1 **1. Supplementary Table 1**. 16s rRNA PCR reaction preparation

Reagent	Volume (µL)	Volume for 5 sample
AmpliTaq reaction Master Mix	6.25	31.5
16s forward primer (27 F)	0.5	2.5
16s Rev primer (1492 F)	0.5	2.5
PCR-grade water	4.25	21.5
Sample	1	1
Total Reaction Volume	12.5	59

2

3 **2. Supplementary Table 2.** Table 2. 16s rRNA PCR program

Cycle	Temperature	Time	Cycle
Initial denaturation	98	5 min	N/A
Denaturation	98	15 s	
Annealing	60	30 s	36 times
Extension	72	90 s	
Final Extension	72	5 min	N/A
Hold	4	Infinite	N/A

4

5 **3. DNA yield and quality**

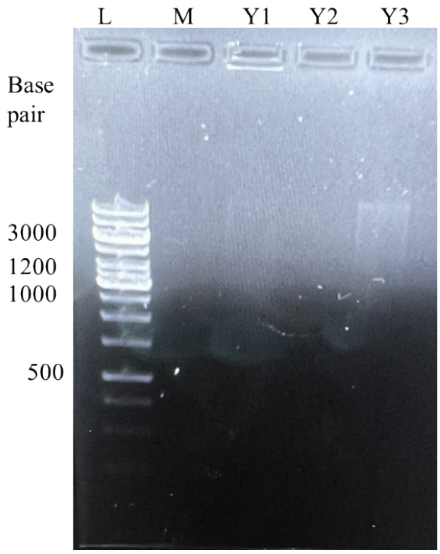
6 High-quality chromosomal DNA was extracted from the microbial cells of both unfermented
7 milk and fermented yoghurt analogues samples using GenElute Bacterial Genomic DNA kit
8 (Sigma-Aldrich). Quantification was performed using a Qubit™ 4 Fluorometer with the
9 dsDNA HS Assay Kit (Invitrogen). The DNA concentrations obtained across 10 samples varied
10 considerably, ranging from 0.144 ng/µL in the unfermented milk base to a maximum of 7.76
11 ng/µL in Yoghurt 2 (Y2) after 14 days of storage (Table 4). DNA samples with concentrations
12 below 1 ng/µL were concentrated using a SpeedVac centrifugal vacuum system to ensure
13 sufficient input for downstream analyses. Variability in DNA yield was attributed to two key
14 factors: (i) procedural inconsistencies in handling a high number of isolates during
15 simultaneous extractions, and (ii) differing microbial cells across inoculated yoghurt
16 formulations and time points.

4. Supplementary Table 3. Yields of DNA isolated from microbial cells by GenElute Bacterial DNA kit

Sample	Concentraction (ng/uL)	After speedvacuum concentration	Volume (µL)
Milk_6 h	0.144	0.5	28
Y1_6 h	11.8	11.8	28
Y3_6 h	0.844	2.12	28
Y3_6 h	0.716	2.01	28
Y1_14 d	0.55	1.5	28
Y2_14 d	7.76	7.76	28
Y3_14 d	2.10	2.1	28
Y1_28 d	2.04	2.04	28
Y2_28 d	6.38	6.38	28
Y3_28 d	4.06	4.06	28

