Effects of high-molecular-weight glutenin subunit on hard-steamed bread quality

**Jing Zhao1**†**,2**, **Tianyi Wang1**†**,2**, **Hui Chen1,2**, **Jiajia Zhao1**, **Ling Qiao 1**, **Bangbang Wu1**, **Yuqiong Hao1**, **Chuan Ge1**, **Juanling Wang2**, **Zhiwei Feng2\***, **Xianghai Meng3\***

1 Institute of Wheat Research, Key Laboratory of Sustainable Dryland Agriculture (Co-construction by Ministry and Province) Ministry of Agriculture and Rural Affairs Shanxi Agricultural University, Linfen, China

2 College of Agriculture, Shanxi Agricultural University, Taigu, China

3 Dryland Farming Institute, Hebei Academy of Agriculture and Forestry Sciences

**\* Correspondence:**

Zhiwei Feng and Xianghai Meng

1. mail address: zhiweifeng@126.com; mengxianghai5229@163.com

|  |
| --- |
| **Supplemental Table 1** Effect of Glu-1 locus on quality traits of steamed bread |
| |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | |  | Loci | **Glu-A1** | | **Glu-B1** | | **Glu-D1** | | |  | Treatment | I1 | I3 | I1 | I3 | I1 | I3 | |  | DOF | 1 | 1 | 2 | 2 | 3 | 3 | | Volume index | Volume(cm3) | 1.99 | 1.45 | 1.29 | 4.82 | 24.50\*\* | 33.78\*\*\* | | Specific volume | 2.2 | 24.29\*\*\* | 21.55\*\* | 22.30\*\* | 2.97 | 15.45\* | | Texture index | Adhesion (mJ) | 1.88 | 3.41 | 58.56\*\*\* | 25.48\*\*\* | 25.48\*\*\* | 46.57\*\*\* | | Cohesiveness (Ratio) | 0.33 | 0.93 | 4.17 | 11.45\* | 4.17 | 11.45\* | | Elasticity (mm) | 1.04 | 0.13 | 43.36\*\*\* | 25.70\*\*\* | 18.38\*\* | 6.55 | | Glueyness (N) | 1.21 | 10.93 | 21.07\*\*\* | 10.96\* | 10.96\* | 27.58\*\*\* | | Chewiness (mJ) | 1.96 | 14.16 | 13.15\*\* | 11.15\* | 11.15\* | 47.92\*\*\* | | Hardness (N) | 0.78 | 9.39 | 7.71 | 36.99\*\*\* | 10.65 | 55.19\*\*\* | | Sensory score | Specific volume | 42.91\*\*\* | 2.52 | 22.46\*\*\* | 2.79 | 16.34\*\*\* | 48.27\*\*\* | | Aspect ratio | 0.48 | 0.54 | 98.76\*\*\* | 41.11\*\*\* | 13.72\*\* | 3.12 | | Surface color | 0.21 | 0.08 | 1.17 | 6.46\* | 0.67 | 6.11 | | Surface structure | 0.37 | 2.36 | 19.93\*\*\* | 33.68\*\*\* | 15.43\*\*\* | 3.61 | | Internal structure | 0.59 | 1.02 | 7.44\* | 9.98\*\* | 0.79 | 5.06 | | Elasticity | 0.28 | 0.79 | 17.78\*\*\* | 2.28 | 3.87 | 3.23 | | Toughness | 0.07 | 1.78 | 12.42\*\*\* | 3.04 | 3.57 | 4.27 | | Stickiness | 0.28 | 1.84 | 7.29\* | 0.24 | 4.46 | 3.27 | | Total points | 3.53\* | 1.11 | 34.50\*\*\* | 25.10\*\*\* | 9.17\* | 6.11 | |
| \*, \*\*, and \*\*\* are significant at 0.05, 0.01, and 0.001, respectively. I1: regime once at overwintering stage, I3: irrigation three times at overwintering, jointing, and filling stages. |

