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Effects of wheat protein compositions on malt quality

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RESEARCH ARTICLE

Abstract

Wheat protein is one of the quality-determining factors of wheat malt. In the present study, wheat protein compositions and malt quality were determined to examine their correlation. During malting, gliadin and glutenin decreased, albumin and globulin increased and the wheat insoluble protein, especially glutelin, determined the dissolved degree of protein. As the wheat insoluble/soluble protein ratio increased, free α-amino nitrogen (FAN) of malt appeared to rise to a peak and then decreased significantly. FAN was the highest when the ratio was 2.23. With the increase in the insoluble/soluble protein ratio, extract, Kolbach index and saccharification time of the malt underwent a significant linear decrease (r=-0.934, 0.973 and 0.950, respectively). The research results indicated that the wheat protein content has to be in the range of 12.72-13.88% to obtain satisfactory wheat malt quality, and the insoluble/soluble protein ratio should be in the range of 1.44-2.23.

Keywords: correlation, malt quality, protein compositions, wheat

1. Introduction

China has the largest beer yield and consumption in the world; in 2012 the beer output was about 490.2 million hectoliters. Barley malt is one of the main raw materials for brewing due to its high enzyme activity (Celus *et al.*, 2006; Silva *et al.*, 2008). However, the price of malting barley is unstable, which greatly influences the production of beer. To meet the demands of beer production, many studies have been conducted in order to find some other suitable crop for malting and brewing. Wheat and sorghum have been used by the brewing industry because of their higher protein and starch content and lower price than barley (Depraetere *et al.*, 2004; Lu and Li, 2006). Wheat is one of the main crops and China has the largest wheat yield in the world. Wheat has been used for centuries to brew traditional (opaque) beer, which differs from European (lager) types because of the large amounts of insoluble materials that remain in it (Agu and Palmer, 1998).

Studies about the effect of barley protein compositions on malt and beer quality are numerous. It is generally agreed that barley grains used for malting should have relatively lower protein levels, because higher protein content is believed to cause some negative effects, such as prolongation of the malting process and deterioration of the malting produce and beer quality (Dai *et al.*, 2007; Robinson *et al.*, 2007a). Robinson *et al.* (2007b) found that the influence of protein on malt and beer quality was complex due to the different protein types present in barley and their different functions in malting and brewing. Albumin, globulin, gliadin and glutelin have been identified in barley, but their functions remain unknown (Silva *et al.*, 2008). The four proteins could be degraded completely or partially by malt protease and transformed into other proteins during germination (Jones and Marinac, 2002). The degradation of malt storage proteins can affect many aspects of beer quality including their clarities (Robinson *et al.*, 2007a), foam stability and haze formation in clear beer (Limure *et al.*, 2009, 2010; Siebert., 1999). The protein composition in wheat is the same as barley including four main categories: glutenin, gliadin, albumin and globulin. Generally, the protein content in wheat is higher than that in barley, which may be disadvantageous for the beer brewing process. During malting, some phenomena, such as compacted starch/protein, limited endosperm hydration

and modified enzymes, were attributed to higher protein levels in wheat (Darlington and Palmer, 1996). High protein content also could result in a decreasing extract and Kolbach index, among other things (Jin *et al.*, 2008).

There were fewer studies investigating the relationship between wheat proteins, malt and beer quality, especially as regards the effects of wheat protein compositions on the malt and beer quality. The objectives of the present study were to investigate the effect of wheat protein compositions on the malt quality and to build the criteria for how to select for malting.

2. Materials and methods

Raw materials

Five wheat (*Triticum aestivum* L.) samples with similar protein content were used. They were obtained from different ecological areas of China. The names, characteristics and ecological areas are listed in Table 1.

Germination

Wheat samples (1 kg) were malted in a micromalting machine (provided by Shandong Taishan Beer Limited Co., Tai'an, China). The detailed process was carried out according to Jin *et al.* (2008) and Jones *et al.* (2000) with some modifications. Seeds were steeped at 16 °C, every 4 h wet steep with 4 h air rest for about 40 h. The samples were ventilated about 15 min in every 60 min. The 1000-grain weight of the wheat was determined during the steeping process and the steep-out moisture of the sample was calculated with the following equation:

$$M = [(M_2\text{-}M_1) \times 100]/M_2$$

Where M is the steep-out moisture (%), M_1 the 1000-grain weight of the wheat in dry matter (g) and M_2 the 1000-grain weight of the steeped wheat in wet matter (g).

