*Article*

# Evolutionary wheat populations in high-quality breadmaking as a tool to preserve agro-food biodiversity

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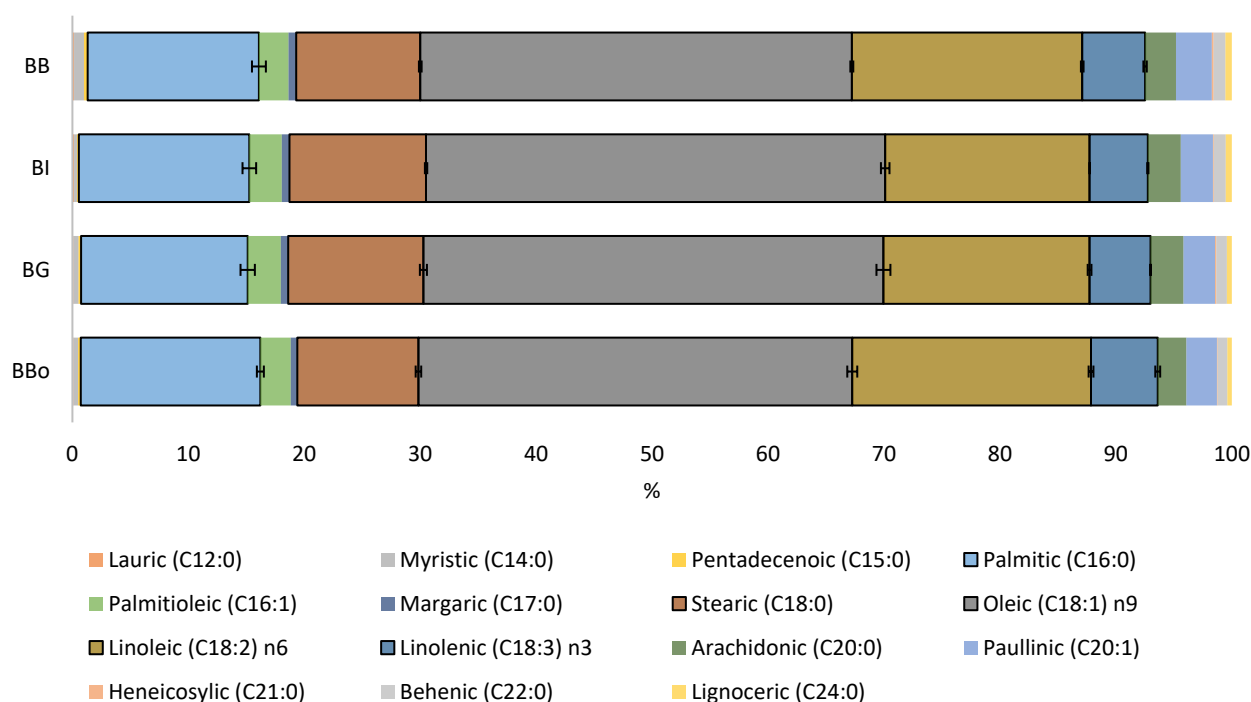verna@unipr.it (G.G.).

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*Figure S1* Fatty acids (FAs) profile of the different breads. Results are reported as cumulative percentage (%) of FAs. BB, bread produced using BIO2 EP; BI, bread produced using ICARDA EP; BG, bread produced using Grossi EP; BBo, bread produced using cv. Bologna.

**Table S1** Mass spectrometry characteristics of nicotinamide, nicotinic acid, thiamine, and folic acid.

| Compound | CID | Monitored ion | Monitored transitions * |
|---|---|---|---|
| | | $[M+H]^+$ | *(m/z)* |
| Nicotinamide | 40 | 123 | 123 → **80**; 123 → 95; 123 → 105; 123 → 122 |
| Nicotinic acid | 37 | 124 | 124 → **80**; 124 → 123; 124 → 105 |
| Thiamine | 22 | 265 | 265 → **122**; 265 → 144 |
| Folic acid | 17 | 442 | 442 → **295**; 442 → 313 |

CID: Collision Induced Dissociation. * The quantifier ion (*m/z*) is reported in bold.

**Table S2** Standard deviation (n=3) of the grain quality parameters of EPs and cv. Bologna.

| Wheat | Test weight (kg/hL) | Thousand kernel weight (g) | Protein content (% d.m.) | Alveograph | | | |
|---|---|---|---|---|---|---|---|
| | | | | W ($10^{-4}$J) | P (mm $H_2O$) | L (mm) | P/L |
| Bio2 EP | 1 | 0 | 1.28 | 13.50 | 1.5 | 9 | 0.07 |
| ICARDA EP | 3 | 0 | 1.72 | 8.50 | 2 | 11.5 | 0.06 |
| Grossi EP | 2 | 1 | 0.53 | 8.50 | 1 | 16.5 | 0.02 |
| Bologna | 0 | 1 | 0.40 | 4.00 | 1.5 | 0.5 | 0.05 |

EP, evolutionary wheat population.

