A new testing method for vital gluten swelling index

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Abstract: Vital gluten is a kind of functional protein, but currently its quality indices are mostly focused on the outer character rather than the inner content. The objective of this study was to develop an improved testing method for the measurement of vital gluten, reported here as the vital gluten swelling index (VGSI). The procedures were optimized using the vital gluten water absorption ratio and the wheat flour protein fraction content. The following procedure is recommended: use 0.9 mL distilled water to hydrate 20 mg vital gluten for 10 min, and then add 0.9 mL of $0.03 \, g \, kg^{-1}$ sodium dodecyl sulfate-lactic acid solution and mix for another 30 min. Finally, centrifuge the mixture at 5000 × g and measure the residue weight. The quality of vital gluten can be simply classified by its swelling index value. A VGSI of $\geq 28\%$ indicates vital gluten of superior grade, between 24% and 28% is a good grade, while $\leq 24\%$ is an inferior grade. Compared with the laborious method of vital gluten water absorption (VGWA), which takes a relatively long time to test one sample, VGSI requires only 47 min to test 24 samples. Further, the VGSI method is more accurate, reliable and repeatable. The coefficient of variation of VGWA of the vital gluten samples is 2.09, while the corresponding value for VGSI was 14.83. The correlation coefficients between VGSI and other vital gluten indices were significant. The research indicates that the method developed is the best to evaluate vital gluten.

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Keywords: vital gluten; vital gluten swelling index; water absorption; insoluble glutenin

INTRODUCTION

Vital wheat gluten has the ability to be highly elastic when water is added. This sets it apart from all other available vegetable proteins. Wheat gluten rapidly absorbs about twice its weight of water. This results in increased yield, dough strength, and extended shelf-life or 'vitality'; hence the name 'vital gluten of wheat'. Vital gluten consists of more than 75% protein and the quality is determined by the protein components. Gliadin and glutenin are the only components that can significantly improve vital gluten processing quality. In these proteins, the former, has low molecular weight and good extensibility, while the latter has high molecular weight and strong elasticity.¹ Vital gluten is the dry matter of the wet gluten. Therefore, the wet gluten content is a crucial material quality control index for the vital gluten industry. However, the higher wet gluten content does not mean a higher vital gluten quality because of their different protein fractions composition.

Several predictive tests of gluten vitality, including water absorption capacity, hydrophilicity, viscosity, elasticity, solvent retention ability, and the proper proportion between gliadin and glutenin, have been investigated.²⁻⁴ International standards usually adopt the Farinograph to test quality of vital gluten. This requires a 300 g sample and 1-2 h to test one specimen (AACC 38-20, 2000).⁵ It is difficult for a small company to control quality and material selection with this technology. The method of vital gluten water absorption (VGWA) is an important index for quality control in China; it is fast and easy to do, and does not need special equipment.⁶ Most of the vital gluten processing companies in China use VGWA as the substitution method for quality control, though with the limitations of low test accuracy and repeatability.

Gluten index is another method to evaluate gluten quality. It is expressed as percentage of wet gluten remaining on the sieve after centrifuging. Gluten index is positively significantly related to the flour swelling index of glutenin.⁷ A value higher than 75% means the flour is strong gluten flour, whereas lower than 30% means poor gluten.⁸ The higher the gluten index, the higher the gluten quality.⁷

China is the country with the highest vital gluten exports, and most companies use the Chinese national standard for quality control. According to the Chinese national standard, vital gluten quality control indices are protein content, color and water absorption capacity. As we know, high protein content does not necessarily mean high protein quality: high-quality vital gluten should have more glutenin and gliadin.

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The color of the vital gluten produced from highquality wheat is pale yellow, while that from lowquality wheat is brownish gray.⁹ Generally, the lighter the color of commercial vital gluten, the better its quality, but there are no recognized standards in this respect. Water absorption content is one of the most important quality indices of vital gluten, but there is a problem of repeatability from one operator to another.⁹ All these methods have drawbacks: the product quality from one company to another was different even though their above quality values were similar. Therefore the vital gluten industry and cereal research demand an economical, rapid and accurate method for the evaluation of vital gluten quality.

The swelling index of glutenin (SIG) has been developed to evaluate insoluble glutenin content. The principle of SIG comes from the sodium dodecyl sulfate (SDS) sedimentation test and modified screening test (MST).¹⁰ The method of calculating the result has changed from the original volume measurement to a new method of weight testing. The test result is more accurate than SDS and MST. The SIG test is affected by the solvent-to-flour ratio and centrifuge speed. SIG values are higher, with a lower ratio, because the solvent is unable to efficiently extract all of the soluble glutenin and a portion of the soluble glutenin remains swollen in the residue.¹¹ Because the vital gluten swelling index was developed by the SIG test, the sample parameters should be determined during the test. The

Table 1. Quality indices related to vital gluten

purpose was to develop a good method that can be used for quality control in the vital gluten processing enterprise.