When the steep-out moisture of the samples reached 45%, the samples were germinated for five days at 16-18 °C. The kilning curve is listed in Figure 1. and specific parameters are as follow: 40 °C for 2 h, 45 °C for 2 h, 50 °C for 2 h, 55 °C for 2 h, 60 °C for 2 h, 65 °C for 1 h, 70 °C for 1 h, 78 °C for 1 h, 82 °C for 1 h. Drying ovens (Shanghai Yiheng Instrument Co., Ltd., Shanghai, China) raised the temperature at a speed of 2 °C/min. After kilning, malt samples were cleaned, and rootlets were removed.

Wheat and wheat malt analysis

The essential indexes of wheat including moisture, 1000-grain weight and germination rate were analysed according to the Chinese national standard methods of wheat (SAC, 2008a) and malting barley (SAC, 2008b). Total protein content was determined by the kjeldahl method according to the Association of Official Analytical Chemists method (AOAC, 2002) and the factor used to convert nitrogen to protein was 5.7.

A modification of sequence-progression fractionation method according to Jin *et al.* (2008) was adopted to finish the protein extraction. The samples were ground with an EBC mill (DLFU W23050; Bühler Technologies GmbH, Ratingen, Germany). A sequential extraction was conducted to determine four proteins. The sequential extraction steps are shown in Figure 2.

Table 1. Characteristics of wheat samples.

Wheat variety	Z-4	Y-5	Y-4	L-2	Y-1
Colour	white	white	white	white	red
Moisture (%)	10.7	9.3	10.2	10.0	9.8
1000-grain weight (g, dry matter)	29	29	33	29	41
Germination rate (%)	95	98	96	92	99
Protein (%, kjeldahl nitrogen)	12.94	12.72	13.52	13.35	13.88
Soluble protein[1] (%)	4.11	2.98	3.36	2.89	3.00
Insoluble protein[2] (%)	5.92	7.09	7.49	7.35	7.96
Insoluble/soluble protein ratio	1.44	2.38	2.23	2.54	2.65
Starch (%)	78.83	80.30	76.51	78.39	82.67
Endosperm structure	soft	soft	soft	soft	soft
Ecological area (province)	Henan	Shandong	Shandong	Shandong	Jiangsu

[1] Soluble protein = albumin + globulin.
[2] Insoluble protein = gliadin + glutelin.

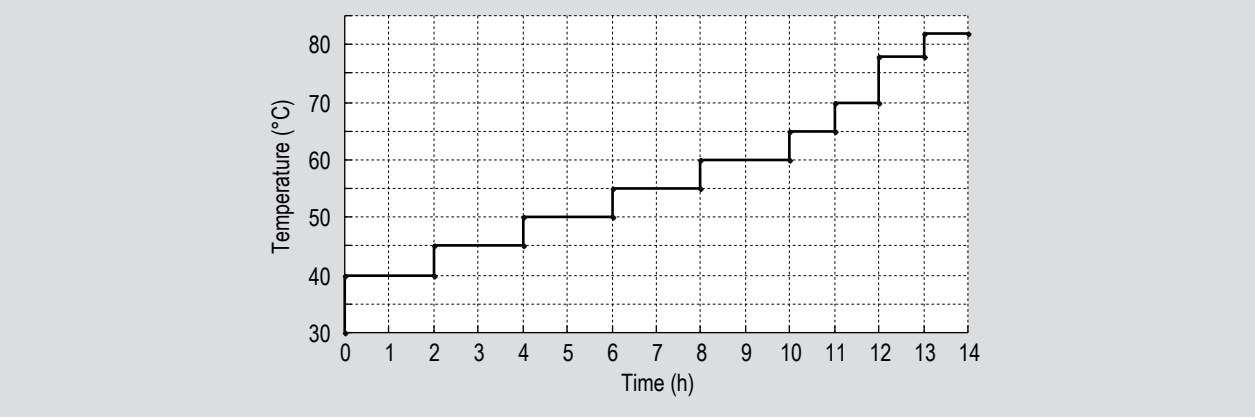

Figure 1. Temperature-time relationship curve during green malt drying.