**Table S3** Standard deviation (n=3) of the nutritional and chemical composition of the bread formulated using the wheat evolutionary population (BB, BI and BG) and bread produced using flour from cv. Bologna wheat (BBo).

| | BB | BI | BG | BBo |
|---|---|---|---|---|
| Carbohydrates (g/100 g) | 1.2 | 1.1 | 1.3 | 1.4 |
| Total dietary fibre (g/100g) | 0.94 | 0.89 | 0.95 | 1.03 |
| Lipids (g/100g) | 0.01 | 0.03 | 0.03 | 0.07 |
| SFA (%) | 0.4 | 0.1 | 0.8 | 0.6 |
| MUFA (%) | 0.2 | 0.2 | 0.6 | 0.6 |
| PUFA (%) | 0.3 | 0.1 | 0.2 | 0.1 |
| Ω-6 / Ω-9 | 0.1 | 0.1 | 0.0 | 0.2 |
| Proteins (g/100g) | 1.3 | 1.7 | 1.1 | 1.6 |
| Mg (mg/100g) | 2.5 | 2.2 | 2.1 | 3.2 |
| Zn (mg/100g) | 0.17 | 0.15 | 0.16 | 0.16 |
| Fe (mg/100g) | 0.21 | 0.13 | 0.17 | 0.21 |
| Se (μg/100g) | 0.20 | 0.20 | 0.22 | 0.22 |
| Thiamine (mg/100g) | 0.01 | 0.01 | 0.01 | 0.12 |

| | | | | |
|---|---|---|---|---|
| Nicotinic acid (mg/100g) | <LOQ | <LOQ | <LOQ | <LOQ |
| Folic acid (μg /100g) | <LOQ | <LOQ | <LOQ | <LOQ |
| Nicotinamide (mg/100g) | 0.01 | 0.11 | 0.01 | 0.10 |

<LOQ Folic acid: 5 μg/100 g; <LOQ Nicotinic acid: 0.01 mg/100 mg, Mg, magnesium; Zn, zinc; Fe, iron; Se, selenium; NAM, nicotinamide; BB, bread produced using BIO2 EP; BI, bread produced using ICARDA EP; BG, bread produced using Grossi EP; BBo, bread produced using cv. Bologna. *: Calories (kJ and kcal) were calculated as sum of nutritive components.

**Table S4** Standard deviation (n=3) of the micronutrients content in flours

| | FB | FI | FG | FBo |
|---|---|---|---|---|
| Mg (mg/100g) * | 2.9 | 2.6 | 2.9 | 4.4 |
| Zn (mg/100g) * | 0.23 | 0.19 | 0.22 | 0.24 |
| Fe (mg/100g) * | 0.27 | 0.13 | 0.27 | 0.28 |
| Se (μg /100g) ** | 0.68 | 0.62 | 0.85 | 0.99 |
| Thiamine (mg/100g) * | 0.01 | 0.00 | 0.08 | 0.03 |
| Nicotinic acid (mg/100g) * | <LOQ | <LOQ | <LOQ | <LOQ |
| Nicotinamide (mg/100g) * | 0.03 | <LOQ | 0.08 | 0.01 |
| Folic acid (μg /100g) ** | 4.4 | <LOQ | <LOQ | <LOQ |

* <LOQ, 0.01 mg/100g; ** <LOQ, 0.5 μg/100 g. FB, BIO2 EP Type 1 flour; FI, ICARDA EP Type 1 flour; FG, Grossi EP Type 1 flour; FBo, cv. Bologna Type 1 flour.