MATERIAL AND METHODS

Samples

Seeds from 16 different Chinese wheat varieties (see Table 1) sown in Shannxi province in 2001 were used in the experiment. After harvesting and adjusting the water content for different kernel hardness, the kernels were milled using a Brabender Junior pilot mill (Brabender OHG, Duisburg, Germany). The flour milling yield was 65%. The flour was stored in a cool room $(-5^{\circ}C)$. Two commercial vital gluten samples came from two local enterprises, one belonging to a good grade and the other to an inferior grade (according to the Chinese National Standard Method).

Analytical methods

Vital gluten swelling index (VGSI)

The VGSI protocol measurement was modified from the swelling index of glutenin developed by Wang and Kovacs.¹¹ After the pre-experiment, a sample size of 18-22 mg seems optimal when 1.8 mL solvent is used for swelling vital gluten. The 18-22 mg vital gluten sample was added to 0.9 mL distilled water in a 2.0mL plastic tube, and mixed thoroughly for 5 s in a single tube vortex stirrer (Vortex Genie2, Scientific Industry, Bohemia, NY, USA). The tubes were quickly capped,

ID	Name	SIG (%)	WGC (%)	GI (%)	MP (%)	SG (%)	IG (%)	VGWA (%)	5000 VGSI (%)	Average of VGSI
1	Xiaoyan 866	4.52ab	32.2a	67.4b	7.22a	0.95b	3.15b	162.8b	23.01c	24.00c
2	Shaanzi 1869	4.11ab	29.1b	95.5a	6.66ab	0.89b	2.78bc	163.6ab	23.59bc	24.73c
3	Xinong 1330	3.60bc	25.0bc	70.4b	6.05b	0.93b	2.06d	163.4ab	22.47c	23.54c
4	Huayu 8	4.91a	26.8b	97.0a	5.77bc	1.46a	3.53a	164.6ab	32.04a	31.36a
5	95-18	4.05b	30.8ab	82.1ab	6.90a	0.88b	3.10b	160.4b	25.20bc	25.56bc
6	Xinong 199	4.85a	29.8ab	95.3a	5.96bc	1.36ab	3.81a	163.4ab	33.79a	31.74a
7	8839-3	4.73a	28.3bc	96.1a	5.29c	1.30b	3.10b	161.8b	28.67b	28.54b
8	Xiaoyan 921	3.99b	29.2b	89.4a	5.88bc	1.63a	2.94b	163.2ab	22.41c	23.77c
9	H-46	3.77bc	23.4c	85.9a	5.10c	0.93b	2.83bc	168.0a	34.50a	34.47a
10	Xiaoyan 137	3.46c	29.2b	51.7c	6.91ab	1.04b	2.21d	154.2c	21.79c	24.22c
11	Nonglin 9823	4.04b	32.4a	54.0c	6.63ab	1.61a	3.10b	152.4c	23.44c	24.79c
12	Xiaoyan 22	4.03b	29.8b	70.5b	6.64ab	0.92b	2.63bc	157.6c	25.20c	26.28b
13	Xiaoyan 6	4.84a	34.1a	84.5ab	6.39b	1.73a	3.59a	157.2c	27.54b	29.15b
14	Shaan 229	4.07b	27.6b	89.9a	6.35b	1.44a	3.04b	161.2b	27.17b	28.28b
15	Xiaoyan 107	4.01b	26.8b	89.9a	5.62bc	1.64a	2.93b	159.4bc	25.88bc	28.54b
16	Gaoyou 503	4.80a	32.8a	94.2a	6.71a	1.41a	3.66a	165.4a	30.05b	30.93ab
17	Good grade vital gluten	-	-	-	6.50b	1.08b	2.97b	160.4b	27.31b	26.77b
18	Inferior grade vital gluten	_	-	-	6.89a	1.07b	2.76bc	154.6c	22.89c	23.51c
Average $(n = 16)$		4.24	29.2	82.1	6.25	1.26	3.03	161.2	27.49	26.67
	ge ($n = 16$)	1.44	10.7	45.3	2.12	0.84	1.75	10.93	15.6	12.71
CV(n = 16)		11.13	9.91	18.10	9.80	25.07	15.93	2.09	14.83	15.46

VGWA, vital gluten water absorption; VGSI, vital gluten swelling index; 5000 VGSI, VGSI value measured at $5000 \times g$ centrifuge force; WGC, wet gluten content; GI, gluten index; MP, monomeric protein content; SG, soluble glutenin content; IG, insoluble glutenin content; average of VGSI, average of VGSI at nine different centrifuge forces; CV, Coefficient of variation.

