

**Table S1.** List and description of biga and Pinsa Romana used in this study.

BIGA				PINSA ROMANA	
Code	Conditions	N	Type of process	Code	
<b>B_24</b>	Ingredients: flour (wheat, rice, and soy), water, baker's yeast. Fermentation, 24 h at 16 °C.	<b>1</b>	Indirect	<b>PR_24</b>	Ingredients: B_24, water, baker's yeast, olive oil, and salt. Leavened for 2 h at 24 °C; 1.5 h processing and resting.
<b>B_48<sub>(1)</sub></b>	Ingredients: flour (wheat, rice, and soy), water, baker's yeast. Fermentation, 48 h at 16 °C.	<b>2</b>	Indirect	<b>PR_48</b>	Ingredients: B_48 <sub>(1)</sub> , water, baker's yeast, olive oil, and salt. Leavened for 2 h at 24 °C; 1.5 h processing and resting.
<b>B_48<sub>(2)</sub></b>	Ingredients: flour (wheat, rice, and soy), water, baker's yeast. Fermentation, 48 h at 16 °C.	<b>3</b>	Indirect	<b>PR_48+SD</b>	Ingredients: B_48 <sub>(2)</sub> , water, sourdough, baker's yeast, olive oil and salt. Leavened for 2 h at 24 °C; 1.5 h processing and resting.
<b>B_48(SD)</b>	Ingredients: flour (wheat, rice, and soy), water, sourdough, baker's yeast. Fermentation, 48 h at 16 °C.	<b>4</b>	Indirect	<b>PR_48(SD)</b>	Ingredients: B_48(SD), water, baker's yeast, olive oil, and salt. Leavened for 2 h at 24 °C; 1.5 h processing and resting.
<b>B_72</b>	Ingredients: flour (wheat, rice, and soy), water, baker's yeast. Fermentation, 72 h at 16 °C.	<b>5</b>	Indirect	<b>PR_72</b>	Ingredients: B_72, water, baker's yeast, olive oil, and salt. Leavened for 2 h at 24 °C; 1.5 h processing and resting.
<b>-</b>	<b>-</b>	<b>6</b>	Direct	<b>PR_RT</b>	Ingredients: flour (wheat, rice and soy), water, baker's yeast, olive oil and salt. Leavened for 2 h at 24 °C; 1.5 h processing and resting.

B\_48<sub>(1)</sub> and B\_48<sub>(2)</sub> biga have the same fermentation conditions and kind of raw ingredients, while they differ only for the percentage (weight/final weight) of each raw ingredient (see Table 1).

**Table S2.** Characterization of biga and sourdough.

	pH	LAB (Log CFU/g)	Yeast (Log CFU/g)
B_24	5.48 ± 0.04	6.40 ± 0.21	7.72 ± 0.14
B_48 <sub>(1)</sub>	5.41 ± 0.02	6.35 ± 0.17	8.00 ± 0.02
B_48 <sub>(2)</sub>	5.42 ± 0.01	6.20 ± 0.25	7.93 ± 0.02
B_48(SD)	3.94 ± 0.01	8.89 ± 0.13	7.22 ± 0.17
B_72	5.55 ± 0.04	7.91 ± 0.06	7.04 ± 0.02
CT_D	5.42 ± 0.02	< 5	8.04 ± 0.04
SD	3.78 ± 0.03	8.06 ± 0.01	7.33 ± 0.13

The data are the means of three independent analysis ± standard deviations (n =3). SD, sourdough; B\_24, biga fermented for 24 h with baker's yeast; B\_48<sub>(1)</sub>, biga fermented 48 h with baker's yeast; B\_48<sub>(2)</sub>, biga fermented 48 h with baker's yeast; B\_48(SD), biga fermented for 48 h with baker's yeast and sourdough; B\_72, biga fermented for 72 h with baker's yeast; CT\_D, control dough made without biga and sourdough.