**Table S5** Standard deviation (n=3) of the total phenolic content (TPC) and phenolic acid (PA) profile in their free (soluble) and bound (insoluble) forms.
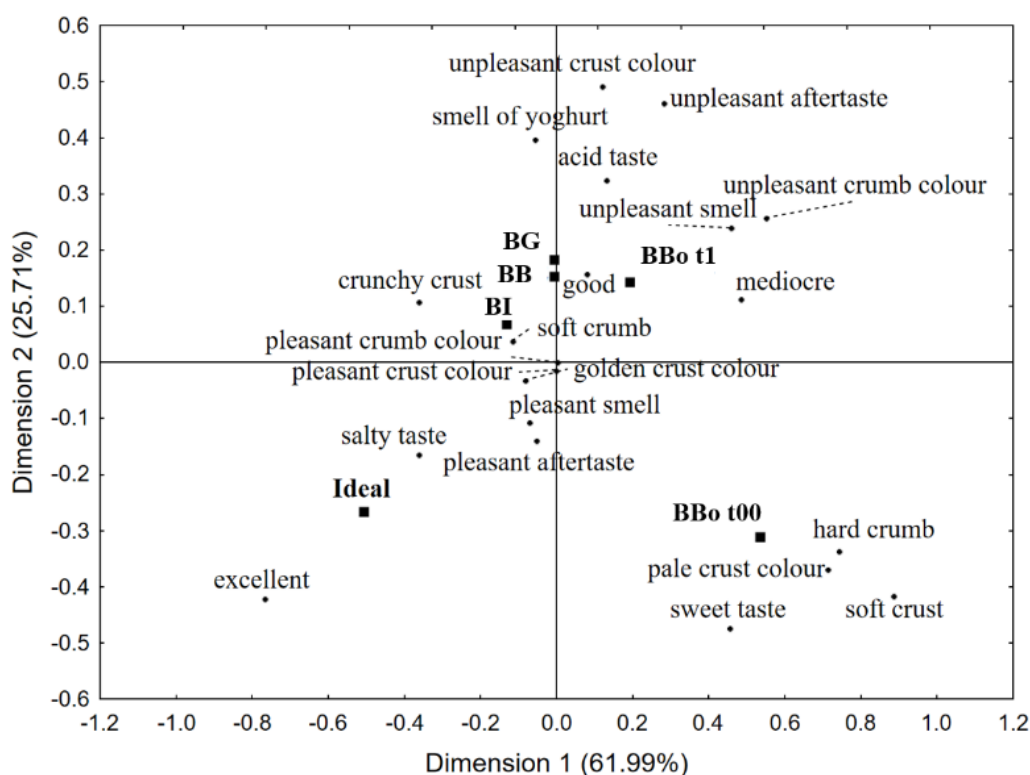
| Sample | TPC | | 4-HB | | p-C | | Caff | | t-Fer | | c-Fer | Sin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Free | Bound | Free | Bound | Free | Bound | Free | Bound | Free | Bound | | Free | Bound |
| Flours | mg GAE/Kg d. m. | | | | | | mg/Kg d.w. | | | | | | |
| FB | 23.98 | 92.15 | 0.01 | <LOQ | <LOQ | 0.01 | 0.0 | 0.03 | 0.06 | 0.12 | 0.08 | 0.04 | 0.11 |
| FI | 13.83 | 38.67 | 0.01 | <LOQ | 0.01 | 0.01 | 0.01 | 0.01 | 0.05 | 0.15 | 0.02 | 0.01 | 0.03 |
| FG | 12.73 | 64.37 | <LOQ | <LOQ | 0.01 | 0.01 | 0.02 | 0.01 | 0.13 | 0.16 | 0.15 | 0.03 | 0.07 |
| FBo | 9.60 | 41.65 | <LOQ | <LOQ | <LOQ | 0.03 | 0.02 | 0.01 | 0.14 | 0.20 | 0.01 | 0.01 | 0.1 |
| Breads | | | | | | | | | | | | | |
| BB | 35.43 | 33.41 | <LOQ | 0.04 | <LOQ | 0.01 | <LOQ | 0.04 | 0.3 | 0.25 | 4.11 | <LOQ | 0.15 |
| BI | 13.86 | 3.06 | <LOQ | 0.1 | <LOQ | 0.11 | <LOQ | 0.01 | 0.21 | 3.00 | 8.23 | 0.11 | 0.07 |
| BG | 8.07 | 4.05 | <LOQ | 0.1 | 0.04 | 0.07 | <LOQ | 0.01 | 0.34 | 1.46 | 1.79 | 0.04 | 0.47 |
| BBo | 0.36 | 16.55 | 0.04 | 0.15 | 0.01 | 0.51 | <LOQ | 0.15 | 0.20 | 9.55 | 2.49 | <LOQ | 1.19 |

<LOQ: 0.05 mg/kg. GAE, Gallic Acid Equivalents; d. m., dry matter; 4-HB, hydroxybenzoic acid; p-C, para coumaric acid; caff, caffeic acid; *t*-fer, *trans*-ferulic acid; *c*-fer, *cis*-ferulic acid; Sin, sinapic acid. FB, BIO2 EP Type 1 flour; FI, ICARDA EP Type 1 flour; FG, Grossi EP Type 1 flour; FBo, cv. Bologna Type 1 flour; BB, bread produced using BIO2 EP; BI, bread produced using ICARDA EP; BG, bread produced using Grossi EP; BBo, bread produced using cv. Bologna.

**Table S6** Standard deviation (n=59) of the sensory scores of breads obtained from acceptability test.

| Bread | Texture | | Colour | | Appearance | Aroma | Taste | Overall acceptability |
|---|---|---|---|---|---|---|---|---|
| | Crust | Crumb | Crust | Crumb | | | | |
| **BI** | 1.54 | 1.36 | 1.58 | 1.39 | 1.14 | 1.30 | 1.50 | 1.25 |
| **BB** | 1.52 | 1.10 | 1.23 | 1.19 | 1.19 | 1.24 | 1.39 | 1.03 |
| **BG** | 1.52 | 1.29 | 1.50 | 1.09 | 1.13 | 1.17 | 1.48 | 1.23 |
| **BBo** | 1.75 | 1.55 | 1.46 | 1.89 | 1.59 | 1.39 | 1.66 | 1.41 |

BB, bread produced using BIO2 EP; BI, bread produced using ICARDA EP; BG, bread produced using Grossi EP; BBo, bread produced using cv Bologna.



**Figure S2** Correspondence analysis of the bread samples and sensory attributes including a Bologna type 00 control bread. BB, bread produced using Bio2 EP; BI, bread produced using ICARDA EP; BG, bread produced using Grossi EP; BBo t00, bread produced using cv. Bologna flour type 00; BBo t1, bread produced using cv. Bologna flour type 1.