|  |
| --- |
| **Supplemental Table 2** Sensory evaluation of steamed bread with different subunit locus variation types |
| |  |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | **Traits** | **Scoring criteria:** | **Treatment** | **Reference sample scores** | **Subunits** | | | | | | | | | **Glu-A1** | | **Glu-B1** | | | **Glu-D1** | | | | | 1 | N | 6+8 | 7+8 | 7+9 | 2+10 | 2+12 | 5+10 | 5+12 | | **Specific volume /(mL/g) (20 score)** | A specific volume greater than or equal to 2.8 is worth a full score of 20; a specific volume less than or equal to 1.8 will be awarded a minimum score of 5 points; the specific volume is between 2.8~1.8, and 1.5 points will be deducted for every 0.1 decrease | I1 | 9.19 A | 6.77 B | 8.05 A | 8.67 A | 6.77 B | 6.80 B | 6.50 B | 7.90 A | 8.11 A | | I3 | 6.73 | 7.08 | 7.26 | 6.77 | 6.95 | 8.50 A | 7.60 B | 5.80 D | 6.78 C | | **Aspect ratio (5 score)** | A maximum score of 5 points is awarded if the aspect ratio is less than or equal to 1.40; a minimum score of 0 is awarded for a score greater than 1.60; between 1.40~1.60, 1 point will be deducted for every 0.05 increase | I1 | 3.42 | 3.72 | 0.00 C | 4.60 B | 4.90 A | 5.00 A | 3.31 B | 4.90 A | 3.11 B | | I3 | 3.35 | 3.65 | 1.46 C | 3.67 B | 4.52 A | 4.20 | 3.39 | 4.10 | 3.33 | | **Elasticity (10 score)** | Good elasticity of finger pressing: 8 ~ 10 points; weak rebound of finger pressing: 6 ~ 7 points; finger compression does not rebound or compresses are difficult: 4 ~ 5 points | I1 | 7.06 | 7.17 | 7.34 | 7.04 | 7.08 | 7.21 | 7.17 | 6.94 | 7.17 | | I3 | 7.40 | 7.32 | 7.17 b | 7.89 a | 7.16 b | 7.26 | 7.09 | 6.90 | 7.64 | | **Surface color (10 score)** | Good glossiness 8 ~ 10 points; slightly darker 6 ~ 7 minutes; gray 4 ~ 5 points | I1 | 7.76 | 7.63 | 7.02 C | 7.45 B | 8.11 A | 8.35 A | 7.04 C | 8.04 AB | 7.75 B | | I3 | 7.29 | 7.67 | 6.44 C | 8.24 A | 7.78 B | 8.00 | 7.28 | 7.33 | 7.67 | | **Surface structure (10 score)** | Smooth surface: 8 ~ 10 points; wrinkled, collapsed, bubbles or burns: 4 ~ 7 points | I1 | 15.18 | 14.76 | 13.70 b | 15.32 a | 15.28 a | 15.33 | 14.64 | 14.67 | 15.03 | | I3 | 14.51 | 15.12 | 13.35 | 15.16 | 15.54 | 16.67 | 14.16 | 14.78 | 15.03 | | **Internal structure (20 score)** | Stomatas fine and uniform :18 ~ 20 points; the stomatas are delicate and basically uniform, with individual bubbles :13 ~ 17 points, the edge and the epidermis are separated, and 1 point is deducted; the stomatas are basically uniform but have one of the following conditions :10 ~ 12 points, too fine, there are slightly more bubbles, the stomatas are uniform but the structure is slightly rough; uneven porosity or very rough structure :5 ~ 9 points | I1 | 7.65 | 7.76 | 7.31 B | 7.33 B | 8.12 A | 8.20 | 7.73 | 7.90 | 7.58 | | I3 | 7.48 | 7.71 | 7.35 | 7.69 | 7.79 | 8.27 | 7.61 | 7.35 | 7.63 | | **Toughness (10 score)** | Strong bite, 8 ~ 10 points; the bite is average; 6 ~ 7 points; poor bite strength, slag off when cutting or chewing dry; 4 ~ 5 points | I1 | 7.91 | 7.84 | 7.29 B | 7.68 B | 8.24 A | 8.32 | 7.66 | 8.17 | 7.79 | | I3 | 8.00 | 7.75 | 7.70 | 7.66 | 7.98 | 7.56 | 7.77 | 8.21 | 7.79 | | **Viscosity (10 score)** | Refreshing and not sticky teeth 8 ~ 10 points; slightly sticky; 6 ~ 7 points chewing is not refreshing, very sticky 4 ~ 5 points | I1 | 7.42 | 7.54 | 7.12 b | 7.34 ab | 7.77 a | 7.94 | 7.46 | 7.84 | 7.34 | | I3 | 7.83 | 7.59 | 7.65 | 7.75 | 7.67 | 7.43 | 7.50 | 7.91 | 7.72 | | **Tasty (5 score)** | The inherent aroma of normal wheat, 5 points; bland taste 4 points, peculiar smell, 2 ~ 3 points | I1 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | I3 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | **Total score (100 score)** | | RF | I1 | 68.19 b | 62.83 B | 70.43 A | 71.28 A | 72.15 a | 66.50 b | 71.35 a | 68.88 ab | | TW | I3 | 68.90 | 63.37 B | 69.83 A | 70.39 A | 72.89 | 67.39 | 67.37 | 68.59 | |
| Lowercase letters indicate P<0.05 and uppercase letters indicate P<0.01. I1: regime once at overwintering stage, I3: irrigation three times at overwintering, jointing, and filling stages. |