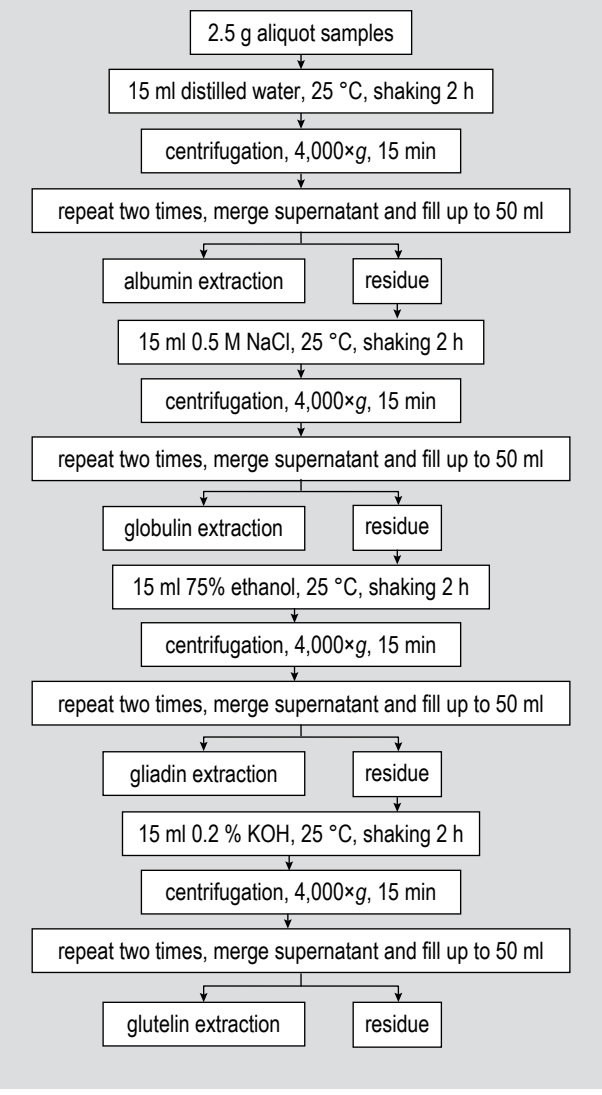

Figure 2. Procedure of sequential extraction.

Dual-wavelength spectrophotometry (ultraviolet-visible spectrophotometer UV-2100; Unico (Shanghai) Instrument Co., Ltd, Shanghai, China) was applied to determine the content of amylose and amylopectin in wheat with iodine as the colouring agent (Jin *et al.*, 2008; Liu and Yu, 2000). The kilned malt samples were analysed according to the analytical methods of the Analytica-European Brewery Convention (1998). The indexes included malt moisture, saccharification time, wort colour, α-amino nitrogen (FAN), total nitrogen, Kolbach index, diastatic power, filtrating time, wort pH and recovery rate.

Statistical analysis

All data in this study were the average values of three parallel determinations. Correlation and variance analysis were carried out using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) and DPS 7.05 (Hangzhou Ruifeng Information Technology Co., Ltd, Hangzhou, China). ^{a}CV (average coefficient of variation) reflected the degree of variation of the same index in different samples. ^{a}CV was calculated with the equation of standard deviation /mean value. The bigger the ^{a}CV value, the more significant was the difference. The D-value was the increment of each protein composition after malting.

3. Results and discussion

Variance of protein content in wheat and malt

The variances of protein content and four protein compositions of five wheat samples are listed in Table 2. The protein content was in the range of 10.07-10.96%. The four protein compositions were glutelin, gliadin, globulin and albumin and the percentage of the four proteins were 37.68-50.18%, 21.33-26.45%, 16.97-27.91% and 10.35-13.06%, respectively. The ^{a}CV among the five samples was also calculated. The protein content of wheat showed the minimum difference with an ^{a}CV value of 0.04. The albumin in wheat showed the maximum difference with an ^{a}CV value of 0.20. Following albumin were glutelin,

Table 2. Variance of wheat protein content (5% level of significance).

Wheat	Protein (%, protein nitrogen)[1]	Albumin (%)	Globulin (%)	Gliadin (%)	Glutelin (%)
Y-1	10.96	1.86	1.14	2.46	5.50
Y-4	10.85	2.10	1.26	2.87	4.62
L-2	10.24	1.83	1.06	2.69	4.66
Z-4	10.03	2.80	1.31	2.14	3.78
Y-5	10.07	1.83	1.15	2.44	4.65
[a]CV[2]	0.04	0.20	0.08	0.11	0.13

[1] Protein = albumin + globulin + gliadin + glutelin.
[2] [a]CV = standard deviation/mean value.

gliadin and globulin with [a]CV values of 0.13, 0.11 and 0.08, respectively. Therefore, the total protein content in the wheat samples was similar, while the content of the four protein compositions showed significant differences.