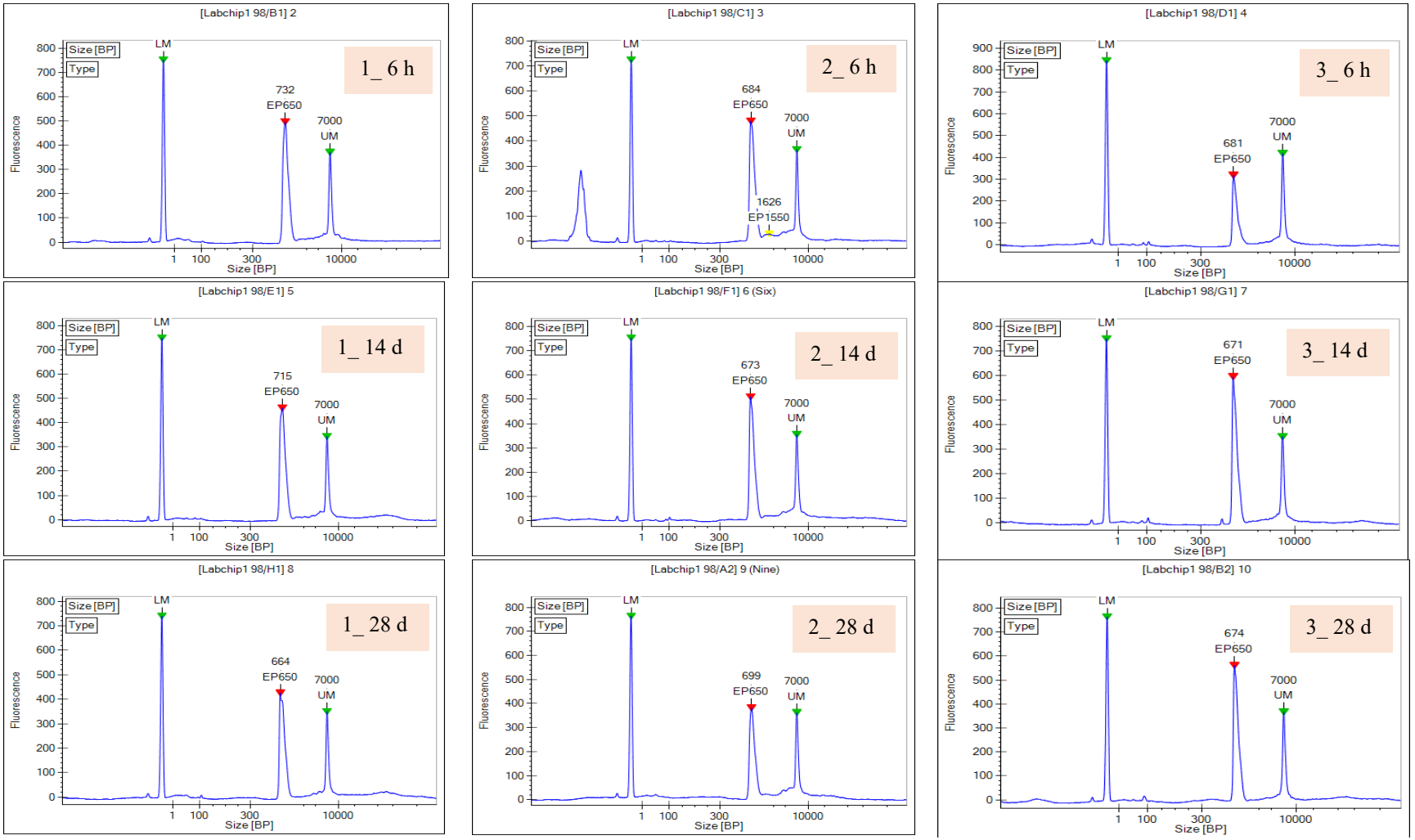
5. DNA integrity and size distribution

Size fractionation of 16S rRNA gene fragments were evaluated by agarose gel electrophoresis on a 0.8% agarose gel containing 2.5 µL per 25 ml of Tris Acetate-EDTA buffer. While smear patterns indicated DNA presence, the concentration was below the threshold for visual detection by gel imaging (Figure 5). However, DNA integrity and fragment size distribution were validated through the Agilent DNA LabChip system.



Supplementary Figure 1. Agarose gel electrophoresis at 0.8% of the PCR products purified from PCR reactions performed using oligonucleotides for bacterial isolates of yoghurt during fermentation and refrigerated storage. The samples were fermented with the following cultures: *L. delbrueckii sub bulgaricus, S. thermophilus* and *Lactobacillus rhamnosus* (Yoghurt Y1); *L. delbrueckii subs. Bulgaricus, Streptococcus thermophilus*, *L. casei* 431 (Yoghurt Y2); *Lactobacillus plantarum*, BB12 (*Bifidobacterium sps)* (Yoghurt Y3), Milk (M) and GeneRular DNA Ladder Mix (L), Band size in base pair (bp) are indicated.