**Table S3.** Free amino acids profile of Pinsa Romana before (mg/kg) and after (mg/L) digestion.

	Pinsa Romana					
	PR_24	PR_48	PR_48(SD)	PR_48+SD	PR_72	PR_RT
Asp	14.35 ± 0.21 <sup>d</sup>	13.43 ± 0.20 <sup>d</sup>	95.45 ± 1.41 <sup>a</sup>	39.09 ± 0.58 <sup>c</sup>	57.23 ± 0.84 <sup>b</sup>	13.82 ± 0.20 <sup>d</sup>
Thr	4.76 ± 0.13 <sup>c</sup>	4.16 ± 0.11 <sup>d</sup>	4.71 ± 0.13 <sup>c</sup>	4.35 ± 0.12 <sup>cd</sup>	7.87 ± 0.21 <sup>b</sup>	10.11 ± 0.27 <sup>a</sup>
Ser	3.12 ± 0.08 <sup>d</sup>	2.06 ± 0.05 <sup>e</sup>	12.56 ± 0.31 <sup>a</sup>	3.79 ± 0.09 <sup>c</sup>	3.98 ± 0.10 <sup>c</sup>	7.66 ± 0.19 <sup>b</sup>
Glu	18.93 ± 0.64 <sup>d</sup>	13.32 ± 0.45 <sup>e</sup>	69.43 ± 2.34 <sup>a</sup>	31.05 ± 1.05 <sup>b</sup>	24.55 ± 0.83 <sup>c</sup>	19.83 ± 0.67 <sup>d</sup>
Gly	12.86 ± 0.29 <sup>b</sup>	10.46 ± 0.24 <sup>c</sup>	1.10 ± 0.03 <sup>d</sup>	12.40 ± 0.28 <sup>b</sup>	22.80 ± 0.52 <sup>a</sup>	22.83 ± 0.52 <sup>a</sup>
Ala	10.95 ± 0.24 <sup>f</sup>	13.6 ± 0.30 <sup>e</sup>	19.56 ± 0.43 <sup>c</sup>	16.07 ± 0.35 <sup>d</sup>	27.95 ± 0.62 <sup>b</sup>	31.41 ± 0.69 <sup>a</sup>
Val	11.03 ± 0.30 <sup>de</sup>	10.04 ± 0.27 <sup>e</sup>	21.85 ± 0.59 <sup>a</sup>	11.95 ± 0.32 <sup>d</sup>	19.20 ± 0.52 <sup>b</sup>	14.79 ± 0.40 <sup>c</sup>
Cys	24.36 ± 0.99 <sup>a</sup>	20.30 ± 0.83 <sup>bc</sup>	21.27 ± 0.87 <sup>bc</sup>	19.00 ± 0.77 <sup>c</sup>	21.49 ± 0.88 <sup>b</sup>	25.01 ± 1.02 <sup>a</sup>
Met	4.46 ± 0.15 <sup>d</sup>	4.02 ± 0.14 <sup>d</sup>	8.80 ± 0.30 <sup>a</sup>	5.58 ± 0.19 <sup>c</sup>	6.24 ± 0.21 <sup>b</sup>	2.47 ± 0.08 <sup>e</sup>
Ile	10.92 ± 0.31 <sup>d</sup>	10.34 ± 0.30 <sup>d</sup>	17.80 ± 0.51 <sup>a</sup>	12.54 ± 0.36 <sup>c</sup>	14.48 ± 0.42 <sup>b</sup>	11.02 ± 0.32 <sup>d</sup>
Leu	2.38 ± 0.08 <sup>d</sup>	1.86 ± 0.06 <sup>d</sup>	37.28 ± 1.28 <sup>a</sup>	5.94 ± 0.20 <sup>c</sup>	12.83 ± 0.44 <sup>b</sup>	2.41 ± 0.08 <sup>d</sup>
Tyr	1.67 ± 0.07 <sup>e</sup>	0.90 ± 0.04 <sup>e</sup>	21.72 ± 0.87 <sup>a</sup>	10.53 ± 0.42 <sup>c</sup>	19.18 ± 0.77 <sup>b</sup>	5.31 ± 0.21 <sup>d</sup>
Phe	1.50 ± 0.06 <sup>d</sup>	1.39 ± 0.06 <sup>d</sup>	28.25 ± 1.13 <sup>a</sup>	6.13 ± 0.25 <sup>c</sup>	14.40 ± 0.58 <sup>b</sup>	2.72 ± 0.11 <sup>d</sup>
GABA	7.53 ± 0.11 <sup>e</sup>	4.58 ± 0.07 <sup>f</sup>	66.92 ± 0.97 <sup>a</sup>	45.65 ± 0.66 <sup>c</sup>	59.67 ± 0.87 <sup>b</sup>	32.87 ± 0.48 <sup>d</sup>
Orn	2.53 ± 0.08 <sup>c</sup>	3.46 ± 0.11 <sup>b</sup>	3.93 ± 0.13 <sup>a</sup>	3.92 ± 0.13 <sup>a</sup>	3.49 ± 0.12 <sup>b</sup>	0.53 ± 0.02 <sup>d</sup>
Lys	7.33 ± 0.18 <sup>c</sup>	4.25 ± 0.11 <sup>e</sup>	5.01 ± 0.13 <sup>d</sup>	4.49 ± 0.11 <sup>de</sup>	10.02 ± 0.25 <sup>b</sup>	12.34 ± 0.31 <sup>a</sup>

