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Determination of Wheat Protein in Beer by FASPEK Wheat/Gluten(Gliadin) ELISA Kit II : 2014 BCOJ Collaborative Work

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CONCLUSIONS

1. Relative reproducibility standard deviation (RSD_R) for determination of wheat protein content using FASPEK Wheat/Gluten(Gliadin) ELISA Kit II ranged from 7.5 to 20.1 %, and was judged acceptable.

2. Recovery of wheat protein was 83.8 %, and was judged acceptable.

RECOMMENDATIONS

1. It was concluded that the ELISA method using FASPEK Wheat/Gluten(Gliadin) ELISA Kit II is capable of determining wheat protein content in beer containing 20 µg/ml or less.

2. The subcommittee recommends that the ELISA method using FASPEK Wheat/Gluten(Gliadin) ELISA Kit II be adopted for inclusion in *the Methods of Analysis of BCOJ*.

3. Discharge the subcommittee.

ABSTRACT

Barley malt is the main ingredient in most Japanese beer, but wheat is also used as an ingredient in some types of beer. It is desired to establish the quantitative method for allergic wheat protein. It will be able to detect wheat protein contamination into wheat free beer through a production line. It will be useful to assess the allergic influence on consumers.

The Consumer Affairs Agency in Japan indicates that the ELISA method is applicable to quantitate allergic wheat protein, and foods with wheat protein contents exceeding 10 µg/g are evaluated positive. The ELISA method has not been conducted with beer in Japan until now.

The BCOJ subcommittee was charged with evaluating the ELISA method. We evaluated wheat protein contents in beer with FASPEK Wheat/Gluten(Gliadin) ELISA Kit II which met the guidelines determined by the Consumer Affairs Agency (1).

PROCEDURE

Six sample pairs (A/B, C/D, E/F, G/H, I/J and K/L) were provided for study.

In the website (2), it is shown that barley malt also gives slightly positive result with FASPEK Wheat/Gluten(Gliadin) ELISA Kit II. Therefore we chose one pair (A/B) as beer not containing wheat to evaluate the value of false positive. We also chose one pair (K/L) which was spiked about 10 µg/ml of wheat protein to sample pair (A/B) to evaluate recovery. The measured value of the spiked wheat proteins was 11.43 µg/ml. Recovery was calculated by the following equation.

$$\text{Recovery}(\%)=\frac{\text{Wheat protein content of sample K/L}-\text{Wheat protein content of sample A/B}}{11.43}\times 100$$

The samples were degassed at 20-30 ˚C and analysis was performed using FASPEK Wheat/Gluten(Gliadin) ELISA Kit II. All procedures were performed based on the manufacturer's manual (3). Sample was finally diluted 400-fold. Assays were performed in triplicate.

Microplate reader with a 450 nm and a 600-650 nm filter was used for measuring absorbance.

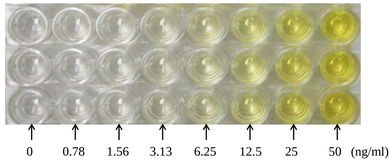

Fig. I The color of the standard solution

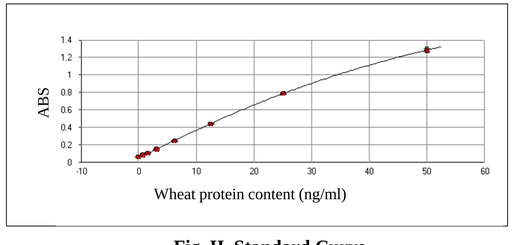

Fig. II Standard Curve

The results were processed according to JIS Z 8401:1999 guidelines (4) and statistical analysis for the processed data was performed according to JIS Z 8402-2:1999 guidelines (5) and the Consumer Affairs Agency Notice.

RESULTS AND DISCUSSION

The results for wheat protein content are shown in Table I. The data of which CV value is over 20 % were excluded from the statistical analysis based on the Consumer Affairs Agency Notice. The other data were checked for outliers using Mandel's *h* and *k* statistics, and Cochran and Grubbs outlier test, and outliers were not detected. The statistical summary of results is shown in Table II.