DNA LabChip analysis revealed consistent amplicon across yoghurt samples and storage conditions (Figure 6). The sample details of DNA LabChip analysis are mentioned in Supplementary Table 3. Yoghurt 1 (Y1) exhibited DNA fragments of approximately 732 bp (base pair) post-fermentation (6 h), decreasing slightly to 715 bp and 664 bp at 14 and 28 days, respectively. Similarly, Yoghurt 2 (Y2) yielded 684 bp fragments after fermentation, followed by 673 bp and 699 bp over storage. Yoghurt 3 (Y3) displayed 681 bp after 6 hours, with slight variation to 671 bp and 674 bp subsequent storage points. These results suggest stable 16S rRNA gene amplification profiles, confirming the reliability of microbial DNA extraction and downstream sequencing protocols.



Supplementary Figure 2. Quantity and size fractionation of microbial DNA using LabChip during yoghurt fermentation and refrigerated storage.

The samples were fermented with the following cultures: *L. delbrueckii sub bulgaricus, S. thermophilus* and *Lactobacillus rhamnosus* (Yoghurt

Y1); *L. delbrueckii subs. Bulgaricus, Streptococcus thermophilus*, *L. casei* 431 (Yoghurt Y2); *Lactobacillus plantarum*, BB12 (*Bifidobacterium sps)* (Yoghurt Y3).

6. **Supplementary Table 4** Sample details for the microbiota dynamics including metagenomic analysis, in lupin-oat based milk and yoghurt analogues fermented by different combinations of probiotic cultures during storage at 4 °C for 28 days.

Sample details

0_hr: Unfermented milk sample

1_6hr, 1_14day, 1_28day: Yoghurt 1 fermented with *Lactobacillus delbrueckii sub bulgaricus, Streptococcus thermophilus* and *Lactobacillus rhamnosus*, samples taken after 6 hr, 14 day and 28 day.

2_6hr, 2_14day, 2_28day: Yoghurt 2 fermented with *Lactobacillus delbrueckii sub bulgaricus, Streptococcus thermophilus* and *Lactobacillus paracasei*, samples taken after 6 hr, 14 day and 28 day.

3_6hr, 3_14day, 3_28day: Yoghurt 3 fermented with *Lactobacillus plantarum* and *Bifidobacterium sps*, samples taken after 6 hr, 14 day and 28 day.

No.	Sample Name	Sample Source	Details
1	1	0_hr	Milk
2	2	1_6 hr	Yoghurt
3	3	2_6 hr	Yoghurt
4	4	3_6 hr	Yoghurt
5	5	1_14 day	Yoghurt
6	6 (Six)	2_14 day	Yoghurt
7	7	3_14 day	Yoghurt
8	8	1_28 day	Yoghurt
9	9 (Nine)	2_28 day	Yoghurt
10	10	3_28 day	Yoghurt