|  |
| --- |
| **Supplemental Table 3** Comparison of steamed bread quality in different HMW-GS |
| |  |  |  |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | **Traits** | **Treatment** | (N,7+8,5+12) | (1,7+8,5+12) | (N,7+8,2+12) | (N,6+8,2+12) | (N,6+8,5+12) | (1,6+8,5+12) | (N,7+9,2+12) | (N,7+9,2+10) | (N,7+9,5+12) | (1,7+9,5+12) | (N,7+9,5+10) | (1,7+9,5+10) | | **Volume** | I1 | 172.4 E | 185.40 BCD | 197.40 A | 189.80 BC | 183.00 CD | 187.20 BCD | 168.00 E | 189.80 BC | 181.80 D | 182.80 CD | 189.60 BC | 192.00 AB | | I3 | 176.6 F | 198.00 B | 190.80 C | 166.40 G | 180.40 EF | 197.60 B | 190.20 C | 203.40 A | 184.40 DE | 187.60 CD | 184.80 DE | 167.20 G | | **Aspect ratio** | I1 | 1.38 CD | 1.33 E | 1.53 A | 1.44 B | 1.45 B | 1.43 B | 1.41 BC | 1.34 DE | 1.33 E | 1.42 BC | 1.41 BC | 1.34 E | | I3 | 1.36 F | 1.53 A | 1.43 BCD | 1.39 CDEF | 1.50 A | 1.43 BC | 1.42 BCD | 1.44 B | 1.38 DEF | 1.37 EF | 1.41 BCDE | 1.53 A | | **Specific volume** | I1 | 1.77 FG | 1.94 ABC | 2.00 A | 1.98 A | 1.94 ABC | 1.97 AB | 1.76 G | 1.88 CDE | 1.83 EF | 1.85 DE | 1.91 BCD | 1.91 BCD | | I3 | 1.82 D | 2.20 A | 1.95 B | 1.86 D | 1.94 B | 2.16 A | 1.91 BC | 1.91 BC | 1.85 DE | 1.87 CD | 1.85 DE | 1.83 DE | | **Hardness (N)** | I1 | 281.90 BCD | 303.97 AB | 292.21 ABC | 270.87 BCD | 259.75 CD | 265.53 BCD | 265.82 BCD | 306.80 AB | 329.89 A | 303.60 AB | 282.07 BCD | 242.53 D | | I3 | 266.26 DE | 264.34 DE | 259.69 DE | 261.92 DE | 238.01 FG | 225.52 G | 271.15 D | 251.11 D | 295.06 EF | 314.93 B | 318.04 B | 399.14 A | | **Adhesion (mJ)** | I1 | 0.09 F | 0.10 F | 0.10 EF | 0.11 DEF | 0.12 CDE | 0.13 CD | 0.21 A | 0.22 A | 0.20 A | 0.14 C | 0.13 C | 0.17 B | | I3 | 0.13 CDE | 0.14 BCD | 0.15 BC | 0.15 B | 0.12 EFG | 0.11 FG | 0.18 A | 0.11 FG | 0.10 G | 0.12 DEF | 0.15 B | 0.13 DE | | **Cohesiveness (Ratio)** | I1 | 0.47 | 0.54 | 0.54 | 0.54 | 0.54 | 0.54 | 0.66 | 0.53 | 0.54 | 0.52 | 0.53 | 0.53 | | I3 | 0.54 B | 0.53 B | 0.53 B | 0.53 B | 0.61 A | 0.63 A | 0.55 B | 0.52 B | 0.52 B | 0.51 B | 0.51 B | 0.52 B | | **Elasticity(mm)** | I1 | 6.66 A | 6.54 AB | 6.56 AB | 6.56 AB | 6.54 AB | 6.50 ABC | 5.66 F | 6.23 DE | 6.30 CDE | 6.37 BCD | 6.11 E | 6.19 DE | | I3 | 6.47 A | 6.45 A | 6.44 A | 6.42 AB | 5.87 F | 6.02 E | 5.80 F | 6.45 A | 6.18 CD | 6.36 AB | 6.30 BC | 6.06 DE | | **Glueyness(N)** | I1 | 133.57 | 163.53 | 158.89 | 148.56 | 146.08 | 144.88 | 137.57 | 148.57 | 138.05 | 117.81 | 116.73 | 98.58 | | I3 | 144.47 B | 142.22 B | 139.57 BC | 141.37 B | 147.55 B | 144.53 B | 114.69 DE | 100.78 E | 117.87 D | 124.53 D | 126.58 CD | 162.72 A | | **Chewiness (mJ)** | I1 | 884.44 | 1058.18 | 1028.91 | 964.13 | 944.47 | 928.38 | 994.24 | 1181.00 | 1113.72 | 958.55 | 910.91 | 780.01 | | I3 | 923.75 CD | 905.78 CDE | 889.11 CDE | 896.28 CDE | 855.70 CDE | 847.12 CDE | 843.18 DE | 827.33 E | 929.73 BC | 1010.31 B | 1012.24 B | 1253.97 A | | **Number of cells** | I1 | 4567.40 CD | 5145.2 A | 3894.4 G | 3947.2 G | 4299.8 EF | 3990.4 G | 4534.60 CDE | 4447.40 DEF | 4898.40 B | 4380.00 DEF | 4243.00 F | 4731.20 BC | | I3 | 4390.4 D | 3174.40 G | 4430.2 D | 4049.00 EF | 3804.80 F | 4047.800 EF | 4299.80 DE | 4705.60 BC | 4872.00 AB | 5111.60 A | 4510.20 CD | 3812.40 F | | **Number of holes** | I1 | 11.49 A | 10.94 A | 3.41 EF | 4.51 DE | 7.02 BC | 5.81 CD | 8.51 B | 2.44 EF | 3.97 DEF | 3.75 DEF | 1.78 F | 4.28 DE | | I3 | 8.14 A | 1.91 E | 5.22 BCD | 5.33 BC | 3.13 DE | 2.41 E | 2.29 E | 3.53 CDE | 7.08 AB | 6.96 AB | 3.40 CDE | 7.08 AB | | **Area of cells** | I1 | 1.52 a | 1.21 ab | 1.15 abc | 1.44 a | 1.33 ab | 1.20 ab | 0.91 abc | 0.55 bc | 0.61 bc | 0.57 bc | 0.40 c | 0.79 abc | | I3 | 1.93 | 1.14 | 0.83 | 1.18 | 0.97 | 1.66 | 0.69 | 0.48 | 0.82 | 0.82 | 0.51 | 1.74 | | **Cell diameter** | I1 | 0.88 E | 0.86 E | 1.08 A | 1.11 A | 0.97 BC | 1.01 B | 0.89 DE | 1.00 B | 0.92 CDE | 0.96 BC | 1.01 B | 0.95 BCD | | I3 | 1.03 CD | 1.35 A | 1.01 DE | 0.97 EF | 1.12 B | 1.08 BC | 1.00 DE | 0.93 FG | 0.88 G | 0.89 G | 0.94 FG | 0.99 DEF | | **Cell volume** | I1 | 27.88 ABC | 24.28 BCD | 23.90 BCDE | 33.96 A | 29.12 AB | 26.16 ABC | 20.00 CDEF | 15.46 EF | 16.08 DEF | 15.00 F | 12.44 F | 17.04 DEF | | I3 | 33.48 A | 28.68 AB | 18.92 BC | 23.00 ABC | 22.58 BC | 18.64 BC | 22.76 BC | 15.50 C | 21.38 BC | 18.70 BC | 14.80 C | 27.16 AB | | **Wall tickness** | I1 | 0.34 CD | 0.32 G | 0.37 A | 0.37 A | 0.35 B | 0.35 B | 0.32 EFG | 0.33 CDE | 0.32 FG | 0.34 CD | 0.34 BC | 0.33 DEF | | I3 | 0.35 C | 0.40 A | 0.35 C | 0.34 DE | 0.36 B | 0.35 C | 0.34 CD | 0.33 EF | 0.32 F | 0.33 F | 0.34 DE | 0.35 C | |
| The lowercase letter after the peer average indicates P<0.05, and the uppercase letter indicates P<0.01. I1: regime once at overwintering stage, I3: irrigation three times at overwintering, jointing, and filling stages. |