The same analysis was conducted on wheat malt. The variance of the protein content and protein compositions changed after malting (Table 3). The [a]CV value of protein compositions of wheat malt became bigger than that of wheat; the [a]CV values of glutelin, globulin, gliadin and albumin were 0.31, 0.30, 0.19 and 0.16, respectively. As the malting process was the same, the changes in variance of malt indexes may have originated from the difference in the four protein compositions.

Changes in four protein compositions after malting

The protein compositions of wheat malt were compared with that of wheat (Figure 3, 4, 5 and 6). From wheat to wheat malt, albumin and globulin increased, while gliadin and glutelin decreased. Albumin and glutelin showed larger variation than globulin and gliadin.

As can be seen in Figure 3, the albumin contents of wheat were in the range of 1.83-2.80% and those in wheat malt were in the range of 5.60-9.26%. There was a 2.80-7.40% increment after malting.

The increment of globulin was smaller than that of albumin (Figure 4). The globulin contents were in the range of 1.06-1.31% in wheat and 1.06-2.06% in wheat malt. So it increased 0.11-0.75% during malting. And even the globulin increment of Y-4 and Y-5, at -0.08% and -0.09%, respectively, was negative but very small.

Another two proteins, gliadin and glutelin, were substantially decreased after malting. Gliadin contents were in the range of 2.14-2.87% in wheat, while they were in the range of 1.38-2.03% in malt. It decreased 0.43-1.37% during malting. Similarly, the glutelin contents of wheat were in the range of 3.78-5.50%, while in wheat malt they were in the range of 0.99-2.30%. A decrement of 1.48-4.51% was observed during malting.

One reason for the above-mentioned changes was that insoluble protein was dissolved into soluble protein during malting. The conclusion appears to be that it was the glutelin that degraded the most.

Table 3. Variance of wheat malt protein content (5% level of significance).

Wheat malt	Protein (%, protein nitrogen)[1]	Albumin (%)	Globulin (%)	Gliadin (%)	Glutelin (%)
Y-1	13.53	9.26	1.25	2.03	0.99
Y-4	12.48	8.34	1.18	1.50	1.46
L-2	12.65	8.34	1.19	1.53	1.59
Z-4	11.42	5.68	2.06	1.38	2.30
Y-5	11.70	6.66	1.06	1.82	2.16
[a]CV[2]	0.06	0.19	0.30	0.16	0.31

[1] Protein = albumin + globulin + gliadin + glutelin.
[2] [a]CV = standard deviation/mean value.

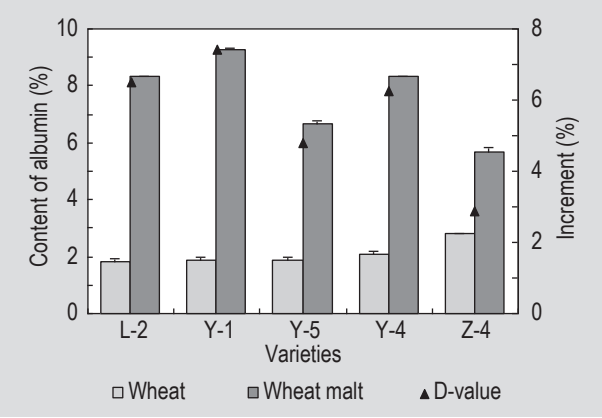

Figure 3. Albumin in wheat and wheat malt.

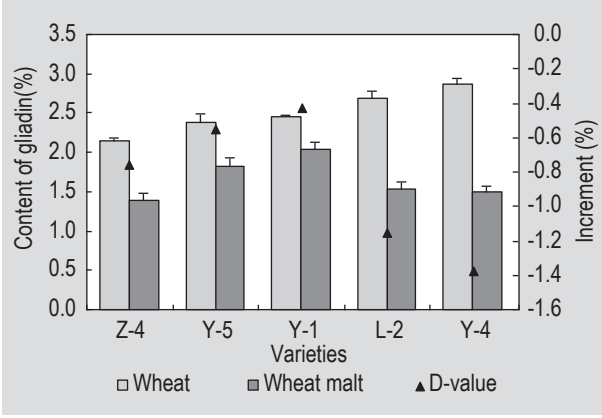

Figure 5. Gliadin in wheat and wheat malt.