Values with different letters are significantly different between samples.

Good-grade vital gluten and inferior-grade gluten are commercial vital gluten samples.

and the mixtures then were placed in a thermomixer (Eppendorf Thermal Mixer Comfort 5355, Eppendorf Instruments, Germany) at 1400 rpm for 10 min with a 5 s vortexing at 25 °C. Following this, 0.9 mL of 0.3% SDS-lactic acid (2% v/v) was added to each tube and vortexed for 30 min, and then were centrifuged at $3000 \times$ g (Eppendorf Centrifuge 5415D, Eppendorf Instruments, Hamburg, Germany) for 5 min. The bulk of the supernatant and foam on the surface were quickly removed with a 1 mL syringe (0.3 mm diameter) connected to a vacuum aspirator pump at 0.097 MPa (SHB-3, Henan Taikang Instruments, China), and the residue recentrifuged at $300 \times g$ for 2 min. The remaining supernatant was then drawn off with a syringe equipped with a fine needle, so as not to touch the residue surface. The tubes and precipitates were weighed, and the VGSI was calculated as the weight of the precipitate divided by the original sample weight (14% moisture base). Centrifugal force was optimized in the above protocol using increases of $500 \times g$ (3500, 4000, 4500, 5000, 5500, 6000, 6500 and $7000 \times g$).

Other quality testing method

The wet gluten content and gluten index were determined by AACC approved method 38-12 (AACC 2000), using the Perten Glutenmatic 2100 (Huddinge, Sweden). Protein content was determined by AACC method 46-10 (AACC 2000), using the Foss Kjeltec 2100 (Hoganos, Sweden). The water absorption capacity of the vital gluten was tested according to the Chinese National Standard Method SN/T 0260, 1993.

Vital gluten sample preparation

The wet gluten specimens of the 16 samples were prepared by AACC method 38-10 (AACC 2000); wet gluten was dried at 35 °C for 24 h in an air oven. This vital gluten powder retains its vital properties when reconstituted. The SIG test was conducted according to the procedure of Wang and Kovacs.¹¹⁻¹³ The wheat protein fraction was extracted and its quantity determined by using the procedure of Wang and Kovacs,¹³ which allowed classification of the protein into monomeric protein, soluble glutenin and insoluble glutenin.

Statistical analysis

All measurements were made twice. The wheat flour was mixed by two repeated milling procedures; the extraction was also done twice. ANOVA correlations were calculated using SAS analysis software.

RESULTS AND DISCUSSION Wheat protein quality indices

Wet gluten content varied from 23.4% to 34.1%, with a mean of 29.2%. The mean and range of the gluten index for the same sample were 82.1% and 45.3, respectively. The gluten index is an important

quality index for wheat flour quality, but wet gluten content has a great impact for the yield of vital gluten processing. Generally speaking, high-quality wheat varieties contain less wet gluten but have a higher gluten index, so it has lower production yield and lower profitability; the lower-quality wheat varieties have more wet gluten but a lower gluten index. The commercial preference is for a pale, cream-colored vital gluten powder with a neutral taste. The protein content of commercial samples is about 12%.⁹

For the 16 tested wheat varieties, SIG ranged from 3.46% to 4.91%, with a mean of 4.24% (Table 1). About 60% of the total protein content of wheat flour was monomeric protein, mainly composed of albumin, globulin and gliadian. Soluble and insoluble glutenin accounted for about 10% and 30%, respectively. The varieties had high insoluble glutenin content, and were also high in gluten index, SIG and VGSI values, but the wet gluten content was low and water absorption ratio was variable. For insoluble glutenin content, 56% of vital gluten samples were higher than the good-grade commercial vital gluten standard sample, and 94% of the samples were higher than the inferior grade commercial vital gluten sample.

Vital gluten quality tested by vital gluten water absorption

For the 16 wheat varieties assessed, VGWA ranged from 152.4% to168.0%, with a coefficient of variation of 2.49 (Table 1). The VGWA of high-quality wheat variety was twice the vital gluten weight after absorbing water. The water absorption of Chinese commercial vital gluten should be 150-180%.9 Water absorption of all of the vital gluten samples in the experiment met the quality demand for vital gluten output. Compared with commercial vital gluten samples, 70% vital gluten samples were higher in VGWA than goodgrade commercial vital gluten standard sample, and 94% of the samples were higher than the inferiorgrade vital gluten. Except for the SIG, MP, SG and IG content, the indices of which were more related to wheat quality, the coefficient of variation for vital gluten water absorption was the lowest (2.09, Table 1), followed by wet gluten content and vital gluten swelling index (9.91 and 14.83, respectively). The highest was gluten index (18.10). The method of gluten index produced more useful data to compare varieties than other vital gluten determining methods because the values were large and the range greater, but currently there is no criterion for vital gluten quality evaluation.