|  |
| --- |
| **Supplemental Table 4** Importance ranking and significance test results of dough rheological properties contribution rate |
| |  |  |  |  |  | | --- | --- | --- | --- | --- | | **Dough rheological properties** | **Importance ranking** | **Relative contribution rate** | **F** | **P** | | X1 | 1 | 50.7 | 5.4 | 0.01 | | X2 | 2 | 24.1 | 2.8 | 0.076 | | X4 | 3 | 7 | 0.8 | 0.408 | | X3 | 4 | 5 | 0.5 | 0.57 | | X8 | 5 | 4.2 | 0.5 | 0.608 | | X6 | 6 | 3.9 | 0.4 | 0.628 | | X5 | 7 | 3.7 | 0.4 | 0.724 | | X7 | 8 | 1.5 | 0.1 | 0.908 | |
| X1-X7 represents protein content, wet gluten content, dough development time, stability time, Zeleny sedimentation value, stretch area, tractility and maximum resistance. |

|  |
| --- |
| **Supplemental Table 5** Dough rheological properties of different subunit variation types |
| |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | **Traits** | **Treatment** | **Glu-A1** | | **Glu-B1** | | | **Glu-D1** | | | | | 1 | Null | 6+8 | 7+8 | 7+9 | 2+10 | 2+12 | 5+10 | 5+12 | | **Test weight (g/L)** | I1 | 725.25±9.84 | 726.50±5.02 | 718.67±6.19 C | 732.33±3.27 A | 726.67±4.77 B | 721.00±0.00 | 724.00±4.05 | 728.00±3.65 | 727.33±8.69 | | I3 | 752.50±25.05 | 759.25±28.11 | 780.33±8.14 A | 784.67±4.63 A | 731.50±5.92 B | 733.00±1.41 b | 767.33±32.04 a | 730.50±4.04 b | 764.67±23.34 a | | **Protein content (%)** | I1 | 12.87±0.44 | 13.00±0.63 | 13.41±0.49 | 12.86±0.50 | 12.78±0.54 | 12.59±0.06 B | 13.63±0.47 A | 12.66±0.60 B | 12.79±0.43 B | | I3 | 13.58±1.95 | 12.83±1.60 | 15.18±0.92 A | 14.08±0.06 B | 11.53±0.47 C | 11.06±0.04 b | 13.55±1.26 a | 11.50±0.67 ab | 13.71±1.77 a | | **Wet gluten content (%)** | I1 | 39.18±3.01 | 41.29±2.18 | 39.87±2.80 A | 41.31±2.32 A | 36.69±1.88 B | 34.60±0.92 C | 40.79±1.78 A | 36.35±1.18 BC | 39.00±3.01 AB | | I3 | 41.05±3.52 | 40.59±2.78 | 41.16±3.80 A | 40.33±1.91 A | 35.06±3.81 B | 34.37±2.48 | 38.92±4.44 | 36.03±6.14 | 38.60±3.84 | | **Dough development time (min)** | I1 | 2.85±0.24 | 2.63±0.23 | 2.58±0.22 