**Table S3. Cont.**

	PR_24	PR_48	PR_48(SD)	PR_48+SD	PR_72	PR_RT
His	1.15 ± 0.03 <sup>c</sup>	1.64 ± 0.05 <sup>b</sup>	1.22 ± 0.04 <sup>c</sup>	1.19 ± 0.04 <sup>c</sup>	2.05 ± 0.06 <sup>a</sup>	1.58 ± 0.05 <sup>b</sup>
Trp	0.29 ± 0.01 <sup>d</sup>	0.02 ± 0.01 <sup>d</sup>	26.88 ± 1.02 <sup>a</sup>	10.59 ± 0.40 <sup>c</sup>	20.56 ± 0.78 <sup>b</sup>	20.22 ± 0.76 <sup>b</sup>
Arg	35.69 ± 0.88 <sup>d</sup>	23.03 ± 0.57 <sup>e</sup>	72.71 ± 1.79 <sup>a</sup>	41.47 ± 1.02 <sup>c</sup>	45.38 ± 1.12 <sup>b</sup>	34.33 ± 0.84 <sup>d</sup>
Pro	14.39 ± 0.73 <sup>d</sup>	18.33 ± 0.93 <sup>c</sup>	20.68 ± 1.05 <sup>c</sup>	25.78 ± 1.31 <sup>b</sup>	32.20 ± 1.64 <sup>a</sup>	24.04 ± 1.22 <sup>b</sup>
Total	190.22 ± 5.16 <sup>d</sup>	161.19 ± 4.35 <sup>e</sup>	557.14 ± 14.72 <sup>a</sup>	311.49 ± 8.16 <sup>c</sup>	425.56 ± 11.29 <sup>b</sup>	295.31 ± 8.03 <sup>c</sup>
Digested (D) Pinsa Romana						
	D-PR_24	D-PR_48	D-PR_48(SD)	D-PR_48+SD	D-PR_72	D-PR_RT
Asp	12.06 ± 3.14 <sup>b</sup>	11.94 ± 1.04 <sup>b</sup>	22.56 ± 0.89 <sup>a</sup>	22.48 ± 0.66 <sup>a</sup>	20.68 ± 2.25 <sup>a</sup>	14.29 ± 2.11 <sup>b</sup>
Thr	32.32 ± 3.13 <sup>c</sup>	32.29 ± 4.64 <sup>c</sup>	56.41 ± 0.90 <sup>a</sup>	51.77 ± 3.95 <sup>ab</sup>	47.14 ± 3.00 <sup>b</sup>	36.49 ± 2.22 <sup>c</sup>
Ser	40.29 ± 1.63 <sup>d</sup>	38.38 ± 4.87 <sup>d</sup>	76.15 ± 0.61 <sup>a</sup>	64.69 ± 7.58 <sup>ab</sup>	59.37 ± 4.54 <sup>bc</sup>	47.10 ± 4.59 <sup>cd</sup>
Glu	46.02 ± 4.91 <sup>d</sup>	47.79 ± 6.29 <sup>cd</sup>	97.97 ± 0.99 <sup>a</sup>	78.01 ± 5.59 <sup>b</sup>	74.41 ± 2.77 <sup>b</sup>	59.30 ± 4.73 <sup>c</sup>
Gly	15.31 ± 0.38 <sup>c</sup>	15.58 ± 2.12 <sup>c</sup>	27.81 ± 0.33 <sup>a</sup>	24.84 ± 2.89 <sup>a</sup>	22.94 ± 1.74 <sup>ab</sup>	18.40 ± 1.63 <sup>bc</sup>
Ala	44.12 ± 4.00 <sup>c</sup>	46.64 ± 5.96 <sup>c</sup>	72.72 ± 3.30 <sup>a</sup>	70.19 ± 6.89 <sup>a</sup>	61.27 ± 3.73 <sup>ab</sup>	48.93 ± 2.50 <sup>bc</sup>
Val	58.25 ± 4.47 <sup>c</sup>	61.91 ± 7.06 <sup>c</sup>	98.88 ± 4.74 <sup>a</sup>	93.85 ± 7.67 <sup>ab</sup>	83.70 ± 3.45 <sup>b</sup>	64.12 ± 3.74 <sup>c</sup>
Cys	16.86 ± 0.49 <sup>c</sup>	19.20 ± 3.74 <sup>bc</sup>	33.94 ± 2.15 <sup>a</sup>	35.04 ± 1.30 <sup>a</sup>	33.17 ± 0.38 <sup>a</sup>	23.51 ± 2.97 <sup>b</sup>
Met	20.70 ± 2.81 <sup>c</sup>	23.78 ± 3.14 <sup>bc</sup>	34.23 ± 3.13 <sup>a</sup>	34.96 ± 1.02 <sup>a</sup>	29.88 ± 0.49 <sup>ab</sup>	22.05 ± 0.95 <sup>c</sup>