Vital gluten quality by VGSI method

VGSI of the samples for the nine different centrifugal forces are quite different (Tables 1 and 2). The highest standard deviation (4.12) and coefficient of variation (15.46) for VGSI were achieved under a centrifugal force of $5000 \times g$.

There was a noticeable difference between the VGSI values of the 16 samples, but no significant

 Table 2. Multiple comparison results of vital gluten swelling index with different centrifugal forces

Centrifugal force	Average of VGSI (%)	Maximum	Minimum	Standard deviation	Coefficient of variation
$3000 \times g$	27.57a	35.23	23.32	3.57	12.95
$3500 \times g$	28.03ab	35.50	24.04	3.40	12.11
$4000 \times g$	28.16ab	34.18	24.48	3.08	10.93
$4500 \times g$	28.08ab	35.80	23.98	3.39	12.06
$5000 \times g$	26.67c	34.50	21.79	4.12	15.46
$5500 \times g$	27.83ab	34.67	23.70	3.25	11.70
$6000 \times g$	27.33ac	33.85	22.82	3.75	13.73
$6500 \times g$	28.02ab	35.39	23.98	3.28	11.70
7000 × g	25.57d	31.52	21.26	3.30	12.81

n = 9; values with different letters are significantly different between samples.

	3000 VGSI	3500 VGSI	4000 VGSI	4500 VGSI	5000 VGSI	5500 VGSI	6000 VGSI	6500 VGSI	7000 VGSI
VGWA	0.42	0.50*	0.49*	0.53*	0.54*	0.45	0.40	0.48*	0.39
SIG	0.40	0.46	0.49*	0.44	0.55*	0.45	0.50*	0.47	0.48*
WGC	-0.27	-0.29	-0.24	-0.25	-0.25	-0.23	-0.24	-0.24	-0.24
GI	0.48*	0.58*	0.60**	0.57*	0.61**	0.55*	0.59**	0.55*	0.57*
MP	-0.58*	-0.59*	-0.56*	-0.54*	-0.57*	-0.53*	0.60**	-0.57*	-0.60**
SG	0.30	0.18	0.23	0.12	0.15	0.21	0.28	0.20	0.34
IG	0.48*	0.52*	0.52*	0.51*	0.59**	0.54*	0.51*	0.53*	0.51*

Asterisks indicate significance at * 5% and ** 1% level.

difference in VGSI at varying centrifugal force ranging from 3500 to $6500 \times g$. The optimal centrifugal force was $5000 \times g$; it produced the best VGSI value to compare varieties (Table 2). For VGSI at $5000 \times g$ centrifugal force, 37% of the vital gluten samples were higher than the good-grade commercial vital gluten standard sample, and 20% of the vital gluten samples were higher than the inferior grade. Based on the analysis results and according to the experience in measuring, we can clarify vital gluten quality using VGSI (Table 1). A VGSI value of higher than 28% is regarded as a superior grade, whereas a value between 24% and 28% is considered good, and a values lower than 24% are considered inferior.

Correlation analysis of VGSI and gluten water absorption and other indices

The VGSI measured was meaningfully correlated with gluten water absorption and other quality indices under conditions of $5000 \times g$ centrifugation. The correlation coefficients between 5000 VGSI and vital gluten water absorption, swelling index of glutenin (SIG), gluten index and insoluble glutenin content were significant and were 0.54, 0.55, 0.61 and 0.59, respectively (Table 3). The VGSI value of other centrifugal forces (except $5000 \times g$) had low or no significant correlations with VGWA and other quality indexes. The research indicates that the method developed is the best to evaluate vital gluten.

CONCLUSION

Compared with the Chinese National Standard Method of VGWA,¹¹ the VGSI has several advantages

as it is easier to learn and quicker to conduct, more accurate, and more reliable and repeatable. As to VGWA, most of the work has to be done manually. The average standard error of the VGWA is 0.17, with a coefficient of variation of 10%; for VGSI the values are 0.04 and 0.16% and are thus superior to older methods.

Compared with the AACC method of Farinograph water absorption, which requires a large flour sample and several hours to test each sample, the VGSI method only requires 47 min to test 24 samples. Second, the VGSI method focuses on the inner content of vital gluten rather than relying on the vital gluten color.

The VGSI method has great merit in the evaluation of gluten quality. It is recommended to adopt the vital gluten processing enterprises and quality control in government laboratories.

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