7. Raw data quality for metagenomic analysis

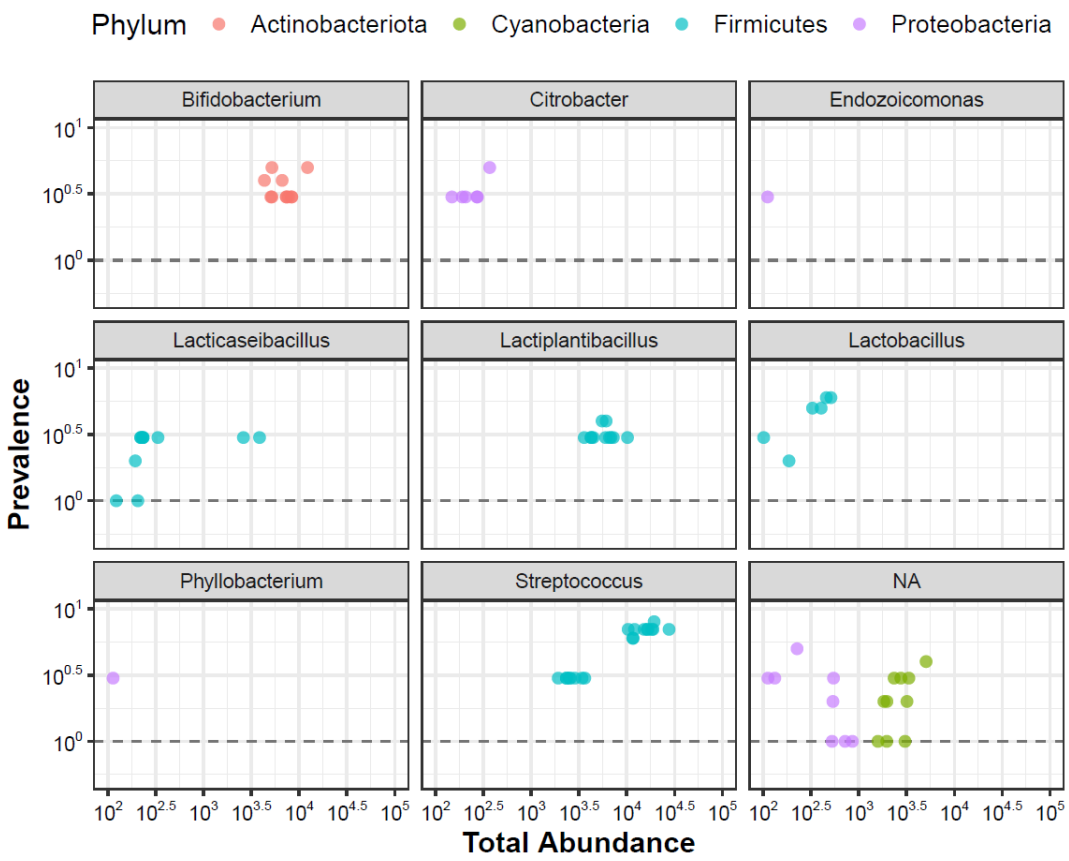
High throughput 16S rRNA amplicon sequencing generated robust datasets across all samples. Detailed sequencing metrics are presented in Table 5. For each sample, key indicators included:

- PE Reads: Paired-end raw counts
- AvgLen (bp): Average read length
- GC Content (%): Proportion of guanine and cytosine bases in total sequences
- Q20/Q30 (%): Quality scores indicating the percentage of bases with Phred scores ≥ Q20 and Q30, reflecting high base-calling accuracy
- Clean Tags: Proportion of reads retained post-filtering for quality and adaptors