**Table S3. Cont.**

	D-PR_24	D-PR_48	D-PR_48(SD)	D-PR_48+SD	D-PR_72	D-PR_RT
Ile	53.02 ± 3.24 <sup>c</sup>	54.20 ± 6.89 <sup>c</sup>	87.40 ± 3.51 <sup>a</sup>	81.57 ± 7.22 <sup>ab</sup>	72.89 ± 2.33 <sup>b</sup>	57.61 ± 2.38 <sup>c</sup>
Leu	105.81 ± 9.30 <sup>c</sup>	116.11 ± 16.98 <sup>bc</sup>	158.83 ± 7.84 <sup>a</sup>	155.39 ± 12.14 <sup>a</sup>	137.75 ± 1.38 <sup>ab</sup>	111.67 ± 0.01 <sup>bc</sup>
Tyr	67.14 ± 6.32 <sup>c</sup>	76.50 ± 10.40 <sup>bc</sup>	107.24 ± 6.75 <sup>a</sup>	104.90 ± 5.77 <sup>a</sup>	92.52 ± 0.69 <sup>ab</sup>	72.59 ± 0.03 <sup>c</sup>
Phe	70.86 ± 9.15 <sup>c</sup>	78.96 ± 10.90 <sup>bc</sup>	109.01 ± 6.54 <sup>a</sup>	109.95 ± 4.00 <sup>a</sup>	96.69 ± 0.01 <sup>ab</sup>	74.39 ± 0.63 <sup>c</sup>
GABA	1.63 ± 0.71 <sup>a</sup>	2.25 ± 0.30 <sup>a</sup>	3.06 ± 0.71 <sup>a</sup>	2.26 ± 1.45 <sup>a</sup>	2.78 ± 0.01 <sup>a</sup>	1.08 ± 0.30 <sup>a</sup>
Orn	0.69 ± 0.04 <sup>c</sup>	1.57 ± 0.33 <sup>ab</sup>	2.19 ± 0.03 <sup>a</sup>	2.00 ± 0.25 <sup>ab</sup>	1.39 ± 0.42 <sup>b</sup>	1.52 ± 0.12 <sup>ab</sup>
Lys	49.28 ± 4.94 <sup>d</sup>	50.75 ± 5.88 <sup>d</sup>	77.37 ± 4.84 <sup>a</sup>	75.07 ± 4.26 <sup>ab</sup>	64.49 ± 0.42 <sup>bc</sup>	53.17 ± 1.55 <sup>cd</sup>
His	25.18 ± 2.06 <sup>c</sup>	26.44 ± 3.27 <sup>c</sup>	42.61 ± 2.09 <sup>a</sup>	39.48 ± 2.30 <sup>a</sup>	33.75 ± 0.81 <sup>b</sup>	27.68 ± 0.86 <sup>c</sup>
Trp	26.79 ± 3.00 <sup>c</sup>	27.07 ± 3.28 <sup>c</sup>	41.59 ± 2.37 <sup>a</sup>	40.51 ± 2.42 <sup>ab</sup>	35.19 ± 0.44 <sup>b</sup>	27.01 ± 0.80 <sup>c</sup>
Arg	72.52 ± 8.88 <sup>c</sup>	81.11 ± 9.47 <sup>bc</sup>	114.04 ± 7.43 <sup>a</sup>	112.21 ± 4.07 <sup>a</sup>	92.06 ± 1.05 <sup>b</sup>	75.45 ± 0.12 <sup>bc</sup>
Pro	4.53 ± 0.13 <sup>d</sup>	4.42 ± 0.29 <sup>d</sup>	11.31 ± 0.97 <sup>a</sup>	6.71 ± 0.33 <sup>b</sup>	6.07 ± 0.47 <sup>bc</sup>	4.95 ± 0.43 <sup>cd</sup>
Total	763.38 ± 71.96 <sup>c</sup>	816.89 ± 110.84 <sup>c</sup>	1275.30 ± 51.92 <sup>a</sup>	1205.88 ± 81.57 <sup>a</sup>	1068.14 ± 26.98 <sup>b</sup>	841.31 ± 32.30 <sup>c</sup>

Values in the same row with different superscript letters differ significantly ( $p < 0.05$ ) based on one-way ANOVA (Tuckey-Kramer).

The data are the means of three independent analysis ± standard deviations (n =3). PR\_24, Pinsa Romana made with biga fermented for 24 h with baker's yeast; PR\_48, Pinsa Romana made with biga fermented for 48 h with baker's yeast; PR\_72, Pinsa Romana made with biga fermented for 72 h with baker's yeast; PR\_48(SD), Pinsa Romana made with biga fermented for 48 h with baker's yeast and

sourdough; PR\_48+SD, Pinsa Romana made with biga fermented for 48 h with baker's yeast, and including sourdough in the final dough. PR\_RT, Pinsa Romana made without biga and sourdough (reference thesis).