b | 2.83±0.23 ab | 2.96±0.36 a | 3.20±0.36 a | 2.77±0.35 b | 3.03±0.27 ab | 2.73±0.28 b | | I3 | 3.00±0.24 | 2.78±0.21 | 2.73±0.24 | 2.97±0.18 | 2.83±0.36 | 3.13±0.32 a | 2.74±0.24 b | 3.10±0.29 a | 2.76±0.27 b | | **Dough stability time (min)** | I1 | 3.37±0.71 | 3.02±0.60 | 2.59±0.20 B | 3.68±0.39 A | 3.62±0.63 A | 4.37±0.49 A | 3.02±0.64 C | 3.72±0.54 AB | 3.27±0.57 BC | | I3 | 3.65±0.52 a | 2.92±0.54 b | 2.76±0.41 B | 3.57±0.55 A | 3.61±0.78 A | 4.63±0.49 A | 2.97±0.56 C | 4.00±0.60 B | 3.18±0.53 C | | **Stretch area (cm2)** | I1 | 50.13±12.57 | 43.13±9.89 | 37.42±7.96 C | 53.50±7.31 B | 59.25±8.03 A | 71.50±2.89 A | 42.33±11.15 C | 58.50±3.63 B | 52.13±9.65 B | | I3 | 54.25±11.95 a | 44.06±10.43 b | 38.33±6.12 C | 56.58±8.38 A | 49.75±8.34 B | 63.50±5.74 A | 43.58±10.52 B | 48.75±4.89 B | 48.58±9.72 B | | **Maximum resistance (EU)** | I1 | 244.13±19.01 | 224.94±27.10 | 226.75±27.86 B | 235.92±24.26 B | 284.96±38.15 A | 336.25±12.01 A | 215.92±19.56 D | 295.13±25.83 B | 253.92±27.19 C | | I3 | 240.88±21.18 A | 215.56±19.63 B | 209.50±15.05 b | 238.50±20.89 a | 246.13±44.87 a | 328.75±16.96 A | 214.58±24.91 C | 251.63±19.45 B | 224.17±19.77 C | | **Tractility (mm)** | I1 | 140.25±22.44 | 136.94±21.82 | 120.42±8.96 C | 155.67±14.72 A | 138.75±9.10 B | 143.50±11.82 | 139.67±22.16 | 132.15±6.20 | 139.00±16.28 | | I3 | 156.75±16.93 a | 139.31±16.02 b | 131.00±11.90 B | 159.25±10.14 A | 136.04±14.44 B | 126.00±6.68 B | 139.08±13.16 AB | 128.50±9.34 B | 147.79±17.84 A | | **Zeleny sedimentation value /mL** | I1 | 24.21±1.38 | 24.94±1.73 | 23.54±0.62 C | 25.84±1.48 A | 24.37±0.88 B | 23.40±1.15 | 25.36±1.95 | 24.78±0.61 | 24.22±0.83 | | I3 | 25.82±1.48 | 24.10±2.07 | 22.96±1.31 B | 26.39±0.63 A | 21.13±0.88 C | 22.43±0.25 | 22.70±2.62 | 21.02±0.15 | 23.71±2.51 | |
| The lowercase letter after the peer average indicates P<0.05, and the uppercase letter indicates P<0.01. I1: regime once at overwintering stage, I3: irrigation three times at overwintering, jointing, and filling stages. |