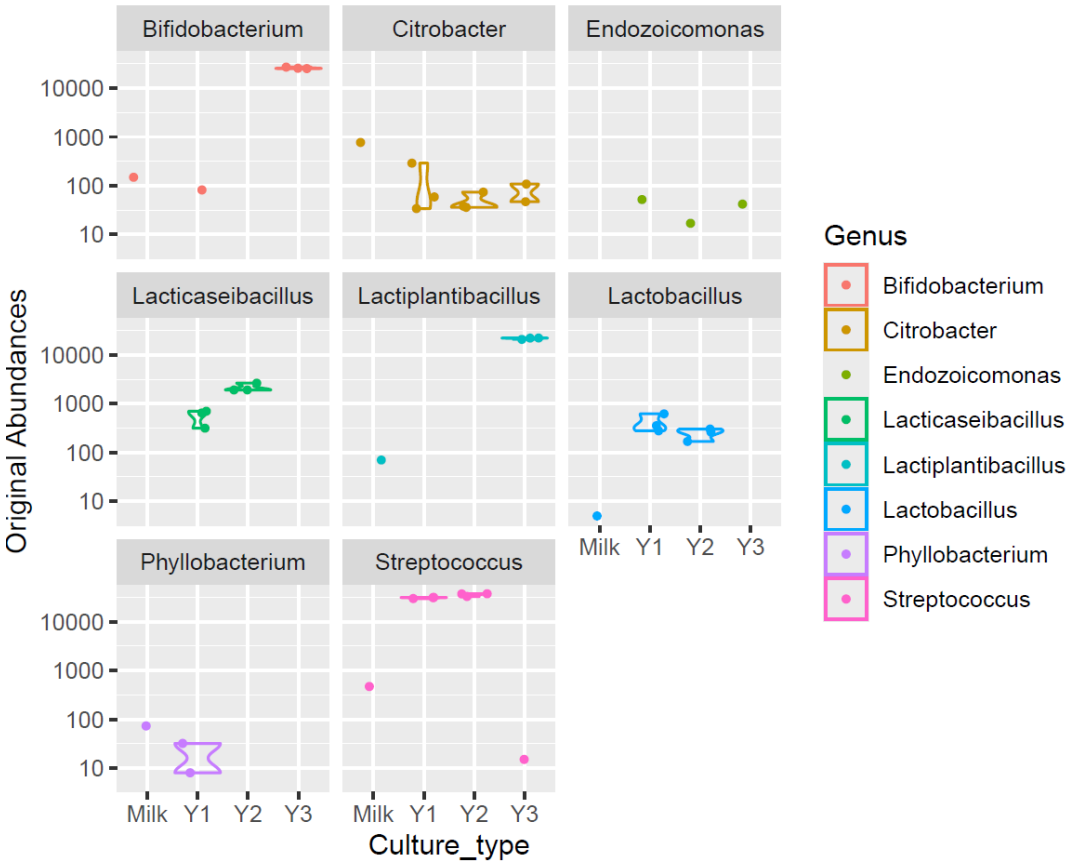
Across all samples, sequencing quality was high, with Q20 and Q30 values consistently exceeding standard benchmarks (>97% and >93%, respectively), ensuring data suitability for reliable taxonomic profiling and comparative microbial community analysis.

8. Supplementary Table 5. Statistics on sequencing data processing

Sample ID	PE Reads	AvgLen(bp)	GC(%)	Q20(%)	Q30(%)	Clean_tags
Bacteria_CG685-001M0001	79,800	247	53.50	97.49	92.87	79,800
Bacteria_CG685-001M0002	80,266	247	53.01	97.52	93.09	80,266
Bacteria_CG685-001M0003	80,229	247	53.06	97.73	93.49	80,229
Bacteria_CG685-001M0004	79,886	247	54.53	97.83	93.69	79,886
Bacteria_CG685-001M0005	80,157	247	53.05	97.83	93.62	80,157
Bacteria_CG685-001M0006	80,049	247	53.05	97.57	93.23	80,049
Bacteria_CG685-001M0007	79,794	247	54.78	97.85	93.73	79,794
Bacteria_CG685-001M0008	79,957	247	53.01	97.56	93.14	79,957
Bacteria_CG685-001M0009	80,165	247	53.05	97.85	93.76	80,165
Bacteria_CG685-001M0010	79,828	247	54.55	97.85	93.80	79,828



(A) Phylum type



(B) Genus type

Supplementary Figure 3. Total abundance of total microbiota composition (A) Phylum type and (B) Genus type of yoghurt after fermentation and refrigerated storage. The samples were fermented with the following cultures: *L. delbrueckii sub bulgaricus, S. thermophilus* and *Lactobacillus rhamnosus* (Yoghurt Y1); *L. delbrueckii subs. Bulgaricus, Streptococcus thermophilus*, *L. casei* 431 (Yoghurt Y2); *Lactobacillus plantarum*, BB12 (*Bifidobacterium sps)* (Yoghurt Y3).

9. Supplementary Table 6 Volatile compounds identified using headspace SPME-GC/MS, in lupin-oat based milk and yoghurt analogues fermented by different combinations of probiotic cultures during storage at 4 °C for 28 days.

