77 (46.75-59.50)	20.09 ±4.23	19.66 (14.33-28.28)	2.04 ±1.04 *,a	1.00 (0.81-1.01)	0.54 *,a (0.44-1.35)	0.05 (0.05-0.19)	1.71 * (1.22-2.29)	1.59 ±1.20	0.05 (0.05-0.17)	0.05 *,a (0.05-0.37)
After fermentation (t=24-h)												
		Molar ratios of SCFA			Molar ratios of Branched-chain SCFA (%) †				Molar ratios of Other SCFA (%) ‡			
	Total VFAs (μmol mL ⁻¹ of sample)	Acetate (%)	Propionate (%)	Butyrate (%)	Branched-chain SCFA (%) §	Iso-butyrate (%)	Iso-valerate (%)	Iso-caproic acid (%)	Other SCFA (%) ¶	Valerate (%)	Caproic acid (%)	Heptanoic acid (%)
NC	25.73 †, b,c (23.56-26.74)	44.55 (44.52-46.54)	17.88 ±6.01	22.66 (21.72-24.52)	9.57 ±0.47 †, ‡, b	3.41 †, ‡ (2.84-3.46)	6.15 †, ‡ (6.06-6.36)	0.07 (0.07-0.38)	3.28 (2.19-4.59)	2.63 ±1.85 †, ‡	0.42 † (0.25-0.87)	0.09 † (0.08-0.24)
INU1	45.53 *,b,c (39.11-52.22)	57.29 (53.84-64.20)	17.04 ±3.33	27.49 (17.61-28.23)	1.33 ±0.29 *,c	0.89 * (0.63-0.93)	0.41 * (0.40-0.47)	0.15 (0.09-0.21)	0.68 b,c (0.55-0.75)	0.56 ±0.22 *	0.03 * (0.03-0.48)	0.03 * (0.03-0.04)
INU2	37.76 *,c (34.64-47.02)	65.60 (62.00-73.24)	14.74 ±3.95	14.24 b (10.86-19.63)	0.89 ±0.22 *,b,c	0.33 * (0.28-0.57)	0.28 * (0.17-0.37)	0.05 (0.05-0.23)	0.52 (0.39-0.63)	0.41 ±0.25 *	0.81 (0.79-1.60)	0.05 b,c (0.04-0.05)
POWS	78.62 *, †, ‡, b,c (71.64-)	37.57 *, †, ‡ (37.35-39.65)	14.96 ±1.16 c	46.08 *, †, ‡, c (43.47-46.39)	1.03 ±0.21 *,c	0.73 * (0.49-0.78)	0.43 * (0.32-0.43)	0.10 (0.06-0.11)	0.43 (0.42-0.76)	0.57 ±0.42 *,c	0.03 * (0.02-0.07)	0.02 *, ‡, c (0.02-0.02)

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	84.36)											
GLBS	48.44 *,b,c (46.45- 49.61)	39.90 †, ‡ (39.64- 40.35)	20.02 ±1.11 c	28.75 ‡, c (27.08- 29.24)	7.59 ±0.67 †, ‡, b,c	1.88 †, ‡, b (1.83- 2.47)	5.08 †, ‡, c (4.98- 5.38)	0.15 (0.10- 0.18)	3.26 †, ‡ (3.07- 5.19)	3.38 ±0.96 †, ‡	0.37 (0.20- 1.22)	0.04 (0.04- 0.29)
POWSE	59.94 *, †, ‡, c (58.56- 60.27)	38.86 *, †, ‡, b (36.50- 39.27)	23.98 ±0.99 *, †, ‡, c	32.00 *, †, c (30.73- 34.52)	3.19 ±1.49 *	1.02 *, ‡ (0.77- 1.19)	1.89 *, †, ‡ (1.37- 2.52)	0.27 (0.17- 0.35)	2.32 b (2.17- 2.65)	2.23 ±0.42	0.14 (0.11- 0.18)	0.06 c (0.05- 0.08)
GLBSE	47.97 * (42.55- 48.09)	43.61 ‡ (41.10- 57.63)	17.07 ±2.38	22.28 b (16.40- 30.75)	4.21 ±3.17 *, †, ‡	2.16 ‡ (1.51- 2.28)	1.02 *, ‡ (0.70- 3.10)	0.26 (0.15- 0.29)	2.06 (1.34- 4.83)	2.34 ±2.06	0.06 (0.05- 1.21)	0.06 (0.05- 0.37)
P overall	0.007	0.045	0.078	0.083	<0.001	0.015	0.010	0.139	0.007	0.073	0.661	0.001

Values are expressed as mean value and SD for parametric; median and interquartile range (Q1 –Q3) for non-parametric data; * Sum of iso-butyrate, iso-valerate and iso-caproic acid; † Sum of valerate, caproic acid and heptanoic acid; *: significantly different compared to [NC] at t=0-h, 8-h or 24-h (p <0.05); †: significantly different compared to [INU1] at t=0-h, 8-h or 24-h (p <0.05); ‡: significantly different compared to [INU2] at t=0-h, 8-h or 24-h (p <0.05); a: significantly different from baseline compared to after 8-h of fermentation (p <0.05) (Paired Samples t-test or Wilcoxon for non-parametric); b: significantly different from after 8-h of fermentation compared to after 24-h of fermentation (p <0.05) (Paired Samples t-test or Wilcoxon for non-parametric); c: significantly different from baseline compared to after 24-h of fermentation (p <0.05) (Paired Samples t-test or Wilcoxon for non-parametric); p-overall refers to the Tests of Between-Subjects Effects and symbols *, †, ‡ to parameter estimates at t=0-h, 8-h and 24-h (Repeated-Measures ANOVA or Friedman test); NC: Negative control; INU1: Inulin 1% (w/v); INU2: Inulin 2% (w/v); POWS: *P. ostreatus* untreated mushroom powder; POWSE: *P. ostreatus* mushroom-extract; GLBS: *G. lucidum* untreated mushroom powder; GLBSE: *G. lucidum* mushroom-extract;

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