

Effects of wort preparing parameters on the composition of soluble dietary fibre in wheat beer

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Abstract

Wheat beer would be a desirable source of quality soluble dietary fibre (SDF). The effects of wort preparing parameters on SDF in wheat beer were investigated in the present work. Non-starch polysaccharides and polyphenols were greatly influenced by wheat malt percentage (WMP), while resistant protein (RP) was augmented by extending resting at 63°C. By verification test, the optimised parameters for high SDF wheat beer were WMP 55%, resting for 20 min at 43°C, 20 min at 50°C, and 20 min at 63°C, in which SDF was 2,178 mg/L. Most of SDF in wheat wort were retained in beer during fermentation and maturation ($R = 0.561^*$). And SDF of wheat beer contained more RP and polyphenols. The present work contributes in controlling SDF content in wheat beer. It also has important guiding significance for beer brewing enterprises to develop new products rich in SDF.

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Introduction

Dietary fibre, including polysaccharides, oligosaccharides, lignin, and associated plant substances (DeVries *et al.*, 2001), is defined as edible part of plants, which is resistant to digestion and absorption in the human small intestine. Based on water solubility, the dietary fibre is classified into insoluble and soluble dietary fibre (SDF) (Chen *et al.*, 2018). SDF has attracted intensive research interests due to its important physiological functions (Wu *et al.*, 2020) such as preventing chronic diseases including hyperglycaemia (Qin *et al.*, 2020), hypertension (Khan *et al.*, 2017), colorectal adenocarcinoma (Mendis *et al.*, 2016), obesity (Wang *et al.*, 2018), hyperlipidaemia (Xue *et al.*, 2019), hypercholesteremia (Yang *et al.*, 2020), and intestinal flora disorder (Wang *et al.*, 2020).

Beer, as the most popular alcoholic beverage in the world (Neto *et al.*, 2017), contains considerable SDF, in which non-starch polysaccharides (NSP) are the dominant component, followed by resistant protein (RP), and polyphenols (PP) (Goñi *et al.*, 2009b). Nowadays, the demand for wheat beer increases all over the world (Mastanjevic *et al.*, 2018). Among NSP in wheat beers, the descending order of content is arabinoxylan, arabinogalactan, β -glucan, and mannose polymers (Li *et al.*, 2020), and wheat beers possess more NSP than barley beers (Li *et al.*,

2019a). Therefore, wheat beer would be a desirable source of quality SDF. According to Elleuch *et al.* (2010), SDF should account for approximately 20 - 30% of our dietary fibre intake, while consumption of 150 mL of beer for a person each day accounts for only 5% SDF intake of the diet (Goñi *et al.*, 2009b). Therefore, increasing the content of SDF in beer is of great significance for human health.

SDF in wheat beer is mainly from malts as well as wort preparation. The percentage of wheat malt and mashing process parameters of wort preparation would have a great influence on SDF content of wheat beer. Arabinoxylan from malts was found to be the main NSP in wheat beer (Li *et al.*, 2017; 2020). Wheat malts possess abundant soluble arabinoxylan (Li *et al.*, 2005), β -(1,4)-endoxyylanase, β -glucanase, and β -d-xylosidase (Jin *et al.*, 2014). Endogenous xylanase from malt is the key enzyme in the degradation of arabinoxylans during wort preparation (Guo *et al.*, 2017). The endoxyylanase in malts exhibited good activity at 40 - 60°C (Kanauchi *et al.*, 2013; Peng *et al.*, 2019), but the optimal temperature of β -D-xylosidase from wheat malts was 70°C (Chai *et al.*, 2015). In addition, water-extract β -glucan in barley malt (0.297%) (Marconi *et al.*, 2014) was higher than that in wheat malt (0.043 - 0.059%) (Vincenzo *et al.*, 2018). Beta-glucanase from barley had good activity at 37 - 45°C, and was rapidly inactivated at 55°C (Lauer *et al.*, 2017). Phenolic

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acids were the main components of PP, of which ferulic acid was the main one (Wannenmacher *et al.*, 2018; Li *et al.*, 2019b). The optimal temperature of the cinnamoyl esterase from barley malt was 30°C (Vanbeneden *et al.*, 2008). About 22% of the wort protein was from enzymatic degradation during mashing, and the malt endoproteinases were stable at 38 - 60°C, and inactivated at 72°C (Jones and Marinac, 2002). Additionally, most of proteins (60 - 76%) retained in beers were RP (Goñi *et al.*, 2009b).

Obviously, increasing SDF content in wheat beer is of great significance to both consumers and beer producers. To date, the effect of wort preparing parameters on SDF in wheat beer has not been reported. To this end, wheat beers with different WMP and mashing process parameters were brewed at a 3-L scale in the present work. Five factors and four levels orthogonal test L16 (4⁵) of wort preparation was designed to investigate the influence of these parameters on SDF and its component NSP, PP, and RP.

Materials and methods

Materials and reagents

Barley and wheat malts were provided by Shandong Taishan Beer Co., Ltd., China. Pepsin (from porcine gastric mucosa, 400 U/mg) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Amyloglucosidase (3300 U/mL) was purchased from Megazyme Inc. (Bray, Ireland). α -Amylase (from bacteria, 35 U/mg) was purchased from Shanghai Macklin Biochemical Co., Ltd., China. Dialysis bag (MWCO 12,000 - 14,000) and Tris-maleate (98%) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd., China. All the other reagents were of analytical grade unless otherwise specified.