Groups	Compound	Unfermented	Fermentation period with different probiotic combinations								
			Y1			Y2			Y3		
		0 h	6 h	14 d	28 d	6 h	14 d	28 d	6 h	14 d	28 d
Alcohols	1-Hexanol	+	+	+	+	+	+	+	+	+	+
	1-Heptyn-3-ol										+
	1-Heptanol				+		+	+		+	+
	1-Octen-3-ol	+	+	+	+	+	+	+	+	+	+
	4-Octanol	+	+	+	+	+	+	+	+	+	+
	3,5-Octadien-2-ol				+	+	+	+	+	+	+
	1-Octanol		+	+	+	+	+	+	+	+	+
	3-Piperidinol							+		+	+
	1-Nonanol								+		
Aldehydes	Hexanal	+	+	+	+	+	+	+	+	+	+
	2-Hexenal, (E)-	+	+	+	+	+	+	+	+	+	+
	Heptanal	+	+	+	+		+	+	+	+	+
	2-Heptenal, (E)-	+	+	+	+	+	+	+	+	+	+
	Benzaldehyde	+	+	+	+	+	+	+	+	+	+
	Octanal				+		+	+	+	+	+
	2,4-Heptadienal, (E,E)-		+		+	+	+	+	+	+	+
	Benzeneacetaldehyde or Phenylacetaldehyde				+		+	+		+	+
	2-Octenal, (E)-	+	+	+	+	+	+	+	+	+	+
	Nonanal	+	+	+	+	+	+	+	+	+	+
	2,4-Octadienal, (E,E)-							+		+	+
	2-Nonenal, (E)-		+	+	+	+	+	+	+	+	+
	Butanal, 3-methyl-							+	+		+
	2,4-Nonadienal, (E,E)-				+		+	+	+	+	+
	2,4-Nonadienal, (E,E)-	+	+	+	+	+	+	+	+	+	+
	2,4-Decadienal	+	+	+	+	+	+	+	+	+	+

	2-Decenal, (E)-	+		+	+	+	+	+	+	+	+	+
	2-Tridecenal, (E)-			+	+	+	+	+	+	+	+	+
Acids	Butanoic acid						+	+	+	+	+	+
	Hexanoic acid				+	+		+	+		+	+
	Hexanoic acid, anhydride					+			+		+	+
	Octanoic acid					+						
	2-Octynoic acid											+
Esters	Cyclopropanecarboxylic acid,butyl ester							+	+	+	+	+
	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	+		+	+	+	+	+	+	+	+	+
Ketones	2-Heptanone	+			+	+		+	+		+	+
	4-Octanone	+		+	+	+	+	+	+	+	+	+
	3,5-Octadien-2-one, (E,E)-								+			
	2-Nonanone				+	+	+	+	+	+	+	+
	Cyclooctanone					+	+	+	+	+	+	+
	β-Damascenone			+	+	+	+	+	+	+	+	+
	3-Methyl-3,5--(cyanoethyl)tetrahydro-4-thiopyranone								+			+
	2,3-Octanedione				+	+	+	+	+	+	+	+
Phenols	Phenol, 4-(1-methylpropyl)-			+	+	+	+	+	+	+	+	+
Alkenes	D-Limonene							+	+	+	+	+
	1,3-Hexadiene, 3-ethyl-2-methyl-	+		+	+	+	+	+	+	+	+	+
Alkanes	6-Methyl-1,7-diazabicyclo[4.1.0]heptane								+			+
	Hexadecane					+	+	+	+	+	+	+
	Nonane, 5-(1-methylpropyl)-						+	+	+	+	+	+
	Undecane, 3-methyl-									+		
	Cyclobutane, 2-ethyl-1-methyl-3-propyl-								+			+
	Nonane, 2,6-dimethyl-							+	+	+	+	+
	Decane, 3,6-dimethyl-			+	+	+	+	+	+	+	+	+
	Silane, cyclohexyldimethoxymethyl-	+										
	Dodecane	+		+	+	+	+	+	+	+	+	+
	Undecane, 2,6-dimethyl-					+	+	+	+	+	+	+

