65

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Validation and Application of Osmolyte Concentration as an Indicator to Evaluate Fermentability of Wort and Malt

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PURPOSE

Fermentability of malt is an important trait of malt quality. Inadequate prediction of malt fermentability or a lack of warning to potential fermentability problems cause downstream production losses within local brewing industry.

Recently, osmolyte concentration (OC), which is a measure of the molar concentration of solutes, has been developed to be a new method for measuring malt or wort quality. When storage compounds such as starch and protein are degraded by enzymes, the molar concentrate will increase and lead to an increase in OC. Since OC can explain the complex degradation of macromolecules during mashing, making it possible to eliminate or replace an expensive, time consuming or impractical measurement to define malt or wort fermentability. Hence, the aim of study was to develop and validate a new method based on the OC to predict the malt fermentability, and determine the effect of mashing parameters on wort OC and the resultant fermentability.

MATERIAL AND METHODS

- **Malts.** Commercial malts (*Hordeum vulgare* L.) were sourced from Tsingtao Brewery Co. Ltd. These malts had the malt quality characteristics as described in Table 1.

Table 1. Quality Characteristics of malts used in this study

Variety	ME	KI	DP	α (U/g malt)	α TS (%)	Total β (U/g malt)	Free β (U/g malt)	β TS (%)	Total LD x10³ (U/g malt)	Free LD x10 ³ (U/g malt)	LD TS (%)
Explorer	81.8	49	277	150	18	733	591	2	304	96	72
Overture	87.8	48	283	224	8	731	737	2	324	155	49
Sebastian	83.7	46	295	132	11	783	732	1.5	305	91	100
Gairdner	81.6	44	277	102	16	813	655	1.7	346	70	100
Metcalfe	82.2	43	327	121	7	675	732	1	236	68	100
Copeland	80.9	40	353	123	11	919	815	1.5	220	83	49
Gairdner	80.8	43	352	134	7	909	926	1.4	307	87	90
Dan'er	78.5	42	287	69	30	813	691	0.6	153	58	100

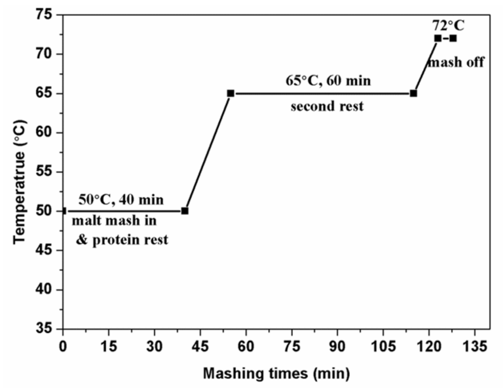

Fig. 1 The mashing protocol used in this study

- **Mashing protocol:** Mashing protocol was shown in figure 1.
- **Determination of OC**
  - **Malt OC (MOC)**: 150 mg of ground malt was placed in a 2-ml centrifuge tube with 750 ul of distilled water, mixed in a mixer and held at 65ºC for 30 min, and then ramped to 100ºC for 5 min to make sure complete starch gelatinazation. Mixed sample were centrifuged at 13,000 g for 10 min, and the supernatant was used to determine osmolyte concentration by freezing point depression osmometer (Loser, Berlin, Germany). Data were expressed as osmolytes per kilogram of malt.
  - **Wort OC (WOC)**: The wort was centrifuged at 13,000 g for 10 min before determining the OC value, and data were expressed as millimoles osmolytes per liter of wort.
- **Determination of fermentability.** The real degree fermentability (RDF) was determined using the modified Wort-5B method and data were expressed as percentage of extract.
- **Sugar determination.** Wort sugars (maltotriose, maltose, glucose, fructose and sucrose) were separated on a column (Rezex ROA, ooH-0138-KO) using HPLC (Waters Alliance 2695, Waters, WA), using 0.005 N sulfuric acid at a flow rate of 0.6 ml/min as the mobile phase.

RESULTS

- **Comparison of MOC with malt parameters and wort parameters**
  - MOC were significantly correlated with α-amylase (r=0.813, P<0.05) and limit dextrinase (LD) (r=0.762, P<0.05), as well as WOC (r=0.795, P<0.05) and wort maltose, glucose and fermentable sugar (r=0.724, 0.779 and 0.744, respectively; P<0.05) (Table 2,3).
  - MOC was significantly correlated with wort RDF (r=0.744, P< 0.01) (Figure 2, Table 2).
- **Comparison of WOC with malt parameters and wort parameters**
  - WOC was significantly correlated with activities of α-amylase and LD, and have no correlation with the DP or β-amylase (Table 2). WOC have a positive significantly correlation with maltotriose, maltose and glucose and fermentable sugars, while have a negative correlation with the dextrin (Table 3).
  - WOC have a positive correlation with fermentable sugars and RDF (Table 3). Compared to MOC, the correlation between WOC and wort fermentable sugars was much stronger than that for MOC and fermentable sugar (MOC versus fermentable sugars: r=0.744, P<0.01 ; WOC versus RDF, r=0.982; P<0.01). Compared to the regression line between MOC and RDF, all points fall quite close regression line between WOC and RDF and evenly distributed above or below the line (Fig. 2).

