

Supplementary materials

Biovalorisation of market surplus bread for development of probiotic-fermented functional beverages

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Table S1. Ingredients of bread variants, information adapted from packaging of bread loafs (Gardenia).

| EWB | FGWB | HCMB |
|---|--|--|
| Wheat flour (unbleached), sucrose, vegetable oil (palm), skimmed milk powder, common salt, dextrose, Baker's yeast, emulsifier, yeast nutrients (ammonium sulphate, sodium chloride, calcium sulphate), thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), iron (hydrogen reduced iron), vitamin C, calcium propionate. | Wholemeal wheat flour with bran and wheat germ, wheat gluten, honey, vegetable oil (palm), skimmed milk powder, Baker's yeast, common salt, dextrose, emulsifiers, yeast nutrients (ammonium sulphate, sodium chloride, calcium sulphate), thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), iron (hydrogen reduced iron), vitamin C, calcium propionate. | Wheat flour (unbleached), sucrose, skimmed milk powder, oat fibre, common salt, dextrose, vegetable oil (palm), calcium carbonate, Baker's yeast, emulsifier, inulin (oligosaccharide), calcium propionate, honey, thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), iron (hydrogen reduced iron), flavouring, lutein, cholecalciferol (vitamin D3) |

EWB: Enriched White Bread; FGWB: Fine Grain Wholemeal Bread; HCMB: Hi Calcium Milk Bread.

Table S2. Nutritional information of bread variants used, information adapted from packaging of bread loafs (Gardenia).

| | EWB | FGWB | HCMB |
|----------------------------|------------|-------------|-------------|
| Energy (kcal/100 g) | 263 | 223 | 252 |
| Protein (g/100 g) | 9.9 | 12.1 | 10.3 |
| Total fat (g/100 g) | 1.9 | 2.7 | 1.5 |
| Saturated fat (g/100 g) | 0.9 | 1.2 | 0.8 |
| <i>Trans</i> fat (g/100 g) | 0.0 | 0.0 | 0.0 |
| Cholesterol (mg/100 g) | 0.0 | 0.0 | 0.0 |
| Carbohydrate (g/100 g) | 54.7 | 38.0 | 53.3 |
| Total sugar | 3.7 | 4.7 | N/A |
| Dietary fibre (g/100 g) | 2.5 | 5.3 | 3.0 |
| Sodium (mg/100 g) | 438 | 274 | 430 |
| Vitamin B1 (mg/100 g) | 0.77 | 0.5 | 0.7 |
| Vitamin B2 (mg/100 g) | 0.48 | 0.3 | 0.4 |
| Vitamin B3 (mg/100 g) | 5.06 | 3.1 | 5.1 |
| Vitamin D3 (µg/100 g) | N/A | N/A | 1.22 |
| Lutein (µg/100 g) | N/A | N/A | 80 |
| Calcium (mg/100 g) | 171.08 | 240.0 | 362 |
| Iron (mg/100 g) | 4.53 | 4.8 | 4.7 |

EWB: Enriched White Bread; FGWB: Fine Grain Wholemeal Bread; HCMB: Hi Calcium Milk Bread.

N/A = Not available (value not declared on packaging).

Table S3. Qualitative observations on bread slurries of different concentrations.



| Total bread solids (%) | 1.25 – 5.0 | 10.0 – 25.0 |
|--------------------------|--|--|
| Qualitative observations | The bread slurries were flowable liquids suitable for beverage applications. Slight sedimentations were observed, the sediments could be dispersed by shaking. | The bread mixtures were solid-like, not flowable, and not suitable for beverage applications. |
| Illustrating images | <div><p>Liquid level</p><p>Sedimentation</p></div> | <div><p>Solid-like Not flowable Not invertible</p></div> |

Table S4. Illustration of phase separation in fermented Enriched White Bread samples without and with additives (3% sweetener, 0.001% stabiliser) after 1 week of incubation at 30 °C.


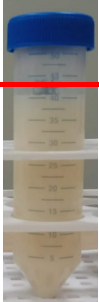
| Without additives | With 3% sweetener + 0.001% stabiliser |
|---|---|
|  |  |

Table S5. Ethanol contents in unfermented bread slurry and fermented bread slurries at beginning and end of shelf life monitoring.

| | Ethanol content (%) | | | |
|----------------|--------------------------|--------------------------|----------------------------------|---|
| | Unfermented bread slurry | <i>L. rhamnosus</i> GG | <i>S. cerevisiae</i> CNCM I-3856 | <i>L. rhamnosus</i> GG + <i>S. cerevisiae</i> CNCM I-3856 |
| Week 0 | 0.09 ± 0.02 ^a | 0.11 ± 0.02 ^a | 0.30 ± 0.02 ^b | 0.25 ± 0.01 ^b |
| Week 6 (5 °C) | - | 0.09 ± 0.01 ^a | 0.24 ± 0.03 ^b | 0.22 ± 0.02 ^b |
| Week 6 (30 °C) | - | 0.10 ± 0.02 ^a | 0.27 ± 0.03 ^c | 0.22 ± 0.02 ^b |

Mean values in the same row with different lowercase letters are significantly different ($p < 0.05$).