Beer preparation

Five factors four levels orthogonal test L16 (4⁵) was applied to design the wort preparing process. Wheat and barley malts were used for wort preparation and wheat beer fermentation. The wort preparing parameters included WMP of 40, 45, 50, and 55%, resting at 37°C (T37) for 0, 10, 20, and 30 min; resting at 43°C (T43) for 0, 10, 20, and 30 min; resting at 50°C (T50) for 0, 10, 20, and 30 min; as well as resting at 63°C (T63) for 0, 10, 20, and 30 min.

The WMP and mashing process parameters are shown in Table 1. Batches 17 and 18 were control samples prepared by all-wheat and all-barley malts, respectively. The details of the beer brewing process

were as follows. After grinding, the mixed malts grist (1,120 g/batch) were saccharified following the procedures in Table 1, in a mashing bath (BGT-8A, Hangzhou Biotech Co., Ltd., China), at a grist to water (Nongfu Spring Co. Ltd., China) ratio of 1:5, with a heating rise rate of 1°C/min. After running the procedure in Table 1, respectively, the mashing temperature was raised to 70°C at 1°C/min, and the saccharification was completed by holding for 5 min at 70°C after iodine reaction disappeared by adding two drops of iodine-potassium iodide solution (1.30 g iodine and 3.50 g potassium iodide was dissolved in 500 mL of deionised water) to the mashing liquid in a colorimetric disc. The wort was obtained by filtering the mash immediately using a 300- μ m aperture stainless steel filter. After adding hop pellets (2 g/kg), the wort was boiled for 15 min (evaporation rate: 10% of the wort volume/h), filtered by a 48- μ m aperture cylindrical cone bottom filter, rapidly cooled to 20°C, and then the original extract was adjusted to 11.5 °P to obtain the hopped wort.

Beer yeast (1 g/L; SafAle WB-06, Fermentis, Belgium) was added to 3 L of hopped wort to ferment in a plastic beer bottle (4 L; Shandong Taishan Beer Co., Ltd., China) with the cap loosely screwed at 20°C in the incubator (SPX-300BSH-II, Shanghai CIMO Medical Instrument Manufacturing Co., Ltd., China). The sugar contents of fermenting wort were measured with a digital sugar meter (LH-B55, Hangzhou Luheng Biological Technology Co., Ltd., China). By the time the sugar contents of the fermenting wort dropped to 6.0 Brix, the bottles' screw cap were tightened, and the bottles were stored in a 5°C incubator for three weeks to obtain the finished beer.

Methods

Viscosity, α -amino nitrogen (α -AN), and pH of wort and beer were measured according to the method of ASBC 6.13, 6.12, and 6.8, respectively. The original extract, real degree of fermentation, and alcohol content were measured by an automatic beer analyser (Alcolyzer, Annton Parr, Austria).

Sample pre-treatment

The samples were centrifuged at 5,000 g (LXJ-IIB, Shanghai Anting Scientific Instrument Factory, Shanghai, China) for 10 min, and then 250 mL of the supernatant was concentrated by a rotary evaporator (45°C, -0.1 Mpa; RE5298 Shanghai Yarong Biochemical Instrument Factory, China) for 50 min to about 100 mL. Before concentration, beer sample was sonicated for 5 min to remove CO₂ by an ultrasonicator (Frequency: 40 kHz, Power: 80%;

Table 1. Wort preparing parameters and physicochemical indicators of wheat wort and beer.