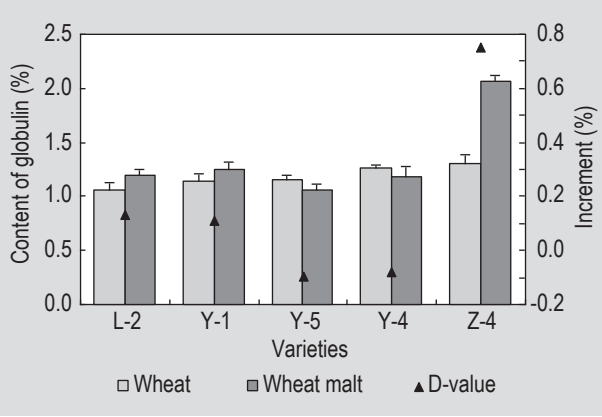

Figure 4. Globumin in wheat and wheat malt.

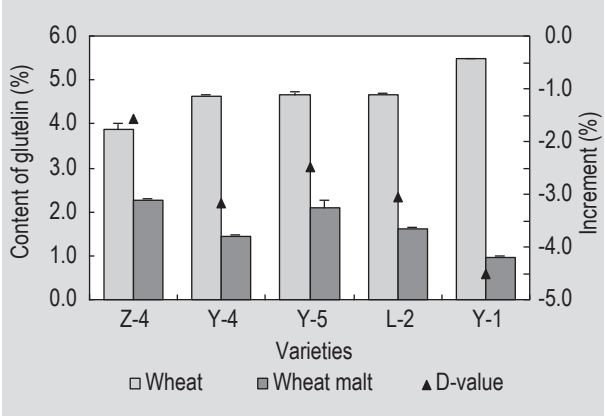

Figure 6. Glutelin in wheat and wheat malt.

Relationship between some protein content indexes of wheat and wheat malt

The relationship between protein compositions of wheat and wheat malt were analysed in Table 4. The protein content, insoluble protein (gliadin + glutelin) and glutelin of wheat showed a more significant correlation with the protein compositions of wheat malt ($P<0.05$). The insoluble protein showed a positive correlation with four indexes of wheat malt, which were albumin (r=0.945, $P<0.05$), protein (protein nitrogen) (r=0.897, $P<0.05$), increase in soluble protein (r=0.939, $P<0.05$) and decrease in insoluble protein (r=0.949, $P<0.05$). Moreover, the insoluble protein showed a negative correlation with the glutelin (r=-0.885, $P<0.05$) of the malt. A similar correlation was found between wheat glutelin and malt protein (Table 4).

Combined with the four protein compositions (Table 2), the content of glutelin was highest and the content difference among the five samples was higher than the three compositions therein. So glutelin played a dominant role and had a more significant effect on the malt compositions than gliadin. On the other hand, a former study found that in malting, the gliadin and glutenin were degraded and transformed into soluble compositions including peptides and amino acids (Jin *et al.*, 2012). According to the above analysis, with regard to the wheat that had a similar protein content, generally glutelin dominated in wheat and its degradation degree determined the dissolved degree of protein.

Effects of wheat protein composition on the characteristics of wheat malt

The characteristics of wheat malts are listed in Table 5. Except for the low FAN and Kolbach index, other indexes could meet the requirements of beer malt in the approved methods of the European Brewery Convention. In addition, the wheat protein (%, kjeldahl nitrogen) content was in the range of 12.72-13.88%, which was higher than that of barley protein. Because of the comparatively higher protein, the Kolbach index should be lower if it was set as an important characteristic of wheat malt. The lower levels of Kolbach index and FAN were due to insufficient breakdown of the protein, which could be improved by prolonging the germination time. There were obvious differences in the saccharification time and diastatic power among the malts by comparing the [a]CV value, which should be due to the

Table 4. Relationship between some protein content indexes of wheat and wheat malt.

