

Title

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

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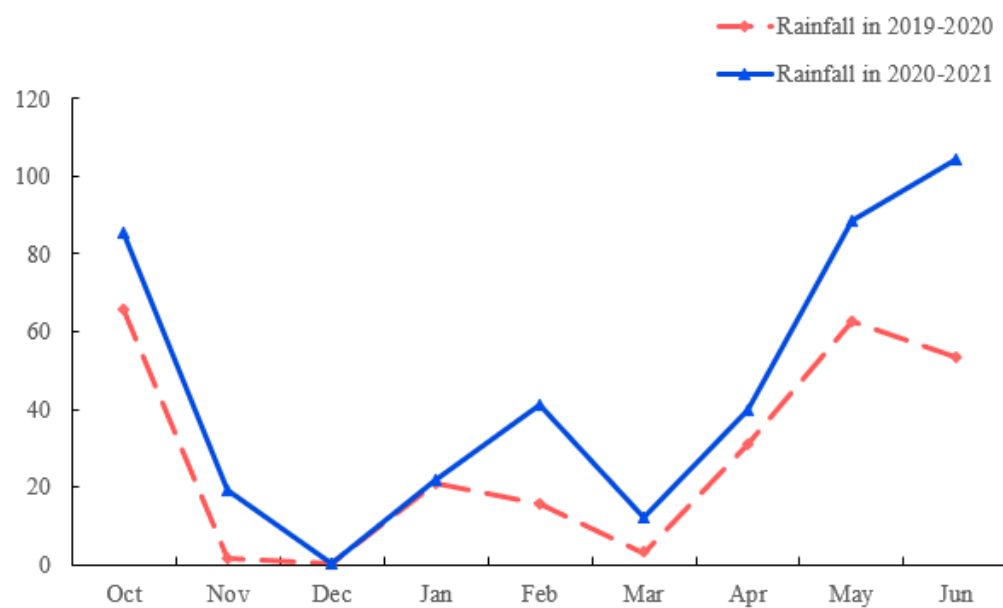
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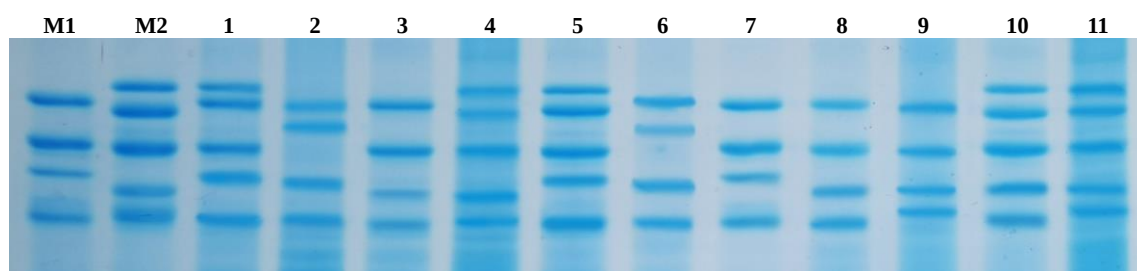
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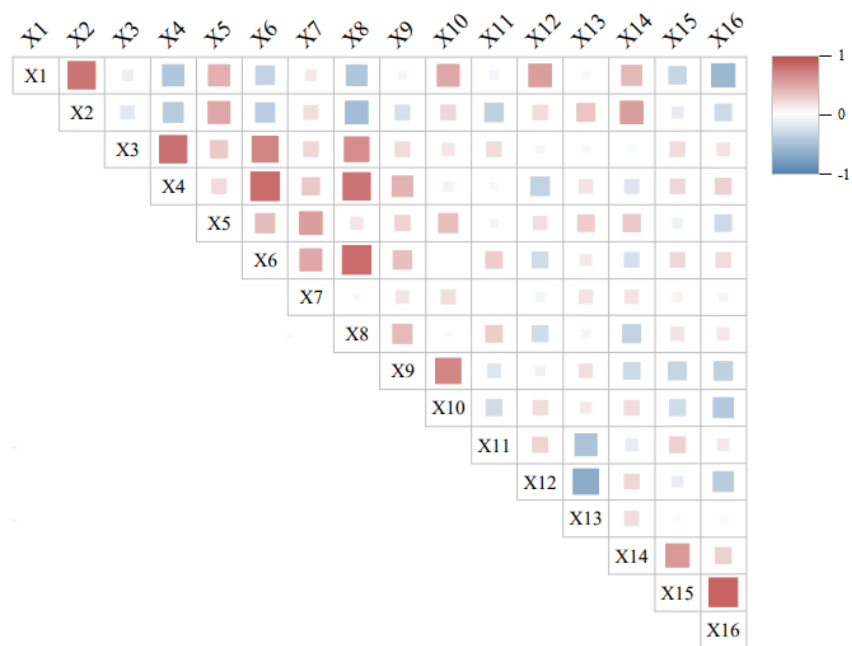


S1 Fig Precipitation during the growth period in 2019-2020 and 2020-2021



S2 Fig SDS-PAGE of HMW-GS in DHs

M1: Chinese Spring (N, 7+8, 2+12); M2: Shiluan02-1 (1,7+9,5+10); 1: Linfen5064 (1, 7+8, 2+12); 2: Nongda3338 (N, 6+8 5+12); 3: Jinmai47 (N, 7+9, 2+12); 4: Jinmai84 (1, 7+9, 5+10); 5~10: partial lines of DHs



S3 Fig Correlation between steamed bread quality traits and rheological properties of dough

X1-X16 represents protein content, wet gluten content, dough development time, stability time, Zeleny sedimentation value, stretch area, tractility, maximum resistance, volume, specific volume, adhesion, cohesiveness, elasticity, glueyness, chewiness and hardness, respectively.

S1 Table Occurrence of HMW-GSs in the DHs

	Glu-A1	Glu-B1	Glu-D1	Number of DHs	Ratio (%)
Linfen5064×Nongda3338	1	7+8	5+12	28	13.46
	1	7+8	2+12	11	5.42
	1	6+8	5+12	24	11.82
	1	6+8	2+12	9	4.43
	N	7+8	5+12	22	10.84
	N	7+8	2+12	65	32.02
	N	6+8	2+12	23	11.33
	N	6+8	5+12	21	10.34
Jinmai47×Jinmai84	1	7+9	5+10	50	18.59
	1	7+9	5+12	49	18.22
	1	7+9	2+10	10	3.72
	1	7+9	2+12	13	4.83
	N	7+9	2+10	25	9.29
	N	7+9	2+12	60	22.30
	N	7+9	5+10	31	11.52
	N	7+9	5+12	31	11.52

S2 Table Effect of Glu-1 locus on quality traits of steamed bread

	Loci	Glu-A1		Glu-B1		Glu-D1	
		I1	I3	I1	I3	I1	I3
	Treatment	I1	I3	I1	I3	I1	I3
	DOF	1	1	2	2	3	3
Volume index	Volume(cm ³)	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***
	Specific volume	42.91***	2.52	22.46***	2.79	16.34***	48.27***
Sensory score	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.

	taste 4 points, peculiar smell, 2 ~ 3 points	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.

S4 Table 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 B	1.74 A
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.

S5 Table 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.

S6 Table 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 (cm ²)	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 A	23.54±0.62 C	25.84±1.48 A	24.37±0.88 B	23.40±1.15	25.36±1.95 a	24.78±0.61 A	24.22±0.83
	I3	25.82±1.48	24.10±2.07 A	22.96±1.31 B	26.39±0.63 A	21.13±0.88 C	22.43±0.25	22.70±2.62 a	21.02±0.15 A	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.