Table 2. The correlation between MOC, WOC and malt parameters

	ME	DP	MOC	WOC	α□	□β	LD	RDF
ME	1							
DP	-.278	1						
MOC	.798*	.199	1					
WOC	**.850****	-.256	**.795***	1				
α	**.914****	-.111	.813*	**.796***	1			
β	-.053	**.855****	.345	-.095	.078	1		
LD	**.899****	-.208	.762*	**.841****	**.970****	.057	1	
RDF	**.850****	-.053	**.867****	**.884****	**.890****	.190	**.890****	1

Table 3. The correlation between MOC, WOC and wort sugar contents

	Dextrin	Maltotriose	Maltose	Glucose	Total sugars	Fermentable sugars	W OC	MOC	RDF
Dextrin	1								
Maltotriose	-.800*	1							
Maltose	-.625	.838**	1						
Glucose	-.614	.856**	.825*	1					
Total sugars	-.305	.735*	.902**	.837**	1				
Fermentable sugars	-.703	.930**	.971**	.917**	.892**	1			
Wort OC	-.797*	.947**	.923**	.928**	.807*	.982**	1		
Wor RDF	-.916**	.852**	.862**	.777*	.605	.887**	.884**	.867**	
Malt OC	-.698	.601	.724*	.779*	.552	**.744***	**.795***	1	1
ME	-.713*	.789*	.881**	.784*	.720*	**0.877****	**0.850***	0.798*	0.85**

Table 4. The correlation between MOC, WOC and wort parameters from different mashing protocol

	Plato	ME	RDF	Alcohol	OC	Dextrin	Maltotriose	Maltose	Glucose	Surcose	Total sugars	Fermentable sugars
Plato	1											
ME	.513	1										
RDF	.640*	.042	1									
Alcohol	.826**	.100	.908**	1								
OC	.521	.044	.912**	.774**	1							
Dextrin	-.309	.139	-.914**	-.686*	-.920**	1						
Maltotriose	.449	-.186	.531	.695*	.534	-.387	1					
Maltose	.639*	.213	.829**	.711*	.654*	-.723*	.044	1				
Glucose	.297	.050	.610*	.503	.859**	-.678*	.561	.219	1			
Surcose	-.163	-.198	.292	.131	.602*	-.518	.351	-.097	.784**	1		
Total sugars	.964**	.353	.712*	.891**	.627*	-.405	.625*	.595	.449	-.037	1	
Fermentable sugars	.706*	.088	.982**	.921**	.942**	-.882**	.583	.794**	.688*	.329	.789**	1

- **The effect of mashing parameters on the WOC, sugars and RDF**
  - WOC showed a stronger correlation with RDF and malt sugar, especially wort fermentable sugars. This provided a simple and fast way to regulate the mash parameters to the optimal wort fermentability. The correlation was higher than described above between various varieties (Table 4).
  - RDF, WOC and fermentable sugars showed a significant increase with increasing mash duration. While ME and Plato was little influenced by mash duration. ME, plato, RDF, total sugars and fermentable sugars showed an increase with increasing mash-in temperature. Peak ME, plato, RDF, total sugars and fermentable sugars was achieved at 65ºC. Increase or decrease in mash temperature beyond 65ºC progressively decrease the ME, plato, RDF, total sugars and fermentable sugars (Fig 3).