Batch	WMP (%)	Rest period(min)				TT (min)	Wheat wort				Wheat beer			
		T37	T43	T50	T63		α -AN (mg/L)	Viscosity (mPa.s)	pH	Alcohol (% v/v)	RDF (%)	Viscosity (mPa.s)	pH	
1	40	0	0	0	0	123	11.46 ± 0.02 ^a	174 ± 9 ^c	1.89 ± 0.00 ^b	5.89 ± 0.00 ^d	4.94 ± 0.01 ^b	67.1 ± 0.0 ^c	1.59 ± 0.00 ^{ab}	4.20 ± 0.00 ^d
2	40	10	10	10	10	110	11.49 ± 0.04 ^a	210 ± 7 ^b	1.87 ± 0.01 ^c	6.02 ± 0.00 ^b	5.06 ± 0.02 ^{ab}	68.5 ± 0.1 ^b	1.60 ± 0.01 ^a	4.34 ± 0.00 ^c
3	40	20	20	20	20	130	11.35 ± 0.03 ^a	196 ± 8 ^b	1.92 ± 0.01 ^a	5.90 ± 0.00 ^c	4.99 ± 0.02 ^{ab}	68.4 ± 0.1 ^b	1.58 ± 0.01 ^{bc}	4.43 ± 0.00 ^b
4	40	30	30	30	30	185	11.63 ± 0.17 ^a	287 ± 1 ^a	1.70 ± 0.01 ^d	6.23 ± 0.00 ^a	5.19 ± 0.11 ^a	69.4 ± 0.4 ^a	1.57 ± 0.01 ^c	4.52 ± 0.00 ^a
5	45	0	10	20	30	115	11.64 ± 0.07 ^{ab}	217 ± 10 ^a	1.86 ± 0.00 ^a	6.19 ± 0.00 ^a	5.21 ± 0.04 ^a	69.6 ± 0.1 ^a	1.60 ± 0.01 ^a	4.47 ± 0.00 ^a
6	45	10	0	30	20	140	11.78 ± 0.08 ^a	194 ± 2 ^b	1.84 ± 0.00 ^b	5.84 ± 0.00 ^c	5.22 ± 0.04 ^a	68.9 ± 0.1 ^b	1.61 ± 0.00 ^a	4.44 ± 0.00 ^b
7	45	20	30	0	10	125	11.71 ± 0.06 ^{ab}	209 ± 3 ^{ab}	1.80 ± 0.00 ^c	5.83 ± 0.00 ^d	5.15 ± 0.04 ^a	68.3 ± 0.1 ^c	1.60 ± 0.01 ^a	4.47 ± 0.00 ^a
8	45	30	20	10	0	130	11.52 ± 0.02 ^b	221 ± 7 ^a	1.77 ± 0.01 ^d	5.96 ± 0.00 ^b	4.98 ± 0.01 ^b	67.3 ± 0.1 ^d	1.59 ± 0.01 ^b	4.43 ± 0.00 ^c
9	50	0	20	30	10	132	11.34 ± 0.06 ^a	239 ± 9 ^a	1.97 ± 0.01 ^a	5.95 ± 0.00 ^b	4.93 ± 0.04 ^a	67.6 ± 0.2 ^a	1.58 ± 0.00 ^b	4.47 ± 0.00 ^a
10	50	10	30	20	0	120	11.43 ± 0.12 ^a	216 ± 9 ^a	1.92 ± 0.01 ^b	5.90 ± 0.00 ^c	4.87 ± 0.10 ^a	66.3 ± 0.6 ^b	1.60 ± 0.00 ^a	4.44 ± 0.00 ^b
11	50	20	0	10	30	123	11.32 ± 0.04 ^a	166 ± 8 ^b	1.81 ± 0.01 ^c	5.95 ± 0.00 ^b	5.01 ± 0.02 ^a	68.9 ± 0.0 ^a	1.58 ± 0.00 ^b	4.37 ± 0.00 ^c
12	50	30	10	0	20	123	11.46 ± 0.00 ^a	222 ± 12 ^a	1.96 ± 0.01 ^a	5.97 ± 0.00 ^a	5.05 ± 0.00 ^a	68.6 ± 0.0 ^a	1.60 ± 0.00 ^a	4.44 ± 0.00 ^b
13	55	0	30	10	20	121	11.23 ± 0.01 ^b	203 ± 5 ^a	1.83 ± 0.01 ^c	5.90 ± 0.00 ^b	4.93 ± 0.00 ^a	68.4 ± 0.0 ^a	1.59 ± 0.00 ^b	4.55 ± 0.00 ^a
14	55	10	20	0	30	123	11.27 ± 0.03 ^b	207 ± 14 ^a	1.82 ± 0.00 ^c	5.89 ± 0.00 ^c	4.96 ± 0.01 ^a	68.5 ± 0.0 ^a	1.61 ± 0.01 ^a	4.41 ± 0.00 ^c
15	55	20	10	30	0	123	11.50 ± 0.06 ^a	210 ± 8 ^a	1.92 ± 0.01 ^b	5.90 ± 0.00 ^b	4.95 ± 0.04 ^a	67.0 ± 0.1 ^c	1.61 ± 0.00 ^a	4.45 ± 0.00 ^b
16	55	30	0	20	10	125	11.45 ± 0.03 ^a	213 ± 0 ^a	1.94 ± 0.01 ^a	5.98 ± 0.00 ^a	4.97 ± 0.01 ^a	67.6 ± 0.0 ^b	1.59 ± 0.00 ^b	4.45 ± 0.00 ^b
17	100	0	10	10	10	94	11.27 ± 0.00	238 ± 10	2.04 ± 0.00	6.08 ± 0.00	4.86 ± 0.01	67.1 ± 0.1	1.67 ± 0.01	4.44 ± 0.00
18	0	0	10	10	10	91	11.43 ± 0.05	183 ± 6	1.71 ± 0.00	5.99 ± 0.00	5.05 ± 0.03	68.8 ± 0.1	1.52 ± 0.02	4.36 ± 0.00

Batches 17 and 18 were control samples prepared with all-wheat malt and all-barley malt. WMP: wheat malt percentage; T37: resting time at 37°C; T43: resting time at 43°C; T50: resting time at 50°C; T63: resting time at 63°C; TT: total saccharification time; OE: original extract; α -AN: α -amino nitrogen; and RDF: real degree of fermentation. Data are mean ± standard deviation of triplicate analyses ($n = 3$). Different lowercase superscripts in the same column with the same wheat malt percentage indicate significant difference by the Tukey's test ($p < 0.05$).

KQ-250DE, Kunshan Ultrasonic Instruments Co., Ltd., China).

Enzymatic hydrolysis and dialysis

The enzymatic hydrolysis of concentrated samples was performed according to Goñi *et al.* (2009a) with slight modifications. First, the sample pH was adjusted to 1.6 with 1 mol/L HCl solution, and then it was mixed thoroughly with 20 mL of 0.1 mol/L HCl-KCl buffer (pH 1.6) and 0.5 mL of pepsin solution (400 U/mL, 1 mg of pepsin was dissolved in 1 mL of 0.1 mol/L HCl-KCl solution, pH 1.6). After incubating at 40°C for 40 min in a water bath (HHW-4, Jintan Shuangjie Experimental Instrument Factory, China) with constant shaking, pH was adjusted to 6.9 with 1 mol/L NaOH. Then 20 mL of 0.1 mol/L Tris-maleate buffer (pH 6.9) and 5 mL of α -amylase solution (2,100 U/mL, 60 mg α -amylase in 1 mL of 0.1 mol/L Tris-Maleate buffer, pH 6.9) were added to incubate at 37°C for 3 h. Finally, pH was adjusted to 4.5 with 1 mol/L HCl solution. Next, 20 mL of sodium acetate buffer (0.2 mol/L, pH 4.5) and 0.4 mL of amyloglucosidase solution were added and incubated in the same shaking water bath at 60°C for 45 min. The enzyme-treated sample was transferred into a dialysis bag (12,000 - 14,000 Da molecular weight cutting off) and dialysed against deionised water at 25°C for 72 h.