Each of the calculated analytical values ranged as follows: RSDr ranged from 2.0 to 4.7 %; RSD_R ranged from 7.5 to 20.1 %; and recovery was 83.8 %. These values were judged acceptable.

It was concluded that the method is capable of determining wheat protein content in beer containing 20 µg/ml or less. The subcommittee recommends that the method should be adopted for inclusion in *the Methods of Analysis of BCOJ*.

Table I
Wheat Protein Content (µg/ml) Determined Using FASPEK Wheat/Gluten(Gliadin) ELISA Kit II

	Sample Pair[a]		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H	I	J	K	L
1	1.84	1.82	8.33	8.68	7.85	7.29	16.71	17.10	14.86	14.60	11.45	11.87
2	1.34[b]	1.08[b]	5.53	5.48	5.62	5.31	14.97	15.09	14.07	13.82	10.72	11.01
3	1.18	1.17	5.05	5.27	5.22	4.86	11.15	11.10	10.33	10.31	9.07	9.21
4	1.64	1.65	7.22	7.27	6.96	7.28	17.47	17.97	14.31	15.09	11.83	12.00
5	1.71	1.61	7.97	8.53	7.15	6.87	16.39	16.01	16.72	16.97	10.70	11.09
6	1.27	1.28	6.45	6.71	6.43	5.90	14.40	14.43	10.76	11.32	11.79	11.35
7	1.62	1.52	6.23	6.16	5.37	5.51	16.08	16.62	12.14	12.39	10.61	10.79
8	1.48	1.68	6.66	6.19	6.17	6.13	15.64	14.70	10.55	11.27	10.66	10.37
9	1.11	1.06	4.78	5.25	5.64	5.32	12.92	12.74	10.58	10.64	10.56	11.01
10	1.02	1.09	5.41	5.57	5.09	5.11	14.16	13.96	10.24	10.93	10.18	10.38
11	1.27	1.13	7.71	7.59	6.25	6.62	15.92	16.47	14.66	14.12	11.29	11.63
12	1.87[b]	2.29[b]	7.24[b]	7.63[b]	8.24	8.64	15.54	15.68	13.56	13.53	12.20	11.82
Mean	1.41	1.40	6.49	6.61	6.33	6.24	15.11	15.16	12.73	12.92	10.92	11.04
Grand Mean		1.41		6.55		6.28		15.13		12.82		10.98

a Data as false positive because sample A/B do not contain wheat
b Outliers identified based on the Consumer Affairs Agency Notice and excluded from the statistical analysis.

Table II
Statistical Summary of Results

	Sample Pair					
	A/B	C/D	E/F	G/H	I/J	K/L
Number of Laboratories	10	11	12	12	12	12
Grand Mean (m)	1.41	6.55	6.28	15.13	12.82	10.98
Repeatability Standard Deviation (S_r)	0.07	0.22	0.24	0.30	0.32	0.23
Relative Repeatability Standard Deviation (RSD_r,%)	4.7	3.3	3.9	2.0	2.5	2.1
Reproducibility Standard Deviation (S_R)	0.28	1.24	1.08	1.84	2.16	0.83
Relative Reproducibility Standard Deviation (RSD_R,%)	20.1	18.9	17.2	12.2	16.9	7.5
Recovery (%)	—	—	—	—	—	83.8

LITERATURE CITED

(1) The Consumer Affairs Agency Notice "Detection method of Allergenic foods" 286,10th September, 2010. http://www.caa.go.jp/foods/pdf/syokuhin495.pdf
(2) Morinaga Institute of Biological Science, Inc. Sensitivity report on website. http://www.miobs.com/product/tokutei/faspek2/reactive.html
(3) Morinaga Institute of Biological Science, Inc. Wheat/Gluten(Gliadin) ELISA Kit II operation manual.
(4) Japanese Industrial Standards. Rules for rounding off of numerical values (Z 8401:1999). In: *JIS Handbook 57: Quality Control 2014.* Japanese Standards Association, Tokyo, Japan. pp. 1539-1540, 2014.
(5) Japanese Industrial Standards. Accuracy (trueness and precision) of measurement methods and results. Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method (Z 8402-2:1999). In: *JIS Handbook 57: Quality Control 2014.* Japanese Standards Association, Tokyo, Japan. pp. 363-395, 2014.