foods

# Supplementary materials

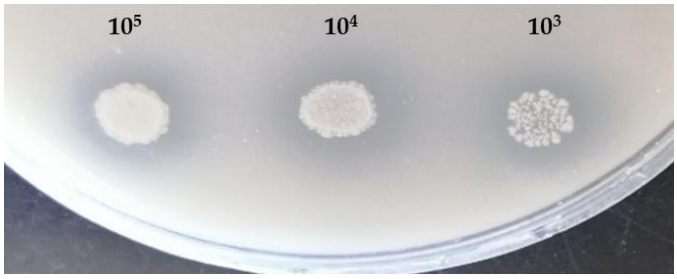


**Figure S1.** ***W. anomalus*** **ME1 strain´s ability to hydrolyze wheat gliadin proteins** on solid medium SM with glucose (2% w/v) and ammonium sulfate (5% w/v) as carbon and nitrogen sources, respectively, and gliadins (0.5% v/v).

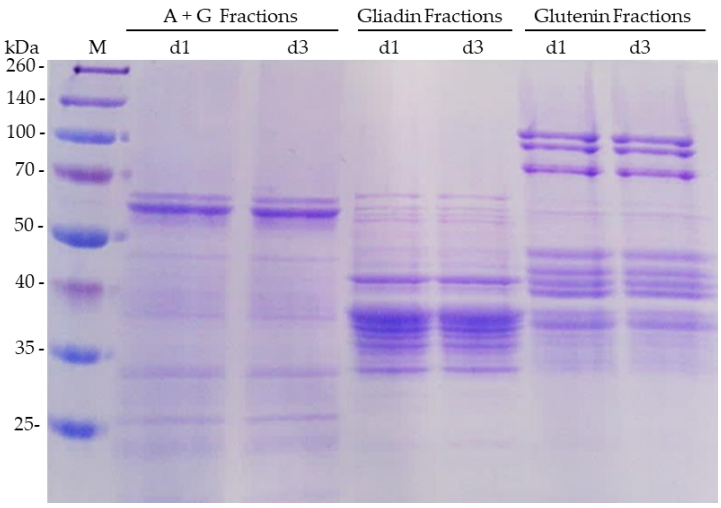


**Figure S2. Validation of the method used to obtain gliadin proteins.** The procedure described in Section 2.2 was applied to 3 samples of V-25 bakery wheat flour. One flour sample was processed per day. The protein fractions obtained on days 1 (d1) and 3 (d3) were resolved by SDS-PAGE (8 μL) and the protein profiles were compared. The gliadin protein fraction extracted with ethanol/water (60/40 v/v) was obtained after subtracting from the flour samples the albumin and globulin proteins (A+G fraction) with a salt solution. Glutenin proteins

extracted from the gliadin-free sediment with propanol-urea-DTT are also shown. M, protein mixture (25-260 kDa) used as the molecular weight marker.

