The Effect of Pitching Rate on Fermentation and Flavour Compounds in High Gravity Brewing

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ABSTRACT

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The effect of pitching rate on fermentation and production of flavour compounds was studied in high gravity wort using a lager yeast. Fermentation performance was followed by monitoring the total sugar content and yeast growth. Volatile compounds were evaluated by analysing higher alcohols, esters and carbonyl compounds at the end of fermentation. Faster fermentation rates and higher yeast counts were observed with the higher pitching levels. Lower amounts of 2- and 3-methyl-1-butanols and higher levels of 2-methyl-1-propanol were found at the increased pitching rates. The concentration of isoamyl acetate was reduced with an increased pitching rate. Higher amounts of diacetyl and 2,3-pentanedione were obtained at the lower pitching levels.

Key words: Fermentation, flavour compounds, high gravity brewing, pitching rate, *Saccharomyces cerevisiae*

INTRODUCTION

High gravity brewing can be described as a procedure which employs wort at higher than normal extract. It subsequently requires diluting the beer, usually with oxygen free water, at a later stage in processing and before packaging. There are a number of advantages to high gravity brewing: increased brewing capacity, hence more efficient use of existing plant facilities, reduced energy, labour, cleaning and effluent costs, improved physical and flavour stability of beer, more alcohol per unit of fermentable extract due to reduced yeast growth, higher adjunct rates, smoother taste and greater flexibility. The disadvantages are as follows: decreased brewhouse material efficiency and hop utilisation, decreased foam stability, problems of flavour match and a negative effect on yeast performance due to osmotic pressure and ethanol concentrations[13–16].

The problems arising from higher than normal gravity could be solved by altering the fermentation temperature, initial oxygen level and/or using higher pitching rates[13,16]. Pitching rates range from 5–20 million cells/mL wort[14]; however during traditional lager production, fermentation is generally initiated by pitching 6–12 million cells/mL wort[4,5,14]. There are various studies on the effects of pitching levels on beer fermentation and flavour compounds, but contradictory results have been reported[2,7,12,16]. Anderson and Kirsop[2] stated that an increased pitching rate decreased the levels of ethyl acetate and isoamyl acetate. Slaughter and McKernan[12] showed that inoculum size affected yeast properties and production of flavour compounds, but seemed to have little effect on ethanol production. They also stated that it was difficult to draw general correlations between all the properties studied and the inoculum size. Suihko et al.[16] reported that as the pitching rate increased, the maximum amount of yeast in the fermenting wort increased and the formation of ethyl acetate and isoamyl acetate decreased, but the effect of pitching rate on the formation of diacetyl and higher alcohols was not clear. Work by Edelen et al.[7] showed that increasing the pitching rate had a significant effect on beer fermentations in terms of shortening fermentation hours, reaching higher yeast peak counts, lowering ester levels and increasing levels of some higher alcohols.

During the alcoholic fermentation of wort, in addition to the major products of ethanol and carbon dioxide, the yeast *Saccharomyces cerevisiae,* excretes a wide range of flavour compounds including higher alcohols (also called fusel oils), esters, carbonyls, sulphur compounds, organic and fatty acids, and a number of miscellaneous compounds. The production of these compounds relates to the overall metabolic balance of the yeast culture. These flavour compounds have a significant effect on the flavour of beer, wine and other alcoholic beverages. Higher alcohols are formed from the catabolic route (Ehrlich pathway) in the presence of amino acids and from the anabolic route from sugars via biosynthesis. In both pathways, the produced keto acid is decarboxylated to the corresponding aldehyde and then the resultant aldehyde is reduced to the corresponding higher alcohol. Esters, which provide fruity and flowery flavours to beers, are mainly produced by yeast from an alcohol fermentation from an activated fatty-acyl CoA molecule and an alcohol catalysed by alcohol acetyltransferase and other enzymes. Carbonyl compounds include aldehyde and ketones. Acetaldehyde is the principal aldehyde produced via decarboxylation of pyruvate. Vicinal diketones, which produce butterscotch flavours in beer, arise as by-products of the pathways leading to valine and isoleucine formation[14,17].