Determination of the soluble dietary fibre content

The dialysis retentate was concentrated to 100 mL by the rotary evaporation (45°C, -0.1 Mpa; RE5298, Shanghai Yarong Biochemical Instrument Factory, China) for 50 min, transferred to a 100-mL volumetric flask, and adjusted to 100 mL to determine the contents of PP, NSP, and RP associated with SDF.

Determination of polyphenols

The contents of PP were determined according to He *et al.* (2013). Briefly, 0.1 mL of dialysis retentate in the 100-mL volumetric flask was diluted to 1 mL with deionised water, and mixed with 5 mL of Folin-Ciocalteu phenol diluent (50 mL of Folin-Ciocalteu phenol reagent was diluted to 500 mL with deionised water). After 1 min, 4 mL of sodium carbonate solution (75 g/L) was added and mixed completely. Reaction was performed at 75°C water bath for 10 min, and samples were immediately cooled to room temperature by running water, then their absorbance at 765 nm was measured. The measurement was compared with a calibration curve of prepared gallic acid standard solutions, and the results were expressed as milligrams of gallic acid

equivalents per litre of sample (mg/L).

Determination of non-starch polysaccharides

NSP was determined according to Englyst and Cummings (1988) with appropriate modification. Briefly, 0, 0.2, 0.4, 0.6, 0.7, 0.8, 1.0, and 1.2 mL of glucose standard solution (1 mg/mL) was pipetted into 25-mL colorimetric tubes, and then deionised water was added to a total volume of 2 mL. Next, 2 mL of 3, 5-dinitrosalicylic acid solution reagent were added and heated in a boiling water bath for 5 min. Then, they were cooled under running tap water immediately, followed by adding deionised water to 25 mL and mixing well. The absorbance at 540 nm was measured to construct the standard curve.

The dialysis retentate was hydrolysed with 1 mol/L sulphuric acid (100°C, 1.5 h), and then neutralised with 4 mol/L NaOH. The hydrolysate (0.5 mL) was transferred into a 25-mL colorimetric tube to follow the steps above. The concentration of reducing sugar was calculated from the standard curve. The results were expressed as milligrams of glucose equivalents per litre of sample (mg/L).

$$\text{Total NSP (mg/L)} = 0.89 \times \text{Reducing sugar content} \quad (\text{Eq. 1})$$

where, 0.89 = conversion factor of monosaccharides to polysaccharides.

Determination of resistant protein

The contents of RP were determined by Bradford assay (Bradford, 1976). After two times dilution, 100 μ L of the dialysis retentate was mixed with 5 mL of Coomassie Brilliant Blue solution, and vortexed immediately. After 3 min, the absorbance at 595 nm was measured. A standard curve was constructed with the bovine serum albumin solutions of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg/mL according to the method described above.

Statistical analysis

Data were processed using SPSS Statistics 21. Variance analysis (ANOVA/Tukey) was performed to compare SDF composition of wort and wheat beers with the same WMP; and the difference of $p < 0.05$ was considered to be significant. Correlation analysis was conducted using Pearson correlation (double-tailed test). The main effect of the mean value was analysed by Minitab 17 to optimise the optimal wort preparing parameters.

Results and discussion

Physicochemical indicators of wheat worts and beers

The physicochemical indicators of 16 batches of wheat worts and corresponding beers are shown in Table 1, and their correlations with wort preparing parameters are shown in Table 2. In the wort samples (Table 1), original extract was 11.23 - 11.78 °P, α -AN was 166 - 287 mg/L, viscosity was 1.70 - 1.97 mPa·s, and pH was 5.83 - 6.23. Among them, wort 4 possessed the highest content of α -AN and pH value but the lowest viscosity, because the rest time in each rest period and TT were longer than the other batches (Table 1). Extending TT could increase α -AN content but decreased viscosity ($R = 0.680^{**}$ and -0.538^* , respectively) while prolonging T43 was conducive to α -AN production ($R = 0.546^*$) (Table 2).

As shown in Table 1, wheat beers were characterised by alcohol (4.87 - 5.22%), RDF (67.0 - 69.6%), viscosity (1.57 - 1.61 mPa·s), and pH (4.20 - 4.55). The viscosity and pH of beers significantly decreased as compared to wort. No correlation was found between beer viscosity and TT, thus indicating that TT had small influence on beer viscosity though TT decreased wort viscosity as stated earlier. Higher pH beer was obtained by prolonging T43 ($R = 0.585^*$) (Table 2). Our previous study found that 43°C was conducive to increase soluble protein level in wheat beer (Wu *et al.*, 2015). Picariello *et al.* (2015) identified 58 proteins from wheat beer, and the isoelectric points of 53 proteins were higher than 5.0. And also, proteins are zwitterions, which act as weak acids and bases by donating and accepting protons in solution. Therefore, proteins with an isoelectric point higher than the pH of beer can

provide buffering capacity for beer pH through protonation (O'Rourke, 2002). Alcohol and RDF were significantly positively correlated with T63 ($R = 0.515^*$, 0.896^{**} , respectively) (Table 2), thus demonstrating again that extending T63 was favourable to produce fermentable sugar (Durand *et al.*, 2009).