Malt\Wheat	Albumin	Globulin	Gliadin	Glutelin	Protein nitrogen	Kjeldahl nitrogen	Soluble protein[1]	Insoluble protein[2]
Albumin	-0.709	-0.555	0.686	0.873	0.837	0.892*	-0.695	0.945*
Globulin	0.956*	0.684	-0.733	-0.711	-0.383	-0.256	0.925*	-0.833
Gliadin	-0.665	-0.498	-0.025	0.862	0.432	0.353	-0.648	0.678
Glutelin	0.557	0.404	-0.549	-0.86	-0.910*	-0.965**	0.540	-0.885*
Protein nitrogen	-0.631	-0.539	0.487	0.903*	0.83	0.926*	-0.629	0.897*
Kjeldahl nitrogen	-0.551	-0.482	0.380	0.886*	0.829	0.937*	-0.551	0.844
Soluble protein[1]	-0.528	-0.435	0.573	0.801	0.865	0.970**	-0.522	0.846
Insoluble protein[2]	0.263	0.182	-0.659	-0.503	-0.814	-0.924*	0.254	-0.640
Increase degree of soluble protein	-0.738	-0.633	0.635	0.889*	0.782	0.871	-0.735	0.939*
Decrease degree of insoluble protein	-0.699	-0.496	0.740	0.854	0.867	0.883*	-0.676	0.949*

[1] Soluble protein = albumin + globulin.
[2] Insoluble protein = gliadin + glutelin.
* Significant correlation at 0.05 level.
** Significant correlation at 0.01 level.

Table 5. Characteristics of wheat malts.

Variety	Z-4	Y-5	Y-4	L-2	Y-1	[a]CV
Saccharification time (min)	15	9	11	10	7	0.26
Wort colour (EBC)	4.0	5.5	5.0	4.5	6.0	0.11
Extract on dry matter (%)	84	81	80	80	80	0.023
FAN (mg/100 g)	99	106	111	105	106	0.057
Total nitrogen (%)	2.151	2.177	2.292	2.317	2.483	0.12
Kolbach index (%)	37	31	31	31	29	0.10
Diastatic power (WK)	323	296	368	428	397	0.16
Wort pH	6.2	6.2	6.3	6.2	6.2	0.0071
Filtrating time (min)	90	-	80	60	50	-
Recovery rate (%)	82.7	84.3	86.2	84.5	84.5	0.011

[a]CV = standard deviation/mean value; FAN = free α-amino nitrogen.
- = measured indexes were incalculable.

difference in the degrading enzymes activities. The wort colour, FAN, total nitrogen, and Kolbach index showed some variance among different malts as well. The variance in the above four indexes were mostly explained by the difference in the protein compositions of wheat (Table 6).

As shown in Table 6, the wheat albumin showed a positive correlation with the extract (r=0.906, P<0.05) and Kolbach index (r=0.940, P<0.05) of wheat malt. With the increase in albumin in the grain, the soluble ingredient would increase in wort. No linear relationship was found between wheat globulin and the characteristics of malt. Wheat globulin exhibited the smallest content change during malting and the smallest effect on the malt indexes.

With the increase in wheat gliadin, FAN had a linear increase (r=0.896, P<0.05). It was maybe because gliadin was more easily degraded by the aminopeptidase during malting.

The glutelin, which had the maximum content level in four wheat protein compositions, showed an important correlation with some characteristics of malt. The glutelin showed a negative correlation with the Kolbach index (r=-0.933, P<0.05), filtrating time (r=-0.905, P<0.05)

Table 6. Effects of wheat protein composition on the characteristics of wheat malt.

Wheat\Malt	Saccharification time	Extract on dry matter	FAN	Total nitrogen	Kolbach index	Filtrating time
Albumin	0.916*	0.906*	-0.657	-0.55	0.940*	0.869
Globulin	0.734	0.677	-0.221	-0.479	0.695	0.868
Gliadin	-0.393	-0.835	0.896*	0.380	-0.644	-0.267
Glutelin	-0.958*	-0.816	0.567	0.886*	-0.933*	-0.905*
Soluble protein[1]	0.902*	0.882*	-0.587	-0.549	0.914*	0.891
Insoluble protein[2]	-0.906*	-0.954*	0.777	0.844	-0.978**	-0.818

FAN = free α-amino nitrogen.
[1] Soluble protein = albumin + globulin.
[2] Insoluble protein = gliadin + glutelin.
* significant correlation at 0.05 level.
** significant correlation at 0.01 level.

and saccharification time (r=-0.958, $P<0.05$). The wheat glutelin showed a positive correlation with total nitrogen of malts (r=0.886, $P<0.05$). The degradation of glutelin during malting increased the soluble protein content of wheat malt. Meanwhile, with the increase in glutelin in wheat, the insoluble protein also increased. So, the Kolbach index depended on the degree of glutelin degradation; the higher the glutelin content in wheat, the lower the Kolbach index of wheat malt (Table 6). The soluble protein and the insoluble protein also had an important influence on the characteristics of wheat malt and the effects were a synergy of albumin and globulin, gliadin and glutelin.