Table S6. Selected volatile organic compounds (VOCs) in unfermented and fermented bread slurries at beginning and end of shelf life monitoring.

| Compounds | LRI | GC-MS/FID peak area (× 10 ⁶) | | | | | | | | | |
|------------------------------|------|--|-----------------------------|----------------------------|----------------------------|----------------------------------|-----------------------------|-----------------------------|---|-----------------------------|-----------------------------|
| | | Unfermented bread slurry | <i>L. rhamnosus</i> GG | | | <i>S. cerevisiae</i> CNCM I-3856 | | | <i>L. rhamnosus</i> GG + <i>S. cerevisiae</i> CNCM I-3856 | | |
| | | | Week 0 | Week 6 (5 °C) | Week 6 (30 °C) | Week 0 | Week 6 (5 °C) | Week 6 (30 °C) | Week 0 | Week 6 (5 °C) | Week 6 (30 °C) |
| <i>Acids</i> | | | | | | | | | | | |
| Acetic acid | 1450 | 0.28 ± 0.08 ^a | 6.55 ± 1.82 ^b | 5.90 ± 1.12 ^b | 34.29 ± 12.71 ^c | 2.21 ± 0.25 ^b | 1.69 ± 0.48 ^b | 5.49 ± 1.44 ^b | 4.20 ± 1.70 ^b | 5.48 ± 1.61 ^b | 21.86 ± 5.42 ^c |
| Propionic acid | 1532 | 4.54 ± 0.87 ^a | 60.07 ± 18.87 ^{bc} | 43.43 ± 12.94 ^b | 97.52 ± 13.77 ^c | 4.68 ± 0.52 ^a | 7.61 ± 1.90 ^a | 17.89 ± 7.34 ^a | 71.02 ± 19.00 ^{bc} | 84.38 ± 9.46 ^c | 47.95 ± 10.84 ^b |
| Isobutyric acid | 1561 | 0.28 ± 0.10 ^a | 0.29 ± 0.02 ^a | 0.32 ± 0.10 ^a | 0.19 ± 0.06 ^a | 0.90 ± 0.19 ^b | 0.13 ± 0.02 ^a | 0.14 ± 0.03 ^a | 0.30 ± 0.06 ^a | 0.32 ± 0.04 ^a | 0.85 ± 0.14 ^b |
| Butyric acid | 1622 | ND | 0.09 ± 0.01 ^a | 0.15 ± 0.08 ^a | 0.28 ± 0.07 ^b | ND | ND | ND | 0.07 ± 0.02 ^a | 0.08 ± 0.02 ^a | 0.08 ± 0.02 ^a |
| <i>Alcohols</i> | | | | | | | | | | | |
| Ethanol | - | 54.70 ± 8.33 ^a | 63.26 ± 21.53 ^a | 57.76 ± 7.43 ^a | 61.39 ± 12.00 ^a | 185.76 ± 91.85 ^b | 277.80 ± 36.77 ^b | 270.95 ± 47.42 ^b | 194.84 ± 17.26 ^b | 203.56 ± 23.17 ^b | 176.16 ± 28.56 ^b |
| Isobutyl alcohol | 1099 | 6.79 ± 0.56 ^{ab} | 4.20 ± 1.05 ^a | 2.55 ± 0.60 ^a | 4.54 ± 0.29 ^a | 16.64 ± 4.93 ^{bc} | 18.30 ± 3.01 ^c | 11.42 ± 1.96 ^b | 6.07 ± 1.03 ^{ab} | 5.32 ± 0.78 ^a | 4.47 ± 0.62 ^a |
| Active amyl alcohol | 1261 | 0.39 ± 0.15 ^a | 0.55 ± 0.06 ^a | 0.11 ± 0.02 ^a | 4.81 ± 1.10 ^b | ND | ND | ND | 0.41 ± 0.13 ^a | 0.43 ± 0.08 ^a | 0.43 ± 0.11 ^a |
| 2-Ethyl-1-hexanol | 1504 | 0.14 ± 0.02 ^a | 0.33 ± 0.13 ^b | 0.22 ± 0.02 ^{ab} | 0.10 ± 0.01 ^a | ND | ND | ND | 0.16 ± 0.03 ^a | 0.14 ± 0.03 ^a | 0.22 ± 0.03 ^{ab} |