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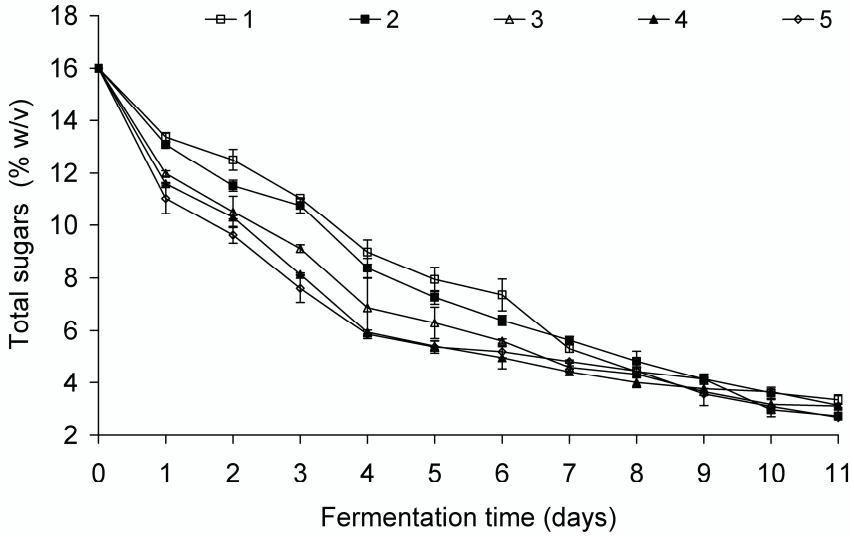

Fig. 1. Total sugar utilization at five different pitching rates. 1) 1×10^7 cells/mL, 2) 2.5×10^7 cells/mL, 3) 5×10^7 cells/mL, 4) 7.5×10^7 cells/mL, 5) 1×10^8 cells/mL.

In this paper, the effect of pitching rate on fermentation and flavour compounds in high gravity wort was studied.

MATERIALS AND METHODS

Yeast and wort

A lager brewing strain of *Saccharomyces cerevisiae* (NCYC 1056) was used throughout. The all-malt wort, 16% (w/v) total sugars, was obtained from the Efes Pilsen Brewery (Adana, Turkey). Pitching yeast was grown aerobically in sterile high gravity wort with 16% (w/v) total sugars at 25°C for 48 h with orbital shaking at 160 rpm. Yeast cells were centrifuged at 4000 rpm for 10 min at 4°C and washed twice with cold sterile water. The pellet was re-suspended in 5 mL of sterile wort. After counting (Thoma haemocytometer) the required amount was added to the fermentation medium[8].

Fermentation procedure

Fermentations were carried out in duplicate, in 1000 mL sterile conical flasks containing 800 mL of sterile high gravity wort. Yeast cells were added to the fermentation vessels at levels of 1×10^7, 2.5×10^7, 5×10^7, 7.5×10^7 and 1×10^8 viable cells/mL wort. All vessels were closed with foam bungs and incubated at 10°C throughout the fermentation. Fermentations were monitored daily by measuring total sugars and performed until about 80% of the total sugars had been consumed.

Fermentation analysis

Yeast cells and viable counts were performed using a Thoma haemocytometer. Viable cells were assessed using the methylene blue stain and ethanol was determined by pycnometer at 20°C after distillation[3]. Total sugars were analysed according to Catley[6] and Amrane and Prigent[1].