Effect of wort preparing parameters on SDF composition in wheat worts

In wort, SDF was 1,923.1 - 2,443.7 mg/L; PP, NSP, and RP were 116.8 - 149.7, 1,511.5 - 1,963.5, and 272.9 - 330.5 mg/L, respectively. NSP was the dominant component and accounted for 77.13 - 81.42% of total SDF (Table 3). NSP and even SDF in wort was increased by improving WMP ($R = 0.684^{**}$ and 0.671^{**} , respectively). In addition, more NSP and RP was found in all-wheat malt wort and beer than that in all-barley malt wort and beer (Table 3). This may be attributed to the soluble arabinoxylan and soluble nitrogen in wheat malt (0.98 and 0.65 - 0.78%, respectively), which were higher than that in barley malt (0.42 - 0.70 and 0.60-0.70%, respectively) (Li *et al.*, 2005; Faltermaier *et al.*, 2014). Unfortunately, according to our previous research (Hu *et al.*, 2018), pure wheat malt beer obtained a poor foaming characteristic. We considered that the reason is the lack of foam-forming 'skeleton protein' in wheat malt, but the protein components from wheat malt kept the foam stable. PP had a significant correlation with T37 and T63 (-0.565^* and 0.642^{**} , respectively) (Table 4). According to Vanbeneden *et al.* (2008), free ferulic acid was released most at 40°C during mashing, and the cinnamoyl esterase was rapidly denatured at temperatures exceeding 60°C. Therefore, shortening

Table 2. Correlation coefficients of physicochemical indicators and wort preparing parameters in wheat wort and beer.

Parameter	Wheat wort				Wheat beer			
	OE	α -AN	Viscosity	pH	Alcohol	RDF	Viscosity	pH
WMP	-0.470	-0.107	0.303	-0.301	-0.474	-0.265	0.336	0.373
T37	0.200	0.305	-0.210	0.145	0.139	0.032	-0.192	0.198
T43	-0.118	0.546*	-0.319	0.057	-0.107	-0.069	-0.192	0.585*
T50	0.252	0.425	0.093	0.301	0.165	0.000	-0.192	0.432
T63	-0.059	0.120	-0.373	0.431	0.515*	0.896**	-0.192	0.322
TT	0.324	0.680**	-0.538*	0.430	0.397	0.303	-0.525*	0.345

OE: original extract; α -AN: α -amino nitrogen; RDF: real degree of fermentation; WMP: wheat malt percentage; T37: resting time at 37°C; T43: resting time at 43°C; T50: resting time at 50°C; T63: resting time at 63°C; TT: total saccharification time; **: significantly correlated at the 0.01 level; and *: significantly correlated at the 0.05 level.

Table 3. SDF composition in wheat wort and beer.