From the above analysis, four protein compositions interconverted in malting, and together affected the characteristics of malt. The insoluble protein, especially glutelin, converted into soluble protein. On the other hand, if the insoluble /soluble ratio was too high, the Kolbach index and extract of the malt would be decreased. So a suitable ratio of insoluble /soluble protein should be found to improve the quality of wheat malt.

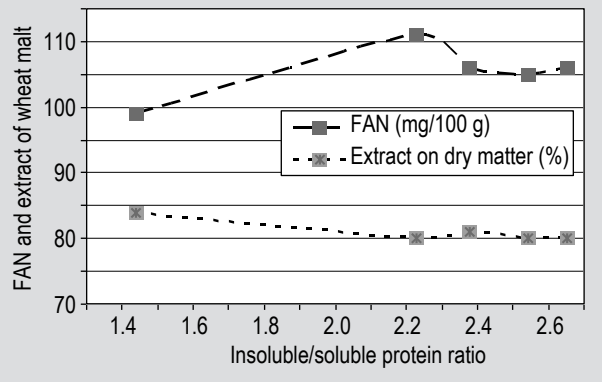

Figure 7. Effects of insoluble/soluble protein ratio on free α-amino nitrogen (FAN) and extract of wheat malt.

Effects of insoluble /soluble protein ratio on the characteristics of wheat malt

The insoluble/soluble protein ratio in the grain also influenced the malt characteristics. As shown in Figure 7, with the increase in the insoluble/soluble protein ratio, FAN increased gradually. After reaching a peak at the ratio of 2.23, FAN dropped quickly and remained constant. The expressed a significant linear decrease with insoluble/soluble protein ratio (r=-0.934, $P<0.05$).

As can be seen in Figure 8, substantial decreases in the Kolbach index and saccharification time were observed. The Kolbach index and saccharification time showed a negative correlation with the insoluble/soluble protein ratio (r=-0.973, $P<0.001$ and -0.950, $P<0.05$, respectively). Wort colour increased with the increase in the insoluble/soluble protein ratio, but showed little variation.

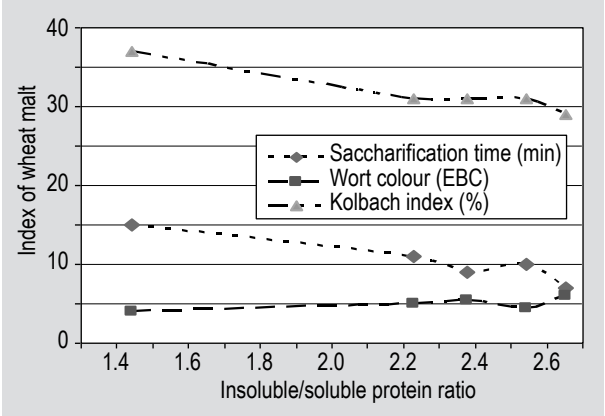

Figure 8. Effects of insoluble/soluble protein ratio on saccharification time, wort colour and Kolbach index of wheat malt.

In summary, to obtain a satisfactory wheat malt quality with high FAN, extract and Kolbach index, the insoluble/soluble protein ratio should be in the range of 1.44-2.23 when wheat protein content is in the range of 12.72-13.88%.

4. Conclusion

The content of different protein compositions resulted in the changes of the malt indexes. A wheat with higher soluble protein and lower insoluble protein content should be selected to acquire more soluble protein in malt.

When the wheat insoluble/soluble protein ratio was 2.23, FAN was the highest. With the increase in insoluble/soluble protein ratio, extract, Kolbach index and saccharification time of the malt showed a significant linear decrease. In this study it was shown that when the wheat protein content is in the range of 12.72-13.88%, the insoluble/soluble protein ratio should be in the range of 1.44-2.23 in order to obtain satisfactory wheat malt quality.

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References

Agu, R.C. and Palmer, G.H., 1998. A reassessment of sorghum for lager-beer brewing. Bioresource Technolog 66: 253-261.