Volatile higher alcohols, esters and carbonyl compounds were analysed by gas chromatograph (HP 5890, Hewlett-Packard, Stockport, UK). Samples were centrifuged at 4°C in capped tubes to remove the yeast cells. The cell-free samples were diluted to 4% ethanol and 5 mL of diluted sample, 2 g of NaCl and 50 µL of internal standard (a mixture of 200 mg/L of 3-heptanone and 18.2 mg/L of hexanedione) were added into vials, sealed and a 1 mL sample was injected into a column by headspace auto sampler (Perkin Elmer, MA, USA). The column was a Chrompack CP-Wax-57-CB (Chrompack, Netherlands) with the following dimensions: 60 m long, 0.25 mm ID and 0.4 µm thick. The column temperature was kept at 43°C for 2 min and increased to 92°C at 1.8°C/min. It was then increased from 92°C to 180°C at 30°C/min and held at that temperature for 4 min. The stream from the column was split 1:1 to Flame Ionisation Detector and Electron Capture Detector. The carrier gas was helium at a flow rate of 2.2 mL/min. The flavour compounds were tentatively calculated by comparing the retention times with those from calibration standard curves on a HP Chemstation data handling system (Hewlett-Packard, UK)[8].

Statistical analysis

Data of flavour compounds were analysed for statistical significance by one-way analysis of variance (ANOVA). Means were compared by Duncan test statistical analysis carried out using the software SPSS 10.0 for Windows[11].

RESULTS AND DISCUSSION

Fermentation

Fig. 1 shows the patterns of total sugar utilisation with the varying pitching rates. Pitching the high gravity wort at different rates had an effect on fermentation. The rate of fermentation increased in order of increased pitching rate. The consumption of 80% of total sugars was reached by the 11th day of fermentation at 10°C in all trials. Other studies[7,16] on fermentation rates in high gravity brewing have reported that higher pitching rates led to faster fermentations

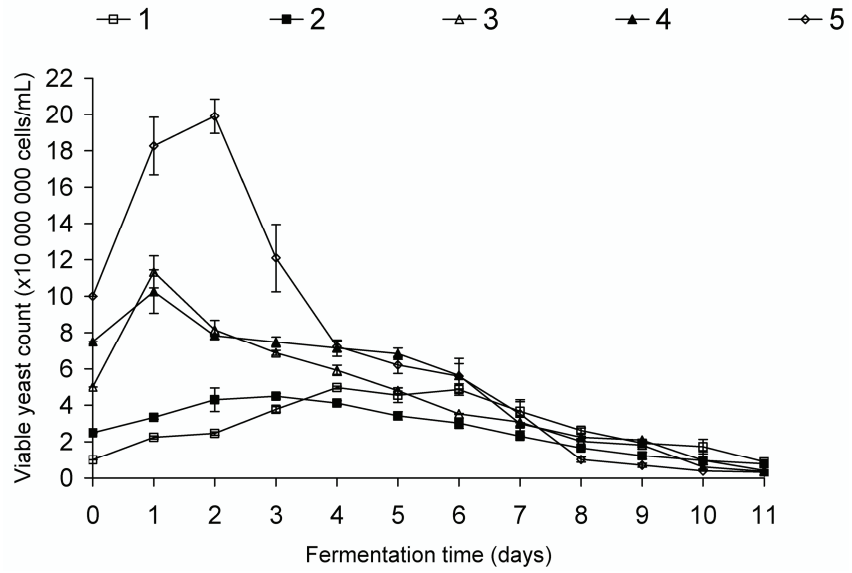

Fig. 2. Yeast growth profile at five different pitching rates. 1) 1×10^7 cells/mL, 2) 2.5×10^7 cells/mL, 3) 5×10^7 cells/mL, 4) 7.5×10^7 cells/mL, 5) 1×10^8 cells/mL.