Batch	Wheat wort					Wheat beer				
	PP (mg/L)	NSP (mg/L)	RP (mg/L)	SDF (mg/L)	PP (mg/L)	NSP (mg/L)	RP (mg/L)	SDF (mg/L)		
1	128.1 ± 1.3 ^a	1511.5 ± 8.0 ^b	283.6 ± 2.9 ^c	1923.1 ± 12.1 ^b	104.2 ± 0.7 ^b	1432.1 ± 20.2 ^c	264.8 ± 3.9 ^c	1801.1 ± 21.7 ^c		
2	128.5 ± 0.9 ^a	1617.7 ± 10.9 ^a	288.1 ± 2.4 ^{bc}	2034.4 ± 11.7 ^a	103.3 ± 1.5 ^b	1493.8 ± 22.9 ^b	266.4 ± 4.1 ^{bc}	1863.5 ± 18.4 ^b		
3	128.0 ± 1.5 ^a	1602.1 ± 19.0 ^a	295.2 ± 4.6 ^{ab}	2025.3 ± 13.7 ^a	114.6 ± 2.6 ^a	1589.8 ± 18.5 ^a	274.6 ± 2.5 ^{ab}	1978.9 ± 21.0 ^a		
4	123.5 ± 0.7 ^b	1526.1 ± 10.8 ^b	301.1 ± 5.7 ^a	1950.6 ± 14.9 ^b	93.3 ± 1.4 ^c	1335.5 ± 24.5 ^d	276.6 ± 3.4 ^a	1705.4 ± 28.5 ^d		
5	145.1 ± 1.3 ^a	1600.3 ± 10.4 ^b	329.4 ± 7.3 ^a	2074.9 ± 15.1 ^b	110.4 ± 0.5 ^a	1505.7 ± 17.2 ^b	314.9 ± 5.0 ^a	1931.1 ± 20.6 ^{ab}		
6	133.1 ± 1.5 ^b	1670.0 ± 20.4 ^a	328.2 ± 3.8 ^a	2131.4 ± 24.7 ^a	100.5 ± 1.3 ^c	1499.7 ± 17.6 ^b	310.2 ± 3.3 ^a	1910.5 ± 16.4 ^b		
7	116.8 ± 1.2 ^c	1564.4 ± 5.9 ^b	284.2 ± 2.4 ^b	1965.4 ± 3.7 ^c	107.3 ± 1.2 ^b	1605.9 ± 29.9 ^a	267.3 ± 1.7 ^b	1980.5 ± 31.6 ^a		
8	120.3 ± 1.9 ^c	1575.9 ± 26.3 ^b	272.9 ± 4.5 ^b	1969.1 ± 28.0 ^c	107.1 ± 1.2 ^b	1624.7 ± 10.5 ^a	252.1 ± 3.9 ^c	1984.0 ± 6.2 ^a		
9	132.1 ± 1.8 ^b	1862.7 ± 12.3 ^b	292.9 ± 3.5 ^b	2287.8 ± 10.3 ^{bc}	113.3 ± 1.1 ^a	1691.6 ± 28.0 ^a	253.3 ± 3.0 ^c	2058.2 ± 27.6 ^a		
10	127.7 ± 0.5 ^c	1876.3 ± 9.1 ^{ab}	311.3 ± 2.0 ^a	2315.3 ± 7.0 ^{ab}	115.3 ± 0.3 ^a	1633.0 ± 17.5 ^b	262.8 ± 4.0 ^b	2011.2 ± 21.0 ^a		
11	144.7 ± 1.4 ^a	1898.2 ± 18.4 ^a	297.5 ± 3.6 ^b	2340.4 ± 22.9 ^a	110.5 ± 1.1 ^b	1443.9 ± 10.2 ^d	282.0 ± 3.2 ^a	1836.4 ± 11.0 ^c		
12	128.5 ± 1.7 ^{bc}	1818.1 ± 10.5 ^c	312.5 ± 3.7 ^a	2259.1 ± 12.5 ^c	108.7 ± 0.5 ^b	1577.2 ± 12.6 ^c	271.3 ± 3.4 ^b	1957.2 ± 10.6 ^b		
13	149.7 ± 1.6 ^a	1963.5 ± 19.4 ^a	330.5 ± 3.2 ^a	2443.7 ± 20.8 ^a	117.72 ^{0a}	1754.6 ± 5.1 ^a	290.5 ± 3.1 ^a	2162.8 ± 4.1 ^a		
14	143.4 ± 1.1 ^b	1856.3 ± 16.4 ^b	278.4 ± 2.1 ^d	2278.1 ± 16.4 ^b	118.1 ± 0.3 ^a	1674.8 ± 18.4 ^b	253.0 ± 5.2 ^c	2045.8 ± 15.8 ^b		
15	119.0 ± 1.6 ^d	1633.4 ± 21.1 ^c	292.0 ± 2.0 ^c	2044.5 ± 20.3 ^c	104.5 ± 2.3 ^c	1584.6 ± 18.4 ^c	247.6 ± 2.6 ^c	1936.7 ± 14.5 ^c		
16	126.2 ± 1.8 ^c	1643.9 ± 18.4 ^c	310.5 ± 4.1 ^b	2080.6 ± 19.0 ^c	110.6 ± 1.5 ^b	1657.8 ± 13.2 ^b	276.1 ± 1.5 ^b	2044.6 ± 14.1 ^b		
17	153.9 ± 0.7	1985.1 ± 24.0	336.2 ± 3.0	2475.2 ± 21.8	120.1 ± 1.4	2158.9 ± 21.3	331.5 ± 3.7	2610.5 ± 24.1		
18	110.4 ± 1.8	1554.6 ± 10.5	266.1 ± 5.4	1931.1 ± 13.7	93.1 ± 1.0	1294.4 ± 8.1	215.7 ± 5.0	1603.3 ± 2.1		
VT	196.7 ± 0.8	1630.4 ± 13.4	466.1 ± 5.1	2293.3 ± 9.1	155.4 ± 1.1	1600.3 ± 28.2	422.0 ± 10.8	2177.7 ± 38.1		
PV	147.2	1872.0	322.9	2342.1	125.7	1834.9	283.8	2244.4		

Batches 17 and 18 were control samples prepared with all-wheat malt and all-barley malt. PP: polyphenols; NSP: non-starch polysaccharides; RP: resistant protein; SDF: soluble dietary fibre; VT: verification test; and PV: predicted value. The content of each indicator has been standardised according to the original extract of 11 °P. Variance analyses of four groups of wort with the same WMP were performed by SPSS Statistics 21. Data are mean ± standard deviation of triplicate analyses (*n* = 3). Different lowercase superscripts in the same column with the same wheat malt percentage indicate significant difference by the Tukey's test (*p* < 0.05).

Table 4. Correlation coefficients of SDF composition and wort preparing parameters in wheat wort and beer.

Wheat wort					Wheat beer				
Indices	PP	NSP	RP	SDF	Indices	PP	NSP	RP	SDF
WMP	0.315	0.684**	0.205	0.671**	WMP	0.565*	0.675**	-0.179	0.668**
T37	-0.565*	-0.277	-0.246	-0.317	T37	-0.341	-0.169	-0.242	-0.229
T43	-0.117	0.163	-0.097	0.132	T43	0.215	0.343	-0.259	0.306
T50	-0.126	-0.097	0.355	-0.058	T50	-0.296	-0.122	0.196	-0.104
T63	0.642**	0.234	0.365	0.294	T63	0.046	-0.255	0.566*	-0.150
TT	-0.264	-0.294	0.007	-0.286	TT	-0.609*	-0.468	0.064	-0.486
PP		0.630**	0.483	0.694**	PP		0.789**	-0.117	0.817**
NSP			0.304	0.991**	NSP			-0.280	0.984**
RP				0.420	RP				-0.109

WMP: wheat malt percentage; PP: polyphenols; NSP: non-starch polysaccharides; RP: resistant protein; SDF: soluble dietary fibre; T37: resting time at 37°C; T43: resting time at 43°C; T50: resting time at 50°C; T63: resting time at 63°C; and TT: total saccharification time. Correlation analysis was carried out by Pearson correlation via SPSS Statistics 21. **: significantly correlated at the 0.01 level; and *: significantly correlated at the 0.05 level.

T37 and prolonging T63 was conducive to PP increase. The main effects of SDF composition in wort are presented in Figure 1. For wort with the highest SDF content, the optimal parameters were WMP, 50%; T37, 0 min; T43, 30 min; T50, 10 min; and T63, 20 min.