Analytica-European Brewery Convention, 1998. Approved methods of the European brewery convention (5th Ed.). Fachverlag, Nürnberg, Germany.

Association of Official Analytical Chemists (AOAC), 2002. Official methods of analysis (4th Ed.). AOAC, Washington, DC, USA.

Celus, I., Brijs, K. and Delcour, J.A., 2006. The effects of malting and mashing on barley protein extractability. Journal of Cereal Science 44: 203-211.

Dai, F., Wang, J.M., Zhang, S.H., Xu, Z.Z. and Zhang, G.P., 2007. Genotypic and environmental variation in phytic acid content and its relation to protein content and malt quality in barley. Food Chemistry 105: 606-611.

Darlington, H.F. and Palmer, G.H., 1996. Homogeneity of the friable flour of malting barley. Journal of the Institute of Brewing 102: 179-182.

Depraetere, S.A., Delvaux, F., Coghe, S. and Delvaux, F.R., 2004. Wheat variety and barley malt properties: influence on haze intensity and foam stability of wheat beer. Journal of the Institute of Brewing 110: 200-206.

Jin, Y.H., Du, J.H., Zhang, K.L., Xie, L. and Li, P.P., 2012. Relationship between Kolbach index and other quality parameters of wheat malt. Journal of the Institute of Brewing 118: 57-62.

Jin, Y.H., Zhang, K.L. and Du, J.H., 2008. Effects of wheat protein on endosperm composites and malt quality. Journal of the Institute of Brewing 114: 289-293.

Jones, B.L. and Marinac, L.A., 2002. The effect of mashing on malt endoprotrolytic activities. Journal of Agricultural and Food Chemistry 50: 858-864.

Jones, B.L., Marinac, L.A. and Fontanini, D., 2000. Quantitative study of the formation of endoproteolytic activities during malting and their stabilities to kilning. Journal of Agricultural and Food Chemistry 48: 3898-3905.

Limure, T., Nami, N., Hirota, N., Zhou, T.S., Hoki, T., Makoto, K., Hayashi, K., Ito, K. and Sato, K., 2010. Construction of a novel beer proteome map and its use in beer quality control. Food Chemistry 118: 566-574.

Limure, T., Nankaku, N., Watanabe-Sugimoto, M., Hirota, N., Zhou, T.S., Kihara, M., Hayyashi, K., Ito, K. and Sato, K., 2009. Identification of novel haze-active beer proteins by proteome analysis. Journal of Cereal Science 49: 141-147.

Liu, X.N. and Yu, Y.B., 2000. Determination of amylase and amylopection in Ginkgo Biloba by dual-wavelength spectrophotometry. Chinese Journal of Spectroscopy Laboratory 17: 506-509.

Lu, J. and Li, Y., 2006. Effects of arabinoxylan solubilization on wort viscosity and filtration when mashing with grist containing wheat and wheat malt. Food Chemistry 98: 164-170.

Robinson, L.H., Healy, P., Stewert, D.C., Eglinton, J.K., Ford, C.M. and Evans, D.E., 2007b. The identification of a barley haze active protein that influences beer haze stability: the genetic basic of a barley malt haze active protein. Journal of Cereal Science 45: 343-352.

Robinson, L.H., Jutter, J., Milligan, A., Lahnstein, J., Eglinton, J.K. and Evans, D.E., 2007a. The identification of a barley haze active protein that influences beer haze stability: cloning and characterisation of the barley SE protein as a barley trypsin inhibitor of the chloroform/methanol type. Journal of Cereal Science 45: 343-352.

Siebert, K.J, 1999. Effects of protein-polyphenol interactions on beverage haze, stabilization, and analysis. Journal of Agricultural and Food Chemistry 47: 353-362.

Silva, F., Noguerira, L.C., Goncalves, C., Ferreira, A.A., Ferreira, I.M.P.L.V.O. and Teixeira, N., 2008. Electrophoretic and HPLC methods for comparative study of the protein fractions of malts, worts and beers produced from Scarlett and Prestige barley (*Hordeum vulgare* L.) varieties. Food Chemistry 106: 820-829.

Standardization Administration of the People's Republic of China (SAC), 2008a. GB1351 Wheat. SAC, Beijing, China.

Standardization Administration of the People's Republic of China (SAC), 2008b. GB7416 Malting barley. SAC, Beijing, China.