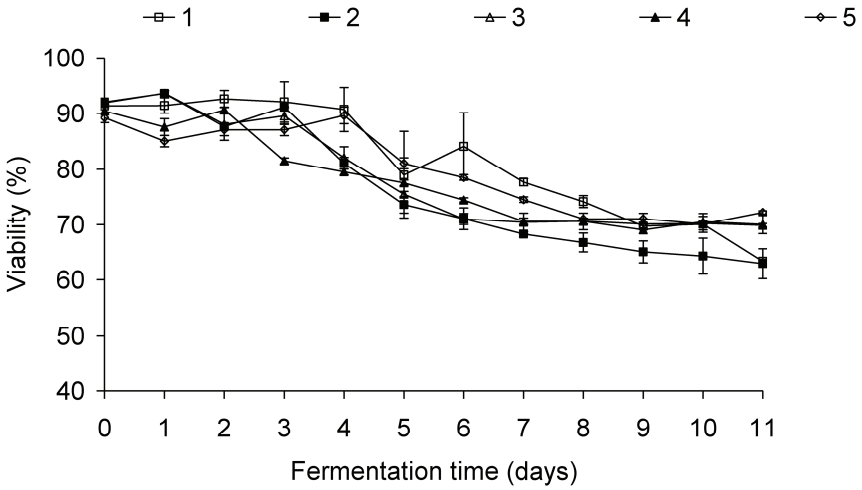

Fig. 3. The percentage viability of yeast cells at five different pitching rates. 1) 1×10^7 cells/mL, 2) 2.5×10^7 cells/mL, 3) 5×10^7 cells/mL, 4) 7.5×10^7 cells/mL, 5) 1×10^8 cells/mL.

In high gravity wort, the ethanol production of the yeast appeared unaffected by the pitching rate. At the pitching level of 2.5×10^7, 5×10^7, 7.5×10^7 and 1×10^8 viable cells/mL, the concentrations of ethanol formed were 6.68, 6.76, 6.87 and 6.83% (v/v), respectively. A slightly lower amount of ethanol (6.46% (v/v)) was obtained when the wort was pitched at 1×10^7 cells/mL. O'Connor-Cox and Ingledew[10] reported that the higher pitching rates (8×10^7 cells/mL) led to higher ethanol concentrations compared to lower pitching rates (1.6×10^7 cells/mL) in high gravity wort (16 ºP) and that the ethanol levels increased from 6.13% (v/v) to 7.63%(v/v) in their experiments.

Viable cell numbers and viability

Fig. 2 illustrates growth profiles of yeast pitched at different rates. With increasing pitching rate, as expected, the maximum viable cell numbers increased. Maximum yeast cell count was 19.9×10^7 cells/mL on day 2 with a pitching rate of 1×10^8 viable cells/mL. Pitching at 5×10^7 and 7.5×10^7 viable cells/mL resulted in maximum numbers of 11.33×10^7 and 10.25×10^7 cells/mL on day 1, respectively. The maximum amount was 4.5×10^7 with a pitching rate of 2.5×10^7 on day 3 and 4.97×10^7 cells/mL with a pitching rate of 1×10^7 viable cells/mL on day 4. After maximum growth, stationary phase was observed. However, after day 6, a decline phase occurred and the yeast viable cell number varied from 3.2×10^6 to 8.7×10^6 cells/mL at the end of fermentation. Edelen et al.[7] and Suihko et al.[16] reported similar growth patterns for the yeasts used in their experiments.

The percentage cell viability was determined using methylene blue and the viabilities can be seen in Fig. 3. At the beginning of the fermentation the viability ranged from 89.2 to 92.0%. A decrease in viability was observed during the fermentation and final values were 63.3, 62.9,

Table I. Effect of five different pitching rates on the concentrations of a number of volatile flavour compounds.

	Pitching rate (viable cells/mL)[y]					
Flavour compounds (mg/L)	**1×10^7**	**2.5×10^7**	**5×10^7**	**7.5×10^7**	**1×10^8**	**Sig.[z]**
Higher alcohols (mg/L)						
n-Propanol	28.05[a,b]	26.01[b,c]	24.09[c]	28.73[a,b]	29.64[a]	*
2-Methyl-1-propanol	26.93[b]	27.23[b]	27.22[b]	31.66[a]	34.27[a]	*
2-Methyl-1-butanol	27.69[a]	25.58[a,b]	21.71[c]	23.59[b,c]	23.76[b,c]	*
3-Methyl-1-butanol	85.59[a]	78.67[a,b]	65.21[c]	69.62[b,c]	68.32[c]	**
Total	**168.26**	**157.49**	**138.23**	**153.6**	**155.99**	
Esters (mg/L)						
Ethyl acetate	13.29	12.13	13.27	13.43	14.19	ns
Ethyl butyrate	0.05	0.06	0.05	0.06	0.05	ns
Isoamyl acetate	0.94[a]	0.69[a,b]	0.26[c]	0.43[b,c]	0.32[b,c]	*
Ethyl hexanoate	0.07	0.056	0.07	0.059	0.056	ns
Ethyl octanoate	0.13[c]	0.14[b,c]	0.14[c]	0.17[a]	0.16[a,b]	*
Total	**14.48**	**13.07**	**13.79**	**14.15**	**14.78**	
Carbonyl compounds (mg/L)						
Acetaldehyde	12.63	9.91	9.2	11.23	15.01	ns
Diacetyl	1.05[a]	0.95[a]	0.58[b]	0.50[b]	0.53[b]	**
2,3 Pentanedione	0.72[a]	0.63[b]	0.43[c]	0.37[c]	0.37[c]	**
Total	**14.4**	**11.49**	**10.21**	**12.1**	**15.91**	
Main Total (mg/L)	**197.14**	**182.05**	**162.23**	**179.85**	**186.68**	