	Undecane, 2,6-dimethyl-					+		+	+	+	+	
	Tridecane				+	+	+	+	+	+	+	
	Hexane, 3,3-dimethyl-	+		+	+	+	+	+	+	+	+	+
Others	Pyridine, 2,3,4,5-tetrahydro-				+		+	+		+	+	
	Isobutyl octyl carbonate							+				
	Piperidine, 3-methyl-							+			+	
	Methylpent-4-enylamine				+	+	+	+	+		+	
	1,3-Propanediamine, N-methyl-								+			
	Estra-1,3,5(10),6-tetraen-17-one, 3-hydroxy-, O-methyloxime				+	+		+	+	+	+	
	1,3-Propanediamine, N-methyl-				+	+	+	+	+	+	+	
	N-(Trimethylsilyl)-N-((trimethylsilyl)oxy)benzamide				+			+	+	+	+	
	N-Methylallylamine				+	+	+	+	+	+	+	
	Benzene, 1,3-bis(1,1-dimethylethyl)-	+		+	+	+	+	+	+	+	+	
	1,3-Propanediamine, N-methyl-								+		+	
	1,3-Propanediamine, N-methyl-										+	
	1,3-Propanediamine, N-methyl-							+	+	+	+	

+ Compound was detected qualitatively

0 h, 6 h, 14 d and 28 d are fermentation periods in hour (h) and days (d)

88 **10. Supplementary Table 7** Non-volatile compounds identified using HPLC-QTOF-MS/MS, in lupin-oat based milk and yoghurt analogues
89 fermented by different combinations of probiotic cultures during storage at 4 °C for 28 days.

90

Class/subclass	Compound name	Unfermented	Fermentation period with different probiotic combinations								
			Y1			Y2			Y3		
		0 h	6 h	14 d	28 d	6 h	14 d	28 d	6 h	14 d	28 d
Negative mode [M-H]⁻											
Flavonoids											
Flavanones	Isobavachin	+	+	+	+	+	+	+	+		+
Flavones	Vaccarin	+	+	+	+	+	+	+	+	+	+
Flavonols	Tiliroside	+	+	+	+	+	+	+	+	+	+
Others: non-phenolic compounds											
Disaccharides	Trehalose	+	+	+	+	+	+	+	+	+	+
Purine nucleosides	Guanosine	+	+	+	+	+	+	+			
Dipeptides	Glutamyltyrosine	+	+	+	+	+	+	+	+	+	+
Dipeptides	Tryptophan	+	+	+	+	+	+	+	+		
Peptides	Pyroglutamyl-Isoleucine 1		+	+	+				+	+	+
Peptides	Pyroglutamyl-Isoleucine 2	+	+	+	+				+	+	+
Hydroxy fatty acids	Hydroxyisocaproic acid								+	+	+
Phenylpropanoic acids	3-Phenyllactic acid						+	+	+	+	+
Yohimbine alkaloids	Syrosingopine	+	+	+	+	+	+	+	+	+	+
Linoleic acids	(10E,15Z)-9,12,13-trihydroxyoctadeca-10,15-dienoic acid										+
Long-chain fatty acids	(Z)-5,8,11-trihydroxyoctadec-9-enoic acid 1	+	+	+	+	+	+	+	+	+	+
Long-chain fatty acids	(Z)-5,8,11-trihydroxyoctadec-9-enoic acid 2	+	+	+	+	+	+	+	+	+	+
Benzanilides	Imatinib	+	+								+
Linoleic acids	(9Z,12E)-15,16-dihydroxyoctadeca-9,12-dienoic acid 1	+	+	+	+	+	+	+	+	+	