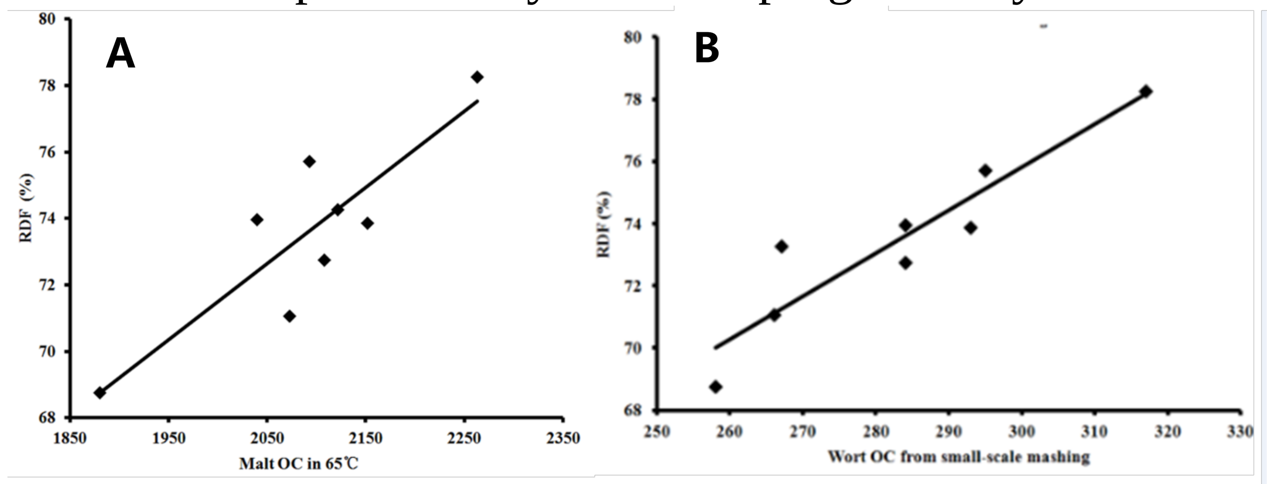

Fig. 2 Correlation and regression line of RDF versus MOC (A) and WOC (B)

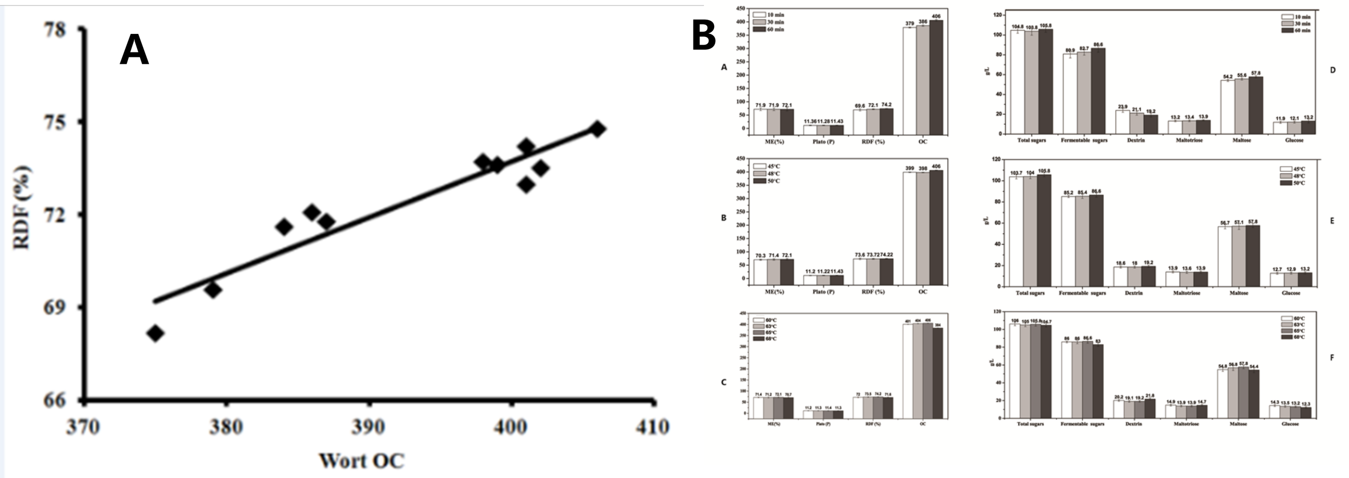

Fig. 3 Regression line of RDF versus WOC (A) for Gairdner with different mashing conditions and the effect of mashing conditions on the wort parameters (B)

CONCLUSIONS

- OC is a measure of the molar concentration of solutes and determined with a vapor pressure osmometer. Once samples are prepared, each measurement of OC takes approx. 2 min and the cost for per OC measurement is <0.01 $ per.
- MOC can be used to predict malt amylolytic enzymes activities, the wort sugar contents and the fermentable sugars. These suggested that OC could be added tool for quick screening of malts for individual amylolytic activities and RDF without the mashing procedure.
- Typically brewers do not seek to produce worts that have maximal fermentability, because unfermentable dextrins and limit dextrins can influence beer mouth-feel and other quality characteristics. To control fermentability, brewers typically adjust either mash temperature or mash duration. Thus, the WOC, which was determined in a simple and fast way, will be a good predictor to control fermentabiltiy.
- In conclusion, the ability of WOC to predict malt fermentability and sugar content allows brewers to keep better control of fermentability in the face of variation of malt quality and quickly adjust mashing conditions for the consistency of wort fermentability.

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