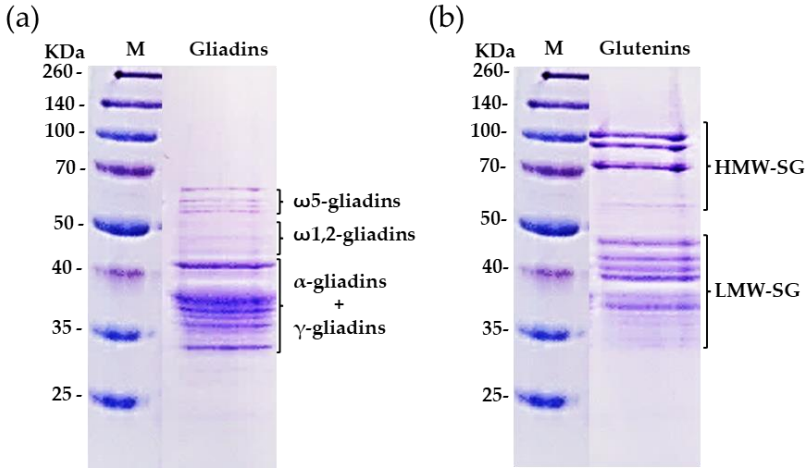


**Figure S3. Gluten proteins of V-25 wheat flour.** The bands belonging to the different types of gliadin (**a**) and glutenin (**b**) proteins were inferred from electrophoretic mobility pattern and molecular weights ($M_r$), according to those described for wheat flour samples in other works [3]. $M_r$ ranges: ω5-gliadins 60 -68 kDa; ω1,2-gliadins 43-60 kDa; α- and γ-gliadins 32-45 Da; HMW-GS (high-molecular-weight-glutenin subunits) 80-120 kDa; LMW-GS (low-molecular-weight-glutenin subunits) 32-45 kDa. M, protein mixture used as $M_r$ marker.

**Table S1. Quantification of gliadin proteins in wheat flour fermentation** from the bands identified by polyclonal antigliadin antibodies.

| Sample | Fermentation time (hours) | Intensity of all bands (arbitrary units) per experiment (Exp) | | | Gliadin hydrolysis percentage per experiment (Exp) | | | Hydrolysis percentage average | Standard deviation (±) |
|---|---|---|---|---|---|---|---|---|---|
| | | Exp 1 | Exp 2 | Exp 3 | Exp 1 | Exp 2 | Exp 3 | | |
| Sc-fermented dough | 0 | 39354795.0 | 37254873.0 | 38456213.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Sc-fermented dough | 24 | 39651672.0 | 38131462.0 | 38651321.0 | 0.8 | 2.4 | 0.5 | 1.2 | 1.0 |
| Sc-fermented dough | 48 | 38873034.0 | 37913032.0 | 38073045.0 | 1.0 | 1.8 | 1.0 | 1.3 | 0.4 |
| Wa-fermented dough | 0 | 38959146.0 | 38052346.0 | 38501571.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Wa-fermented dough | 24 | 19748473.0 | 19026173,0 | 18250785.5 | 49.3 | 50.0 | 52.6 | 50.6 | 1.7 |
| Wa-fermented dough | 48 | 19535562.0 | 19615581.0 | 19050643.0 | 49.9 | 48.5 | 50.5 | 49.6 | 1.1 |

Sc, Hércules baker's yeast; Wa, the *Wickerhamomyces anomalus* yeast strain ME1

**Table S2. Quantification of gliadin proteins in bread making** from the bands identified by the monoclonal anti-α-gliadin antibody [14D5] (ab367299).

| Sample | Fermentation time (hours) | Intensity of all bands (arbitrary units) per experiment (Exp) | | | Gliadin hydrolysis percentage per experiment (Exp) | | | Hydrolysis percentage average | Standard deviation (±) |
|---|---|---|---|---|---|---|---|---|---|
| | | Exp 1 | Exp 2 | Exp 3 | Exp 1 | Exp 2 | Exp 3 | | |
| Uninoculated dough | 24 | 2593188.0 | 2194236.0 | 1994760.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Bread control | | 1366794.0 | 1739556.0 | 1242540.0 | 37.7 | 20.7 | 47.3 | 35.2 | 13.5 |
| Sc-fermented dough | 0 | 2776971.2 | 2598633.6 | 2547680.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Sc-fermented dough | 24 | 2710476.4 | 2710476.4 | 2683640.0 | 5.3 | 4.3 | 2.4 | 4.0 | 1.5 |
| Sc-made bread | | 1275716.0 | 1177584.0 | 981320.0 | 61.4 | 54.7 | 54.1 | 56.7 | 4.1 |
| Wa-fermented dough | 0 | 2655180.0 | 2413800.0 | 2413800,0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Wa-fermented dough | 24 | 1368640.0 | 1263360.0 | 1052800.0 | 56.3 | 47.7 | 48.5 | 50.8 | 4.8 |
| Wa-made bread | | 640360.0 | 548880.0 | 457400.0 | 81.0 | 77.23 | 75.8 | 78.1 | 2.7 |

Sc, Hércules baker's yeast; Wa, the *Wickerhamomyces anomalus* yeast strain ME1