Oxidized fatty acids	Unknown fatty acid	+		+	+	+	+	+	+	+	+	+
Linoleic acids	(9Z,12E)-15,16-dihydroxyoctadeca-9,12-dienoic acid 2	+		+	+	+	+	+	+	+	+	+
Linoleic acids	(9Z,12E)-15,16-dihydroxyoctadeca-9,12-dienoic acid 3	+		+	+	+	+	+	+	+	+	+
Linoleic acids	9-hydroxy-10,12-octadecadienoic acid 1	+		+	+	+	+	+	+	+	+	+
Linoleic acids	9-hydroxy-10,12-octadecadienoic acid 2	+		+	+	+	+	+	+	+	+	+
Long-chain fatty acids	Lichesterylic acid 1	+		+	+	+	+	+	+	+	+	+
Long-chain fatty acids	Lichesterylic acid 2	+		+	+	+	+	+	+	+	+	+
Octadecanoids	9-hydroxyoctadeca-10,12-dienoic acid	+		+	+	+	+	+	+	+	+	+
Long-chain fatty acids	12-Hydroxyoctadecanoic acid								+	+	+	+
Lipids	Unknown lysophosphatidylethanolamine	+		+					+		+	+
Long-chain fatty acids	9-stearolic acid			+	+	+	+	+	+	+	+	+
Long-chain fatty acids	Hexadecanoic acid	+		+	+	+	+	+	+	+	+	+
Positive mode [M+H]$^+$												
Flavonoids												
Flavones	Luteolin-4'-O-glucoside			+	+	+	+		+	+		+
Flavones	Vaccarin	+		+	+	+	+	+	+	+	+	+
Flavones	Apigenin 6,8-digalactoside	+		+	+	+	+	+	+	+	+	+
Flavanones	tris(2,4-ditert-butylphenyl) phosphate	+		+	+	+	+		+	+	+	+
Phenolic acids												
Simple phenolic acids	Phthalic acid											+
Others: non-phenolic compounds												
Pyridine alkaloids	Trigonelline	+		+	+	+	+		+	+	+	+
Piperidines	4-Hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine	+		+	+		+		+	+	+	+
Purine alkaloids	Adenine	+			+	+	+	+	+	+	+	
Dipeptides	L-Prolyl-L-isoleucine	+		+	+	+	+	+	+	+	+	+
Bipyridines and oligopyridines	Anabasamine	+		+	+	+	+	+	+	+	+	+
Dipeptides	L-Prolyl-L-isoleucine	+		+	+	+	+		+	+	+	+

Dipeptides	Glutamyltyrosine	+		+	+	+	+	+	+	+	+	+
Triterpenoids	Dehydrotumulosic acid			+			+					
Lysine alkaloids	Matrine	+		+	+	+	+	+	+	+	+	+
Purine alkaloids	Deethylatrazine	+		+	+	+	+	+	+	+	+	+
Stilbenoids	Erianin	+		+	+	+	+	+	+	+	+	+
Steroids	Halistanol sulfate 2	+		+	+	+	+	+	+	+	+	+
Alkaloids	Anileridine			+	+	+	+	+	+	+	+	+
Furofuranoid lignans	Eudesmin	+		+	+	+	+	+	+	+	+	+
Glycerophospholipids	Unknown lysophosphatidylcholine 1	+		+		+					+	
Glycerophospholipids	Unknown lysophosphatidylcholine 2	+		+	+	+	+	+	+	+	+	
1,3-Aminoalcohols	D-ribo-Phytosphingosine										+	
Linoleic acids	Octadeca-9,12,15-trienoic acid							+	+		+	
N-Acylethanolamines	Linoleoyl Ethanolamide									+	+	
Isoquinoline alkaloids	Thalsimine	+		+	+		+	+	+	+	+	+
Phenylmethylamine	Piptamine	+		+	+	+	+	+	+	+	+	+
N-Acylethanolamines	Oleoyl Ethanolamide										+	
Glycerophosphocholines	(3-hexadecanoyloxy-2-hydroxy-propyl) 2-(trimethylammonio)ethyl phosphate											+
Benzoic acid esters	Dioctylphthalate	+		+	+	+	+	+	+			+
Piperidine alkaloids	2-[5-amino-10-hydroxy-17-[2-(5-hydroxy-3-imidazol-1-yl-6-methyl-2-piperidyl)ethyl]-13-(hydroxymethyl)-7-methyl-4,8-diazatricyclo[17.3.1.1^{3,7}]tetracosa-1(22),19(23),20-trien-10-yl]acetic acid										+	+

+ Compound was detected qualitatively

0 h, 6 h, 14 d and 28 d are fermentation periods in hour (h) and days (d)