Effect of wort preparing parameters on the SDF composition in wheat beers

In beers, SDF was 1,705.4 - 2,162.8 mg/L; PP, NSP, and RP was 93.3 - 118.1, 1,335.5 - 1,754.6, and 247.6 - 314.9 mg/L, respectively (Table 3), but they all decreased in comparison to that in wort. NSP was also the main component of SDF ($R = 0.984^{**}$) (Table 4) in beers, and accounted for 77.97 - 82.19% of SDF. The proportion was slightly higher than that in wort because proteins and polyphenols were the main components of precipitation formed during fermentation and maturation (Steiner *et al.*, 2010).

The correlation coefficients among SDF and its composition, and wort preparing parameters of wheat beers are also listed in Table 4. Similar to that in wort, NSP and SDF contents in beers were most heavily impacted by WMP ($R = 0.675^{**}$ and 0.566^* , respectively), while RP was most heavily impacted by T63 (0.668^{**}) (Table 4). PP was significantly related to NSP, SDF, WMP, and TT ($R = 0.789^{**}$, 0.817^{**} , 0.565^* , and -0.609^* , respectively). Figure 1 demonstrates the main effect of wort preparing parameters on SDF composition in beers. To increase SDF content in wheat beer, the optimal parameters were WMP, 55%; T37, 0 min; T43, 20 min; T50, 20 min; and T63, 20 min. In contrast to the optimal parameters of wort, a higher WMP was adopted for

beer because wheat malt was the main source of SDF in the wheat beer. And shorter T43 was adopted for beer in comparison to the optimal parameters of wort. Although 43°C was conducive to increasing solubility of arabinoxylan and protein, unfortunately, some of them were lost during fermentation (Steiner *et al.*, 2010). The decrease in pH was considered to be the main cause of protein precipitation during fermentation (Steiner *et al.*, 2011). At the same time, increasing the fermentation temperature might also reduce the loss of cold solid protein during the fermentation process. In addition, longer T50 was adopted for beer in comparison to the optimal parameters of wort. Our previous study reported that T50 was conducive for the production of arabinoxylan during mashing for wheat beer (Li *et al.*, 2017).

The correlation of SDF composition among wheat wort and beer is listed in Table 5, indicating that the majority of PP, NSP, and SDF were retained in the beers after fermentation and maturation ($R = 0.511^*$, 0.540^* , and 0.561^* , respectively). In particular, the most of RP in wort was retained in beers ($R = 0.815^{**}$). As a result, SDF level in wheat beer could be effectively optimised by adjusting wort preparing parameters especially increasing WMP.

Wheat beer contained a higher SDF content than lager and dark beers (1.87 - 2.02 g/L), and had higher PP and RP than lager beer (Goñi *et al.*, 2009b). High content of soluble protein in wheat wort and beer (Hu *et al.*, 2018) would be the reason that more RP was found in the samples in Table 3. In addition, PP accounted for 5.26 - 6.02% of SDF in wheat beer which was significantly higher than the proportion of SDF from other foods, such as cereals