[y] According to the Duncan test means within columns followed by the same letter are not significantly different[11].
[z] Sig: Significance. * and ** display the significance at 5% and 1% respectively by least significant difference. ns: not significant.

69.9, 70.0 and 72.2% with pitching rates of 1×10^7, 2.5×10^7, 5×10^7, 7.5×10^7 and 1×10^8 viable cells/mL wort. Yeast cell viability was low at the end of the fermentation in this study and serial repitching in brewing is not advisable[9] with viabilities of less than 85%.

Production of volatile flavour compounds

The effects of different pitching rates on the concentrations of higher alcohols, esters and carbonyl compounds in high gravity wort are illustrated in Table I. Increasing the pitching rate in high gravity wort led to an increase in the concentration of 2-methyl-1-propanol (isobutanol) and to a decrease in the concentration of 2-methyl-1-butanol (active amyl alcohol) and 3-methyl-1-butanol (isoamyl alcohol). The formation of n-propanol (1-propanol) was not affected by the various pitching rates. Edelen et al.[7], reported similar trends for 3-methyl-1-butanol and 2-methyl-1-propanol. Slaughter and McKernan[12] studied the effect of pitching rate along with pantothenate concentration. They stated that there was a slight tendency for the level of isoamyl alcohols and n-propanol to rise with pitching rate. Slaughter and McKernan[12] also reported that the concentration of 2-methyl-1-propanol decreased with increasing pitching rate, but that there was no evidence of a differential influence of pitching rate with regard to the concentration of pantothenate.

The volatile esters determined in this study were ethyl acetate, isoamyl acetate (3-methylbutyl acetate), ethyl butyrate, ethyl hexanoate (ethyl caproate) and ethyl octanoate (ethyl caprylate). The pitching rate had no clear effect on the concentrations of esters with the exception of isoamyl acetate. Pitching at the lower rates produced a higher concentration of isoamyl acetate. The results obtained for isoamyl acetate in this study are in agreement with the findings of Anderson and Kirsop[2], Edelen et al.[7] and Suihko et al.[16], but for ethyl acetate the results are in disagreement with the findings of Anderson and Kirsop[2] and Suihko et al.[16]. However, Slaughter and McKernan[12] have reported that the concentration of ethyl acetate rose or declined at different pitching rates depending on the pantothenate concentration.

With regard to the carbonyl compounds, the amount of acetaldehyde was not affected by the pitching rates used in these experiments. However at lower pitching rates, the samples had higher concentrations of diacetyl and 2,3-pentanedione.

CONCLUSIONS

As expected, increasing the pitching rates led to faster fermentation rates and higher yeast cell counts. Formation of 2-methyl-1-propanol increased with increasing pitching rate, but the formation of 2- and 3-methyl-1-butanol decreased. The pitching rate did not alter ester formation in these experiments, with the exception of isoamyl acetate, where the level declined with an increased pitching rate. Lower pitching rates led to higher levels of diacetyl and 2,3-pentanedione. The effect of pitching rate on flavour compounds clearly merits further investigation.

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