| Furfuryl alcohol | 1674 | ND | ND | ND | ND | 0.15 ± 0.02 ^b | 0.07 ± 0.02 ^a | 0.06 ± 0.00 ^a | ND | ND | ND |
| 2-Phenethyl alcohol | 1944 | ND | ND | ND | ND | 6.42 ± 2.77 ^a | 13.36 ± 3.13 ^b | 14.32 ± 4.67 ^b | ND | ND | 19.75 ± 4.35 ^b |
| <i>Ketones and Aldehydes</i> | | | | | | | | | | | |
| Diacetyl | - | 8.83 ± 0.35 ^a | 13.12 ± 4.78 ^a | 12.04 ± 0.68 ^a | 12.67 ± 0.97 ^a | ND | ND | ND | ND | ND | ND |
| Hexanal | 1076 | 2.80 ± 0.89 ^a | 18.33 ± 4.14 ^b | 15.37 ± 5.42 ^b | 21.39 ± 6.58 ^b | ND | ND | ND | 15.76 ± 4.41 ^b | 17.36 ± 3.74 ^b | 22.03 ± 5.44 ^b |
| 2-Heptanone | 1178 | 0.82 ± 0.20 ^a | 0.97 ± 0.34 ^a | 0.80 ± 0.39 ^a | 1.02 ± 0.20 ^a | 0.82 ± 0.22 ^b | 0.47 ± 0.09 ^a | 0.48 ± 0.06 ^a | 0.76 ± 0.32 ^a | 1.24 ± 0.35 ^a | 0.97 ± 0.31 ^a |
| 2-Octanone | 1278 | ND | 0.07 ± 0.03 ^a | 0.11 ± 0.02 ^a | 0.08 ± 0.02 ^a | 0.26 ± 0.07 ^b | 0.13 ± 0.03 ^a | 0.11 ± 0.01 ^a | 0.04 ± 0.01 ^a | 0.04 ± 0.01 ^a | 0.03 ± 0.01 ^a |
| Acetoin | 1291 | 3.14 ± 0.39 ^b | 4.56 ± 1.58 ^b | 3.11 ± 0.13 ^b | 3.49 ± 0.69 ^b | 0.35 ± 0.10 ^a | 0.31 ± 0.10 ^a | 0.37 ± 0.08 ^a | 1.74 ± 0.35 ^{ab} | 1.71 ± 0.34 ^{ab} | 1.96 ± 0.32 ^{ab} |
| 2-Octenal | 1428 | 0.36 ± 0.10 ^b | 0.23 ± 0.08 ^{ab} | 0.44 ± 0.26 ^{ab} | 0.30 ± 0.06 ^b | ND | ND | ND | 0.24 ± 0.03 ^{ab} | 0.20 ± 0.04 ^a | 0.34 ± 0.07 ^b |
| Furfural | 1471 | 0.11 ± 0.03 ^b | 0.08 ± 0.01 ^b | 0.08 ± 0.02 ^b | 0.04 ± 0.01 ^a | ND | ND | ND | 0.08 ± 0.00 ^b | 0.10 ± 0.01 ^b | 0.08 ± 0.03 ^b |
| Butyrolactone | 1644 | ND | ND | ND | ND | 0.05 ± 0.01 ^a | 0.34 ± 0.03 ^b | 0.40 ± 0.06 ^b | ND | ND | ND |
| <i>Esters</i> | | | | | | | | | | | |
| Ethyl heptanoate | 1319 | ND | ND | ND | ND | ND | 0.05 ± 0.02 ^a | 0.06 ± 0.03 ^a | ND | ND | ND |
| Ethyl octanoate | 1425 | 0.37 ± 0.05 ^{ab} | 0.40 ± 0.16 ^{ab} | 0.33 ± 0.12 ^{ab} | 0.81 ± 0.26 ^c | 0.66 ± 0.21 ^{bc} | 0.48 ± 0.08 ^b | 0.35 ± 0.13 ^{ab} | 0.43 ± 0.06 ^{ab} | 0.37 ± 0.03 ^{ab} | 0.24 ± 0.05 ^a |

Results expressed as mean \pm standard deviations from independent experiments ($n = 3$). LRI: experimental linear retention index determined on a DB-FFAP column relative to C10-C40 alkane standard. ND = Not detected. Lowercase letters indicate significant differences ($p < 0.05$) in the same row.