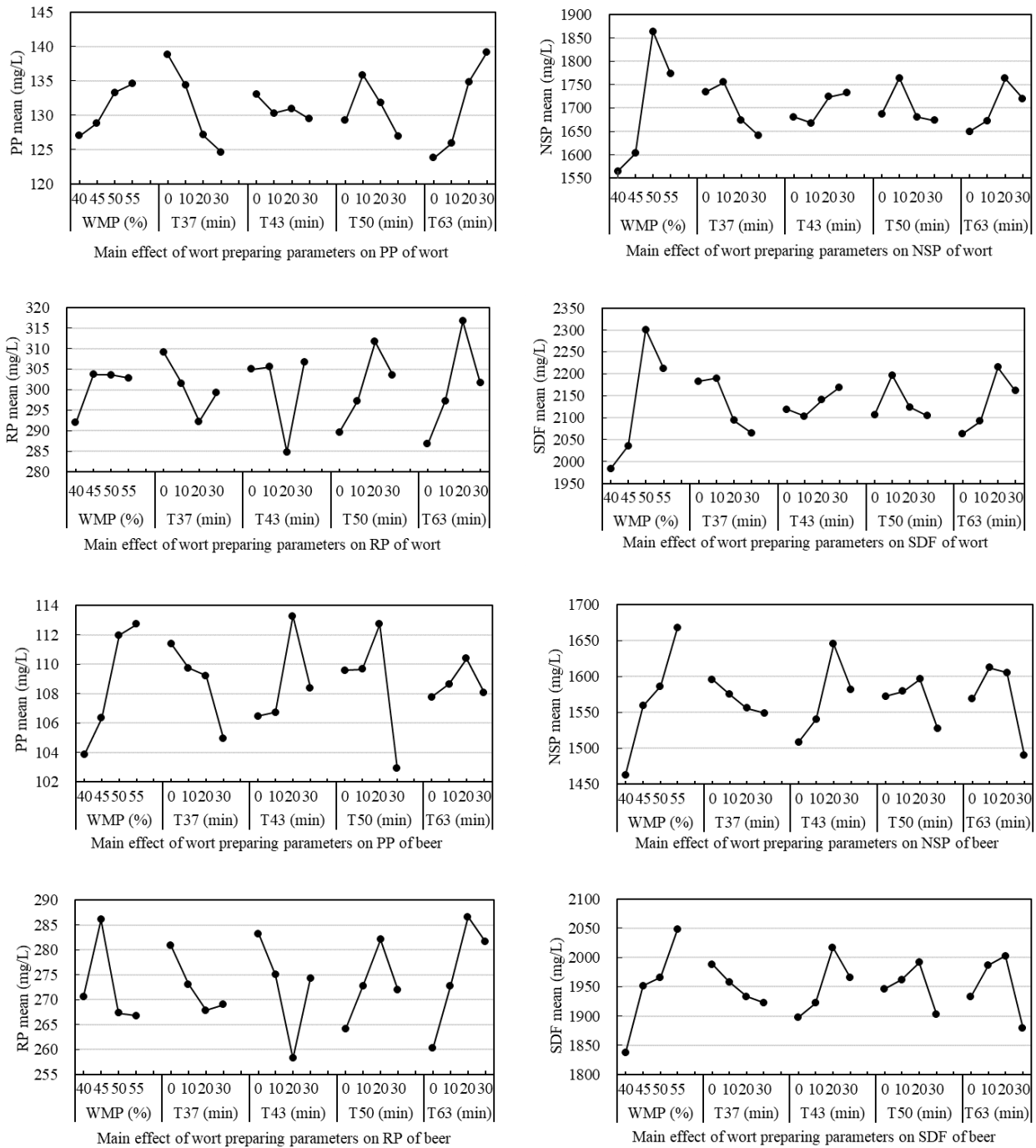


Figure 1. Main effects of wort preparing parameters on SDF composition in wheat wort and beer. PP: polyphenols; NSP: non-starch polysaccharides; RP: resistant protein; SDF: soluble dietary fibre; WMP: wheat malt percentage; T37: resting time at 37°C; T43: resting time at 43°C; T50: resting time at 50°C; and T63: resting time at 63°C. The main effect of wort preparing parameters on SDF composition in wheat wort was obtained by taking the mean value of the corresponding components in wort that matched wort preparing parameters as the ordinate and wort preparing parameters as the abscissa.

(0.11%), vegetables (0.12%), and fruits (0.12%) (Goñi *et al.*, 2009a). Therefore, SDF in wheat beer might have better physiological characteristics because it contained more RP and PP. Further study would be performed to explore the functional properties and impact on beer quality of SDF and its components. The optimisation of SDF content in

wheat beer changed the content of various components in wort and beer, which might affect the final quality of beer. For example, the temperature increase without any pause during mashing will decrease the concentrations of histidine and tryptophan (Carvalho *et al.*, 2018), and the composition and content of amino acids would affect

Table 5. Correlation coefficients of SDF composition in wheat wort and beer.

	Indicator	Wheat wort			
		PP	NSP	RP	SDF
Wheat beer	PP	0.511*	0.681**	0.077	0.666**
	NSP	0.152	0.540*	0.044	0.511*
	RP	0.515*	-0.024	0.815**	0.100
	SDF	0.270	0.569*	0.190	0.561*

PP: polyphenols; NSP: non-starch polysaccharides; RP: resistant protein; and SDF: soluble dietary fibre. Correlation analysis was performed by Pearson correlation via SPSS Statistics 21. **: significantly correlated at the 0.01 level; and *: significantly correlated at the 0.05 level.

the final quality of beer (Aredes *et al.*, 2021). In addition, zinc ions will form complexes with proteins and be removed together during boiling process (Sterczyńska *et al.*, 2020). Calcium and magnesium ions can promote the formation of salt bridges and flocculation of negatively charged proteins, and can also reduce the surface charge of proteins to promote precipitation. Therefore, the influencing factors of SDF in wheat beer need to be further explored.

Verification tests

Verification test and prediction results of high SDF wheat wort and beer by the optimised parameters are shown in Table 3. The content of SDF in wheat wort and wheat beer was 2,293.25 and 2,177.71 mg/L, respectively, which was consistent with the predicted values, though the three compositions had some difference from the predicted values. NSP content in the verification test was lower than the predicted value, while PP and RP contents were higher than the predicted value. The verification test removed T37, which would prevent PP and RP from degrading to micromolecules. It would promote more PP dissolution by resting for 20 min at 43, 50 and 63°C, respectively; while it would promote more RP to dissolve in the wort and beer by resting for 20 min at 50 and 63°C, respectively (Figure 1). The content of NSP and SDF in the verification test was still lower than that of all-wheat malt beer, which proved again that NSP and even SDF were most affected by WMP (Figure 1).

Conclusion

The wort preparing parameters affected the physicochemical indexes of wort and beer. Extending TT increased α -AN content but decreased viscosity, while prolonging T43 was conducive to α -AN production. No correlation was found between beer viscosity and TT, though TT decreased wort

viscosity. Hence, a higher pH beer was obtained by prolonging T43.

Wheat beer was an ideal source of quality SDF. The primary SDF composition in wheat beer was NSP (77.97 - 82.19%), followed by RP (12.31 - 16.31%) and PP (5.26 - 6.02%). NSP was most influenced by WMP, PP was affected by both WMP and mashing process, while RP was most affected by mashing process. Furthermore, the all-wheat malt beer possessed more SDF, in which NSP, RP, and PP were more than that in the all-barley beer. The optimised wort preparing parameters for high wort SDF was quite different from that for wheat beer because NSP, RP, and PP would precipitate in fermentation and the subsequent maturation, and the majority of SDF, NSP, RP, and PP in wort remained in beer. Therefore, a wheat beer with ideal level of SDF could be obtained by modulating WMP and mashing process parameters in beer brewing. Further studies would be performed to explore the functional properties of SDF in wheat beer, especially the effects on beer quality and benefits on human health.

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